The *Escherichia coli* SRP and SecB targeting pathways converge at the translocon

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Two distinct protein targeting pathways can direct proteins to the *Escherichia coli* inner membrane. The Sec pathway involves the cytosolic chaperone SecB that binds to the mature region of pre-proteins. SecB targets the pre-protein to SecA that mediates pre-protein translocation through the SecYEG translocon. The SRP pathway is probably used primarily for the targeting and assembly of inner membrane proteins. It involves the signal recognition particle (SRP) that interacts with the hydrophobic targeting signal of nascent proteins. By using a protein cross-linking approach, we demonstrate here that the SRP pathway delivers nascent inner membrane proteins at the membrane. The SRP receptor FtsY, GTP and inner membranes are required for release of the nascent proteins from the SRP. Upon release of the SRP at the membrane, the targeted nascent proteins insert into a translocon that contains at least SecA, SecY and SecG. Hence, as appears to be the case for several other translocation systems, multiple targeting mechanisms deliver a variety of precursor proteins to a common membrane translocation complex of the *E.coli* inner membrane.
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Introduction

In eukaryotes, protein targeting to the membrane of the endoplasmic reticulum (ER) is initiated by the co-translational recognition of targeting signals by the signal recognition particle (SRP) (reviewed in Rapoport *et al.*, 1996). The SRP is a ribonucleoprotein particle composed of six proteins assembled on an RNA scaffold (7S RNA). Upon recognition of a targeting signal by the 54 kDa SRP subunit (SRP54), translational elongation of the nascent polypeptide chain is inhibited. The ribosome-bound nas-

cent chain (RNC) in complex with SRP is then targeted to the membrane-associated α-subunit of the SRP receptor (SRα), and the RNC is subsequently released from the SRP. This targeting process requires the binding of GTP to both SRP54 and SRα (Connolly and Gilmore, 1989; Rapiejko and Gilmore, 1997). The released nascent chain enters the translocon, the ribosome makes a tight seal with the translocon, translation is resumed and the nascent chain inserts co-translationally into the aqueous trans-location channel (Rapoport *et al.*, 1996; Johnson, 1997). The hydrolysis of GTP at SRP54 and SRα is required to dissociate the SRP–SRα complex and recycle these targeting factors (Connolly *et al.*, 1991).

Protein targeting to the *Escherichia coli* inner membrane can occur via the Sec pathway and the SRP pathway. The extensively studied Sec pathway uses a cytosolic chaperone, SecB, that binds post-translationally or at a late co-translational stage to the mature region of pre-proteins (Kumamoto and Francetic, 1993). The SecB–pre-protein complex is targeted to the membrane where SecA is activated for high-affinity recognition of SecB and pre-protein by binding to the membrane-embedded translocon (reviewed in Driessen *et al.*, 1998). SecB is released from the pre-protein as the ATPase SecA mediates post-translational translocation through the SecYEG translocon by ATP-driven cycles of insertion and de-insertion (Economou and Wickner, 1994; Economou *et al.*, 1995).

Although SecA is not an integral part of the *E.coli* translocon, it is considered part of the dynamic structure of the translocon at certain stages in the translocation process (Driessen *et al.*, 1998). In eukaryotes, SecA homologues have only been identified in chloroplasts (Nakai *et al.*, 1994; Yuan *et al.*, 1994). In contrast, the core structure of the mammalian and *E.coli* translocons appears to be conserved: both complexes are hetero-trimeric, consisting of Sec61α, β and γ (Sec61 complex) and of SecY, SecE and SecG (SecYEG complex), respectively. SecE and Sec61γ, and especially SecY and Sec61α, share significant sequence similarity (Rapoport *et al.*, 1996). Sec61α is in close proximity to translocating proteins during co-translational translocation (High *et al.*, 1993; Mothes *et al.*, 1994). In *Saccharomyces cerevisiae*, the homologous Sec61 complex is involved in both co- and post-translational translocation. In addition, another trimeric translocon (Ssh1 complex) has been identified that probably functions exclusively in co-translational protein transport (Rapoport *et al.*, 1996; Wilkinson *et al.*, 1997).

