**Bacillus subtilis** Histone-like Protein, HBsu, Is an Integral Component of a SRP-like Particle That Can Bind the Alu Domain of Small Cytoplasmic RNA*

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Small cytoplasmic RNA (scRNA) is metabolically stable and abundant in *Bacillus subtilis* cells. Consisting of 271 nucleotides, it is structurally homologous to mammalian signal recognition particle RNA. In contrast to 4.5 S RNA of *Escherichia coli*, *B. subtilis* scRNA contains an Alu domain in addition to the evolutionarily conserved S domain. In this study, we show that a 10-kDa protein in *B. subtilis* cell extracts has scRNA binding activity at the Alu domain. The in vitro binding selectivity of the 10-kDa protein shows that it recognizes the higher structure of the Alu domain of scRNA caused by five consecutive complementary sequences in the two loops. Purification and subsequent analyses demonstrated that the 10-kDa protein is HBsu, which was originally identified as a member of the histone-like protein family. By constructing a HBsu-deficient *B. subtilis* mutant, we showed that HBsu is essential for normal growth. Immunoprecipitating cell lysates using anti-HBsu antibody yielded scRNA. Moreover, the co-precipitation of HBsu with (His)6-tagged Ffh depended on the presence of scRNA, suggesting that HBsu, Ffh, and scRNA make a ternary complex and that scRNA serves as a functional unit for binding. These results demonstrated that HBsu is the third component of a signal recognition particle-like particle in *B. subtilis* that can bind the Alu domain of scRNA.

The first step of secretory pathway needs protein factors that distinguish secretory proteins from cytoplasmic proteins. In higher eukaryotes, the first distinction between secreted and cytoplasmic proteins occurs at the ribosome upon the specific association of SRP with the signal sequence (1, 2). SRP purification from canine pancreas is composed of a 7 S RNA (7 SL RNA, referred to here as SRP RNA) and six proteins (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) (3). cDNAs for each protein have been cloned (4–9). The SRP proteins are associated with RNA as either monomers (SRP19 and SRP54) or heterodimers (SRP9/SRP14 and SRP68/SRP72). SRP54 is the best characterized component of SRP. SRP54 interacts with both SRP RNA and signal sequences and binds GTP (10–12). Studies using SRP constituted with either a subset of the SRP proteins or components modified with N-methyl maleimide indicate that SRP19 is required for SRP54 to associate with SRP RNA, SRP9/SRP14 is required for elongation arrest, and modification of SRP68/SRP72 by N-methyl maleimide prevents the close interaction of SRP with SRP receptor and inhibits translocation-promoting activity (13). On the other hand, the targeting of bacterial preproteins to the inner membrane also seems to involve other cytoplasmic pathways that converge at the membrane SecYEG translocon (14–18). One pathway unique to *Escherichia coli* involves a secretion-specific chaperone like SecB. This chaperone guides preproteins, such as proOMPα, to a membrane-associated receptor, SecA, which provides a link to the translocon complex (19). A second targeting pathway in prokaryotes involves chaperones that are not specific for secretory proteins. Roles for these chaperones become important when normal targeting pathways are impaired. DnaJ and DnaK are required for the residual transport of SecB-dependent proteins (20).

In addition to these pathways, a third pathway has been proposed because components of the SRP and SRP receptor have been identified in a wide variety of species (21). In *Bacillus subtilis*, scRNA, Ffh, and Srb (FtsY), which are homologous to eukaryotic SRP RNA, SRP54, and the α-subunit of SRP receptor, respectively, have been identified (22–25). Like SRP54, Ffh can be cross-linked to a variety of functional signal sequences derived from both Gram-negative and -positive bacteria (26, 27). Moreover, we demonstrated that the depletion of SRP components or SRP receptor causes defects in preprotein translocation (23, 24, 28).

