A Transcription Factor with a Leucine-Zipper Motif Involved in Light-Dependent Inhibition of Expression of the \textit{puf} Operon in the Photosynthetic Bacterium \textit{Rhodobacter sphaeroides}

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In the purple nonsulfur photosynthetic bacterium \textit{Rhodobacter sphaeroides} the synthesis of components of the photosystem is regulated in response to oxygen tension and light intensity. We have purified and cloned a \textit{trans}-acting protein (SPB) that binds to the promoter region of the \textit{puf} operon, which encodes the apoproteins of light-harvesting complex I and the reaction center. The SPB was composed of a single polypeptide with an apparent molecular mass of 15.0 kDa. The nucleotide sequence of the \textit{spb} gene was determined. The gene encoded 104 amino acid residues, which correspond to a molecular mass of 11.5 kDa. SPB exhibited 53% homology to \textit{HvrA} in \textit{Rhodobacter capsulatus}. The deduced amino acid sequence indicated that SPB contained a region with homology to the leucine-zipper motif of c-JUN, a transcription factor in eukaryotes, and SPB also had a DNA-binding domain on the amino-terminal side of the leucine-zipper motif. The leucine-zipper motif of SPB might contribute to the formation of a dimer. Northern analysis indicated that \textit{spb} was constitutively and monocistronically transcribed in \textit{R. sphaeroides}, irrespective of growth conditions. Structural and functional differences between SPB and \textit{HvrA} are discussed.

Key words: DNA-binding protein — Leucine-zipper motif — \textit{puf} operon — \textit{Rhodobacter sphaeroides} — Transcription factor.

Purple photosynthetic bacteria, such as \textit{Rhodobacter sphaeroides}, can grow phototrophically in darkness or in the light. The photosynthetic apparatus consists of three pigment-protein complexes, the reaction center (RC), and light-harvesting complexes I (LHI) and II (LHII). The accumulation of the LHI and LHII apoproteins depends on the expression both of the \textit{puf} operon, which encodes LHI apoproteins and the \textit{L} and \textit{M} subunits of the RC (Bauer and Marrs 1988, Zhu and Kaplan 1985, Zhu et al. 1986), and of the \textit{puc} operon, which encodes LHII apoproteins (Kiley and Kaplan 1987, Lee et al. 1989). Earlier studies have shown that the levels of \textit{puf}- and \textit{puc}-specific transcripts are higher in cells grown in darkness under low oxygen tension than under high oxygen tension (Clark et al. 1984, Klug et al. 1984, 1985). Recently, we demonstrated that the levels of transcripts from both operons were lower in cells grown under low oxygen tension in the light than in darkness (Shimada et al. 1992). We also showed that blue light (approximately 450 nm) had the highest inhibitory effect on the expression of these two operons (Shimada et al. 1992).

Studies in vitro of \textit{Rhodobacter capsulatus} have revealed that the -57 to -30 nucleotide sequence, with dyadic symmetry, of the \textit{puf} operon acts as an oxygen-dependent regulatory promoter and functions as a protein-binding site (Narro et al. 1990, Klug 1991). Two different DNA-protein complexes were detected in extracts of cells grown under aerobic conditions in darkness, whereas only one DNA-protein complex was found in extracts of cells grown under semi-aerobic conditions in darkness (Klug 1991). Dephosphorylation of the proteins in the latter extracts resulted in the formation of two DNA-protein complexes in vitro. Klug (1991) proposed a model for the regulation by oxygen of the \textit{puf} operon that resembles a "two component system". At low oxygen tension, the phosphorylated PPBP (\textit{puf} promoter-binding protein) binds to the right arm of the sequence with dyadic symmetry and allows transcription. At high oxygen tension, PPBP is dephosphorylated, binds to both arms of the symmetric sequence and lowers the rate of initiation of transcription.

As part of an effort to identify the cellular factor(s) involved in the oxygen-dependent regulation of the \textit{puf} and \textit{puc} operons, \textit{trans}-acting mutants have been isolated and
the mutated genes have been characterized. Sganga and Bauer (1992) and Mosley et al. (1994) identified the regA and regB genes of *R. capsulatus*, which are involved in trans-activation of the expression of the photosynthetic apparatus under anaerobic conditions. The RegA protein exhibits strong sequence homology at the amino-terminus to the known response-regulators of "two component systems" (Stock et al. 1989) but lacks any sequence similarity in the carboxy-terminal region, which normally binds to DNA. It was proposed, therefore, that RegA might be an activator of another regulatory protein and not a DNA-binding protein itself (Sganga and Bauer 1992). The amino acid sequence of RegB exhibits some similarity to those of the prokaryotic family of histidine sensor kinases (Mosley et al. 1994). In *R. sphaeroides*, *prrA* and *regA*, which exhibit a high degree of homology to *regA* of *R. capsulatus*, have been cloned (Eraso and Kaplan 1994, Phillips-Jones and Hunter 1994).

