**Streptomyces lividans** contains a minimal functional signal recognition particle that is involved in protein secretion

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The bacterial version of the mammalian signal recognition particle (SRP) is well conserved and essential to all known bacteria. The genes for the *Streptomyces lividans* SRP components have been cloned and characterized. FtsY resembles the mammalian SRP receptor and the *S. lividans* SRP consists of Ffh, a homologue of the mammalian SRP54 protein, and scRNA, which is a small size RNA of 82 nt in length. Co-immunoprecipitation studies confirmed that Ffh and scRNA are probably the only components of the *S. lividans* SRP and that pre-agarase can co-immunoprecipitate with Ffh, suggesting that the SRP is involved in targeting secretory proteins.

The mammalian small size RNA (SRP RNA) is longer than 300 nt and contains two domains. The small domain (Alu domain) binds to SRP proteins 9 and 14 (Bui & Strub, 1999). The *Bacillus subtilis* SRP system is similar to that of *E. coli* but its SRP RNA is larger (generally known as scRNA; 271 nt long) than in *E. coli* (generally known as 4·5S RNA; 114 nt long); it contains an Alu domain and the SRP harbours the *B. subtilis* histone-like (HBsu) protein as an additional component, interacting with the Alu domain of the scRNA (Nakamura et al., 1999). The large domain (domain IV) of the small size RNA is fully conserved in mammalian and bacterial cells and is involved in the interaction with both the Ffh and the hydrophobic nascent polypeptide chain (Batey et al., 2000). The interaction between Ffh and the 4·5S RNA represents the minimal functional SRP, all the bacterial SRP components being essential for cell growth.

Whereas in *E. coli* the SRP and the SecA/SecB-based secretion system(s) belong to two different targeting pathways functioning in a substrate-specific manner (Beck et al., 2000), a functional interaction has been detected between SecA and Ffh in *B. subtilis* (Bunai et al., 1996). These differences between Gram-positive and Gram-negative bacteria may indicate the existence of different mechanisms for targeting secretory proteins. *B. subtilis* does not seem to have a SecB protein and the signal peptides of the *B. subtilis* secretory proteins seem to be more hydrophobic and longer than those of *E. coli*. Thus, the *B. subtilis* SRP system may be responsible for the targeting of both membrane and secretory proteins (Oguro et al., 1996).

Gram-positive bacteria belonging to the genus *Streptomyces* are soil bacteria with mycelial growth that undergo a complex biochemical and morphological differentiation before...
forming exospor formation (Chater, 1998). Streptomyces produce and secrete large quantities of proteins (Gilbert et al., 1995) and *Streptomyces lividans*, in particular, has often been used as a host for the secretion production of heterologous proteins (Gilbert et al., 1995; Van Mellaert & Anné, 1994).

The genome sequence of *Streptomyces coelicolor* A3(2) (GenBank accession no. AL645882), an organism closely related to *S. lividans*, revealed the absence of a *secG* gene in this organism. The *S. coelicolor* genome is almost identical to that of *S. lividans*, from which *secG* appears to be missing as well (M. San Roman & R. P. Mellado, unpublished data), thereby rendering the study of the SRP complex very attractive. The identification and characterization of the *S. lividans* SRP system components are described in this study as a first step in exploring the possible role played by the *S. lividans* SRP in secretion.

**METHODS**

**Bacterial strains, plasmids and media.** *S. lividans* TK21 (Hopwood et al., 1985) was used as the wild-type strain and was cultured in liquid NMMP medium or on solid R5 medium and harvested as described by Hopwood et al. (1985). *S. lividans* TK21[M15(pREP4)] (Palacin et al., 2002) is an *S. lividans* TK21 mutant strain in which the major signal peptidase (SipY) is inactive and which carries the *S. coelicolor* agarase gene *dagA* on a multicopy plasmid. This strain overproduces agarase and has been used in co-immunoprecipitation experiments. Thiostrepton (5 μg ml⁻¹) or kanamycin (10 μg ml⁻¹) was added to the media when required. *B. subtilis* 168 (trpC2) was provided by C. Anagnostopoulos (INRA, Jouy-en-Josas, France) and was cultured in Luria Broth (LB). The procedures for the growth of *B. subtilis* were as described by Harwood & Cutting (1990). *E. coli* K514 (Murray, 1983) was cultured in LB (Sambrook et al., 1989) and used for plasmid propagation. Ampicillin (100 μg ml⁻¹) was added to the media when necessary.