SRP RNAs have been identified in all cells analyzed to date. Larsen and Zwieb (29) proposed that mammalian SRP RNA consists of eight helices (numbered 1–8). Mammalian SRP RNA (7SL RNA) is subdivided into two domains. The Alu domain, consisting of helices 1–5 (helix 1 is unique to *B. subtilis* and Archaeabacteria), seems to have arose during evolution to the Alu interspersed repetitive sequences found in the human genome (30); the S domain, comprising part of helix 5 and helices 6–8, contains 150 nucleotides of core sequence lacking Alu repeat similarity. An evolutionary comparison has revealed that almost all Archaeabacteria and eukaryotes, except yeast, contain SRP RNA consisting of eight helices. The nucleotide sequence in the region corresponding to helix 8 is highly conserved among SRP RNA homologues. On the other hand, the Alu domain contains the two smallest subunits, SRP9 and SRP14, and their sequences comprise about 100 nucleotides from the 5′ end and 50 nucleotides from the 3′ end of SRP RNA, respectively. Moreover, phylogenetic analyses indicate that SRP RNA is the evolutionary progenitor of the repetitive Alu sequences (31). Functional analysis of mammalian SRP RNA demonstrated that the Alu domain is required for elongation arrest, because SRP particles lacking this domain are deficient in this function (13). In contrast to the structural integrity...
observed among eukaryotes and Archaea bacteria SRP RNAs, the length and secondary structure of eubacterial SRP RNA vary. E. coli 4.5 S RNA consists of 114 nucleotides that can fold into a single hairpin corresponding to helix 8 and part of helix 5 (32). Almost all SRP RNA from Gram-negative bacteria have a secondary structure similar to that of E. coli (29). On the other hand, the secondary structure of SRP RNAs from Gram-positive bacteria differs from that of E. coli. Bacillus subtilis scRNA is considered to be a member of the SRP RNA family (22). It is transcribed as a 354-nucleotide precursor and then processed into a single hairpin corresponding to helix 8 and part of helix 5 (32). The structural features of SRP RNA and eubacterial phylogeny based upon the 16S RNA sequence reveal a discrepancy (21). Thermus thermophilus has been placed in the earli- est branches of the tree, whereas bacilli are thought to have arisen considerably later in eubacterial evolution. The question then arose as to the advantages conferred upon Bacillus and other Gram-positive bacteria by the preservation of helices 1–5 if eukaryotic RNAs represent the prototype structure. To identify the structural requirements for B. subtilis scRNA, we constructed mutants in which individual helices were deleted and assayed their importance in vivo. The results showed that helices 1–4 and part of helix 5 are not essential for vegetative growth but are needed for the formation of heat-resistant spores (35). Moreover, we propose that scRNA combined with Ffh to form a SRP-like particle functions in the translocation of sporulation-specific proteins such as penicillin-binding protein 5* to the interspace between the mother cell and the prespore. To understand the function of the Alu domain of B. subtilis scRNA, it is first necessary to identify protein(s) that can bind this region.

Here we identified B. subtilis HBsu (10 kDa) protein with respect to its binding affinity for the Alu domain of scRNA (referred to as the scRNA Alu domain) that consists of helices 1–4 and part of helix 5. Using several mutants of scRNA, we found that the HBsu protein recognizes the secondary structure of these helices rather than the specific nucleotide sequences. We also show that HBsu, Ffh, and scRNA make a stable complex in vivo and that scRNA functions as a backbone for complex formation. We discuss the structural similarity of HBus to that of the SRP9/SRP14 heterodimer demonstrated by Birse et al. (36).

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—Table I lists the plasmids and bacterial strains used. The E. coli and B. subtilis strains were cultivated in Luria Bertani medium. Antibiotics were added at the following concentrations: chloramphenicol, 5 μg/ml; ampicillin, 50 μg/ml; and tetracycin 10 μg/ml. IPTG was added at 2 mM unless otherwise indicated.

Plasmid Construction—Mutant sc103 is thought to have helices 1–5. Nucleotides 80 and 249 of sc103 are linked with the sequence GGAA. Plasmids pTUE1331 (sc103), 1333 (sc73*), and 1334 (sc51) were constructed by annealed fragments into pSP64, synthetic oligonucleotides were designed to create HindIII and BamHI sites at the 5’ and 3’ end,
respectively.

Plasmids pTUE1335 and pTUE1336 were constructed for in vitro transcription of hammerhead ribozyme and B. subtilis trna (AGC). These RNAs are thought to have double-stranded structures and were used in the gel retardation assay as competitors. The two annealed oligonucleotides, each having one RNA were cloned into the HindIII-BamHI sites of pSP64.

The entire coding region of the B. subtilis HBsu gene (hbs) was amplified by the polymerase chain reaction using synthetic oligonucleotide primers C and D (C, 5'-GGAGATCTTTTTCCGGCAACTGCGTCTTTT-AAGC-3'; D, 5'-GGGGGTCTAGAAGACAGATGCACTCAG-3') and genomic DNA as a template. The 106-bp DNA product was cut with Smal-XbaI and inserted into the SmaI-XbaI site of pUC19, which allows the overexpression of hbs was used as a template (26). The PCR product was purified and used to construct recombinant plasmid designated pTUE1338 and used to construct B. subtilis strain UT1681.

