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Enhancing Effect of *Bacillus subtilis* Ffh, a Homologue of the SRP54 Subunit of the Mammalian Signal Recognition Particle, on the Binding of SecA to Precursors of Secretory Proteins *In Vitro*[1]

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The precursors of β-lactamase fusion proteins having the signal peptide of *Bacillus subtilis* alkaline protease (pAprE-BlaH6) or penicillin binding protein 5* (pPBP5*-BlaH6) accumulated in *B. subtilis* cells in the absence of SecA or Ffh. Using the five purified precursors of secretory proteins including the two fusion proteins, *B. subtilis* Ffh and SecA, we analyzed the protein targeting mechanism of *B. subtilis in vitro*. *B. subtilis* SecA recognized the completely translated precursors of secretory proteins to which Ffh also bound. Moreover, *B. subtilis* SecA-precursor complex formation was enhanced 15- to 30-fold when the precursor and Ffh were incubated first and then SecA was added, but not *vice versa*. We also found that *B. subtilis* SecA directly interacted with Ffh *in vitro*. These results indicate that *B. subtilis* SecA and Ffh interact to function cooperatively in a protein translocation pathway including other protein factors, and that Ffh, as well as SecB in *Escherichia coli*, enhances the binding of SecA to presecretory proteins in *B. subtilis* cells.

Key words: *Bacillus subtilis*, Ffh, protein secretion, SecA, signal recognition particle.

In mammalian cells, the signal recognition particle (SRP) plays a central role in the recognition and targeting of presecretory proteins to endoplasmic reticulum membranes (*1*). SRP is a ribonucleoprotein complex composed of one RNA (SRP 7S RNA) and six proteins (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) (*2*). SRP54 consists of a structurally distinct N-terminal G-domain and a C-terminal M-domain (*3, 4*). Lütcke *et al.* (*5, 6*) reported that the M-domain of SRP54 binds to a signal peptide of a nascent presecretory protein, probably through direct interaction with the hydrophobic region in the signal peptide. This causes elongation of the nascent polypeptide chain on the ribosome to be arrested (*2, 6*). The complex formed through this interaction is targeted and then binds to a hetero dimeric SRP receptor (SRα and SRβ) on the endoplasmic reticulum membrane, followed by translocation to the membrane (*2, 6, 7*).

On the other hand, a nascent presecretory protein is thought to be recognized by molecular chaperones such as SecB in *Escherichia coli* (*8*), and the function of SecB is to carry the precursor of a secretory protein to membrane bound SecA, which is the peripheral ATPase subunit of translocase. This process involves SecA binding to the precursor of the secretory protein-SecB complex through the direct interaction of SecB as well as the leader and mature domains of the presecretory protein (*9*). The precursors of secretory proteins are then translocated across the cytoplasmic membrane through a SecA ATPase-dependent translocase consisting of SecE, SecG, SecY, and other membrane proteins (*9, 10*). It has been revealed that SecA promotes protein translocation during cycles of SecA insertion and deinsertion on SecYEG on the membrane (*11, 12*). Therefore, it has been considered that the protein translocation system of *E. coli* differs from those of mammalian cells. However, other evidence has revealed similarity between the protein translocation pathways of eukaryotes and prokaryotes, although a homologue of prokaryotic SecA has not been identified in mammalian cells. Homologues of SRP 7S RNA and SRP54 have been identified in animals, plants, yeasts, eubacteria, archaebacteria, and chloroplasts (*13-15*). *E. coli* 4.5S RNA and Ffh protein, which are the homologues of SRP 7S RNA and SRP54, respectively, form a complex that can specifically interact with the signal peptide of nascent secretory proteins (*16*). Furthermore, Luirink *et al.* (*16*) proposed that the role of *E. coli* SRP is that of a chaperone that is specific for the signal sequence of nascent proteins and which maintains them in a translocation-competent conformation. The Ffh/4.5S ribonucleoprotein complex tightly binds to FtsY, a homologue of the SRP-receptor α-subunit (SRα) in a GTP-dependent manner (*13*). Therefore SRP, SRP receptor and Sec61α (a mammalian homologue of the SecY subunit) (*17*) are thought to be basically conserved in the protein translocation pathways of prokaryotic cells.

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Abbreviations: SRP, signal recognition particle; scRNA, small cytoplasmic RNA; Bla, *E. coli* β-lactamase; AprE, *B. subtilis* alkaline protease; PBP5*, *B. subtilis* penicillin binding protein 5*; CGTase, cyclodextrin glucanotransferase of alkalophilic *Bacillus* sp. #1011; Ni-NTA, nickel nitrilotriacetate; PMSF, phenylmethylsulfonyl fluoride; IPTG, isopropyl 1-thio-β-D-galactopyranoside; PVDF, polyvinylidene difluoride; ECL, enhanced chemiluminescence; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

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Recent studies indicated important roles for *E. coli* SRP and FtsY in the assembly of inner membrane proteins as opposed to the Sec machinery mediating the posttranslational targeting of secretory proteins (*18–21*). However, little is known about the passage of presecretory proteins from SRP to the Sec machinery.

Bacillus subtilis is a Gram-positive bacterium that secretes higher levels of many enzymes into the culture media than *E. coli*. Homologues of *E. coli* SecA, SecE, and SecY have been identified in this organism, as have an SRP-like particle and its receptor (Srb renamed FtsY in the *B. subtilis* genome project, see Ref. *30*) (*22–28*). *B. subtilis* SecA has been isolated as a homodimer of 94 kDa subunits possessing ATPase activity that is stimulated in the presence of presecretory proteins and inverted membrane vesicles (*25, 29*). A *B. subtilis* SecA (*secA341*ts) mutant accumulates the precursor proteins of α-amylase and β-lactamase at 45°C (*25*). *B. subtilis* SecA cannot complement the depletion of *E. coli* SecA either *in vivo* or *in vitro* (*25*). Homologues of *E. coli* SecB were not found in *B. subtilis* on a computer search for the complete nucleotide sequence of its chromosomal DNA (*30*).

Small cytoplasmic RNA (scRNA) is a 271 nts homologue of SRP 7S RNA (300 nts), which has a secondary structure similar to that of mammalian SRP 7S RNA (*31*). It differs from *E. coli* 4.5S RNA in size (114 nts) and secondary structure. Nakamura *et al.* (*32*) reported that *B. subtilis* Ffh, a homologue of SRP54 protein, forms a complex with scRNA in *B. subtilis* cells, and we found a protein that bound to scRNA recently (Yahagi, Yamasaki, Nakamura, and Yamane; unpublished observations). Depletion of Ffh or scRNA inhibits the secretion of extracellular α-amylase and β-lactamase by *B. subtilis* (*24, 26*). The *B. subtilis* SRP-like particle is essential for normal cell growth and protein translocation (*26*). Bunai *et al.* (*33*) and Takamatsu *et al.* (*34*) reported that *B. subtilis* Ffh can intrinsically bind to precursors of β-lactamase (pBlaH6) and its fusion proteins, which have the signal peptide of *E. coli* outer membrane protein A (pOmpA-BlaH6), *B. subtilis* alkaline protease (pAprE-BlaH6), *B. subtilis* penicillin binding protein 5* (pPBP5*-BlaH6), or alkalophilic *Bacillus* sp. #1011 cyclodextrin glucanotransferase (pCGTase-BlaH6) at the site containing the h1 hydrophobic region of the M-domain. Therefore, both SecA and Ffh participate in the translocation of a presecretory protein in *B. subtilis*. However, little is known about the functional relationship between *B. subtilis* SecA and Ffh.

