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REVIEW

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Mechanisms of gene expression controlled by pressure in deep-sea microorganisms

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Abstract A pressure-regulated operon has been cloned and sequenced from deep-sea barophilic *Shewanella* strains. To understand pressure-regulated mechanisms of gene expression, a regulatory element upstream of the pressure-regulated operon from *Shewanella* sp. strain DSS12 was studied. Regions A and B were classified by sequence analysis. A unique octamer motif, AAGGTAAG, was found to be repeated in tandem 13 times in region B. An electrophoretic mobility shift assay demonstrated that a σ^{54}-like factor recognizes region A and other unknown factors recognize region B. Different shift patterns of the protein–DNA complexes were observed when extracts of cells cultured at 0.1 MPa or 50 MPa were incubated with a DNA probe specific for region B. These results indicate that the deep-sea strain DSS12 expresses different DNA-binding factors under different pressure conditions.

Key words Barophilic bacteria · Deep-sea bacterium · Electrophoretic mobility shift assay (EMSA) · Gene expression · Pressure-regulated operon · *trans*-acting factors

Introduction

The deep sea is an extreme environment typified by high pressure and low temperature. Microorganisms in the deep sea are adapted to this environment, and they may have unique mechanisms of gene expression controlled by high pressure. To investigate the deep-sea adaptation mechanisms in bacteria, we have isolated a number of bacteria displaying barophilic (piezophilic) and barotolerant (piezotolerant) growth properties from deep-sea sediment samples obtained by means of the manned and unmanned submersibles, *Shinkai* 6500 and *Kaiko*, operated by the Japan Marine Science and Technology Center (Kato et al. 1995, 1996a,b, 1998; Nogi et al. 1998). Such deep-sea bacteria have unique systems for gene expression controlled by hydrostatic pressure, and we have reviewed the subject of pressure regulation in these deep-sea bacteria (Bartlett et al. 1995; Kato and Horikoshi 1996; Kato and Bartlett 1997).

The moderately barophilic *Shewanella* sp. strain DSS12 isolated from the Ryukyu trench (depth, 5110 m), grows optimally at 30 MPa and 8°C, but also grows at atmospheric pressure (0.1 MPa) and 8°C (Kato et al. 1995). Therefore, this strain is useful as a model for comparison of various feature of bacterial physiology under high and low hydrostatic pressure conditions. An operon identified as a pressure-regulated operon, whose promoter was activated by growth under high pressure, was recently cloned and characterized from this strain. We have reported that gene expression from this operon, which has five transcription initiation sites, is controlled at the transcriptional level by elevated pressure (Kato et al. 1997). However, the molecular basis of transcription of this operon remains to be elucidated. One way to elucidate the basis of gene expression under these conditions is via detailed characterization of *trans*-acting factors that function through binding to the promoter region of a gene. Gene expression is regulated by interactions between *trans*-acting factors such as transcription factors and the promoter region.

In this paper, we report the analysis of a regulatory element found upstream of the pressure-regulated operon of the deep-sea barophilic *Shewanella* sp. strain DSS12. In this study, we have also investigated several factors capable of binding to the operon under different pressure conditions by means of an electrophoretic mobility shift assay (EMSA).

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Sequence analysis of the pressure-regulated operon and adjacent regions

The expression of genes in the pressure-regulated operon (ORF 1 and ORF 2) from the moderately barophilic *Shewanella* sp. strain DSS12 is controlled positively at the transcriptional level by elevated pressure (Fig. 1). Five transcription initiation sites have been identified by primer extension analysis in the region upstream of the pressure operon (Fig. 2) (Kato et al. 1997). Nucleotide sequence analysis of this region indicated the existence of two potential regulatory regions as follows:

1. Similarity between a consensus sequence for σ^{54} binding and part of the region designated region A was detected (homology greater than 90%) when the 5′-flanking region of the second transcription initiation site was compared with several consensus sequences of *Escherichia coli* σ factors. However, typical promoter consensus sequences were not found in the flanking regions of other transcription initiation sites. These findings suggest that this strain may have not only a new type of promoter sequences, but also a previously unknown type of sigma factor.
2. A unique octamer motif, AAGGTAAG, was found to be tandemly repeated 13 times in the region designated region B containing the third to fifth transcription initiation sites just upstream of region A. A palindromic sequence, AGTTAAAGATTAAACT, was found in a region downstream of the tandemly repeated sequence. The significance of this repeat sequence and palindromic structure in relation to gene expression is unclear. The detailed structures of the upstream region of the pressure operon are summarized in Fig. 3.