Recently, a light-responsive gene for a trans-activator, hvrA, was identified in *R. capsulatus* by Buggy et al. (1994). They indicated that HvrA was a trans-activator of the expression of genes for LHI and RC but not for LHI and genes involved in the synthesis of photopigment. McGlynn and Hunter (1992) purified a protein that bound to the upstream region of the *puc* operon. The purified protein was a single polypeptide with an apparent molecular mass of 22.5 kDa on SDS-polyacrylamide gel electrophoresis and 26.0 kDa in gel filtration. However, a protein that binds to the *puf* operon has not been purified from either *R. sphaeroides* or *R. capsulatus*. We showed previously that, in *R. sphaeroides*, a single DNA-protein complex can be detected in extracts of cells grown under aerobic conditions in darkness and in extracts of cells grown under semi-aerobic conditions in the light; no complex can similarly be found in extracts of cells grown under semi-aerobic conditions in darkness. Furthermore, a single DNA-protein complex was detected in extracts of cells grown in darkness under semi-aerobic conditions when extracts were treated with a phosphatase. These observations suggest the existence of a protein that binds to the promoter region of the *puf* operon in *R. sphaeroides* to regulate the expression of the *puf* operon via phosphorylation and dephosphorylation of itself or some other molecule(s) (Shimada et al. 1993). We here report the purification of such a protein, which we designated SPB (*R. sphaeroides* *puf* operon-binding protein) and the cloning of the corresponding gene. SPB contained a region homologous to the leucine-zipper motif of c-JUN, a transcription factor in eukaryotes, as well as a DNA-binding domain on the amino-terminal side of the leucine-zipper motif.

**Materials and Methods**

**Organism and growth conditions—Rhodobacter sphaeroides**

strain 2.4.1. was used throughout this study. The cells were cultured at 34°C in 1,000 ml of the medium of Cohen-Bazire et al. (1957) in a 5,000-ml Sakaguchi flask on a reciprocal shaker operated at 95 strokes per min until the culture reached an absorbance of 0.6–0.8 at 660 nm. Under these growth conditions the harvested cells were almost colorless.

**Preparation of a soluble fraction**—The culture was centrifuged at 10,000 × g for 10 min at 4°C, and the cell pellet was washed with 15 mM Tris-HCl (pH 8.3 at 4°C) that contained 0.1 mM dithiothreitol, 0.1 mM EDTA, 50 mM NaCl, 50 mM phenylmethylsulfonyl fluoride and 50 mM N-tosyl-l-phenylalanine chloromethyl ketone, as protease inhibitors, and resuspended in 50 ml of the same buffer. The cells were disrupted by two passages through a French pressure cell. The cell debris was sedimented by centrifugation at 12,000 × g for 30 min at 4°C, and the resultant supernatant was centrifuged at 257,000 × g for 3 h at 4°C. Solid ammonium sulfate was added to the supernatant to 30% saturation and the mixture was stirred for 30 min on ice and then centrifuged at 17,000 × g for 30 min at 4°C. The concentration of ammonium sulfate in the supernatant was increased to 60% saturation. The precipitate was collected by centrifugation and dissolved in 50 mM N-EDT (pH 8.3, 4°C) and then the column was washed with 60 ml of the same buffer. Protein was eluted with a 100-ml linear gradient of 50–600 mM N-EDT (pH 8.3, 4°C) at 1.3 ml min⁻¹, and 2-ml fractions were collected. Active fractions, identified by gel shift assays (below), were dialyzed against one liter of 50 mM N-EDT (pH 7.5, 4°C) overnight at 4°C.

**Affinity chromatography on DNA-cellulose**—The protein sample was mixed with 1.2 ml of native calf thymus DNA-cellulose (Pharmacia LKB Biotechnology) that had been equilibrated with 50 mM N-EDT (pH 8.3, 4°C) and then the column was washed with 60 ml of the same buffer. Protein was eluted with a 100-ml linear gradient of 50–600 mM N-EDT (pH 8.3, 4°C) at 1.3 ml min⁻¹, and 1-ml fractions were collected.

