ABBREVIATIONS

ACP	acyl carrier protein
ATP	Adenosine 5'-triphosphate
bp	Base pair(s)
CTP	Cytidine 5'-triphosphate
dsRBD	Double stranded-RNA binding domain
ER	Endoplasmic reticulum
GTP	Guanosine 5'-triphosphate
IM	Inner membrane
IMS	Inter membrane space
IPTG	Isopropyl-1-thio-β-D-galactoside
kb	Kilobase pair(s)
kDa	Kilo Dalton
LB	Luria-Bertani
nt	Nucleotide(s)
NTP	Nucleoside 5'-triphosphate
ORF (*orf*)	Open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PVDF	polyvinilidene difluoride
RNP	Ribonucleoprotein
scRNA	Small cytoplasmic RNA
SDS	Sodium dodecyl sulfate
SRP	Signal recognition particle
SRα	SRP receptor α-subunit
SRβ	SRP receptor β-subunit

ABSTRACT

In mammalian cells, the signal recognition particle (SRP) and the SRP receptor play a central role in targeting presecretory proteins to the membrane of the endoplasmic reticulum (ER). SRP is a ribonucleo-protein complex composed of one RNA (SRP 7S RNA) molecule and six proteins of 9, 14, 19, 54, 68, and 72 kDa. SRP interacts with the signal sequence of nascent polypeptide emerging from ribosomes and the complex formed is targeted and bound to a hetero-dimeric receptor consisting of SRα and SRβ on the ER. In contrast, it has been considered that the chaperones and Sec proteins play a pivotal role in targeting and translocation of secretory proteins in *Escherichia coli*. However, recently, the molecular homologues to the components of mammalian SRP have been identified in prokaryotes. In *E. coli*, 4.5S RNA and *E. coli* Ffh protein have been identified as homologues to the SRP 7S RNA and SRP54 of mammalian SRP, respectively. Furthermore, FtsY exhibits a homology with SRα. In *Bacillus subtilis*, small cytoplasmic RNA (scRNA) and *B. subtilis* Ffh protein have been also identified as homologues to the SRP 7S RNA and SRP54, respectively. Depletion of either the SRP RNA or SRP54 homologues in *E. coli* or *B. subtilis* has defects in the export of secretory proteins. These evidences suggest that the SRP-SRP receptor mediated targeting system is also involved in protein secretion in prokaryotes. To investigate the *B. subtilis* SRP-SRP receptor mediated targeting system in detail, I cloned a *B. subtilis* gene encoding a homologue of mammalian SRα and characterized its operon structure and gene products.

To clone a *B. subtilis ftsY* gene encoding a homologue of the mammalian SRα, the PCR was performed against *B. subtilis* chromosome using synthesized oligonucleotides for the GTP-binding elements which were conserved in SRα and its homologues. The *ftsY* gene was cloned using this PCR product as a probe. The nucleotide sequence of the entire 1220-bp DNA fragment was determined. The sequence revealed one possible complete open reading frame (ORF) in this region, as well as the C-terminal portion of other ORF. In the complete 987-bp *orf*, a sequence

identical to the PCR fragment was located. As the gene encodes a homologue of mammalian SRα, it was designated as *srb* (SRP receptor of *B. subtilis*), and it was renamed *ftsY* after completion of whole genome sequence analysis of *B. subtilis*. The deduced amino acid sequence of the FtsY protein shared 26.6, 36.2 and 49.7% identity with those of mammalian SRα, archaebacterial DPα and *E. coli* FtsY, respectively. Downstream of the stop codon of the *ftsY* gene, there was an ρ-independent terminator but no promoter sequence upstream from the gene. This suggests that *ftsY* consists of an operon with another upstream *orf*(s). To analyze the gene organization in the potential operon and the biological function of FtsY, a 6098-bp DNA containing *ftsY* was sequenced. In addition to *ftsY*, it was identified that four complete (*orf2* to *orf5*) and one partial (*orf1*) ORFs in this sequence. Primer extension analysis and Northern blot analysis revealed that *ftsY* (*orf5*) constitutes an operon with two additional *orfs*, *orf3* and *orf4*. A database search of known proteins revealed that *orf3* encodes a homologue of *E. coli* RNase III (36.0% identical amino acids) and *orf4* encodes a homologue of yeast Smc1 (26.6% identical amino acids). Therefore *orf3* and *orf4* were designated as *rncS* and *smc*, respectively. Then I constructed a *B. subtilis* mutant in which *ftsY* expression was able to be induced by IPTG. The depletion of FtsY caused a defect in the cell growth and the cells became filamentous, broken and twisted shapes. The depletion of FtsY caused accumulation of several secretory proteins in the cell. Furthermore, a fusion protein, which has *B. subtilis* PBP5* signal peptide region and β-lactamase mature region, was accumulated in the FtsY depleted cell. PBP5* which is concerning with the synthesis of cortex in spore is translated in mother cell and then localized to forespore. These results suggest that the FtsY protein is concerning not only with the secretion of extracellular proteins but also with the localization of spore-forming proteins.

To analyze the effect of deleting FtsY on sporulation, I investigated the expression of *ftsY* in sporulation. In vegetative cells, *ftsY* is transcribed together with two upstream genes, *rncS* and *smc*, that are under the control of the major transcription factor σ^A. In contrast, another 1.7 kilonucleotide *ftsY* mRNA was found during sporulation, and it was defect in the sporulating cells of σ^k and GerE mutants. Therefore, *ftsY* is solely expressed from a σ^K and GerE controlled

promoter that is located immediately upstream of *ftsY* inside the *smc* gene during sporulation. To examine the role of FtsY during sporulation, I constructed the *B. subtilis* ISR39 strain, a *ftsY* conditional mutant in which *ftsY* expression can be shut off during spore formation but not during the vegetative state. Spores of ISR39 have the same resistance to heat and chroloform as the wild type, while its resistance to lysozyme was obviously reduced. Electron microscopy showed that the outer coat of ISR39 spores was not completely assembled. The coat protein profile of the *ftsY* mutant spores was different from that of wild type spores. The amounts of CotA, and CotE were reduced in spore coat proteins of FtsY mutant spores and the molecular mass of CotB was reduced. In addition, CotA, CotB, and CotE existed normally at T_8 of sporulation in the *ftsY* mutant cells. In addition, immunoelectron microscopy localized FtsY on the inner and outer coats of wild type spores. These results suggested that FtsY has pivotal roles in assembling coat proteins onto the coat layer during spore morphgenesis.