This study showed that *B. subtilis* SecA binding to presecretory proteins is enhanced 15- to 30-fold when the precursors are initially incubated with *B. subtilis* Ffh *in vitro*. We also found that *B. subtilis* SecA directly interacted with Ffh *in vitro*. We therefore propose that *B. subtilis* SecA and Ffh interact to function cooperatively in a protein translocation pathway including other protein factors.

MATERIALS AND METHODS

Strains—We used *B. subtilis* strain 168 (*trpC2*) as the wild type strain, TB301 (*trpC2, secA341*) as the *secA* ts mutant (*35*), DF46 (*trpC2, spac-1:: ffh, penP-lacI*, Cm^r) as a conditional mutant of Ffh (*26*), and *E. coli* strains JM109 (*recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1,* λ^-, Δ(*lac-proAB*), F′ [*traD36, proAB*$^+$, *lacI*^{q}, *lacZ*ΔM15]) (*36*), BA13 (F$^-$, *araD136,* Δ*lacU169, relA, rpsL, thi, secA13*^{amber}, *supF*^{ts}) (*37*), M15 (*Nal*^{s}, *Str*^{s}, *rif*^{s}, *lac*I$^-$, *ara*$^-$, *gal*$^-$, *mtl*$^-$, F$^-$, *recA*$^+$, *uvr*$^+$) (Qiagen, Chatsworth, CA), and MM52 (F$^-$, *araD136,* Δ(*lac*)*U169, relA, rpsL, thi, secA51*(Ts)) (*38*).

Antisera—We raised rabbit anti-*B. subtilis* SecA (*25*), rabbit anti-*B. subtilis* Ffh (*26*), and rabbit anti-CGTase antisera (*39*). Rabbit anti-β-lactamase and rabbit anti-catalase antisera were purchased from 5′-3′ Inc. Co. and Poly Science Inc., respectively.

Cell Lysate Preparation—We constructed *E. coli-B. subtilis* shuttle plasmids pTUBE1234 and pTUBE1235 encoding *B. subtilis* alkaline protease-β-lactamase fusion protein (AprE-BlaH6) and *B. subtilis* penicillin binding protein 5*-β-lactamase fusion protein (PBP5*-BlaH6), respectively (*37*). The mature region of β-lactamase (40 to 283 amino acids) was fused downstream of the signal peptide of each secretory protein. In these plasmids, the gene encoding each recombinant β-lactamase exists downstream of the tetracycline resistant gene in the same direction and should be constitutively expressed. *B. subtilis* 168 or TB301 transformants carrying plasmid pTUBE1234 or pTUBE1235 were cultured in 5 ml of Luria-Bertani medium (L-broth) supplemented with 10 μg/ml of tetracycline at 30°C for 4 h. Thereafter, the transformants were incubated for a further 2 h at 30 or 42°C. *B. subtilis* DF46 transformants were cultured in 10 μg/ml of tetracycline at 37°C in the presence or absence of 1 mM IPTG until the absorbance at 660 nm reached about 0.3. The cells were harvested by centrifugation, suspended in 100 μl buffer I (25 mM Tris-HCl, pH 8.0, 10 mM EDTA, 50 mM glucose, 1% lysozyme), incubated at room temperature for 5 min, and then boiled in the presence of 0.4 M Tris-HCl, pH 6.8, 2% SDS, 5% mercaptoethanol, and 10% glycerol for 5 min before use as the cell lysate preparation.

Purification of Recombinant β-Lactamases with a 6× His Tag Using Ni-NTA Agarose Beads—We constructed *E. coli* plasmids pTUE1251, pTUE1253, pTUE1254, pTUE1255, and pTUE1256 encoding β-lactamase of *E. coli* plasmid pUC18 (BlaH6), *E. coli* OmpA-β-lactamase fusion protein (OmpA-BlaH6), *B. subtilis* alkaline protease-β-lactamase fusion protein (AprE-BlaH6), *B. subtilis* penicillin binding protein 5*-β-lactamase fusion protein (PBP5*-BlaH6), and alkalophilic *Bacillus* sp. #1011 cyclodextrin glucanotransferase-β-lactamase fusion protein (CGTase-BlaH6), respectively (*33*). The mature region of β-lactamase (40 to 283 amino acids) was fused downstream of the signal peptide of each secretory protein and six consecutive histidine residues (6×His tag) were connected to the C-terminal of the β-lactamase region, as shown schematically in the structures of the fused β-lactamases predicted from their nucleotide sequences (Fig. 1). These fusion genes were placed under the control of the *tac* promoter for overexpression with IPTG. To purify the precursors of β-lactamase and other fusion proteins, the constructed plasmids were transformed into an *E. coli secA* mutant (*secA13*^{amber}) (BA13). *E. coli* BA13 transformants carrying pTUE1251, pTUE1253, pTUE1254, pTUE1255, and pTUE1256 were cultured in 200 ml of L-broth containing 50 μg/ml of ampicillin at 30°C for 2 h. Thereafter, 1 mM IPTG was added and the transformants were incubated for a further 2 h at 42°C. The precursors of β-lactamase fusion proteins in cell lysates in buffer II (50 mM Na_2HPO_4-NaH_2-

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PO_4, pH 7.8, 300 mM NaCl, 1% SDS) were purified with Ni-NTA agarose beads (Qiagen Inc., Chatsworth, CA). The samples were then mixed with buffer III (50 mM Na_2HPO_4-NaH_2PO_4, pH 7.8, 300 mM NaCl, 8 M urea) containing 200 mM imidazole. To prepare the mature forms of the five fusion proteins, *E. coli* BA13 transformants carrying pTUE1251, pTUE1253, pTUE1254, pTUE1255, and pTUE1256 were cultured in 200 ml of L-broth supplemented with 50 μg/ml of ampicillin at 30°C for 2 h, and then 1 mM IPTG was added for a further 3 h culture. The mature forms of the β-lactamases were likewise purified. The estimated purity of the proteins was over 90%, as judged on laser densitometry of a Coomassie Brilliant Blue-stained gel using NIH image software.

Expression and Purification of B. subtilis SecA—The *B. subtilis* SecA protein was purified as described (*25*). An *E. coli* MM52 transformant carrying pTUE855 (*25*), in which *B. subtilis* secA was under the control of the *tac* promoter, was incubated at 30°C for 6 h in 2.5 liters of L-broth containing 50 μg/ml of ampicillin. After induction with 1 mM IPTG for 4 h, the cells were harvested by centrifugation and then suspended in 10 mM potassium phosphate buffer (pH 7.2) (80 ml). The cells were disrupted by sonic oscillation, and then the cell debris was removed by centrifugation. The precipitate obtained on saturation with 40 to 50% $(NH_4)_2SO_4$ was dialyzed against 10 mM potassium phosphate buffer (pH 7.2) and then applied to a hydroxyapatite column (2.5 by 12 cm) equilibrated with the same buffer. SecA was eluted with a linear gradient of 10 to 250 mM potassium phosphate buffer (pH 7.2). The purity of the preparation was over 90%, as judged on laser densitometry of a Coomassie Brilliant Blue-stained gel using NIH image software.