Downstream of the pressure-regulated operon, other pressure-regulated genes were discovered. The first gene

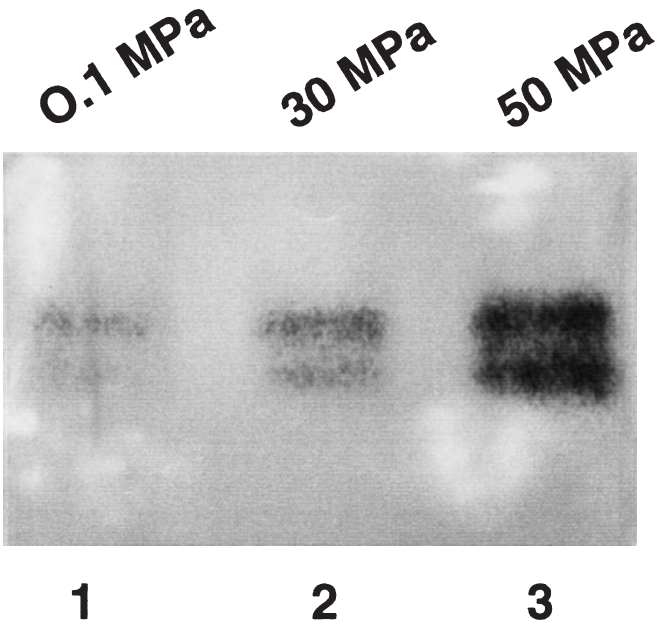
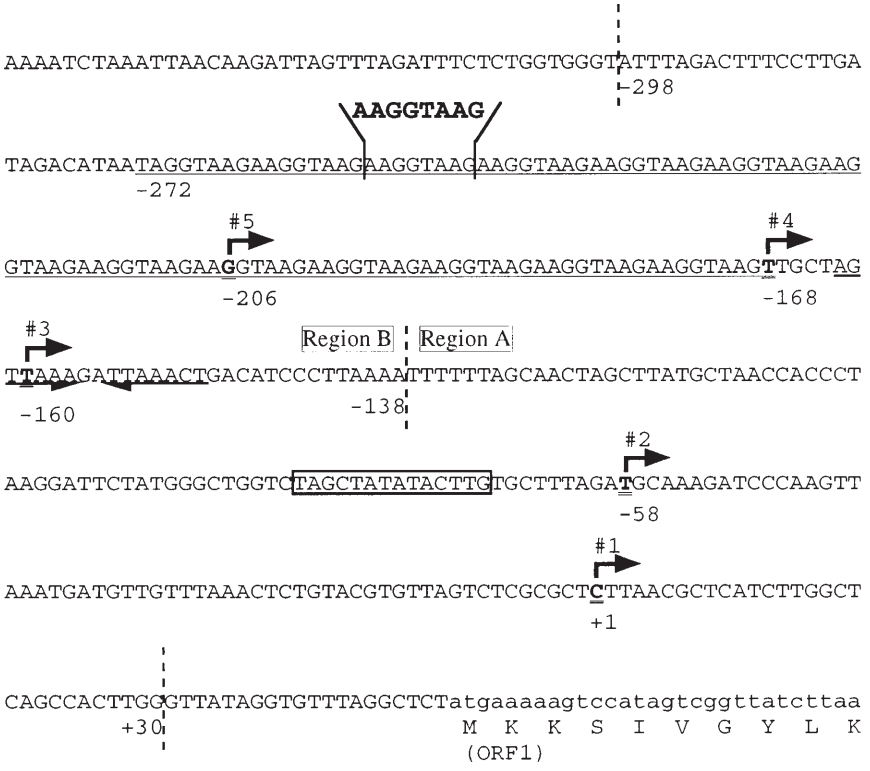