**Preparative electrophoresis**—Preparative disc electrophoresis was performed according to the protocol of the supplier of the apparatus (model NA-1800; Nihon Eido, Tokyo, Japan). The gel column of the apparatus (5.0 cm i.d. × 2.5 cm) was filled with an SDS-polyacrylamide gel (15%) gel, and a stacking gel was stacked on the lower layer (Laemmli 1970). Two milliliters of the protein sample were mixed with 2.0 ml of the sample buffer for electrophoresis [125 mM Tris-HCl (pH 6.8), 4.4% SDS, 13.0% sucrose, 11.0% β-mercaptoethanol, 0.0001% Coomassie Brilliant Blue R-250] and the mixture was incubated for 30 min at room temperature. The mixture was then subjected to electrophoresis at a constant voltage of 100 V at 4°C and 1-ml fractions were collected.

**Denaturation and renaturation of SPB**—SPB was denatured and renatured as described by Wada et al. (1990) and Inomata et al. (1992) with a slight modification. Protein in 100 µl of sample obtained by preparative electrophoresis was precipitated by addition of 500 µl of ice-cold acetone and 10 µg of bovine serum albumin.
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4°C.

by fresh buffer and the solution was dialyzed for a further 2 h at 4°C. The buffer was then replaced with the renaturation buffer (denaturation buffer without 6 M guanidine hydrochloride) for 2 h at 4°C. The buffer was then replaced by Tween 20 and 10 μg bovine serum albumin. The dialyzate was mixed with the DNA-cellulose and packed into a column, and then the unbound proteins were washed out with 50 mM N-EDT (pH 7.5, 4°C). The bound protein that had bound to the S-Sepharose was eluted with a linear gradient of 50–600 mM N-EDT (pH 8.3, 4°C). SPB was eluted between 270 and 600 mM N-EDT.

Analysis of proteins—The concentration of proteins was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard. Proteins were subjected to electrophoresis on an SDS-polyacrylamide (15%) gel and stained with silver as described by the supplier of the silver-staining kit (Silver Stain Plus Kits; Bio Rad, Hercules, U.S.A.).

Analysis of the amino-terminal sequence of SPB—The amino-terminal amino acid sequence was analyzed by the method of Matsudaira (1987) and Hirano and Watanabe (1990).

Manipulation of DNA, cloning and sequencing of the *spb* gene—Isolation of chromosomal DNA from *R. sphaeroides* and cloning of the *spb* gene were performed by the methods of Iba et al. (1987). A mixture of the following oligonucleotides, which corresponded to the amino-terminal amino acid sequence, was synthesized taking codon usage into account:

5'-ATG-GA(C)-ATC-GA(G)-CT(G)-GA(C)-3'

The oligonucleotide mixture was used as a probe for the cloning of the *spb* gene. DNA sequencing was performed with a BcaBEST™ Dideoxy Sequencing Kit (Takara Shuzo Co., Ltd., Kyoto, Japan).

Preparation of RNA and Northern hybridization—Isolation of total RNA from *R. sphaeroides* and Northern hybridization were performed by the methods of Shimada et al. (1992). The *Bgl*-SacII restriction fragment (Fig. 5, A) and the *PstI*-KpnI restriction fragment (Fig. 1) were used as specific probes for the *bchA* gene and the *puf* operon, respectively. The oligonucleotide 5'-AACCAATGCCTCACGCTTGTGGCGGCCC CGCTCA ATTTCCTTTGAGTTT-3' was used as the probe for 16S rRNA (Dryden and Kaplan 1990).

**Results**

Purification of SPB—To examine the binding of SPB to the promoter region of the *puf* operon, we performed gel shift assays. The probe DNA for gel shift assays was the *SmaI*-*PstI* fragment from the upstream region of the *puf* operon, as described previously (Fig. 1; Shimada et al. 1993). Most of the activity of SPB was detected in the precipitate obtained between 30 and 60% saturation with ammonium sulfate from the supernatant after ultracentrifugation (257,000 × g, 3 h). The precipitate was resuspended in buffer, dialyzed, and applied to a column of S-Sepharose that was then washed with 50 mM N-EDT. Then the protein that had bound to the S-Sepharose was eluted with a linear gradient of 50–600 mM N-EDT (pH 8.3, 4°C). The active fractions were dialyzed against 50 mM N-EDT (pH 7.5, 4°C). The bound protein was purified by affinity chromatography on native calf thymus DNA-cellulose. The dialyzed was mixed with the DNA-cellulose and packed into a column, and then the unbound proteins were washed out with 50 mM N-EDT (pH 7.5, 4°C).