Expression and Purification of B. subtilis Ffh with a 6× His Tag—*B. subtilis* Ffh with a 6×His tag was purified according to the method of Nakamura *et al.* (*32*). The *B. subtilis* Ffh protein cloned in pTUE815 (*32*) was expressed in *E. coli* M15 harboring pREP4 (Qiagen Inc., Chatsworth, CA). Cells were harvested and suspended in 10 ml of buffer IV (20 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 150 mM NaCl, 10% glycerol, 0.05% NP40, 0.1 mM PMSF). Glass beads were added and then the mixture was vigorously vortex-mixed. The supernatant separated from the glass beads was centrifuged. The precipitate was dissolved in 10 ml of buffer V (0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0, 8 M urea) and centrifuged again. Thereafter, Ni-NTA agarose beads were added to the supernatant. The Ni-NTA agarose beads were finally eluted with buffer VI (same composition as buffer V but the pH was 6.3) containing 200 mM imidazole. The eluted protein was sequentially dialyzed against buffer VII (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 50% glycerol, 0.1 mM PMSF) containing 6, 4, 2, and 0 M urea. The estimated purity of the protein was 95%, as judged on laser densitometry of a Coomassie Brilliant Blue-stained gel using NIH image software.

Ligand Affinity Blotting—Ligand affinity blotting was performed according to a slight modification of the procedure described by Snyders *et al.* (*40*). The purified *B. subtilis* Ffh or catalase dissolved in buffer VII was resolved by PAGE in the presence of 0.1% SDS and then blotted onto a PVDF membrane. Thereafter, the blotted membrane was coated with skim milk to block nonspecific binding and washed with buffer VIII [20 mM Tris-HCl, pH 8.0, 154 mM NaCl, 0.2% Tween 80 (v/v)]. *B. subtilis* SecA or catalase dissolved in 10 mM potassium acetate (pH 7.2) was applied to this membrane. We detected *B. subtilis* SecA bound to transblotted Ffh on the membrane using anti-SecA antiserum, followed by ECL (Pharmacia-Amersham).

Identification of Ffh-SecA Complexes (Tag-Precipitated Assay)—Buffer VII containing 0.2 nmol of *B. subtilis* Ffh with a 6×His tag was mixed with 0.1 nmol of the purified *B. subtilis* SecA dissolved in 10 mM potassium acetate (pH 7.2), followed by incubation for 15 min at 20°C. Ffh-SecA complexes were pelleted using Ni-NTA agarose beads, washed 3 times with 1 ml of buffer IX (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1 mM EDTA, 2% Triton X-100), and then boiled for 5 min in the presence of 0.4 M Tris-HCl (pH 6.8), 2% SDS, 5% mercaptoethanol, and 10% glycerol. The sample was resolved by PAGE in the presence of 0.1% SDS and then blotted onto a PVDF membrane. We detected SecA on the membrane using anti-SecA antiserum, followed by ECL.

Identification of the Complexes of SecA and Precursors of β-Lactamase Fusion Proteins—Samples containing precursors of β-lactamase or its derivatives denatured with 8 M urea were mixed with the purified *B. subtilis* SecA protein dissolved in 10 mM potassium acetate (pH 7.2), followed by incubation for 15 min at 20°C. SecA-precursor complexes that formed in the mixture were sedimented using anti-SecA antiserum and protein-A-Sepharose beads (Sigma, St. Louis). The pellets were washed three times with buffer IX, and then boiled for 5 min in the presence of 0.4 M Tris-HCl, pH 6.8, 2% SDS, 5% mercaptoethanol, and 10% glycerol. Samples were resolved by PAGE in the presence of 0.1% SDS and then blotted onto a PVDF membrane. We detected β-lactamase and its derivatives on the membrane using anti-β-lactamase antiserum, followed by ECL.

General DNA Manipulations—DNA manipulations and *E. coli* transformation were performed according to Sambrook *et al.* (*41*). *B. subtilis* was transformed by the method of Wilson and Bott (*42*).

RESULTS

Translocation of β-Lactamase Fusion Proteins in B. subtilis Cells Depends upon SecA and Ffh—To confirm that the translocation of the β-lactamase fusion proteins is dependent on both Ffh and SecA in *B. subtilis* cells, we examined the accumulation of their precursors under Ffh and SecA deficient conditions. Plasmids pTUBE1234 and pTUBE1235 encoding the precursors of AprE-BlaH6 and PBP5*-BlaH6, respectively (Fig. 1), were transformed into *B. subtilis* 168, TB301 containing *secA341*ts (*35*), and DF46, in which the entire *ffh* gene on the chromosome is only expressed in the presence of IPTG (*26*). The TB301 and 168 transformants were cultured at 30°C overnight, and then seeded (4%, by volume) into fresh medium for 3 h at 30°C. The cultured medium of one sample of each transformant was shifted to 42°C, and then the strains were incubated for 2 h at 42°C. The remaining cultures were incubated at 30°C. Cell lysates were prepared as described under "MATERIALS AND METHODS," and separated by SDS-PAGE, and then the precursor and mature forms of AprE-BlaH6 and PBP5*-BlaH6 were detected by immuno-

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blotting. At the permissive temperature, at which *B. subtilis* SecA is expressed, no precursor accumulated in either strain when β-lactamase fusion proteins were detected by immunoblotting using anti-β-lactamase antiserum (Fig. 2, lanes 1, 3, 5 and 7). In contrast, SecA was undetectable and the precursors of both pAprE-BlaH6 and pPBP5*-BlaH6 were detected at the non-permissive temperature in TB301 (Fig. 2, lanes 6 and 8), but not in 168 (Fig. 2, lanes 2 and 4). About 74% of AprE-BlaH6 and 85% of PBP5*-BlaH6 were found to be in the precursor form in TB301. We similarly examined *B. subtilis* DF46 transformants that were cultured for a few hours to reach an absorbance at 660 nm of 0.3 in the presence or absence of IPTG. Cell lysates were prepared and accumulation of the precursor forms of β-lactamase fusion proteins was examined (Fig. 2, lanes 9–12). In the absence of IPTG, the level of Ffh was reduced and both precursors were detected (Fig. 2, lanes 10 and 12), as in the absence of SecA. About half the levels pAprE-BlaH6 and pPBP5*-BlaH6 were found to be in the precursor form. CGTase-BlaH6 was also found in the precursor form in both TB301 and DF46 (data not shown). Therefore, the translocation of these three fusion proteins requires both SecA and Ffh in *B. subtilis* cells.

