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Transcriptional Induction of Purple Membrane and Gas Vesicle Synthesis in the Archaebacterium *Halobacterium halobium* Is Blocked by a DNA Gyrase Inhibitor

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We have investigated the expression of the bacteriorhodopsin gene (*bop*) and the gas vesicle protein gene (*gvpA*) in the extremely halophilic archaebacterium *Halobacterium halobium*, using primer-directed reverse transcription of RNA to quantify message levels. The level of *gvpA* gene transcript was found to increase about 5-fold from early to mid-logarithmic growth phase, while the level of *bop* gene transcript increased about 20-fold from mid-logarithmic to stationary phase. Transcriptional induction of both the *gvpA* and *bop* genes was significantly reduced by aeration and almost completely blocked by the DNA gyrase inhibitor novobiocin.

Halobacterium halobium is an extremely halophilic archaebacterium that grows in hypersaline environments (13). The bacteria synthesize purple membrane, a two-dimensional hexagonal lattice of a single integral membrane protein, bacteriorhodopsin (17), which contains a covalently bound retinal chromophore and functions as a light-driven transmembrane proton pump (1, 28). *H. halobium* also synthesizes intracellular gas-filled vesicles, which impart buoyancy and thus increase the availability of light and oxygen to the cells (5, 22). The gas vesicle membrane is lipid-free and contains a single major protein (22). Cultures of *H. halobium* grown at low oxygen concentrations accumulate very high levels of purple membrane and gas vesicles (22, 28, 38). Under such conditions, purple membrane can cover 50% of the cell surface and the bacteria can float. The light-driven proton-pumping activity of bacteriorhodopsin can drive ATP synthesis, which allows *H. halobium* to grow phototrophically (13, 16, 31).

Genetic studies on purple membrane and gas vesicles have focused on the structure of the genes and mRNAs (7, 10, 14) and on high-frequency insertion mutations that occur spontaneously (2, 6–10, 21, 24, 25, 29, 35). Three genes important for purple membrane synthesis have been identified on the *H. halobium* chromosome: *bop*, specifying the purple membrane apoprotein (14), and two genes of possible regulatory function, *brp* (2) and *bat* (25), which are located just upstream of *bop* and transcribed in the opposite direction. Insertions into *brp* or *bat* result in a loss of *bop* gene expression and also a loss of carotenoid pigments (24, 29; our unpublished results). The gas vesicle protein gene *gvpA* has also been cloned and localized to pNRC100, a 190-kilobase-pair plasmid (8; W.-L. Ng and S. DasSarma, *in* F. Rodriguez-Valera, ed., *General and Applied Aspects of Halophilic Microorganisms*, in press). Sequencing and analysis of mutants have shown that the *gvpA* gene is clustered together with three other genes necessary for wild-type levels of gas vesicle synthesis: *gvpC*, which is located downstream, and *gvpD* and *gvpE*, which are upstream and transcribed divergently (6, 8, 21). Additionally, a nearly silent chromosomal gas vesicle locus has been identified (20, 21). Thus, many *H. halobium* genes have been identified and sequenced but their roles in regulation of purple membrane and gas vesicle synthesis remain unclear.

In contrast to the limited knowledge on regulation of gene expression in *H. halobium*, a considerable amount of information is available for eubacteria. Of particular interest to us is the possible correlation between increased DNA supercoiling and anaerobiosis. For example, topoisomerase mutants of *Salmonella typhimurium* have been reported that show altered growth characteristics under strict aerobic or anaerobic conditions (40), and anaerobic induction of the nitrogenase genes of *Rhodopseudomonas capsulata* and *Klebsiella pneumoniae* is blocked by DNA gyrase inhibitors, such as novobiocin (23). These studies and others (reviewed in reference 30) suggest that regulation of gene expression by oxygen is mediated in part by changes in the superhelical density of DNA in eubacteria.

We have recently initiated an investigation into the regulation of bacteriorhodopsin and gas vesicle protein gene expression in *H. halobium*. In this initial report, we show that the *bop* and *gvpA* genes are transcriptionally regulated and that mRNA levels are substantially reduced by aeration of cultures or by addition of novobiocin to the medium.