The more recently discovered SRP pathway in *E.coli*, on the other hand, involves cytosolic factors that strongly resemble components involved in protein targeting to the eukaryotic ER membrane (Luirink and Dobberstein, 1994; Wolin, 1994). Thus, a small SRP has been identified that consists of a 4.5S RNA and a 48 kDa GTPase designated

P48 (or Ffh for <u>f</u>ifty-<u>f</u>our <u>h</u>omologue), which are homologous to the eukaryotic 7S RNA (Poritz *et al.*, 1988) and SRP54 (Bernstein *et al.*, 1989; Römisch *et al.*, 1989), respectively. The SRP was shown *in vitro* to interact by virtue of P48 with RNCs of several secreted and membrane proteins, with a preference for substrates that expose particularly hydrophobic targeting signals (Valent *et al.*, 1995, 1997). In agreement with these findings, recent evidence indicates that proteins equipped with strongly hydrophobic targeting signals (e.g. integral inner membrane proteins) are particularly dependent on the SRP for efficient membrane assembly *in vivo* (Mac Farlane and Müller, 1995; De Gier *et al.*, 1996; Ulbrandt *et al.*, 1997). Moreover, a putative SRP receptor (FtsY) has been identified based on sequence similarity with SRα (Bernstein *et al.*, 1989; Römisch *et al.*, 1989). FtsY interacts with the SRP *in vitro* in a GTP-dependent manner (Miller *et al.*, 1994; Kusters *et al.*, 1995) and is essential for the efficient secretion of certain proteins (Luirink *et al.*, 1994) and the biogenesis of inner membrane proteins *in vivo* (Seluanov and Bibi, 1997).

Until now, a connection between the *E.coli* Sec and SRP pathways had not been established. In this study, we demonstrate that the two pathways merge at the inner membrane. Proteins targeted by the SRP are shown to interact co-translationally with the *E.coli* inner membrane. Release of the SRP from the RNC occurs at the membrane and requires both FtsY and GTP. After release from the SRP, the nascent chains insert into the membrane at a translocon that contains SecA, SecY and SecG.

Results

Short nascent inner membrane proteins associate with E.coli inner membranes

In a previous study (Valent *et al.*, 1997), we have used an *E.coli in vitro* translation system in combination with bifunctional cross-linking reagents to investigate the molecular interactions of short nascent pre-secretory and membrane proteins in the cytosol. A direct interaction of the SRP with nascent polypeptides that expose particularly hydrophobic targeting signals was demonstrated, suggesting that inner membrane proteins are the primary physiological substrates of the *E.coli* SRP. In addition, the cytosolic chaperone trigger factor (TF) was found to interact with all nascent polypeptides long enough to protrude from the ribosome (Valent *et al.*, 1995, 1997; Hesterkamp *et al.*, 1996), indicating that TF has a general affinity for nascent polypeptides and is positioned near the nascent chain exit site on the *E.coli* ribosome.

In the present study, we investigated the later stages in SRP-mediated protein targeting and membrane insertion. First we examined whether short nascent membrane proteins are able to bind to import-competent *E.coli* inverted inner membrane vesicles (INVs) (De Vrije *et al.*, 1987). As model targeting substrates we used leader peptidase I (Lep), a polytopic membrane protein that removes N-terminal signal peptides from exported proteins at the periplasmic side of the cytoplasmic membrane (reviewed in Dalbey, 1991), and FtsQ, a type II cytoplasmic membrane protein involved in cell division (Carson *et al.*, 1991), which both interact with the SRP *in vitro* (Valent

et al., 1995, 1997). In addition, Lep insertion has been shown to depend on the SRP *in vivo* (De Gier *et al.*, 1996).