Plasmid pUC19 (Norlander et al., 1983) was used to construct a *BamHI* library of gel-purified *S. lividans* TK21 chromosomal DNA fragments and to propagate cloned DNA sequences in *E. coli*. Plasmid pFF14 carries a 14 kb long *BamHI* chromosomal DNA fragment containing the *ffh* gene.

*E. coli* M15 is an *E. coli* K-12 derivative that harbours plasmid pREP4 (Villarejo & Zabin, 1974) and was purchased from Qiagen. The *E. coli* expression plasmid pQE30 (Qiagen) can provide a high level of expression of proteins carrying a hexahistidine (His₆) tail at their amino ends. Plasmid pQE30 was used to propagate His₆–Ffh coding sequences in *E. coli* M15. Ampicillin (100 μg ml⁻¹) and kanamycin (25 μg ml⁻¹) were added to the media when needed.

**DNA manipulation and PCR amplification.** General recombinant DNA manipulation was carried out as described previously (Hopwood et al., 1985; Sambrook et al., 1989). Restriction endonucleases and DNA modifying enzymes were obtained from Boehringer Mannheim, Promega and EcoGene. *S. lividans* TK21 chromosomal DNA was used as a template for PCR amplification by incubation at 95 °C for 3 min, followed by 30 cycles of incubation at 95 °C (1 min), 45 °C (1 min) and 72 °C (2 min), with a final extension cycle at 72 °C for 10 min.

**Expression and purification of His₆–Ffh for antibody preparation.** Oligonucleotides H13 (5’-CGCGAGATCCACCAGTCCGCTCTTGCGCAG-3’) and H16 (5’-CCAAACGTTGCCCATGACTTCTTGGA-3’) were used as primers to amplify the *Ffh* coding sequences. The PCR-amplified DNA fragment was fused in-frame to the His₆ tail as indicated in the QIAexpressionist manual (Qiagen). Ffh insoluble protein was recovered from a 500 ml culture of *E. coli* cells, as indicated in the QIAexpressionist manual. The cleared lysate supernatant containing the His₆-tagged protein was loaded onto an equilibrated Ni-NTA spin column. The protein was extracted from the column by centrifugation as indicated in the QIAexpressionist manual. All buffers used were as described in the manual.

To raise polyclonal anti-Ffh antibodies in rabbits, purified Ffh preparations (50 μg in 500 μl) were mixed with 500 μl complete Freund’s adjuvant and injected intramuscularly (2 × 1 ml) into a Hollander rabbit (Pfd: HOL) at intervals of 3 weeks. A blood sample was taken 2 weeks after applying the last injection, and the serum, collected by centrifugation (5 min, 150 g), was prepared as described by Dunbar & Schwobel (1990). Polyclonal antibodies were obtained against agarase (DagA), as described previously (Parro & Mellado, 1994).

**Intracellular protein analysis and Western blot experiments.** Total intracellular proteins were visualized by Coomassie blue staining of 12-5% SDS/polyacrylamide gels (Laemmli, 1970). For the Ffh Western blot analysis, intracellular proteins were separated by 12-5% SDS-PAGE and transferred to Immobilon PVDF membranes (Millipore) as described by Timmons & Dunbar (1990). Protein concentration in the different samples was determined as described by Bradford (1976) using standard bovine gamma globulin (Bio-Rad). The transferred material was incubated with antibodies raised against Ffh or DagA. Peptides reacting with the antibodies were revealed by incubation with [35S]-labelled protein A [0-1 μCi ml⁻¹ (3-7 kBq ml⁻¹)] from *Staphylococcus aureus* (Amersham) as described by Timmons & Dunbar (1990). Membranes were exposed to Agfa Curix RP2 film at -70 °C.

**Transcriptional analysis.** RNA was isolated as described previously (Parro et al., 1991) but with some modifications (Kedzierski & Porter, 1991). Total RNA (30 μg) was transferred to nylon membranes (Hybond-N⁺; Amersham) and used for Northern blot analysis as described by Sambrook et al. (1989). Nylon membranes were incubated overnight at 65 °C in 0.5 M sodium phosphate pH 7.2/10 mM EDTA/7% (w/v) SDS.