Fig. 2 Elution of SPB from the DNA-cellulose affinity column. The concentration of protein was monitored by measuring the absorbance at 280 nm (•••••). Activity was assayed by the retardation of the mobility as described in Materials and Methods (○○○○).
proteins were eluted with a linear gradient of 50-1,000 mM N-EDT (pH 7.5, 4°C). The elution profile of the DNA-cellulose column is shown in Figure 2. The strongest absorption at 280 nm was detected at fraction 6, corresponding to about 300 mM N-EDT (pH 7.5, 4°C), and a small peak was detected at fraction 17, at about 750 mM N-EDT (pH 7.5, 4°C). Between fractions 14 and 19, SPB activity was eluted with a profile similar to that of the protein (indicated by the absorbance at 280 nm). Fraction 17, exhibiting the highest activity, contained proteins that migrated as two major bands, with molecular masses of 14.4 and 15.0 kDa, during SDS-polyacrylamide gel electrophoresis (Fig. 3A, top).

To determine whether these two proteins (14.4 and 15.0 kDa) were subunits of SPB, we performed gel shift assays with the two proteins. The proteins in the active fractions from DNA-cellulose (fractions 15 through 18) were purified by preparative disc electrophoresis. The proteins in fractions obtained by the preparative disc electrophoresis were denatured and then renatured as described in Materials and Methods. The activities of the renatured proteins were analyzed by gel shift assays. The renaturation by the guanidine hydrochloride method (see Materials and Methods) indicated that the recovery of the activity of SPB was more than 50%. The renatured protein of 15.0 kDa bound to the DNA probe, whereas the protein of 14.4 kDa did not (Fig. 3A). To confirm that the 15.0-kDa protein was SPB, we performed a competition assay. When salmon

Fig. 3 Results of SDS-polyacrylamide gel electrophoresis of fraction 17 in Fig. 2 (A, top), DNA-binding activity of renatured SPB (A, bottom) and the gel shift assay with a competitor (B). (A) Proteins in fraction 17 from the DNA-cellulose column were separated by preparative disc electrophoresis, and then the proteins were denatured and then renatured as described in Materials and Methods. The numbers represent the molecular masses of each protein deduced from their mobility on the SDS-polyacrylamide gel (top). (B) The gel shift assay was performed using the indicated protein fractions. All samples included SPB that had been purified by preparative disc electrophoresis (Fig. 3A, 15.0 kDa). Lane 1, no addition of competitor, as a control; lanes 2 and 3, addition of the synthetic oligomer (oligo; 10 and 30 ng, respectively); lanes 4 and 5, addition of sonicated salmon sperm DNA (ssDNA; 10 and 30 ng, respectively) as a negative control.

Fig. 4 Silver staining of an SDS-polyacrylamide gel after electrophoresis of various fractions. Lane 1, 20 µl from a 35-ml solution of the precipitate obtained between 30 and 60% saturation with ammonium sulfate; lane 2, 30 µl from 48 ml of the eluate from the S-Sephase column; lane 3, 30 µl from 4 ml of the eluate from the DNA-cellulose column; lane 4, 30 µl from 200 µl of the sample from preparative disc electrophoresis; lane 5, no sample was applied. The positions of size markers (kDa) are shown. The 15.0-kDa polypeptide in the purified preparation is indicated. The bands between 43.0 kDa and 94.0 kDa are due to artifacts of the silver-staining procedure.
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sperm DNA was used as a competitor DNA in the gel shift assay, the shifted band did not completely disappear. However, when the oligomer whose sequence was the same as that of the SPB binding-site (Shimada et al. 1993) was used as the competitor, the shifted band completely disappeared, indicating that the protein of 15.0 kDa specifically bound to the probe DNA (Fig. 3B). Therefore, the 15.0-kDa protein was identified as SPB. Figure 4 shows the silver-staining pattern of fractions at different stages of purification after SDS-PAGE of each fraction. After preparative gel electrophoresis, a single band of a 15.0-kDa protein was obtained.