RNCs were prepared by translating truncated mRNAs in an *E.coli* membrane-free cell extract (Valent *et al.*, 1997). The truncated mRNAs encode polypeptides of ~100 amino acids to allow optimal exposure of the N-terminal targeting sequence. The purified RNCs were incubated with INVs and subjected to flotation gradient analysis under high salt conditions. After centrifugation, four fractions were collected and analysed by SDS–PAGE and phosphor imaging. The top fraction contains floated membranes and targeted RNCs, whereas the untargeted RNCs remain in the bottom fractions. In Figure 1A, the percentage of membrane-associated (floated) RNCs is shown. Approximately 30% of the nascent Lep (101Lep) and FtsQ (108FtsQ) fractionated with the INVs, indicating efficient membrane association. These data indicate that nascent membrane proteins can interact with *E.coli* inner membranes *in vitro*.

We have demonstrated previously that the interaction of the SRP with nascent pre-secretory proteins correlates with the hydrophobicity of the exposed targeting sequence (Valent *et al.*, 1995, 1997). To determine the effect of targeting sequence hydrophobicity on membrane association of nascent polypeptides, PhoA derivatives with mutated signal sequence core regions were used (Doud *et al.*, 1993). A nascent PhoA construct (91PhoA) carrying a strongly hydrophobic signal sequence (9Leu, 1Ala) that interacts efficiently with the SRP (Valent *et al.*, 1995, 1997) showed significant membrane association. In contrast, a 91PhoA construct exposing a moderately hydrophobic (4Leu, 6Ala) signal sequence that does not interact with the SRP (Valent *et al.*, 1995, 1997) showed no significant membrane association (Figure 1A).

We conclude that the membrane association of short nascent pre-secretory and membrane proteins correlates with the hydrophobicity of the targeting signal and hence their ability to interact with the SRP. This suggests that the SRP plays a role in the targeting of these proteins.

Membrane-targeted nascent proteins interact with Sec translocon components

To probe the molecular environment of membrane-associated 108FtsQ, we used an unbiased cross-linking approach. Nascent polypeptides were incubated with INVs to allow targeting, and subsequently treated with the bifunctional cross-linking reagent disuccinimidyl suberate (DSS) (Figure 1B, lanes 1 and 2). DSS is the membrane-permeable analogue of BS^3, a cross-linking reagent that we used previously to probe interactions of untargeted RNCs (Valent *et al.*, 1997). After cross-linking, the samples were extracted with alkaline sodium carbonate buffer to separate integral membrane (Figure 1B, lane 3) from peripheral and soluble cross-linked complexes (Figure 1B, lane 4).