Oligonucleotides scRNA1 (5’-GCCCCCAACACGCTTTCGA-3’) and scRNA2 (5’-GAGTGCAGGAGACTGCG-3’) were used to amplify a 493 bp long DNA fragment from the *S. lividans* chromosome containing the *scRNA* gene. The amplified DNA fragment (5 ng) was used as template to extend 10 pmol of primer scRNA4 (5’-TCCGGCCC-TGGGGAGTTCTGAT-3’) with 5 U of Sequencing Grade *Tag* DNA polymerase (finol DNA Cycle Sequencing System; Promega) in the presence of *finol* DNA Sequencing 1 × Buffer (Promega) and 10 μCi of [α₃²P]dCTP (10 μCi ml⁻¹, 3000 Ci mmol⁻¹; Amersham). The labelled DNA complementary to the scRNA was used as a specific probe for Northern blot analysis.

High-resolution S1 nuclease protection experiments were conducted as described previously (Sambrook et al., 1989; Barthelemy et al., 1986; Parro et al., 1998) using 50 μg total RNA. The DNA molecular size ladders were enzymically derived by the dideoxy chain termination method (Sanger et al., 1977) by extension of primer scRNA4, 5’-labelled with 40 μCi [α₃²P]dCTP (10 μCi ml⁻¹, 3000 Ci mmol⁻¹; Amersham), using the 493 bp long DNA fragment as template. The

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The recombinant bacterium was grown at 37 °C with vigorous shaking and induced with 1 mM IPTG; the culture was allowed to grow for 5 h, as indicated in the QIAexpressionist manual (Qiagen). Ffh insoluble protein was recovered from a 500 ml culture of *E. coli* cells, as indicated in the QIAexpressionist manual. The cleared lysate supernatant containing the His₆-tagged protein was loaded onto an equilibrated Ni-NTA spin column. The protein was extracted from the column by centrifugation as indicated in the QIAexpressionist manual. All buffers used were as described in the manual.
RESULTS

Cloning of the S. lividans SRP system components

On sequence comparison among conserved bacterial Ffh proteins, degenerate oligonucleotides H5 (5'-CGCGGATG/CAGAGTCGACGACGAC/CGG(C/G)/CCAG-3') and H4 (5'-CGCGGATTCG/CGCtC/GCC/CGCGGCGG/C/GTGTCG/C AGTCG-3') were designed for use as probes to screen for clones containing putative S. lividans Ffh coding sequences in an S. lividans genomic BamHI library made in the E. coli plasmid pUC19. Plasmid pFF14 carrying an approximately 14 kb long BamHI fragment containing the Ffh coding sequences was selected. Chromosome walking indicated that the DNA fragment containing ffh was located adjacent to the 8-3 kb long BamHI fragment previously shown by us to contain four different signal peptidease genes (Parro et al., 1999). The ffh gene (GenBank accession no. AF071565) is likely to form a two-gene operon with gldD (Fig. 1a). As expected, the deduced amino acid sequence of the S. lividans Ffh protein showed a high degree of homology when aligned to sequences of other bacterial Ffh proteins (not shown).

Using the SRP database (http://psyche.uthct.edu/dbs/SRPDB/SRPDB.html), oligonucleotides scRNA1 and scRNA2 were designed to directly amplify the putative scRNA coding DNA sequence from the S. lividans genome. The amplified

Detection of scRNA associated with FH and GTPase activity.

Bacteria from mid-exponential phase S. lividans cell cultures were harvested, concentrated four-fold in buffer I (50 mM triethanolamine pH 7.5/100 mM potassium acetate/1 mM EDTA/0–1 % (w/v) Triton X-100/1 mM DTT) and broken up with a French press [1000 p.s.i. (6.9 MPa)]. Aliquots (300 µl) from the cell lysate were pre-incubated at 4 °C for 5 min with 0.5 % (w/v) protein A-Sepharose CL-4B (Sigma). The cleared lysate was incubated for 12 h with anti-Ffh serum at 4 °C before adding protein A-Sepharose again, and incubation continued for a further 2 h. Immunoprecipitated material was collected by centrifugation and recovered in 20 µl buffer I.

The scRNA contained in the immunoprecipitated material was recovered by heating to 65 °C in 1:1 mixtures of 4 M guanidine thiocyanate/phenol. The denatured RNA was analysed by Northern blot.