The band of the 15.0-kDa protein was electrophalted on a polyvinylidene difluoride membrane, cut out from the membrane, and the amino-terminal amino acid sequence of the protein was determined, and found to be:

\[ \text{NH}_2\text{-Met-Asp-Ile-Asp-Leu-Asp-Ser.} \]

**Cloning of the spb gene**—The amino-terminal amino acid sequence of SPB was used to design oligonucleotides that were then synthesized, as described in Materials and

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**Fig. 5** Restriction map and sequence analysis of *spb*. (A) Restriction map of a 6.5-kb *SmaI* fragment of *R. sphaeroides* DNA. The hatched box represents the *spb* gene. (B) Nucleotide and predicted amino acid sequences for the *spb* gene. The restriction sites (BglII and SacII) used for preparation of the probe for Northern hybridization are noted. The underlined sequence (from nt 1 to nt 18) is the region that hybridized to the synthetic oligonucleotide probe (see Materials and Methods). The recognition sites of BglII and SacII are shown at nt 52 and nt 243, respectively.
Methods, and used for the cloning of the \( spb \) gene from a genomic library of \( R. sphaeroides \) 2.4.1. The \( spb \) gene was cloned and its nucleotide sequence was determined. Figure 5(A) shows the restriction map of the 6.5-kb \( Smal \) fragment that included the \( spb \) gene. The \( spb \) gene was subcloned in the \( BamHI \) fragment. The sequence of the clone had an open reading frame (starting from an ATG codon) that encoded 104 amino acid residues, which corresponded to a protein of 11.5 kDa (Fig. 5B). The deduced amino acid sequence of SPB exhibited 53\% homology to that of \( HvrA \) which is a light-responsive trans-activator of the \( puf \) and the \( puh \) operons in \( R. capsulatus \) (Buggy et al. 1994). Furthermore, SPB contained a region with homology to the leucine-zipper motif of c-JUN.

Expression of the \( spb \) gene—In order to examine the expression of the \( spb \) gene under different growth conditions, we performed Northern hybridization with a \( BglI-SacII \) (Fig. 5B) restriction fragment as a specific probe for the \( spb \) gene. Figure 6 shows the results of Northern hybridization for the \( spb \) gene, as well as for the \( puf \) operon, in cells grown under different environmental conditions. As shown in Figure 6(A) the \( spb \) gene was expressed under all conditions tested. The length of the transcript of the \( spb \) gene was about 400 nucleotides (nt). By contrast, two transcripts of the \( puf \) operon (2,600 and 600 nt) were detected in cells that had been grown semi-aerobically in darkness, whereas they were not detected in cells that had been grown either semi-aerobically in the light or aerobically in darkness (Fig. 6B). 16S rRNA was expressed under all conditions (Fig. 6C).

Discussion

In this study, we purified SPB and cloned its gene. The level of expression of the \( puf \) and the \( puc \) operons in \( R. sphaeroides \), as well as in \( R. capsulatus \), is reduced by increases in oxygen tension and in light intensity. Furthermore, the level of expression of the two operons is specifically reduced by blue light (450 nm; Shimada et al. 1992). The two operons seem, therefore, to be regulated by an identical or similar mechanism that involves receptors, a signal transduction pathway, a cis-element and a trans-acting factor. However, there are some differences, as described below. In \( R. sphaeroides \), the \( puf \) operon-binding protein is a 15.0-kDa protein (this report) and the \( puc \) operon-binding protein is a 26.0-kDa protein (McGlynn and Hunter 1992). The protein-binding sequence of the \( puf \) operon (5'-GCGATCCGGCGCG-3') exhibits no homology to that of the \( puc \) operon (5'-CAAAATTGTCCCTTTCTGA-3') (McGlynn and Hunter 1992, Shimada et al. 1993), suggesting that the mechanisms for regulation of expression by the cis-element and the trans-acting factor of the \( puf \) operon and the \( puc \) operon, respectively, might be different.

Northern hybridization indicated that the \( spb \) gene was expressed constitutively under all growth conditions tested (Fig. 6A). Gel shift assays indicated that SPB did not bind to the promoter region of the \( puf \) operon in cells grown in darkness under semi-aerobic conditions, whereas it did bind after cell-free extracts had been treated with bacterial alkaline phosphatase (Shimada et al. 1993). These results suggest that the DNA-binding activity of SPB might be regulated by a phosphorylation-dephosphorylation reaction and not by control of the transcription and/or translation of the \( spb \) gene. The results of Northern hybridization support this assumption.