The 3' part of the B. subtilis hbs gene encoding the +1 to +29 amino acids of HBsu was amplified by PCR using the synthetic oligonucleotide primers C and D (C, 5'-GAGATTTAAGACAGATGCACTCAG-3'; D, 5'-GGGGGTCTAGAAGACAGATGCACTCAG-3') and genomic DNA as a template. The 106-bp DNA product was cut with Smal-XbaI and inserted into the SmaI-XbaI sites of pTUE1336, was used as a template (25). The PCR product was purified and cut with EcoRI and then inserted into the EcoRI site of pDHS8. The resulting plasmid, designated pTUE1339, was used to construct B. subtilis strain UT1682.

The constructs were confirmed by physical mapping and DNA sequencing.

In Vitro Synthesis of RNAs—Probe RNAs were transcribed from BamHI-linearized plasmids using 35 units of SP6 RNA polymerase (Takara Shuzo Ltd.) in the presence of [γ-32P]CTP (400 Ci/mmol; Amersham International) using the Riboprobe Gemini system II Buffers Kit (Promega). The sample was precipitated twice by ethanol, and then purified RNAs were resolved in sterile deionized water. The concentrations of radiolabeled RNAs were determined from the specific activity of the [γ-32P]CTP incorporated into the transcripts. Before use, RNAs were denatured by incubating them for 15 min at 65 °C, followed by a slow cooling to room temperature. Competitor RNAs were also transcribed from the HBsu gene (1t o o)

**FIG. 1. Ffh and scRNA co-sediment in sucrose density gradient.** Crude cell extracts were layered over a 5–15% sucrose gradient and centrifuged for 10 h at 40,000 × g. The gradient fractions were collected and divided into two aliquots. One aliquot was analyzed by Western blot using an antibody against Ffh (A), and total RNA from the other aliquot was analyzed by Northern blot using a scRNA-specific probe (B). Sedimentation values of the protein standards (cytochrome oxidase, 5.8 S; fibrinogen, 8.0 S; catalase, 11 S) are indicated.
purification of a B. subtilis Alu Domain-binding Protein

Identification of a Protein That Binds to the scRNA Alu Domain in B. subtilis Cell Lysates—Fig. 1 shows that Ffh and scRNA co-sedimented on sucrose density gradients with a sedimentation coefficient of 8 S, which is greater than would be expected for a particle containing only Ffh and scRNA (data not shown). Thus, the B. subtilis complex probably contains another protein component(s). Moreover, the protein component(s), if present, could bind the Alu domain consisting of helices 1–5 because Ffh binds the S domain comprising helix 5. To identify the Alu domain-binding protein(s) in B. subtilis cell extract, we performed a RNA mobility shift assay. B. subtilis cell extracts were incubated with 32P-labeled sc103 (Fig. 2B) containing helices 1–5 synthesized in vitro. One distinct band appeared, and the amount of complex formed was dependent on the amount of protein added (Fig. 3, lanes 2–5). The mobility shift band corresponding to this band was also obtained when a smaller volume of fraction 7 and 8 was used (data not shown). To identify which protein in the fraction is responsible for Alu domain binding activity, fractions containing activity from the DEAE-Sepharose CL-6B column (Fig. 4A, fractions 5–10) were individually concentrated to ~50 µl and separated on a denaturing SDS gel. The separated proteins were blotted onto a polyvinylidene difluoride membrane and hybridized with 32P-labeled sc103 transcribed in vitro. After washing the membrane to remove excess probe, a 10-kDa protein interacted with the probe, and the amount of the positive band peaked at fraction 7 (Fig. 4B). When aliquots of these fractions were resolved by SDS-PAGE, the gel stained with Coomassie Blue demonstrated that a single predominant protein band with an apparent molecular weight of about 10,000 was responsible for the positive band in the Northwestern blot (Fig. 4, B and C). The protein was blotted onto a polyvinylidene difluoride membrane and excised, and the amino acids were sequenced.