Measurement of *bop* and *gvpA* gene message levels. We used primer extension assays to measure the mRNA levels for the *bop* and *gvpA* genes in wild-type *H. halobium* NRC-1. The primers for each gene were synthetic decapentameric deoxyribonucleotides which were complementary to the corresponding mRNAs (sequences 5′-CCTGCGATACCCCCT-3′ and 5′-CAAGCCTGAAGAATC-3′). The primers were ^{32}P labeled by T4 polynucleotide kinase and extended by using avian myeloblastosis virus reverse transcriptase on *H. halobium* RNA template which was prepared by phenol extraction. The size of the *bop* cDNA was 45 nucleotides, whereas the *gvpA* cDNA was 47 nucleotides. These sizes are consistent with the previously mapped transcription start sites (7, 10). Each primer extension reaction mixture contained the same amount of crude RNA, which was quantitated by measuring the A_{260} and by ethidium staining after agarose gel electrophoresis. Control experiments showed that the extent of cDNA synthesis is proportional to the abundance of mRNA in the reaction mixture (data not shown).

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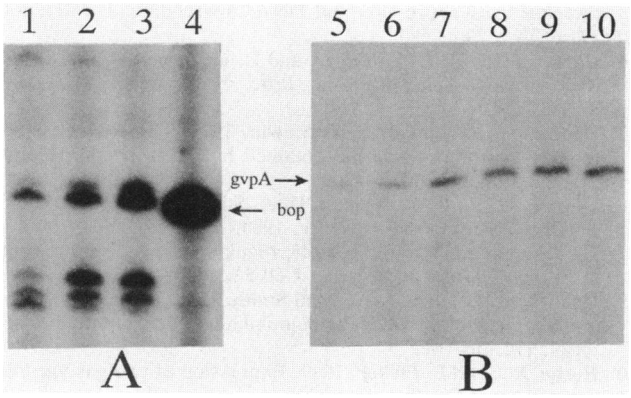

FIG. 1. Quantitation of *bop* and *gvpA* mRNA accumulation during culture of *H. halobium*. (A) *H. halobium* RNA isolated at various growth stages was analyzed by primer extension, using the *bop* primer. Analysis was carried out on RNA isolated from batch cultures at A_{600} values of 0.6 (lane 1), 1.3 (lane 2), 2.3 (lane 3), and 2.5 (lane 4). (B) *H. halobium* RNA isolated at various growth stages was analyzed by primer extension, using the *gvpA* primer. Analysis was carried out on RNA isolated from batch cultures at A_{600} values of 0.04 (lane 5), 0.3 (lane 6), 0.6 (lane 7), 1.3 (lane 8), 2.3 (lane 9), and 2.5 (lane 10).

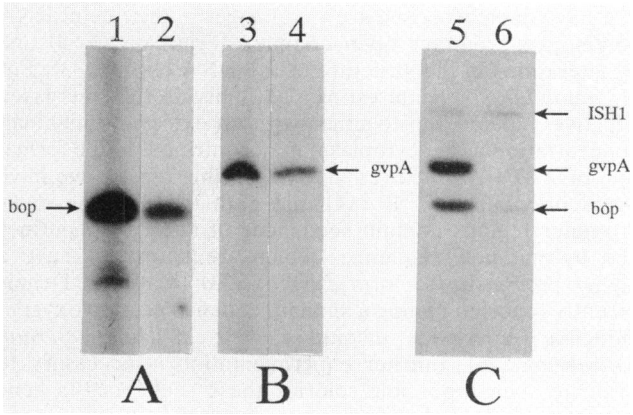

FIG. 2. Quantitation of *bop* and *gvpA* mRNA accumulation in aerated cultures (A and B) and a novobiocin-treated culture (C) of *H. halobium*. *H. halobium* RNA was isolated at an A_{600} of 2.4 from standard cultures (lanes 1 and 3) and aerated cultures (lanes 2 and 4) and analyzed by primer extension, using the *bop* primer (lanes 1 and 2) or the *gvpA* primer (lanes 3 and 4). For the novobiocin experiment, a culture of *H. halobium* at an A_{600} of 0.2 was divided and grown further without addition of novobiocin (lane 5) or with 1.5 μg of novobiocin per ml (lane 6). When the cultures reached an A_{600} of 1.8, RNA was extracted and analyzed by primer extension reactions with the *bop* and *gvpA* primers as well as an IS*H1* primer.

Initially, we analyzed *H. halobium* RNA isolated from batch cultures grown under standard laboratory conditions (9), which show the highest levels of purple membrane and gas vesicles at stationary phase (22, 38). The *bop* mRNA was found to be highly induced, about 20-fold, from the mid-logarithmic phase to the stationary phase (Fig. 1A). The *gvpA* mRNA was also found to be induced but to a lesser extent (about fivefold), and the abundance increased from early to mid-logarithmic phase, at which point it remained approximately constant until the stationary phase (Fig. 1B).