The Ffh protein present in the immunoprecipitate was visualized by Western blot analysis using anti-Ffh serum. To determine the GTPase activity associated with the immunoprecipitated Ffh, half of the immunoprecipitated material was incubated at 37 °C for 1 h with 100 µCi [γ-32P]GTP (10 mCi ml⁻¹, 3000 Ci mmol⁻¹; Amersham) in a final volume of 20 μl of 20 mM Tris/HCl pH 7.5/2 mM MgCl2/10 % (w/v) glycerol/0–1 mg ml⁻¹ BSA/1 mM GTP. One-tenth of the reaction (equivalent to 15 µl cell lysate) was applied to a PEI-cellulose sheet (Aldrich) and developed with 0.5–5 M potassium phosphate pH 3.5. The radioactively labelled GDP released by the GTPase hydrolytic action was revealed by exposure to Agfa Curix RP2 film at −70 °C.

Co-immunoprecipitation of FH and pre-DagA. Aliquots (300 µl) from French press lysates of S. lividans TK21Y62/pPAGAs5 cell cultures harvested at the transition to the stationary phase of growth were immunoprecipitated by incubation with anti-Ffh serum or anti-DagA serum, as described above. The presence of pre-agarase in the anti-Ffh immunoprecipitated material or Ffh in the anti-DagA immunoprecipitate was determined by Western blot analysis using serum anti-DagA or anti-Ffh, respectively.

Fig. 1. Restriction maps of cloned DNA containing the ffh, ftsY and scRNA genes. The sizes of the different gene coding sequences and the lengths of the encoded RNAs and polypeptides are indicated. (a) Restriction map of the 14 kb BamHI fragment cloned from the S. lividans TK21 chromosome. Only relevant restriction endonuclease sites are indicated. The chromosomal distance (3055 bp) between ftsY and the 14 kb long DNA fragment containing ffh is indicated. The 14 and 7.8 kb long BamHI fragments containing ffh and the four sip genes, respectively, are adjacent in the S. lividans genome. (b) Genes adjacent to the scRNA gene are indicated.
DNA was sequenced and proved identical to the equivalent region of the *S. coelicolor* genome. The relative position of the scRNA gene in the *S. lividans* genome is indicated in Fig. 1(b). The putative scRNA sequence was 82 nt long (GenBank accession no. AY081854) and was shown to share all those structural features that characterize the equivalent *E. coli* RNA molecule (Fig. 2).

On sequence comparison among conserved bacterial FtsY proteins, oligonucleotides ft3 (5'-CGATCCCGGGGTTT-CCAGGGGCC-3') and ft2 (5'-CGATCAAGCAACCTCC-GCCACCGG-3') were designed to directly amplify the *ftsY* gene from the *S. lividans* genome. The 1536 bp long amplified DNA fragments were propagated in the *E. coli* plasmid pUC19. Plasmid pFTY containing the *ftsY* gene was selected.

The *ftsY* gene (GenBank accession no. AY140960) is also located near the *sip* genes, although not as closely as *ffh* (Fig. 1a). As expected, the deduced FtsY amino acid sequence showed a high degree of homology when aligned with sequences of other bacterial FtsY proteins (not shown).

**Transcriptional analysis of the SRP components**

Submerged cultures of *S. lividans* TK21 were incubated at 30°C in NMMP medium supplemented with 0.5% (w/v) mannitol as carbon source. They grew exponentially with a doubling time of ~4.2 h. The transition to stationary phase occurred 25–30 h after inoculation, at biomass dry weights of ~2.5 mg ml⁻¹. Transcriptional analysis of the *ftsY* gene by Northern blot confirmed that *ftsY* is transcribed as a single gene transcriptional unit, and the *ftsY* transcript was detected throughout the different phases of bacterial cell culture growth (Fig. 3a), apparently being more abundant at the early-exponential phase of growth, as confirmed by RT-PCR analysis (not shown).