The 10-kDa Protein Is HBsu—At the amino-terminal region of the 10-kDa protein, the only sequence obtained was MNKLTELINAVAEASELSKRD. A search of the nonredundant protein database using the BLAST search program revealed that this 10-kDa protein is identical to histone-like protein (HBsu) (SwissProt accession number P08821) (40). The identity of substrates to determine which helices would serve as binding sites for this protein. We constructed two mutants that were deletions of helix 5. We divided helix 5 into three subdomains (referred to as helices 5-1, 5-2, and 5-3; Fig. 2). In addition to helices 1 and 2, helix 5-3 and part of helix 5-2 were also deleted from sc73 and sc73* substrates. In sc73*, five nucleotides (AGCGG) in the loop connected to helix 3 were changed to UGCCC. As a result, nucleotide complementation between the two loops connected to helices 3 and 4 was disrupted to diminish the pseudoknot structure between them (Fig. 2). Mutant sc51 was composed of two subdivisions of helix 5 (helix 5-1 and 5-2), corresponding to domain II. Of these three mutants, only sc73 formed the same amount of binding complexes as sc103 (Fig. 3, lanes 1–10), whereas sc51 had no activity (Fig. 3, lanes 16–20). The sc73* mutant showed a significant band, but the efficiency was reduced to 20% of that of sc103 (Fig. 3, lanes 11–15).

Purification of the 10-kDa Protein—A B. subtilis cell lysate was fractionated with saturated ammonium sulfate (65–90%), and the pellet was loaded onto a DEAE-Sepharose CL-6B column (Pharmacia). As shown in Fig. 4A, a shift band with the same mobility as that shown in Fig. 3 was found in fractions 6–12 (C2). Moreover, in fractions 7 and 8, more mobility shift band (C1) was obtained. This band, designated C1, may be caused by the binding of homodimeric protein to the radiolabeled RNA probe, because this band disappeared and only band C2 was obtained when a smaller volume of fraction 7 and 8 was used (data not shown). To identify which protein in the fractions is responsible for Alu domain binding activity, fractions containing activity from the DEAE-Sepharose CL-6B column (Fig. 4A, fractions 5–10) were individually concentrated to ~50 µl and separated on a denaturing SDS gel. The separated proteins were blotted onto a polyvinylidene difluoride membrane and hybridized with 32P-labeled sc103 transcribed in vitro. After washing the membrane to remove excess probe, a 10-kDa protein interacted with the probe, and the amount of the positive band peaked at fraction 7 (Fig. 4B). When aliquots of these fractions were resolved by SDS-PAGE, the gel stained with Coomassie Blue demonstrated that a single predominant protein band with an apparent molecular weight of about 10,000 was responsible for the positive band in the Northwestern blot (Fig. 4, B and C). The protein was blotted onto a polyvinylidene difluoride membrane and excised, and the amino acids were sequenced.

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this 10-kDa protein as HBsu was confirmed by immunoblotting using rabbit anti-B. subtilis HBsu antisem (data not shown). However, we could not exclude the possibility that a low level of contaminating protein in the fraction was responsible for the band shift. To confirm that HBsu is identical to the 10-kDa protein, we expressed His-tagged HBsu in E. coli and purified it using a Ni-NTA column. The binding activity of the purified HBsu was tested by gel mobility shift assay (A) and by Northwestern blotting (B). C, Sepharose CL-6B fractions were concentrated, separated by denaturing SDS-PAGE, and stained with Coomassie Blue, and then the proteins were sequenced. C1 and C2 indicate the position of RNA-protein complex. FP indicates a protein-free RNA probe.

In Vivo Complex Formation of HBsu with Ffh via scRNA—Data from our in vitro studies indicate that HBsu can form a complex with scRNA in cells. To determine whether or not HBsu interacts directly with scRNA in vivo, we immunoprecipitated B. subtilis cell extracts with anti-HBs antisem and determined the presence of scRNA within the HBsu immune complex by Northern blotting with the 32P-labeled DNA fragment encoding mature scRNA. scRNA was immunoprecipitated with the anti-HBs antiserum but not with rabbit preimmune antiserum (Fig. 6A, lanes 2 and 3), demonstrating that B. subtilis scRNA associates with HBsu in vivo. Because scRNA is known to be associated with another protein, Ffh, it is more likely that Ffh-scRNA-HBsu forms a ternary complex. To examine this notion, we constructed the B. subtilis mutant UT1682 in which chromosomally encoded Ffh is tagged with six consecutive histidine residues at the carboxyl terminus (Fig. 6B). A cell lysate of this mutant was mixed with Nt-NTA resin, and then a complex containing His-tagged Ffh was precipitated. The complex was washed, and then we examined whether or not scRNA and HBsu were present. Fig. 6C shows that both HBsu and scRNA were complexed with Ffh(His)6-Ni-NTA resin. Moreover, the amount of HBsu diminished when lysates were incubated with ribonuclease A and T1 before precipitation (Fig. 6C, lane 5). These data suggest that HBsu-scRNA-Ffh form a complex in vivo and that scRNA serves as a functional unit (backbone) for ternary complex formation. We have recently shown that B. subtilis scRNA can bind elongation factor G (38). However, the complex with Ffh (His)6-NTA resin did not contain elongation factor G (data not shown).

