**srb: a Bacillus subtilis Gene Encoding a Homologue of the α-Subunit of the Mammalian Signal Recognition Particle Receptor**

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**Abstract**

We cloned a *Bacillus subtilis* gene (*srb*) encoding a homologue of the mammalian signal recognition particle receptor α-subunit (SRα). The gene is 987 bp in length and encodes a 329-amino acid protein. The deduced amino acid sequence of the protein shared 26.6, 36.2 and 49.7% identity with those of mammalian SRα, archaebacterial DPα and *Escherichia coli* FtsY, respectively. The protein contains three conserved GTP-binding elements like the other three SRP receptor proteins, though the N-terminal portion of the putative *B. subtilis* protein was shorter than the others. Secondary structure prediction showed that an amphipathic α-helix is positioned in the N-terminal region. A defect in *srb* inhibited cell growth and protein translocation.

**Key words:** *Bacillus subtilis*; signal recognition particle (SRP); SRP receptor

Small cytoplasmic RNA (scRNA) and Ffh protein of *Bacillus subtilis* are homologues of SRP 7S RNA and SRP54 proteins of the mammalian signal recognition particle (SRP), respectively, which function in targeting pre-secretory protein to the endoplasmic reticulum (ER). The scRNA and Ffh form a complex in vivo and defects in them inhibit secretory protein translocation in *B. subtilis*. Therefore, it is suggested that the SRP-like particle, of which the structure and function are similar to those of mammalian SRP, is also present and functional in *B. subtilis*. On the other hand, an SRP receptor consisting of SRα and SRβ binds SRP to ER in mammalian cells. In *Escherichia coli*, 4.5S RNA and Ffh associate to form a stable ribonucleoprotein complex, which interacts with FtsY, a homologue of mammalian SRα.

To confirm the presence of an SRP-SRP receptor system for targeting secretory protein in *B. subtilis* in a manner similar to mammalian cells and *E. coli*, we cloned the *B. subtilis srb* gene encoding a homologue of mammalian SRα and *E. coli* FtsY, we then characterized the predicted motifs of its amino acid sequence.

To verify the existence of a *B. subtilis* gene encoding a homologue of mammalian SRα and *E. coli* FtsY, *B. subtilis* chromosomal DNA was subjected to Southern hybridization using the *E. coli* ftsY gene as a probe after the DNA was digested with several restriction enzymes and electrophoresed on agarose gel. However, no positive bands were identified. We then investigated the GTP-binding consensus elements which are highly conserved in the SRP receptors (Fig. 1). The amino acid sequences of element I of mammalian SRα, archaeabacterial DPα, and *E. coli* FtsY were completely identical, whereas they differed from those of SRP54 and Ffh proteins of *E. coli* and *B. subtilis*. Furthermore, their sequences in element II were also identical. Based upon these amino acid sequences of elements I and II, we synthesized two oligonucleotides, PG1 [5'-ggtgt(c/t)aatgg(c/t)gttggIaa-3'] and PG2 [5'-cagacg(a/g)ccIgc(c/t)gt(a/g)tc-3']. The polymerase chain reaction (PCR) using these synthesized DNA as primers generated a 262-bp DNA fragment as the major product. This fragment was sequenced and its putative amino acid sequence appeared to have 47.2% identity with the region between elements I and II of *E. coli* FtsY. Therefore, this 32P-labeled fragment was used as a probe for hybridization with the *B. subtilis* chromosomal DNA after digestion with several restriction enzymes. A single band of about 1.9 kb in the *Aor51HI* hybridized. The *Aor51HI*-digested chromosomal DNA from *B. subtilis* 168 was resolved by agarose gel electrophoresis. Thereafter, 1.5- to 2-kb fragments were extracted from the gel, ligated with λgt10 arms, packaged in vitro, and transfected into *E. coli* NM514, then 64 positive phage clones carrying the 1.9-kb *Aor51HI* fragment were isolated. Physical mapping of a purified phage DNA with several restriction enzymes, revealed that the insert in the isolated phage contained the 1.2-kb *EcoT11*I-SnaBI fragment that specifically hybridized with the probe [Fig. 2 (A)].
We determined the nucleotide sequence of the entire 1220-bp EcoT14I-SnaBI fragment. The nucleotide and deduced amino acid sequences are shown in Fig. 2 (B). Sequencing revealed one possible complete open reading frame (ORF) in this region, as well as the C-terminal portion of another. In the complete 987-bp orf encoding 329 amino acid residues (MW=36.4 kDa), we located a sequence identical to the probe used for hybridization, from 449 to 711. This gene was designated as srb (SRP receptor of B. subtilis). Upstream of the initiation codon of the srb gene, there was a Shine-Dalgarno sequence but no promoter region, suggesting that srb constitutes an operon with another ORF(s) upstream. Downstream of the stop codon of the srb gene, there was a p-independent terminator in the region from 1061 to 1092. The sequence reported here has been deposited in the GSDB, DDBJ, EMBL and NCBI nucleotide databases under the accession number D49781.