In the Na_2CO_3 pellet, two major 108FtsQ cross-linking adducts appeared at ~120 and ~41–44 kDa. The 120 kDa product was immunoprecipitated using anti-SecA (Figure 1A, lane 10), indicating that it represents a membrane-integral complex of the radiolabelled nascent FtsQ (12 kDa) and SecA (102 kDa). The fuzzy 42 kDa product was found exclusively in the Na_2CO_3-resistant fraction and immunoprecipitated with anti-SecY (Figure 1B, lane 9).
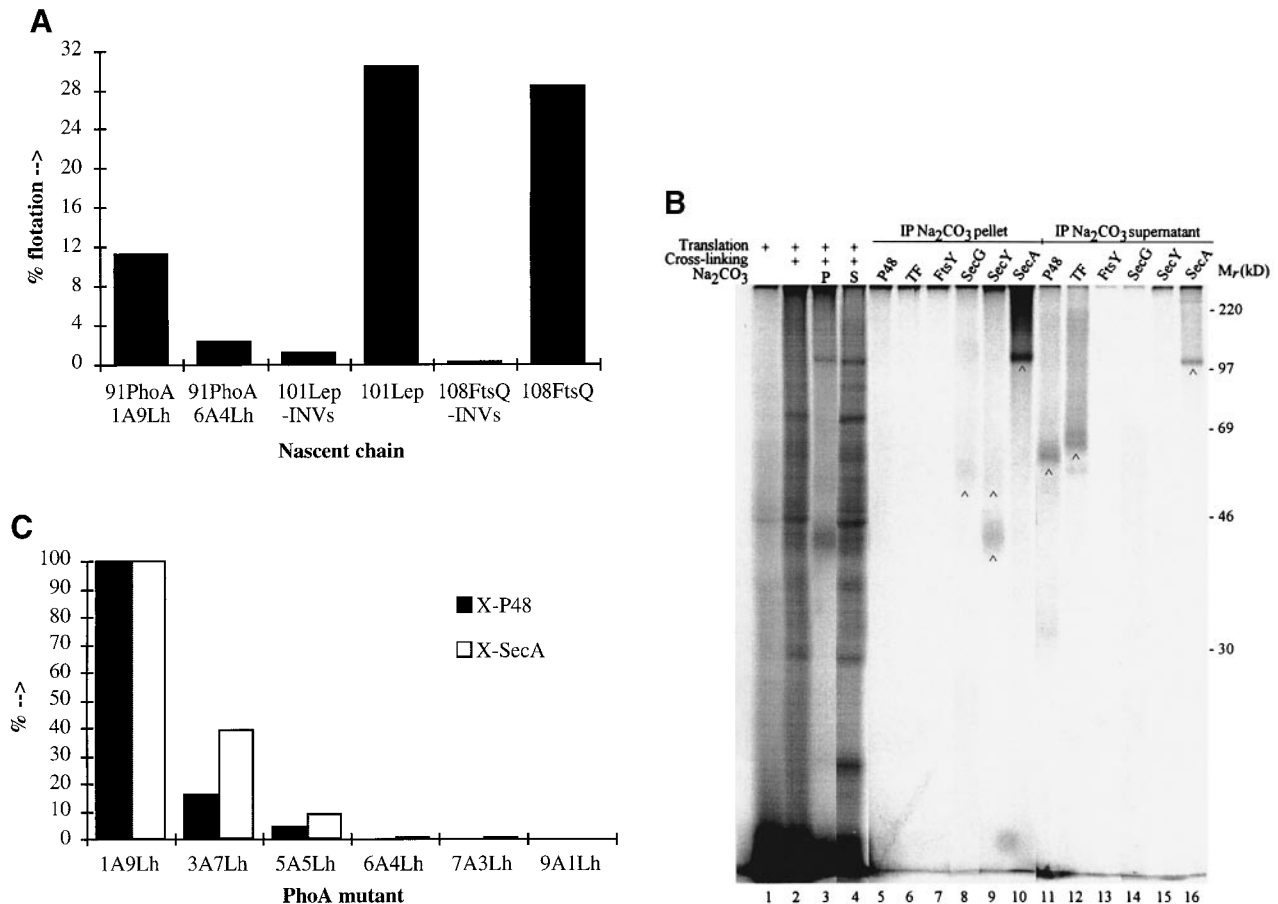
Fig. 1. Short nascent pre-secretory (PhoA) and inner membrane proteins (Lep and FtsQ), associate with INVs and interact with the translocon components SecA and SecY. (**A**) The indicated RNCs were produced, incubated with or without INVs and subjected to flotation gradient analysis as described in Materials and methods. The percentage of RNCs in the top (membrane) fraction is shown. (**B**) 108FtsQ RNCs were incubated with INVs and treated with DSS. After quenching, soluble and peripheral cross-linking complexes were extracted from the membranes with Na_2CO_3 as described in Materials and methods. Both pellet (P) and TCA-precipitated supernatant (S) fractions were examined by immunoprecipitation (IP) for the presence of cross-linking adducts with the indicated proteins. Immunoprecipitated protein complexes are indicated by an arrow. The M_rs of marker proteins are indicated on the right. (**C**) Mutant 91PhoA nascent chains were examined as described in (B). The total amount of cross-linking to SecA and P48 after immunoprecipitation was determined by phosphor imaging. Maximum cross-linking was set at 100%.