Fig. 1. **Schematic representation of the structures of β-lactamase fusion proteins.** The N-terminal 39 amino acid residues of pUC18 β-lactamase were replaced with the N-terminal region of *E. coli* OmpA, *B. subtilis* AprE, PBP5*, or alkalophilic *Bacillus* sp. #1011 CGTase by gene manipulation. Arrowheads indicate potent cleavage sites on each signal peptide. Gray boxes are amino acid residues derived from the sequence encoded by the multi-cloning site of the expression vector. These precursors have a 6×His tag at their C-termini and can be conventionally purified with Ni-NTA agarose beads. The hydrophobic core region of signal peptide is boxed.

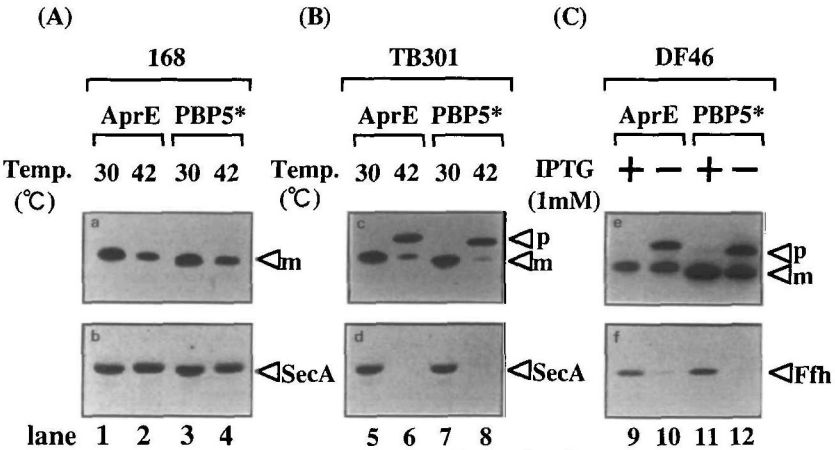

Fig. 2. **Dependence of AprE-BlaH6 and PBP5*-BlaH6 translocation on SecA and Ffh in *B. subtilis* cells.** Plasmids pTUBE1234 and pTUBE1235 encoding pAprE-BlaH6 (lanes 1, 2, 5, 6, 9, and 10) and pPBP5*-BlaH6 (lanes 3, 4, 7, 8, 11, and 12), respectively, were introduced into *B. subtilis* 168 (wild type), TB301 and DF46. (A) and (B) Transformants of 168 and TB301 were cultured at 30°C (lanes 1, 3, 5, and 7) or 42°C (lanes 2, 4, 6, and 8). Total proteins (10 μg each) of cell lysates were resolved by SDS-PAGE. The upper panels (A-a and B-c) are autograms of immunoblots using anti-β-lactamase antiserum. The lower panels (A-b and B-d) are immunoblots using anti-*B. subtilis* SecA antiserum. Arrowheads indicate the precursor (p) and mature (m) forms of the fusion proteins in the upper panels (A-a and B-c), and SecA in the lower panels (A-b and B-d). (C) DF46 transformants were cultured at 37°C in the presence (lanes 9 and 11) and absence (lanes 10 and 12) of IPTG. Total proteins (10 μg each) were resolved by SDS-PAGE. The upper panel (C-e) is an immunoblot using anti-β-lactamase antiserum. The lower panel (C-f) is an immunoblot using anti-*B. subtilis* Ffh antiserum. Arrowheads indicate the precursor (p) and mature (m) forms of the fusion proteins in the upper panel (C-e) and Ffh in the lower panel (C-f).

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Analysis of Binding of B. subtilis SecA to Precursors of β-Lactamase Fusion Proteins In Vitro—Direct evidence for the SecA interaction with presecretory proteins (OmpF-Lpp, prosapolipoprotein) in *E. coli* was obtained by chemical cross-linking with EDAC (*43*). However, whether or not *B. subtilis* SecA can bind to precursors of secretory proteins has not yet been examined in *B. subtilis*. We therefore purified precursor proteins of β-lactamase and its derivatives, in which the signal peptide region was replaced with that of *E. coli* OmpA, *B. subtilis* AprE, PBP5*, or an alkalophilic *Bacillus* sp. #1011 CGTase (Fig. 1). The precursor of BlaH6 denatured with 8 M urea was mixed with *B. subtilis* SecA dissolved in 10 mM potassium acetate (pH 7.2) in the presence of bovine serum albumin in a 1:11 ratio, and incubated as described under "MATERIALS AND METHODS." Complexes were sedimented using anti-*B. subtilis* SecA antiserum and protein-A-Sepharose beads. The precursor of BlaH6 precipitated with SecA was separated by SDS-PAGE and detected by immunoblotting with anti-β-lactamase antiserum. A single band with a molecular mass corresponding to that of pBlaH6 was obtained in the presence but not in the absence of SecA (Fig. 3, lanes 3 and 4). Corresponding bands of other precursor proteins, pOmpA-BlaH6, pAprE-BlaH6, pPBP5*-BlaH6, and pCGTase-BlaH6, were also detected (Fig. 3, lanes 5, 7, 9, and 11). In contrast, positive bands did not appear when mature BlaH6 replaced the precursor protein (Fig. 3, lane 1). Bands were undetectable when the mature forms of the other four β-lactamase derivatives were added to the reaction mixtures (data not shown). These results indicated that *B. subtilis* SecA recognizes the completely translated precursors of secretory proteins denatured with 8 M urea.

Figure 3 indicates that *B. subtilis* SecA recognizes the denatured presecretory proteins with signal peptides derived from both Gram-negative and -positive bacteria, and this coincided with the finding that the translocation of *B. subtilis* α-amylase, pUC18 β-lactamase and *E. coli* OmpA is dependent on SecA in *B. subtilis* (*25, 44*). However, the *E. coli secA* mutant cannot grow at non-permissive temperatures when the wild-type *B. subtilis secA*$^+$ gene is introduced (*25*).

B. subtilis Ffh Enhances Complex Formation between SecA and Precursor Proteins—We investigated the relationship between Ffh and SecA in the protein translocation pathway of *B. subtilis* cells. The effect of Ffh on the formation of SecA and precursor protein complexes was examined. We also studied the effect of SecA on the formation of Ffh and precursor protein complexes. In the first analysis, 0.003 nmol Ffh and 0.2 nmol precursor of pPBP5*-BlaH6 denatured with 8 M urea were incubated for 15 min at 20°C, and then 0.2 nmol SecA was added and the mixture was further incubated for 15 min at 20°C. SecA-pPBP5*-BlaH6 complexes formed in this reaction mixture were sedimented using anti-*B. subtilis* SecA antiserum and protein-A-Sepharose beads, and then resolved by SDS-PAGE. We detected pPBP5*-BlaH6 on immunoblotting using anti-β-lactamase antiserum. The band density was 10-fold that of the complex formed when the pPBP5*-BlaH6 precursor was incubated without Ffh (Fig. 4, lanes 1 and 2). The formation of Ffh-precursor protein complexes was not enhanced by incubating SecA and precursors before adding Ffh (data not shown). These results indicated that *B. subtilis* Ffh can enhance the interaction of SecA and pPBP5*-BlaH6.