Northern hybridization revealed that the length of the transcript of the \( spb \) gene was approximately 400 nt. The coding region of the \( spb \) gene consisted of 312 bp and, therefore, the gene may be transcribed in a monocistronic manner. In \( R. capsulatus \) and \( R. sphaeroides \), genes related to regulation or photosynthesis are organized as gene clusters (e.g., a photosynthesis-related gene cluster and a regulatory gene cluster; Buggy et al. 1994, Coomber and Hunter 1989, Coomber et al. 1990). Therefore, the upstream and/or downstream region of the \( spb \) gene might encode regulatory genes related to the expression of SPB. We are presently examining the 6.5-kb DNA fragment (Fig. 5A) and analyzing the relationships of the gene products.
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**Fig. 7** Amino acid sequence homology between SPB and c-JUN and ‘helical wheel’ diagrams for SPB and c-JUN. (A) The amino acid sequence of SPB is represented from the 30th to the 68th amino acid, and that of c-JUN from chicken is represented from the 250th to the 288th amino acid (Nishimura and Vogt 1988). Residues marked with stars and dots indicate identical and similar residues, respectively. Leucine residues are shown in black boxes. (B) The ‘helical wheel’ diagrams of SPB and c-JUN are shown in (a) and (b), respectively.

SPB includes a region with similarity to the leucine-zipper motif of c-JUN (Fig. 7A), a eukaryotic trans-acting factor (Bohmann et al. 1987, Nishimura and Vogt 1988, Pabo and Sauer 1992), and to the leucine-zipper motifs of prokaryotic trans-acting factors (Maxon et al. 1990, Chakerian et al. 1991). The leucine-zipper motif consists of periodic repetitions of leucine residues at every seventh position over a total of eight helical turns. The motif of SPB is, however, slightly different from that in eukaryotes. Figure 7(B) shows ‘helical wheel’ diagrams (Branden and Tooze 1992) for c-JUN and SPB. c-JUN has leucine residues on the same side of the helical wheel. The homologous region of SPB has leucine residues on the same side of the helical wheel, although the interval between leucine residues is not seven residues in every case. The abundance of basic (arginine and lysine) and hydrophobic (alanine and leucine) residues upstream of the leucine-zipper motif in SPB is characteristic of other leucine-zipper proteins (Fig. 5; Pabo and Sauer 1992). A sequence on the amino terminal side of the leucine-zipper motif of SPB also exhibits homology to the sequence adjacent to the leucine-zipper motif of c-JUN (Fig. 7). This sequence might be thought to be a DNA-binding domain. Therefore, the region might be a prototype of leucine-zipper motif of transcription factors. The SPB-binding site was between nt —709 and —722 in the upstream region of the *puf* operon, and it had a palindromic sequence, 5'-CGGATCC-GGCCCGG-C-3'. (The DNA sequence is numbered in relation to the start codon of *pufB*, with the first base of the initiation codon designated +1 and the base preceding it designated —1; Shimada et al. 1993). Similar to many transcription factors in bacteria (Branden and Tooze 1992), SPB probably forms a dimer that binds to this palindromic DNA sequence. The leucine-zipper motif of SPB might contribute to the formation of such a dimer. However, we did not examine whether the SPB protein forms a homodimer or a heterodimer.

SPB exhibited no homology to RegA and RegB in *R. capsulatus* or to RegA and PrrA in *R. sphaeroides*, all of which are regulatory factors (sensor regulators) that control high-level induction of the *puf*, *puh*, and *puc* operons (Eraso and Kaplan 1994, Sganga and Bauer 1992, Mosley et al. 1994, Phillips-Jones and Hunter 1994). SPB exhibited 53% homology to HvrA in *R. capsulatus*, which has a helix-turn-helix motif and is considered to bind to the promoter regions of the *puh* and *puf* operons (Buggy et al. 1994). SPB is different from HvrA in that SPB is a negative regulator, whereas HvrA is a trans-acting activator (Shimada et al. 1993, Buggy et al. 1994). In *R. sphaeroides*, SPB bound to a cis-element of the *puf*-operon when the operon was not expressed, whereas SPB did not bind to the cis-element when the operon was expressed (Shimada et al. 1993). Another difference between HvrA and SPB is that...
SPB is transcribed monocistronically (Fig. 6), whereas HvrA is transcribed polycistronically (Buggy et al. 1994). If we assume that SPB is a counterpart of HvrA in R. sphaeroides, these functional differences between the two transcription factors might represent the difference between the final steps in the signal transduction pathways in the two species of bacteria.

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