Northern blot analysis confirmed that the scRNA gene is transcribed as a single gene transcriptional unit, with scRNA being detected throughout the different phases of bacterial cell culture growth. The transcription initiation site of the *S. lividans* scRNA has been mapped by high-resolution S1 nuclease protection experiments (Fig. 3b, left panel). No pre-scRNA was detected. The scRNA was radioactively labelled at its 5’ end and its size was determined by protection from the S1 nuclease digestion (Fig. 3b, right panel). Protected fragments of 76–82 nt in size were detected. The presence of more than one protected band may be due to the S1 nuclease ‘nibbling effect’ described previously (Christie & Calendar, 1983; Mellado et al., 1986); hence, the largest size has been taken as the full-length for the *S. lividans* scRNA.

The *ffh* gene is likely to form part of an operon containing at least two genes, as deduced from the sequencing data. RT-PCR analysis using oligonucleotides H8 and H12 as primers revealed that the *ffh* transcript was present throughout the different phases of bacterial cell culture growth (Fig. 3c), the transcript being considerably more stable than the *glnD* transcript (not shown), suggesting a differential post-transcriptional processing of the *glnD* and *ffh* messengers.

A putative downstream box that is complementary to the 16S rRNA (Wu & Janssen, 1996; Sprengart et al., 1996) was identified in the *ffh* transcript, which may ensure its translation. Table 1 shows the putative downstream boxes of *ffh* and signal peptidase (*sipW* and *sipZ*) transcripts (Parro et al., 1999) compared to that of the viomycin phosphotransferase (*vph*) transcript (Wu & Janssen, 1996) and the *S. lividans* 16S rRNA complementary sequence.

**Structural components of the SRP**

Ffh DNA coding sequences were fused in-frame to a His₆ coding sequence at its amino end and propagated in pQE30, so that *S. lividans* Ffh could be over-synthesized in *E. coli,*
purified on Ni-NTA columns and used to raise rabbit polyclonal antibodies against the purified protein. As expected, the antibodies cross-reacted with Ffh proteins from *E. coli* and *B. subtilis*, thereby confirming the high degree of homology observed between them (not shown).

Anti-Ffh antibodies were used to immunoprecipitate *S. lividans* TK21 cell lysates. The presence of Ffh in the resulting immunoprecipitated material was further analysed by Western blot experiments and its capacity to hydrolyse GTP was analysed by TLC. The presence of accompanying scRNA in the immunoprecipitate was analysed by Northern blot experiments. A unique protein of the expected molecular size was detected reacting with the anti-Ffh serum (Fig. 4a); GTPase activity was also present in the immunoprecipitate (Fig. 4b) and an scRNA of the expected mobility was detected as well (Fig. 4c).

The results obtained clearly indicate that Ffh and scRNA are the main components of the *S. lividans* SRP. The *S. lividans* scRNA is very small in size (82 nt long) and lacks the Alu domain; therefore, it seems very unlikely that the *S. lividans* scRNA would have the capacity to bind to other proteins apart from Ffh. Thus, the structure of the *S. lividans* SRP is that of the minimal functional SRP and resembles that of *E. coli*.

**SRP is involved in protein secretion**

*S. lividans* TK21Y62(pAGAs5) is an *S. lividans* TK21 mutant strain in which the major signal peptidase (SipY) is inactive and which carries the *S. coelicolor* agarase gene *dagA* on the multicopy plasmid pAGAs5 (Palacin *et al*., 2002). This strain is able to overproduce agarase, although pre-agarase processing takes place at a much lower speed than in *S. lividans* TK21(pAGAs5) and, as a result, pre-agarase tends to accumulate intracellularly at a higher level. To determine if this minimal functional SRP was involved in targeting secretory proteins, *S. lividans* TK21Y62(pAGAs5) cell lysates were immunoprecipitated with anti-Ffh antibodies or anti-agarase antibodies. The presence of either agarase or Ffh in the immunoprecipitated material was analysed by Western blot using the corresponding antiserum in each case. Anti-agarase antibodies precipitated the pre-agarase present inside the cell (Fig. 5a, lane 1), whereas anti-Ffh antibodies

![Fig. 3. Transcriptional analysis of the **ffh**, **ftsY** and **scRNA** genes.](image)