HBsu Is Required for Cell Growth—To understand the function of HBsu, we constructed plasmid pTUE1338, which would allow replacement of the native HBsu with the IPTG-inducible spac-1 promoter. In the resulting transformed strain, UT1681, the gene expression of HBsu is regulated by IPTG. The transformants were able to grow as well as wild type cells in the presence of IPTG (Fig. 7). However, if this transformant is inoculated in the absence of IPTG, the growth is greatly inhibited, indicating that HBsu is essential for normal growth. These results are consistent with those of Mica and Marahiel (41).

**DISCUSSION**

To date, only Ffh has been identified as a protein component of a SRP-like particle in bacteria. Homologues of SRP9/SRP14 that can interact with the Alu domain of SRP RNA have not yet been identified. The present study demonstrated that the B. subtilis histone-like protein, HBsu, is an integral component of a ribonucleoprotein complex containing at least Ffh and scRNA.
and that HBsu can recognize the predicted secondary structure of the Alu domain. HBsu of B. subtilis belongs to a widespread family of histone-like proteins in bacteria (40, 42, 43), and its function is essential for cell growth (41). This family represents a group of small, basic, and abundant proteins that bind DNA (44). Histone-like proteins play a role in DNA condensation. Like HU, HBsu binds DNA. In contrast to HU, which predominantly forms heterodimers, HBsu binds DNA as a homodimer (45). Because the function of HBsu in the SRP-like particle remains to be resolved, several arguments favor the notion that HBsu is a true component of the B. subtilis riboprotein complex. (i) The immunoprecipitation of cell lysates using anti-HBsu antiserum yielded scRNA (Fig. 6A). Moreover, immunoprecipitation identified only scRNA as the RNA species in the immunoprecipitates (data not shown). (ii) Fig. 6C shows that HBsu co-precipitation with (His)_6-tagged Ffh depended on the presence of scRNA, suggesting that scRNA serves as a functional unit (backbone) in this complex. (iii) After fractionation on ω-amino pentyl-agarose and DEAE-Sepharose CL-6B that had been used to purify mammalian SRP, scRNA, Ffh, and HBsu were still co-eluted by a relatively high salt concentration (250 mM KOAc acetate), suggesting that the three constituents formed a complex in a salt-resistant manner.

Although the mechanism of the interaction of B. subtilis HBsu with the scRNA Alu domain was unknown, the crystal structure of the monomeric and homodimeric B. steatorrhophilum DNA-binding protein HU (BstHU) relative to the mesophilic homologue B. subtilis HBsu was determined at a resolution of 3 Å, and a model of nucleic acid-HU interaction was proposed (45). Based on the proposed model, the secondary structure of the BstHU monomer is represented as a1-a2-β1-β2-β3-α3 with two distinct halves (Fig. 8A). The amino-terminal half consists of two a-helices connected by a broad turn (β1) to create a V-shaped supersecondary structure. The carboxy-terminal half consists mainly of a three-stranded antiparallel β-sheet that spans the top of the V formed by the two helices. The two halves of the structure are connected by a β-turn between a2 and strand 1, which has a highly conserved glycine residue. Fig. 8B shows that the amino acid residues among B. subtilis HBsu, TonN, and B. steatorrhophilum HU are highly conserved (more than 90% amino acid sequence homology). Therefore, a similar supersecondary structure could be drawn for B. subtilis HBsu. Two monomers wrap around each other to produce a novel dimeric structure. The base of the molecule is formed by the two V-shaped helical supersecondary structures packed such that the two α2 helices are in contact roughly at a right angle, and the two α1 helices are on either side. Moreover, the visible parts of the arms, together with the two symmetry-related strand 3 between them, create a concave surface on the dimer. The helical depression has a diameter of 25 Å, indicating that the protein electrostatically interacts with the DNA. A mammalian heterodimer consisting of 9- and 14-kDa polypeptides (SRP9/SRP14) folds with a strikingly similar concave structure, regardless of the low amino acid sequence similarity among B. subtilis HBsu, SRP9, and SRP14. Birse et al. (36) showed that SRP9 and SRP14 are structurally homologous, containing the same α-β-β-α fold. According to their model, the heterodimer has a pseudo 2-fold symmetry and is saddle-like, comprising a rigidly curved six-stranded amphiathic β-sheet with the four helices packed on the convex side, and the