When the predicted amino acid sequence of the B. subtilis Srb protein was aligned with those of E. coli FtsY, archaeobacterial DPα and mammalian SRα, the C-terminal two-thirds of Srb containing elements I, II and III of the GTP-binding motif (G-domain) showed a high degree of similarity. The region of sequence identity among the four proteins included residues 1-329 of Srb, residues 180-497 of E. coli FtsY, residues 66-369 of Sulfolobus solfataricus DPα and residues 302-638 of mammalian SRα (Fig. 3). There was 49.7, 36.2 and 26.6% sequence identity between Srb and FtsY, S. solfataricus DPα and mammalian SRα, respectively. The amino acid sequence of element III of Srb was TKLD, which corresponds to the conserved sequence (TKXD) in the SRP54 family. Moreover, the sequence of element I was completely identical to the conserved sequence (GVNGVGTK/S) in SRP receptor, indicating that Srb is a member of the SRP54 family. In contrast to the homology in the C-terminal portion, the length of the N-terminal portion varied (Fig. 4). The N-terminal portion of the Srb protein was shorter and about one-fourth that of mammalian SRα. Mammalian SRα possesses charged amino acid clusters in its N-terminal region. SRα might interact with 7S RNA in SRP through this region.9 In the Srb protein, these charged amino acid clusters were not observed.

A secondary structure prediction showed an amphipathic α-helix structure positioned in the N-terminal region of Srb (from 26 to 43) (Fig. 5). This amphipathic α-helix structure was also found in SRα (from 310 to 327), DPα (from 93 to 110) and FtsY (from 202 to 219) (Figs. 4 and 5). Furthermore, the amphipathic α-helix regions were located about 100 amino acids upstream from element I of the four proteins. It is not known what this conserved structural feature means. On the other hand, eukaryotic and prokaryotic SRP54 possess an amphipathic α-helix structure in their C-terminal methionine-rich domain.3'10 Therefore, it is likely that the SRP receptor binds to SRP through each amphipathic helix structure, where hydrophobic regions react in a face-to-face manner like the leucine zipper.11'12 Alternatively, it has been reported that polypeptides, in which an amphipathic α-helix structure is contained, can bind to a lipid bilayer surface.13 Although the subcellular localization of Srb in B. subtilis cells has not yet been determined, the analogy of E. coli FtsY implies that Srb may be located peripherally on the surface of the plasma membrane.

Mammalian SRα forms a complex with membrane-integrated SRβ,14 and interacts with the membrane through its N-terminal hydrophobic region.9 In E. coli FtsY, S. solfataricus DPα and B. subtilis Srb, there are no significant hydrophobic segments in the corresponding region. Therefore, it is more plausible that SRα homologues of bacterial cells bind directly to the membrane.
Figure 2. Physical map (A) and nucleotide sequence of 1220 bp and the deduced amino acid sequence (B). (A) One orf (srb) was predicted from the nucleotide sequence in Fig. 2-B. The thick bar indicates the position of PCR product used as the probe in Southern hybridization. The arrow indicates the direction of transcription and translation in the ORF. (B) The putative ribosome-binding site (SD) is underlined. An inverted repeat of putative transcription termination signals is indicated by converging arrows. The three GTP-binding elements (boxes I to III) are indicated. Southern hybridization was performed as follows. DNA digested with restriction enzymes were separated by agarose gel electrophoresis and transferred onto GeneScreen Plus nylon membranes (Du Pont/NEN Research Products, Boston, MA). PCR amplified DNA fragment between GTP-binding element I and element II was labeled with [a-32P]dCTP using a random primer DNA labeling kit (Takara Shuzo Co. Ltd., Kyoto, Japan). For hybridization at high stringency, the membranes were prehybridized for 1 h at 65°C in a solution of 1 M NaCl, 10% dextran sulfate, 0.5% sodium dodecyl sulfate (SDS) and denatured herring sperm DNA (0.5 mg/ml). Labeled probe was then added to the mixture and hybridized for 12 to 18 h at 65°C. The membranes were washed for 1 h at 65°C with 2×SSC (1×SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 1% SDS. Plaque hybridization was performed following the same procedure under high stringency after plaques were fixed to the nylon membrane. DNA was sequenced by dideoxy chain-termination as described by Sanger et al. 15 with the Taq Dye primer Cycle Sequencing Kit and a model 373A DNA sequencer (Applied Biosystems Inc.).
**Figure 3.** Homology of the putative srb gene product with other proteins of SRP receptors. The alignment of the primary sequences of *B. subtilis* Srb (Srb), *E. coli* FtsY (FtsY), *S. solfataricus* DPa (DPa) and mammalian SRα (SRα) is indicated. Putative GTP-binding elements (boxes I to III) are indicated. The position in the amino acid sequence of the first amino acid on every line is shown. The amino acid residues showing identity to Srb are indicated by a black background. Gaps for maximum matching are indicated by dashes. Asterisks indicate the ends of amino acid sequences.

**Figure 4.** Schematic alignment of the primary structure of Srb of *B. subtilis* and SRP receptors. The boundaries defining the GTP-binding domain (G-domain) containing the elements I, II and III are indicated. The lightly shaded areas show the regions of an amphipathic α-helix structure (indicated by arrow). Diagonal hatching at the N-terminus of SRα indicates the membrane anchoring region.
However, we cannot discard the possibility that other components which support binding of SRα homologues are located in the plasma membrane.

Functional analysis has shown that the depletion of the Srb protein in *B. subtilis* causes a defect in normal cell growth and aberrant morphology (data not shown). Furthermore, depletion of Srb in *B. subtilis* led to a defect of the translocation of some extracellular proteins (data not shown). We therefore suggest that *B. subtilis* contains a gene encoding a homologue of mammalian SRα.

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