Effect of aeration on *bop* and *gvpA* gene expression. To determine the effect of oxygen on *bop* and *gvpA* mRNA accumulation, we compared the message levels in cultures that were grown under increased aeration over standard cultures at late logarithmic phase. Aerated cultures contained substantially lower levels of both *bop* and *gvpA* mRNAs compared with the standard cultures (Fig. 2A and B), indicating that aeration can reduce the accumulation of the two messages.

Effect of novobiocin on *bop* and *gvpA* gene expression. We next examined the effect of novobiocin on the accumulation of *bop* and *gvpA* mRNA and, as a control, on the message for the long open reading frame in the *H. halobium* insertion sequence IS*H1* (35). Novobiocin was added to a culture of *H. halobium* at early logarithmic phase, and after the culture reached late logarithmic phase RNA was extracted and analyzed by primer extension (Fig. 2C). The results show that novobiocin blocks the accumulation of the *bop* and *gvpA* messages. The effect of novobiocin is specific to the *bop* and *gvpA* genes since transcription of an unrelated gene, the insertion sequences IS*H1* long open reading frame, was largely unaffected.

Our results reported above are similar to those reported for several genes in eubacteria. With *R. capsulata*, Kranz and Haselkorn studied anaerobic induction of the nitrogen fixation genes by construction of a *nifH*::*lacZ* fusion (23). They showed that the fusion gene is regulated separately by oxygen and fixed nitrogen. They also found that anaerobic induction of *nif* genes is blocked by inhibitors of DNA gyrase in *R. capsulata* and also *K. pneumoniae*. More recently, *K. pneumoniae nifLA* gene expression was shown to be inhibited in vivo by a high oxygen concentration and a DNA gyrase inhibitor, and the corresponding promoter was shown to require negative supercoiling for transcriptional activity in vitro (11). It has been noted that the expression of several other genes is affected by the addition of DNA gyrase inhibitors, including *gyrA* and *gyrB* (15, 27), *proU* (18), and several genes encoded in the chloroplast genome (39).

The target of novobiocin in halobacteria is likely to be a eubacterium-type DNA gyrase. A DNA gyrase gene, similar to the eubacterial gene, has recently been cloned by gene transfer from a novobiocin-resistant halophilic archaebacterial mutant (19). Studies carried out by Sioud et al. on a small plasmid in *Halobacterium* sp. strain GRB showed directly that superhelical density decreases at a low concentration of novobiocin in the medium (36, 37). Novobiocin has also been reported to block DNA replication, but analysis of the copy numbers of both the *bop* gene and the plasmid-encoded *gvpA* gene indicated that the copy numbers remained unchanged in our experiments (data not shown). Novobiocin also decreases the growth rate by a factor of about 2, and thus we cannot rule out the possible contribution of the changed growth rate on gene expression.

In previous genetic studies on purple membrane-deficient mutants, Betlach and co-workers identified a putative regulatory gene, named *bat*, upstream of the *bop* gene (2, 25). Interestingly, our comparison of the *bat* gene product predicted from the DNA sequence with the National Biomedical Research Foundation protein data base, using the FASTA program (34), indicates a region of significant similarity (34% identity and 59% similarity over 97 amino acid residues with no gaps) to the *nifL* gene product in *K. pneumoniae* (12). Since the *nifL* gene product is thought to function as an oxygen sensor in *K. pneumoniae* (12), the *bat* gene product may serve a similar function in *H. halobium*.

Two mechanisms for gene regulation by DNA supercoiling

that have been proposed are via changes in the rate of RNA polymerase-promoter open complex formation (3, 4) and by alterations in the structure of the DNA (18, 32, 33). For *H. halobium*, one interesting possibility is that increased negative supercoiling together with an extremely high salt concentration in the cytoplasm may contribute to the formation of Z DNA structures in vivo. In this regard, we have noted previously (7, 10, 14) that both the *bop* and *gvpA* promoter regions contain sequences of alternating purines and pyrimidines, sequences which are known to have a higher propensity to form Z DNA (33). May and Dennis recently reported finding a similar sequence near an oxygen-inducible superoxide dismutase gene in *Halobacterium cutirubrum* (26). Further experimentation is necessary to evaluate such a possible role for these sequences in gene regulation in *H. halobium*.

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