Cross-linking of nascent chains to Sec61α, the mammalian homologue of SecY (Rapoport *et al.*, 1996), results in similarly smeared adducts (Laird and High, 1997). It should also be noted that although SecY has a predicted M_r of 49 kDa, it usually migrates as an ~35 kDa product upon SDS–PAGE (Akiyama and Ito, 1986). In addition, a faint cross-linking product of ~50 kDa was immuno-precipitated with anti-SecY (Figure 1B, lane 9) and anti-SecG (Figure 1B, lane 8) which presumably represents SecG cross-linked via SecY to a 108FtsQ–SecY adduct.

In the Na_2CO_3 supernatant, the cross-linking pattern was more complex (Figure 1B, lane 4). Immunoprecipitation revealed the presence of adducts to the SRP protein constituent P48 and to TF (Figure 1B, lanes 11 and 12) that have been observed previously with 108FtsQ synthesized in the absence of INVs (Valent *et al.*, 1995, 1997). In addition, a fraction of the 108FtsQ–SecA complex was detected in the supernatant (Figure 1B, lane 16). This may represent cross-linking between 108FtsQ and the membrane-peripheral form of SecA, since it was not observed upon cross-linking in the absence of INVs (Valent *et al.*, 1997). Almost all other radiolabelled products were lost upon purification of RNCs through a high salt sucrose cushion (Valent *et al.*, 1997; also Figure 2A, compare

lanes 2 and 3), indicating that these were not RNC-specific cross-linking products. No cross-linking adducts with integral membrane proteins SecY or SecG were detected in the Na_2CO_3 supernatant fraction (Figure 2B, lanes 14 and 15). A direct cross-linking between RNC and FtsY was not detected. Immunoprecipitation did not reveal any interactions of 108FtsQ with other targeting factors and chaperones known to interact with nascent polypeptides, such as DnaK/J and SecB (reviewed in Bukau *et al.*, 1996). The cross-linking pattern obtained with 101Lep was remarkably similar to that observed with 108FtsQ (data not shown).

Our principal conclusion from this initial analysis is that nascent FtsQ and Lep form translocation intermediates that are in close proximity to the translocon components SecA, SecY and SecG. The simplest interpretation of these results is that the SRP mediates co-translational targeting to the translocon which is also used by proteins whose targeting is dependent on SecB.