Fig. 1. Northern blotting analysis of RNAs from the moderately barophilic *Shewanella* sp. strain DSS12 grown under varied pressure conditions. The 0.45 kb *Hin*dIII-*Eco*T22I DNA fragment (containing a part of ORF1 and -2) was labeled with digoxygenin and used as a probe

Fig. 2. Nucleotide and deduced amino acid sequences of the region upstream of the pressure-regulated operon from strain DSS12 (accession number: D83386). Transcriptional start points are indicated with *bold characters* and # *[number]*. *E. coli* σ^{54} recognized the consensus sequence indicated in the *box*. A palindromic sequence is *underlined with arrows*. The sequence repeated in tandem 13 times is *underlined*. The regions designated as regions A and B (see Fig. 3) are shown between *vertical dotted lines*



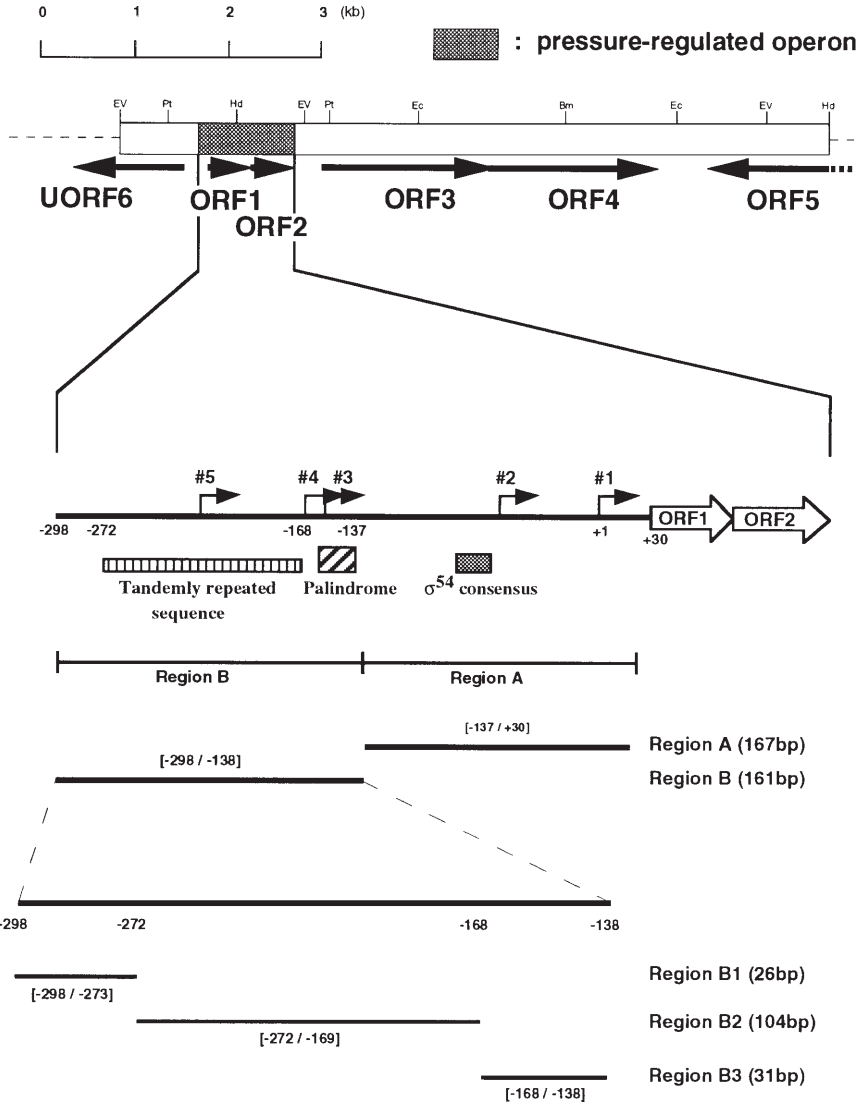


Fig. 3. Structure of the pressure-regulated operon and the region upstream of the pressure-regulated operon in strain DSS12. Tandemly repeated, palindromic, and σ^{54} consensus sequences are shown in *boxes*. Region A spans positions −137 to +30; region B spans positions −298 to −138; and regions B1, B2, and B3 span positions −298 to −273, −272 to −169, and −168 to −138, respectively