We examined the effect of the Ffh concentration upon the ability of Ffh to enhance SecA-precursor complex formation by using the precursors of PBP5*-BlaH6 and AprE-BlaH6 (Fig. 5). Prior incubation of Ffh with the precursor of pPBP5*-BlaH6 or pAprE-BlaH6 increased the maximal amount of complexes about 30- and 15-fold, respectively (Fig. 5, A and B) within 15 min compared with that in the absence of Ffh. On the other hand, when an equal amount of bovine serum albumin, which does not interact with the precursor proteins, was added instead of *B. subtilis* Ffh, the

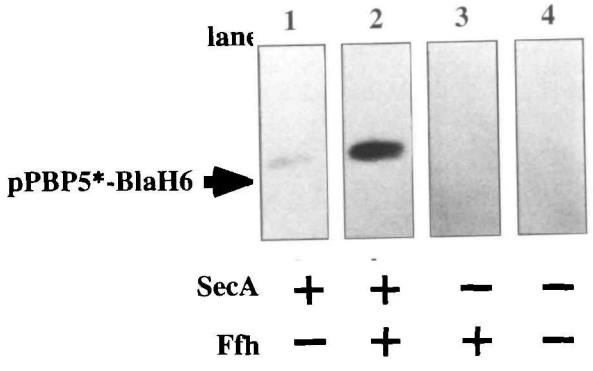

Fig. 4. **Interaction between SecA and the precursor of pPBP5*-BlaH6 enhanced by Ffh.** Purified *B. subtilis* Ffh (0.003 nmol) dissolved in buffer VII (lanes 2 and 3) or buffer VII (lanes 1 and 4) was mixed with 0.20 nmol of pPBP5*-BlaH6 denatured with 8 M urea. SecA (0.2 nmol) dissolved in 10 mM potassium acetate (pH 7.2) (lanes 1 and 2) or 10 mM potassium acetate (pH 7.2) (lanes 3 and 4) was then added to these samples, followed by incubation for 15 min at 20°C. The pPBP5*-BlaH6 bound to SecA was pelleted using anti-SecA antiserum and protein-A-Sepharose beads. The precipitated pPBP5*-BlaH6 was resolved by SDS-PAGE, and then detected by immunoblotting using anti-β-lactamase antiserum.

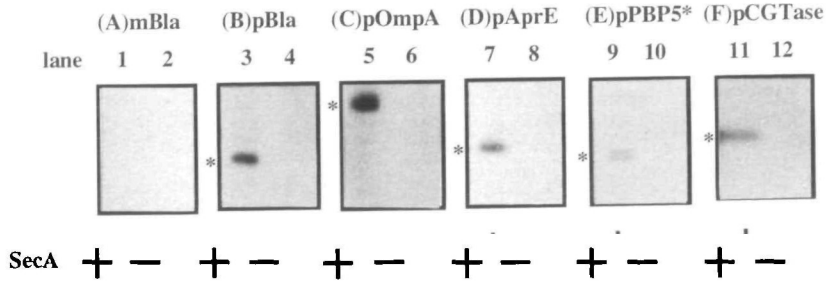

Fig. 3. **Presecretory protein binding activity of *B. subtilis* SecA.** Mature β-lactamase [mBlaH6 (lanes 1 and 2) (A)], and precursors of β-lactamase [pBlaH6 (lanes 3 and 4) (B)], OmpA-β-lactamase fusion protein [pOmpA-BlaH6 (lanes 5 and 6) (C)], of AprE-β-lactamase fusion protein [pAprE-BlaH6 (lanes 7 and 8) (D)], PBP5*-β-lactamase fusion protein [pPBP5*-BlaH6 (lanes 9 and 10) (E)], or CGTase-β-lactamase fusion protein [pCGTase-BlaH6 (lanes 11 and 12) (F)] (0.2 nmol each) denatured with 8 M urea were mixed with (lanes 1, 3, 5, 7, 9, and 11) or without (lanes 2, 4, 6, 8, 10, and 12) 0.40 nmol of the purified *B. subtilis* SecA protein. The β-lactamase bound to SecA was pelleted using anti-SecA antiserum and protein-A-Sepharose beads. The β-lactamase in the pellet was resolved by SDS-PAGE, and then detected by immunoblotting using anti-β-lactamase antiserum.

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formation of SecA-precursor protein complexes did not increase. When the efficiency of complex formation between SecA and precursor proteins was assessed using EDAC, the similar enhancing effect was observed (data not shown), suggesting that Ffh can directly enhance the binding ability of SecA as to precursor proteins rather than that the interaction of Ffh and SecA increases the amount of Ffh-precursor protein complexes immunoprecipitated with anti-*B. subtilis* SecA antiserum. During the incubation of Ffh and precursors, several concentrations of scRNA were added. However, enhancement of the SecA-precursor complex formation by Ffh was not obviously affected (unpublished observations).

Formation of the B. subtilis Ffh-SecA Complex In Vitro—Since *B. subtilis* Ffh enhanced the formation of SecA-presecretory protein complexes, we investigated, by

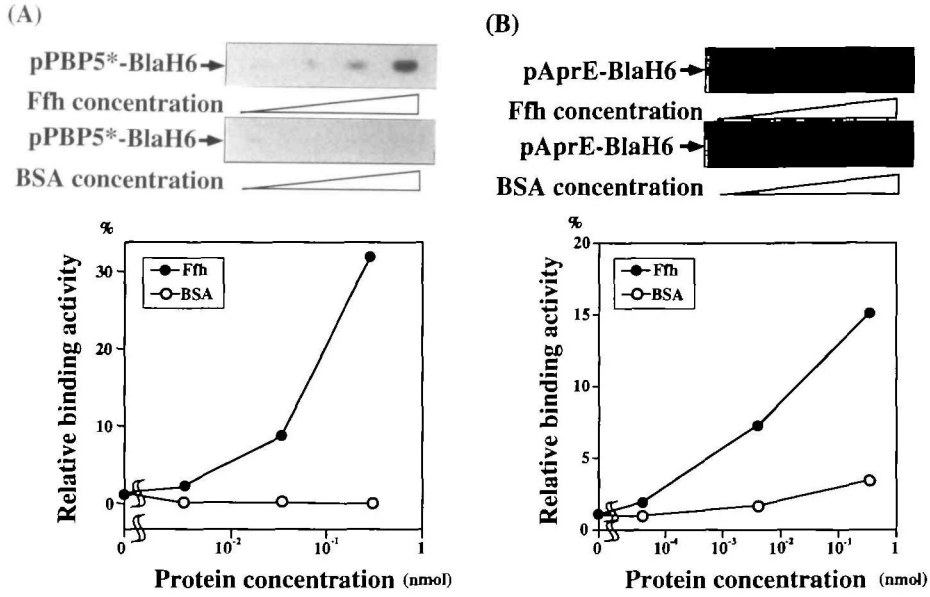

Fig. 5. **Analysis of the dependency on the concentration of Ffh of the Ffh-enhanced interaction between SecA and precursor.** Samples containing 0.20 nmol pPBP5*-BlaH6 (A) or 0.20 nmol pAprE-BlaH6 (B) denatured with 8 M urea were mixed with 0, 4.0×10^{-5}, 4.0×10^{-3}, and 4.0×10^{-1} nmol or 0, 3.0×10^{-4}, 3.0×10^{-3}, and 3.0×10^{-2} nmol, respectively, of the purified *B. subtilis* Ffh dissolved in buffer VII, followed by incubation for 15 min at 20°C. SecA (0.2 nmol) dissolved in 10 mM potassium acetate (pH 7.2) was added to these samples, followed by further incubation for 15 min at 20°C. The precursor of pAprE-BlaH6 or pPBP5*-BlaH6 bound to SecA was pelleted using anti-SecA antiserum and protein-A-Sepharose beads, resolved by SDS-PAGE, and detected by immunoblotting using anti-β-lactamase antiserum. The density of each band was quantified using NIH image software and plotted for comparison. The relative binding activity of Ffh as to each presecretory protein, defined as the ratio of the amount of presecretory protein co-immunoprecipitated with *B. subtilis* SecA to the total amount of the presecretory protein added first, was plotted against the concentration of Ffh or BSA.