The interaction of the SRP with nascent proteins has been shown to correlate with the hydrophobicity of the targeting sequence (Valent *et al.*, 1995, 1997). This prompted us to examine the membrane interactions of mutant 91PhoA carrying signal sequences with incre-
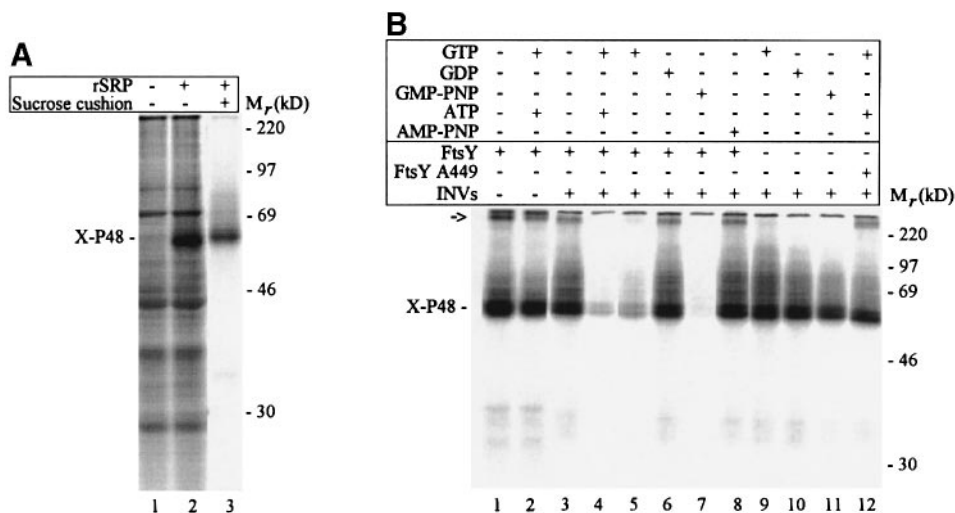
Fig. 2. Requirements for the release of SRP from RNCs. (**A**) 108FtsQ RNCs were incubated in the presence or absence of 350 nM of rSRP. SRNCs were prepared by purifying an equal portion of rSRP-saturated RNCs through a high salt sucrose cushion. SRP–RNC interactions were monitored by cross-linking with DSS. Quenched samples were TCA-precipitated and analysed by SDS–PAGE. The RNC–P48 adduct (X-P48) is indicated on the left. The M_rs of marker proteins are indicated on the right. (**B**) SRNCs were incubated for 5 min at 25°C with or without INVs (1.25 mg/ml protein), (mutant) FtsY (1 μM) or nucleotides (30 μM each), as indicated. After 5 min on ice, samples were treated with DSS and Na_2CO_3 as described in Materials and methods. The TCA-precipitated Na_2CO_3 supernatant is shown. The RNC–P48 adduct (X-P48) and RNC, P48 and FtsY containing adduct (arrow) are indicated on the left. The M_rs of marker proteins are indicated on the right.

mental differences in hydrophobicity (Doud *et al.*, 1993). Cross-linking efficiencies can be compared directly since these constructs are identical except for the hydrophobic core region of the signal sequence. Thus, the potential cross-linking lysine residues are flanking the core region in each construct. The quantified results shown in Figure 1C demonstrated a correlation between the hydrophobicity of the exposed signal sequence and cross-linking to P48 in the Na_2CO_3 supernatant, consistent with previous results obtained in the absence of membranes (Valent *et al.*, 1995, 1997). In addition, the efficiency of cross-linking to TF was similar for all constructs (data not shown; see also Valent *et al.*, 1997). A striking correlation between the hydrophobicity of the signal sequence and cross-linking to SecA (Figure 1C) was observed that coincides with cross-linking to P48 in the Na_2CO_3 supernatant (Figure 1C) and association of the mutant nascent chain with the membrane (Figure 1A). Approximately 10% of the cross-linked SecA was detected in the Na_2CO_3 supernatant. SecY adducts could not be identified for any PhoA construct used (data not shown), perhaps reflecting a different interaction with the translocon or an unfavourable positioning of cross-linking residues.

Taken together, the data suggest a connection between the SRP targeting machinery and the Sec translocon.

SRP is released from RNCs at the membrane by FtsY which is in the GTP-bound state

The experiments described above demonstrated that membrane-inserted 108FtsQ RNCs are not associated with the SRP in contrast to untargeted RNCs (Figure 1B, compare lanes 5 and 11). This implies that the SRP is released before, or concomitant with, membrane insertion and association with the translocon. To investigate the requirements for dissociation of the SRP–RNC complex and membrane insertion, we used 108FtsQ since it exhibits strong cross-linking to P48 (Valent *et al.*, 1995, 1997; Figure 1B, lane 11) and associates efficiently with SecA

and SecY (Figure 1B, lanes 9 and 10). In order to analyse SRP binding directly (i.e. without immunoprecipitation), reconstituted *E.coli* SRP (rSRP) was added after translation to saturate RNCs with SRP. A substantial increase in cross-linking efficiency was observed (Figure 2A, compare lanes 1 and 2), indicating effective rSRP–RNC interaction. The rSRP–RNC complexes remained intact after purification over a high salt sucrose cushion, demonstrating the stability of the interaction (Figure 2A, lane 3; see also Valent *et al.*, 1997). These purified complexes were designated SRNCs and used to study the requirements for release of the SRP from the RNCs.