was identified as *cydD* (ORF 3 in Fig. 3). In *E. coli*, CydD is required for assembly of the cytochrome *bd* complex, one of the components of the aerobic respiratory chain (Poole et al. 1994). *E. coli cydD* mutants display increased sensitivity to high pressure, but display wild-type levels of sensitivity to high pressure when bearing the DSS12 *cydD* on a plasmid (Kato et al. 1996c). We have observed that the cytochrome *bd* complex protein was detectable by spectrophotometric analysis only on high-pressure cultivation of strain DSS12 (Tamegai et al. 1998). Further, *cydD* seems to function to allow cell growth under high-pressure conditions. The *cydD* gene is known to be necessary for expression of the components of respiratory systems. Considering these results, it seems possible that regulation of the respiratory system in strain DSS12 is responsive to high hydrostatic pressure, and the respiratory system appears to play a significant role in cell growth under high-pressure conditions.

Electrophoretic mobility shift assay

We have reported that levels of transcripts (mRNA) from these five transcription initiation sites (Figs. 2 and 3) are increased by elevated pressure and that the region upstream of the operon may have a role in regulating expression of the operon (Kato et al. 1997), and it seems likely that some pressure-regulated DNA-binding proteins might bind to this region under high-pressure conditions. To analyze whether such DNA-binding proteins exist in strain DSS12, an electrophoretic mobility shift assay (EMSA) was performed.

Cell lysates for the EMSA were prepared from strain DSS12 grown in Marine Broth 2216 at 0.1 MPa (atmospheric pressure) and at 50 MPa, at 8°C. DNA probes as mentioned in Fig. 3 (regions A, B, B1, B2, and B3) were ^{32}P-labeled at the 5′-end, and the EMSA experiments using

these lysates and probes were performed according to the procedure reported by Toku and Tanaka (1996).

The complex formed by *E. coli* σ^{54} protein and region A was identified (Fig. 4A, lane 4). A similar complex was observed when region A was incubated with the lysate prepared from cells cultured at 0.1 MPa (Fig. 4A, lane 2) or 50 MPa (Fig. 4A, lane 3). The band showing retarded mobility in lane 3 is more intense than that in lane 2. These results indicate that a σ^{54}-like factor is present in strain DSS12 and that the level of production of this factor under high-pressure conditions are greater than under atmospheric pressure conditions.

A similar analysis was performed to determine whether the protein(s) bind to region B. Figure 4B shows that some proteins capable of binding to region B were present (lanes 2 and 6) and that the shift pattern of each band showing retarded mobility was different. This result indicates that different factors capable of recognizing region B are expressed in this strain under different pressure conditions. The results of competition experiments also indicate that factors in the lysate prepared from cells grown at atmospheric pressure recognize region B as a whole (lanes 3–5), whereas factors found in the lysate of cells grown at high pressure bind to particularly the B2 and B3 subregions