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exposed concave surface being lined with positively charged residues. The SRP9/SRP14 heterodimer may be the latest member of a growing family of small α/β RNA-binding proteins. These striking structural similarities indicate that saddle-like folding in the HBsu homodimer is important for scRNA Alu domain binding and that curvature of the HBsu β-sheet is suitable for the tertiary structure of the RNA maintained by the five base pairings between the two loops. However, we have not examined whether or not HBsu dimerization is required for binding. Nonetheless, mutational analysis supported this notion because nucleotide changes diminishing the formation of this tertiary interaction notably reduced the ability of HBsu to bind the scRNA Alu domain (Fig. 3, sc73*). The dsRNA binding consensus sequence (KGFG-F-V-F) conserved among E. coli RNaseIII, human TAR-binding protein, and Drosophila staufen protein was found between β strands 1 and 2 in HBsu (Fig. 8) (46).

The present results raise the question of the significance of the interaction between HBsu and its RNA ligand. We demonstrated that physiological defects of the depletion of scRNA can be compensated by E. coli 4.5 S RNA that completely lacks the Alu domain (23). Moreover, depleting scRNA of its Alu domain did not affect vegetative growth. Therefore, the essential nature of HBsu protein would appear to be unrelated to its role in B. subtilis SRP function. However, we also showed that the growth rates of the scRNA mutant without the Alu domain are inhibited at the end of the vegetative phase (35). The frequency of heat-resistant spores was concomitantly reduced to 20% of the level in the wild type. Secretory proteins, including α-amylase and alkaline protease in B. subtilis, are synthesized at the end of vegetative growth and actively secreted into the culture medium. Moreover, during sporulation, many proteins are synthesized in the mother cell, translocated across the membrane, and localized in the interspace between the mother cell and the forespore. The penicillin-binding protein PBP5* is representative of proteins located in the outer forespore membrane. This is specifically synthesized in the mother cell at the late stage of sporulation and is required for the heat-resistant spore formation. Bunai et al. (26, 47) demonstrated that Ffh protein that is a component of B. subtilis SRP can bind the signal sequence of PBP5*. Based on these data, B. subtilis SRP function appears to be required for translocation during the

**Fig. 8.** Structure-based amino acid sequence alignment comparing histone-like proteins from Gram-positive and -negative bacteria. A, schematic representation of the tertiary structure of B. stearothermophilus DNA-binding protein II dimer. The protein model was generated by Molscript using crystallographic data from Tanaka et al. (45). The protein backbone is depicted by ribbons. B, amino acid sequence alignment of bacterial histone-like proteins: HBsu B. sub., B. subtilis HBsu; YonN B. sub., B. subtilis YonN; HBS B. ste., B. stearothermophilus DNA-binding protein II; NS-1 E. coli, E. coli NS-1 (HUα); NS-2 E. coli, E. coli NS-2 (HUβ). The secondary structure distribution is based on an electron density map of B. stearothermophilus DNA-binding protein II (45) and is represented by β-strands (horizontal black bars) and α-helices (horizontal gray bars). Consensus of at least 50% identical charged residues is denoted by □; residues contributing to the hydrophobic core are depicted by ■.
late stage of vegetative growth and during sporulation. The loss of function that arises when the Alu domain is deleted suggests that the Alu domain, perhaps including HBsu, is required for B. subtilis SRP function. We also demonstrated the requirement for the Alu domain by showing that Clostridium perfringens scRNA that has this domain can compensate for both vegetative growth and spore formation in B. subtilis cells depleted of scRNA (48).

Brown et al. (49) demonstrated that the stable assembly of yeast SRP in vivo relies on the presence of all subunits. The levels of sc1R1 RNA (SRP RNA) and of other SRP proteins in yeast were significantly reduced in strains lacking any one of SRP14p, SRP21p, SRP68p, or SRP72p. In contrast to yeast, B. subtilis vegetative growth and spore formation in strains lacking any one of the scRNA that has this domain can compensate for both SRP function. We also demonstrated the requirement for the Alu domain of the scRNA Alu-domain-binding protein II. We are grateful to N. Foster for critical reading of the manuscript.

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