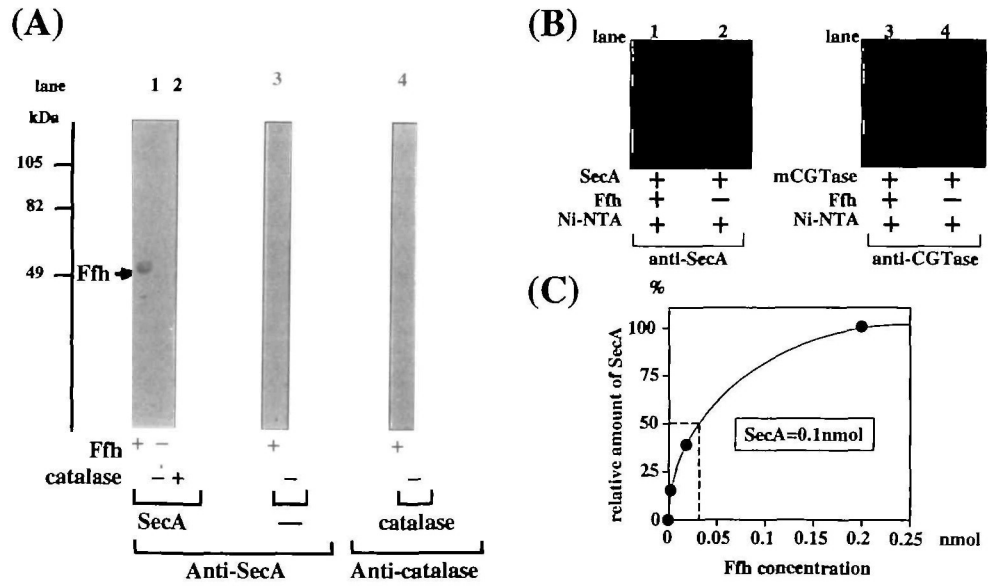

Fig. 6. **Detection of complex formation between *B. subtilis* Ffh and SecA by ligand affinity blotting, and isolation of Ffh-SecA complexes *in vitro*.** (A) Ligand affinity blotting: Purified *B. subtilis* Ffh (lanes 1, 3, and 4) or catalase (lane 2) was resolved by SDS-PAGE and then blotted onto a PVDF membrane. SecA (lanes 1 and 2) or catalase (lane 4) was applied to the membrane. Lane 3 was washed with 10 mM potassium acetate (pH 7.2). Trans-blotted Ffh on the membrane was detected using anti-SecA (lanes 1, 2, and 3) or anti-catalase (lane 4) antiserum. The arrow indicates the position of Ffh. (B) Tag-precipitated assay: Purified Ffh with a 6×His tag (0.2 nmol) was mixed with *B. subtilis* SecA (0.1 nmol) (lanes 1 and 2) or mature CGTase (0.1 nmol) (lanes 3 and 4). Complexes were pelleted using Ni-NTA agarose beads and then purified. The samples were resolved by SDS-PAGE, blotted onto a PVDF membrane, and detected using anti-SecA (lanes 1 and 2) or anti-CGTase (lanes 3 and 4) antiserum, followed by ECL. (C) The dependency of the Ffh-SecA complex formation on the concentration of Ffh utilizing tag-precipitation: Samples containing 0.10 nmol of SecA dissolved in 10 mM potassium acetate were mixed with 0, 0.002, 0.02, and 0.2 nmol of the purified *B. subtilis* Ffh dissolved in buffer VII, followed by incubation for 15 min at 20°C. Complexes were pelleted using Ni-NTA agarose beads and then purified. Samples were resolved by SDS-PAGE and blotted onto a PVDF membrane, and the complexes were detected using anti-SecA antiserum, followed by ECL. The density of each band was quantified using NIH image software and plotted.

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ligand affinity blotting, whether or not SecA and Ffh could form complexes (Fig. 6A). A band was detected with anti-*B. subtilis* SecA antiserum when purified Ffh was blotted onto a PVDF membrane (Millipore) after SDS-PAGE and then SecA was applied to the membrane. The position of the band corresponded to the molecular mass of *B. subtilis* Ffh (Fig. 6A, lane 1). In contrast, bands were undetectable when either catalase was blotted onto the membrane (Fig. 6A, lane 2) or Ffh was blotted onto the membrane and then catalase was applied and detected using anti catalase antiserum (Fig. 6A, lane 4).

Furthermore, we examined SecA-Ffh binding *in vitro* by means of a tag-precipitated assay using purified *B. subtilis* Ffh with a 6×His tag attached to Ni-NTA agarose beads. A band corresponding to *B. subtilis* SecA was detected for the sample mixed with *B. subtilis* Ffh (Fig. 6B, lane 1), whereas no bands were detected in the absence of Ffh (Fig. 6B, lane 2). A band corresponding to the negative control, mature CGTase, was not detected even in the presence of the *B. subtilis* Ffh protein (Fig. 6B, lanes 3 and 4). These results indicated that *B. subtilis* Ffh and SecA directly interact to form Ffh-SecA complexes.

To analyze the dependency of the Ffh-SecA complex formation on the concentration of Ffh, *B. subtilis* SecA was precipitated with various amounts of Ffh. The amount of precipitated SecA increased with the Ffh concentration; the half maximal concentration of *B. subtilis* Ffh protein was about 0.03 to 0.1 nmol of the SecA protein (Fig. 6C).

DISCUSSION

Luirink *et al.* (*16*) proposed that the role of the *E. coli* SRP is that of a chaperone, which specifically binds to the signal sequence of nascent proteins and which maintains them in a translocation-competent conformation. We found that the processing and translocation of the precursors of β-lactamase fusion proteins used in this study depend on SecA and Ffh, and that Ffh can enhance the binding activity of SecA as to precursors 15- to 30-fold, which occurred even in the absence of nucleotides. These results suggested that *B. subtilis* SRP-like particle functions as a protein translocation-specific molecular chaperone for SecA. Furthermore, most pAprE-BlaH6 and pPBP5*-BlaH6 accumulated in the precursor forms in *B. subtilis* under the conditions of SecA depletion, whereas about half of pAprE-BlaH6 and pPBP5*-BlaH6 were found as precursors under Ffh-deficient conditions (Fig. 2). These findings show that the effect of SecA on the translocation of proteins is more important than that of Ffh, and suggest that *B. subtilis* Ffh and other targeting pathways of the protein translocation machinery gather at the translocon including SecA.