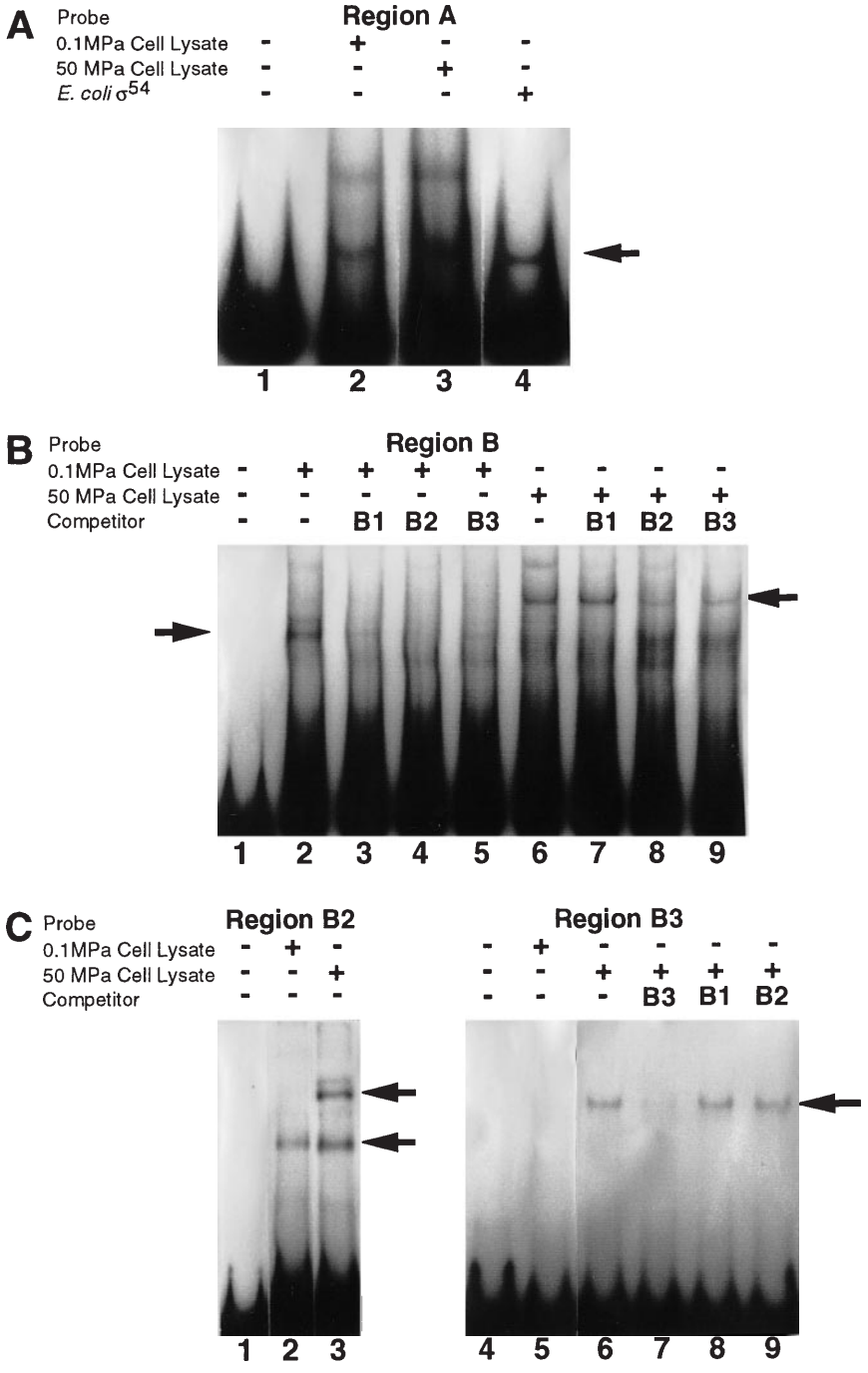


Fig. 4A–C. Electrophoretic mobility shift assays confirm binding of constituents of lysates prepared from DSS12 cells cultured at 0.1 MPa or 50 MPa and binding of *E. coli* σ^{54} to the region upstream of the pressure-regulated operon. Labeled probes, region A, region B, region B2, and region B3, were incubated with lysates of cells grown at 0.1 MPa or 50 MPa, and the complexes formed were separated on a native 5% or 10% polyacrylamide gel. As competitors, a 100-fold excess of each of the following unlabeled oligonucleotides was added to the binding reaction. **A** Lysates of DSS12 cells grown at 0.1 MPa and 50 MPa, and *E. coli* σ^{54}, binding to region A; **B** lysates of DSS12 cells grown at 0.1 MPa and 50 MPa, binding to region B; **C** lysates of DSS12 cells grown at 0.1 MPa and 50 MPa, binding to regions B2 and B3. The complexes are indicated by *arrows*