**Table 1.** Putative downstream boxes for **ffh**, **sipW** and **sipZ** compared to that of **vph**

Translation start codons AUG and GUG are shown in bold. The putative downstream boxes are underlined. Numbers in parentheses indicate the relative nucleotide positions in the *S. lividans* 16S rRNA anti-downstream box (anti-db; Wu & Janssen, 1996).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td><strong>ffh</strong> mRNA</td>
<td>GUGACCCUGCCUCGCUCUGACGGCAGGGCAGGACACUUUGCCUGCCGG</td>
</tr>
<tr>
<td><strong>sipW</strong> mRNA</td>
<td>AUGGACACCAAAACACAGGACAGCAGGCGACGUCUCUCGCCGCC</td>
</tr>
<tr>
<td><strong>sipZ</strong> mRNA</td>
<td>AUGGGUGGGGAGGACAGACGACGACGUGUCUCCCGCGC</td>
</tr>
<tr>
<td><strong>vph</strong> mRNA</td>
<td>AUGGGAAUCUUGAGACGCAAGGCGAUCGCAGCUCCUGCGU</td>
</tr>
<tr>
<td><em>S. lividans</em> anti-db</td>
<td>3’(1470) GCCGUACGGUGGG 5’(1456)</td>
</tr>
</tbody>
</table>
only precipitated the pre-agarase bound to the Ffh (Fig. 5a, lane 2). Anti-Ffh antibodies precipitated the Ffh present inside the cell (Fig. 5b, lane 2), whereas anti-agarase antibodies only precipitated a comparatively small amount of Ffh bound to pre-agarase (Fig. 5b, lane 1). Differences in relative band intensity, as revealed by Western blot analysis, could also reflect differences in the relative specificity of each antibody. The fact that both antibodies co-immunoprecipitated the other protein indicates that an interaction of Ffh and pre-agarase does occur inside the cell, therefore suggesting that the SRP system is involved in the S. lividans protein secretion pathway.

**DISCUSSION**

*S. lividans* and *B. subtilis* are two Gram-positive bacteria which produce a considerable amount of secretory proteins and both bacteria have been widely used for the production and secretion of proteins of industrial interest. Regarding the secretion mechanism, both bacteria share interesting features, namely, they have more than one gene for type I signal peptidases, five located chromosomally in *B. subtilis*, two of them being the major ones (Tjalsma et al., 1998), and four in *S. lividans*, one of them being major (Palacin et al., 2002). Moreover, neither *B. subtilis* nor *S. lividans* have an equivalent to the *E. coli* SecB protein. The *B. subtilis* SRP system seems able to substitute the SecB deficiency targeting membrane and secretory proteins (Oguro et al., 1996); therefore, the identification of the SRP components in *S. lividans* and the search as to whether they were involved in protein secretion was an obvious task.

The *S. lividans* SRP system consists of a functional SRP, a ribonucleoprotein, composed of Ffh, and an 82 nt long RNA. The small size of this RNA, as occurs in *E. coli*, seems to leave no room for other proteins to adhere to the complex, although at present this cannot be totally ruled out. Fig. 6 shows a comparison of the *E. coli*, *B. subtilis* and *S. lividans* SRP structures. FtsY, the receptor protein, also forms part of the *S. lividans* SRP system and its expression takes place throughout the cellular growth similar to that of the scRNA and Ffh proteins. So far, all attempts made by us to obtain mutants in any of the three corresponding genes have failed, thus indicating the possible essential nature of the SRP components in *S. lividans*. Interestingly enough, transcription of *S. lividans* signal peptidase genes seems to occur in the shape of polycistronic messengers that are specifically processed (Parro et al., 1999), as appears to be the case for *ffh* transcription. The *ffh* transcript seems to contain an internal downstream box (Wu & Janssen, 1996) to ensure translation of the processed messenger, a...
feature also shared with the sip transcripts (Table 1; Parro et al., 1999).

The S. lividans SRP, despite being minimal in composition, seems to be involved in protein secretion, interacting with pre-agarase and in the pre-protein co-immunoprecipitate interacting with Ffh (Fig. 5). The length and hydrophobicity of the signal peptides of S. lividans secretory proteins seem to be more similar to those of B. subtilis than to those of E. coli. It has been argued that these signal peptide characteristics may allow the B. subtilis SRP system to target both secretory and membrane proteins (Herskoviits et al., 2000); therefore, it seems reasonable to assume that the B. subtilis and S. lividans SRP systems are performing the same intracellular assignments.

The construction of a His6-tagged FtsY protein, currently under way, will help in the design of experiments to elucidate the role played by the FtsY receptor in SRP-mediated secretion in S. lividans.

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