E. coli SecA interacts with presecretory proteins by recognizing the positive charge at the amino terminus of the signal peptide (*43*). Although the presecretory protein recognition mechanism of *B. subtilis* SecA remains to be resolved, we showed that *B. subtilis* SecA, as well as Ffh, recognizes denatured presecretory proteins with the signal sequences of AprE and PBP5* derived from *B. subtilis*, CGTase derived from *Bacillus* sp. #1011, β-lactamase derived from pUC18, and OmpA derived from *E. coli* (Fig. 3, B, C, D, E, and F).

Hartl *et al.* (*9*) revealed that SecB increases the efficiency of SecA-dependent productive binding of preproteins to *E. coli* inner membrane vesicles. Therefore, to determine whether or not *B. subtilis* Ffh functions as a specific molecular chaperone like *E. coli* SecB, we determined whether or not the formation of SecA-precursor protein complexes is enhanced by prior incubation of Ffh and the precursor. Approximately 15- and 30-fold more pAprE-BlaH6 and pPBP5*-BlaH6, respectively, bound to SecA compared with in the absence of Ffh under these conditions (Figs. 4 and 5). In contrast, the ability of Ffh to bind precursors was not enhanced by prior incubation of SecA and the precursor (data not shown). Therefore, the dominant function of Ffh in SRP-like particles will be to enhance the formation of SecA-precursor complexes. Ffh may maintain presecretory proteins in an unfolded state, which is necessary for SecA interaction.

To confirm that Ffh serves as a molecular chaperone for SecA in the translocation pathway in *B. subtilis*, whether or not the two machinery proteins directly interact should be examined because the protein translocation-specific molecular chaperone of SecB directly interacts with SecA in *E. coli* (*9, 45*). We verified that SecA and Ffh interact *in vitro* by means of ligand affinity blotting and a tag-precipitated assay. *B. subtilis* Ffh directly bound to SecA in both assays (Fig. 6, A and B). Ligand affinity blotting showed that the *B. subtilis* Ffh protein transblotted onto a PVDF membrane after SDS-PAGE separation could bind to the SecA protein (Fig. 6A). On the contrary, the Ffh protein did not bind to SecA on the membrane (data not shown). These results suggest that the homodimeric structure of *B. subtilis* SecA is important for the Ffh interaction, because the sodium dodecyl sulfate on SDS-PAGE destroys the homodimer structure of SecA.

Valent *et al.* (*46*) recently reported that the inner membrane proteins recognized by SRP are inserted into a translocon that contains at least SecA, SecY, and SecG, which involves the release of the precursor from SRP by FtsY and GTP in *E. coli*. Our finding that SecA and Ffh cooperatively function in the protein translocation pathway in *B. subtilis* are consistent with this report. We have not yet defined the interaction between SecA or Ffh and inner membrane proteins. *E. coli* Ffh may function as a molecular chaperone by directly interacting with SecA in these targeting cascades. Based upon the results of these experiments, we propose that the *B. subtilis* SRP-like particle couples with the bacterial protein translocation pathway consisting of SecA, SecE, and SecY in *B. subtilis* to produce extracellular enzymes.

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REFERENCES

1. Walter, P. and Blobel, G. (1982) Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature* **299**, 691-698
2. Walter, P. and Johnson, A.E. (1994) Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* **10**, 87-119
3. Bernstein, H.D., Poritz, M.A., Strub, K., Hoben, P.J., Brenner, S., and Walter, P. (1989) Model for signal sequence recognition from amino-acid sequence of 54K subunit of signal recognition particle. *Nature* **340**, 482-486
4. Römisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M., and Dobberstein, B. (1989) Homology of 54K protein of signal-recognition particle, docking protein and two *E. coli*