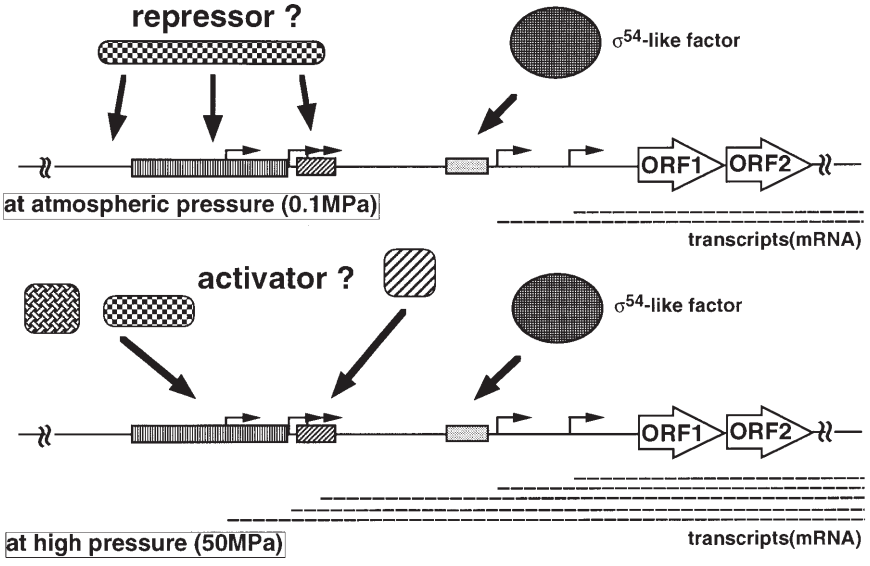


Fig. 5. Summary of binding of *trans*-acting factors to the region upstream of the pressure-regulated operon of the deep-sea barophilic *Shewanella* sp. strain DSS12 under atmospheric pressure (0.1 MPa) and high-pressure (50 MPa) conditions. *Vertical shading*, sequence tandemly repeated 13 times; *diagonal hatching*, palindrome structure; *dotted shading*, σ^{54} consensus sequence

(lanes 7–9). Moreover, the subregions designated as B2 and B3 (Fig. 3) within region B were analyzed with respect to the ability to function as an internal site for DNA binding. As shown in Fig. 4C, while only a single band shift was observed (lane 2) when region B2 (tandemly repeated sequence) was incubated with the lysate prepared from cells cultured at 0.1 MPa, two bands with retarded mobility were detected (lane 3) when the lysate prepared from cells cultured at 50 MPa was examined. This finding may indicate that different factors recognizing the same repeat sequence are expressed in this strain under different pressure conditions. When region B3 containing the palindromic sequence was incubated with the lysate prepared from cells cultured at 50 MPa, a band shift was detected (lane 6), but no such band shift was observed in the case of the lysate prepared from cells cultured at 0.1 MPa (lane 5). This result shows that the factor capable of recognizing the palindromic structure is expressed only under high-pressure conditions. Competition experiments indicate that this factor particularly recognizes only the B3 region, because the band with retarded mobility was eliminated in the presence of excess unlabeled competitor (Fig. 4C, lanes 7–9).

Interactions of the factors binding to the upstream region under both pressure conditions are summarized in Fig. 5. Because transcripts from region B are not detectable in cells grown at atmospheric pressure (0.1 MPa) (Kato et al. 1997), the factors binding to region B might function as a transcriptional repressor. In contrast, in cells grown at high pressure (50 MPa), all transcripts are detectable (Kato et al. 1997), suggesting that the factors expressed under these conditions might act as transcriptional activator.

Conclusion

The regulatory element upstream of the pressure-regulated operon in deep-sea *Shewanella* sp. strain DSS12 was found to consist of two regions, A and B. EMSA analysis demonstrated that a σ^{54}-like factor recognizes region A and other unknown factors recognize region B. Different shift patterns of protein–DNA complexes were observed using cell lysates prepared from cells of strain DSS12 cultured at 0.1 MPa or 50 MPa. A factor present in cells grown at 0.1 MPa binds to region B, and a factor in cells grown at 50 MPa binds particularly to a unique octamer motif tandemly repeated 13 times (AAGGTAAG; region B2). These results indicate that the deep-sea barophilic strain DSS12 expresses different DNA-binding factors under different pressure conditions.

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