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proteins with putative GTP-binding domains. *Nature* **340**, 478-482
5. Lütcke, H., High, S., Römisch, K., Ashford, A.J., and Dobberstein, B. (1992) The methionine-rich domain of the 54 kDa subunit of signal recognition particle is sufficient for the interaction with signal sequences. *EMBO J.* **11**, 1543-1551
6. Lütcke, H. (1995) Signal recognition particle (SRP), a ubiquitous initiator of protein translocation. *Eur. J. Biochem.* **228**, 531-550
7. Bacher, G., Lütcke, H., Jungnickel, B., Rapoport, T.A., and Dobberstein, B. (1996) Regulation by the ribosome of the GTPase of the signal-recognition particle during protein targeting. *Nature* **381**, 248-251
8. Collier, D.N., Bankaitis, V.A., Weiss, J.B., and Bassford, P.J., Jr. (1988) The antifolding activity of SecB promotes the export of the *E. coli* maltose-binding protein. *Cell* **53**, 273-283
9. Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J.P., and Wickner, W. (1990) The binding cascade of SecB to SecA to SecY/E mediates preprotein targeting to the *E. coli* plasma membrane. *Cell* **63**, 269-279
10. Douville, K., Price, A., Eichler, J., Economou, A., and Wickner, W. (1995) SecYEG and SecA are the stoichiometric components of preprotein translocase. *J. Biol. Chem.* **270**, 20106-20111
11. Economou, A., Pogliano, J.A., Beckwith, J., Oliver, D.B., and Wickner, W. (1995) SecA membrane cycling at SecYEG is driven by distinct ATP binding and hydrolysis events and is regulated by SecD and SecF. *Cell* **83**, 1171-1181
12. Nishiyama, K., Suzuki, T., and Tokuda, H. (1996) Inversion of the membrane topology of SecG coupled with SecA-dependent preprotein translocation. *Cell* **85**, 71-81
13. Miller, J.D., Bernstein, H.D., and Walter, P. (1994) Interaction of *E. coli* Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor. *Nature* **367**, 657-659
14. Althoff, S., Selinger, D., and Wise, J.A. (1994) Molecular evolution of SRP cycle components: functional implications. *Nucleic Acids Res.* **22**, 1933-1947
15. Li, X., Henry, R., Yuan, J., Cline, K., and Hoffman, N.E. (1995) A chloroplast homologue of the signal recognition particle subunit SRP54 is involved in the posttranslational integration of a protein into thylakoid membranes. *Proc. Natl. Acad. Sci. USA* **92**, 3789-3793
16. Luirink, J., High, S., Wood, H., Giner, A., Tollervey, D., and Dobberstein, B. (1992) Signal-sequence recognition by an *Escherichia coli* ribonucleoprotein complex. *Nature* **359**, 741-743
17. Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U., and Rapoport, T.A. (1992) A mammalian homolog of SEC61p and SECYp is associated with ribosomes and nascent polypeptides during translocation. *Cell* **71**, 489-503
18. Macfarlane, J. and Müller, M. (1995) The functional integration of a polytopic membrane protein of *Escherichia coli* is dependent on the bacterial signal-recognition particle. *Eur. J. Biochem.* **233**, 766-771
19. de Gier, J.-W.L., Mansournia, P., Valent, Q.A., Phillips, G.J., Luirink, J., and von Heijne, G. (1996) Assembly of a cytoplasmic membrane protein in *Escherichia coli* is dependent on the signal recognition particle. *FEBS Lett.* **399**, 307-309
20. Seluanov, A. and Bibi, E. (1997) FtsY, the prokaryotic signal recognition particle receptor homologue, is essential for biogenesis of membrane proteins. *J. Biol. Chem.* **272**, 2053-2055
21. Ulbrandt, N.D., Newitt, J.A., and Bernstein, H.D. (1997) The *E. coli* signal recognition particle is required for the insertion of a subset of inner membrane proteins. *Cell* **88**, 187-196
22. Nakamura, K., Nakamura, A., Takamatsu, H., Yoshikawa, H., and Yamane, K. (1990) Cloning and characterization of a *Bacillus subtilis* gene homologous to *E. coli secY*. *J. Biochem.* **107**, 603-607
23. Sadaie, Y., Takamatsu, H., Nakamura, K., and Yamane, K. (1991) Sequencing reveals similarity of the wild-type *div*+ gene of *Bacillus subtilis* to the *Escherichia coli secA* gene. *Gene* **98**, 101-105
24. Nakamura, K., Imai, Y., Nakamura, A., and Yamane, K. (1992) Small cytoplasmic RNA of *Bacillus subtilis*: functional relationship with human signal recognition particle 7S RNA and *Escherichia coli* 4.5S RNA. *J. Bacteriol.* **174**, 2185-2192
25. Takamatsu, H., Fuma, S., Nakamura, K., Sadaie, Y., Shinkai, A., Matsuyama, S., Mizushima, S., and Yamane, K. (1992) *In vivo* and *in vitro* characterization of the *secA* gene product of *Bacillus subtilis*. *J. Bacteriol.* **174**, 4308-4316
26. Honda, K., Nakamura, K., Nishiguchi, M., and Yamane, K. (1993) Cloning and characterization of a *Bacillus subtilis* gene encoding a homolog of the 54-kilodalton subunit of mammalian signal recognition particle and *Escherichia coli* Ffh. *J. Bacteriol.* **175**, 4885-4894
27. Jeong, S.M., Yoshikawa, H., and Takahashi, H. (1993) Isolation and characterization of the *secE* homologue gene of *Bacillus subtilis*. *Mol. Microbiol.* **10**, 133-142
28. Oguro, A., Kakeshita, H., Honda, K., Takamatsu, H., Nakamura, K., and Yamane, K. (1995) *srb*: a *Bacillus subtilis* gene encoding a homologue of the α-subunit of the mammalian signal recognition particle receptor. *DNA Res.* **2**, 95-100
29. Nakane, A., Takamatsu, H., Oguro, A., Sadaie, Y., Nakamura, K., and Yamane, K. (1995) Acquisition of azide-resistance by elevated SecA ATPase activity confers azide-resistance upon cell growth and protein translocation in *Bacillus subtilis*. *Microbiology* **141**, 113-121
30. Kunst, F. and others (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390**, 249-256
31. Poritz, M.A., Strub, K., and Walter, P. (1988) Human SRP RNA and *E. coli* 4.5S RNA contain a highly homologous structural domain. *Cell* **55**, 4-6
32. Nakamura, K., Nishiguchi, M., Honda, K., and Yamane, K. (1994) The *Bacillus subtilis* SRP54 homologue, Ffh, has an intrinsic GTPase activity and forms a ribonucleoprotein complex with small cytoplasmic RNA *in vivo*. *Biochem. Biophys. Res. Commun.* **199**, 1394-1399
33. Bunai, K., Takamatsu, H., Horinaka, T., Oguro, A., Nakamura, K., and Yamane, K. (1996) *Bacillus subtilis* Ffh, a homologue of mammalian SRP54, can intrinsically bind to the precursors of secretory proteins. *Biochem. Biophys. Res. Commun.* **227**, 762-767
34. Takamatsu, H., Bunai, K., Horinaka, T., Oguro, A., Nakamura, K., Watabe, K., and Yamane, K. (1997) Identification of a region required for binding to presecretory protein in *Bacillus subtilis* Ffh, a homologue of the 54-kDa subunit of mammalian signal recognition particle. *Eur. J. Biochem.* **248**, 575-582
35. Takamatsu, H., Nakane, A., Sadaie, Y., Nakamura, K., and Yamane, K. (1994) The rapid degradation of mutant SecA protein in *Bacillus subtilis secA341* (ts) mutant causes a protein translocation defect in the cell. *Biosci. Biotech. Biochem.* **58**, 1845-1850
36. Yanish-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103-119
37. Oliver, D.B., Cabelli, R.J., and Jarosik, G.P. (1990) SecA protein: autoregulated initiator of secretory precursor protein translocation across the *E. coli* plasma membrane. *J. Bioenerg. Biomembr.* **22**, 311-336
38. Oliver, D.B. and Beckwith, J. (1981) *E. coli* mutant pleiotropically defective in the export of secreted proteins. *Cell* **25**, 765-772
39. Kimura, K., Ishii, Y., Kataoka, S., Takano, T., and Yamane, K. (1990) Expression of the β-cyclodextrin glucanotransferase gene of an alkalophilic *Bacillus* sp. #1011 in *Escherichia coli* cells and characterization of the synthesized enzyme. *Agric. Biol. Chem.* **54**, 641-648
40. Snyders, S., Ramamurthy, V., and Oliver, D. (1997) Identification of a region of interaction between *Escherichia coli* SecA and SecY proteins. *J. Biol. Chem.* **272**, 11302-11306
41. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
42. Wilson, G.A. and Bott, K.F. (1968) Nutritional factors influenc-

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ing the development of competence in the *Bacillus subtilis* transformation system. *J. Bacteriol.* **95**, 1439-1449
43. Akita, M., Sasaki, S., Matsuyama, S., and Mizushima, S. (1990) SecA interacts with secretory proteins by recognizing the positive charge at the amino terminus of the signal peptide in *Escherichia coli*. *J. Biol. Chem.* **265**, 8164-8169
44. Meens, J., Frings, E., Klose, M., and Freudl, R. (1993) An outer membrane protein (OmpA) of *Escherichia coli* can be translocated across the cytoplasmic membrane of *Bacillus subtilis*. *Mol. Microbiol.* **9**, 847-855
45. Breukink, E., Nouwen, N., van Raalte, A., Mizushima, S., Tommassen, J., and de Kruijff, B. (1995) The C terminus of SecA is involved in both lipid binding and SecB binding. *J. Biol. Chem.* **270**, 7902-7907
46. Valent, Q.A., Scotti, P.A., High, S., de Gier, J.-W.L., von Heijne, G., Lentzen, G., Wintermeyer, W., Oudega, B., and Luirink, J. (1998) The *Escherichia coli* SRP and SecB targeting pathways converge at the translocon. *EMBO J.* **17**, 2504-2512

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