By analogy with the eukaryotic system, the most likely candidate for a membrane receptor/release factor for the SRP is FtsY since it displays sequence similarity to the α-subunit of the mammalian SRP receptor (SRα) (Bernstein *et al.*, 1989; Römisch *et al.*, 1989) and interacts with rSRP *in vitro* in a GTP-dependent process (Miller *et al.*, 1994; Kusters *et al.*, 1995). For this reason, release of the SRP from 108FtsQ was studied by varying the presence of FtsY (Luirink *et al.*, 1994) and nucleotides in the presence of wild-type INVs. FtsQ RNCs were saturated with rSRP and purified over a high salt sucrose cushion. These SRNCs were incubated in the presence or absence of INVs, FtsY and nucleotides (Figure 2B). Addition of INVs, FtsY and GTP was required for efficient loss of P48 cross-linking (Figure 2B, lanes 1–5 and 9). This indicates an FtsY-mediated dissociation of the SRP from the nascent chain at the membrane. GDP could not substitute for GTP in this reaction (Figure 2B, lane 6), reminiscent of the requirement for GTP in the analogous reaction in the mammalian system (Connolly and Gilmore, 1989).

To examine whether binding of GTP to FtsY is required for the release of the SRP from the RNCs, a mutant FtsY was used that is unable to bind GTP because of a point mutation in G4, the fourth consensus region for GTP binding (Kusters *et al.*, 1995). This mutant, FtsY A449,

did not mediate the release of SRP from the nascent chain (Figure 2B, lane 12), indicating that binding of GTP to at least FtsY is a prerequisite for the release of SRP from the nascent polypeptide.

Hydrolysis of GTP was not required for the release of the SRP from RNCs. When using wild-type FtsY in the presence of the non-hydrolysable GTP analogue GMP-PNP, release of the SRP from RNCs was even more pronounced than when GTP was used (Figure 2B, compare lanes 5 and 7). GTP hydrolysis is essential for the release of the SRP from FtsY *in vitro* (Miller *et al.*, 1994). It is possible that GMP-PNP locks the SRP in an SRP–FtsY complex, thus preventing SRP from undergoing cycles of nascent chain binding and release. This would lower the amount of SRP available to re-associate with the RNCs. AMP-PNP, a non-hydrolysable ATP analogue, was completely inactive in SRP release (Figure 2B, lane 8), showing the nucleotide specificity of the reaction. In the absence of added FtsY, only a slight release was observed (Figure 2B, compare lanes 5 and 7 with 9 and 11), reflecting the activity of the endogenous FtsY present in the INVs.

FtsY differs from SRα in that it is located not only in the target membrane but also in the cytosol (Luirink *et al.*, 1994). It is thought that FtsY is only functional when bound to the inner membrane (Zelazny *et al.*, 1997). Interestingly, in the absence of INVs (Figure 2B, lane 2), a soluble high molecular weight complex was observed (Figure 2B, arrow) that contains both P48 and FtsY since it was not detected when the SRP was released efficiently from the RNCs (Figure 2B, lanes 4 and 7) or when FtsY was not included (Figure 2B, lanes 9–11). This conclusion was verified by immunoprecipitation using antibodies directed against both P48 and FtsY (data not shown) and suggests that FtsY is able to interact with RNC–SRP complexes in the cytosol, independently of the presence of INVs. In the presence of FtsY A449 (Figure 2B, lane 12) or the absence of GTP (Figure 2B, lane 3), the same complex was formed (Figure 2B; arrow), suggesting that the binding of FtsY to the SRP–RNC complex also does not require GTP.

Taken together, we conclude from these results that cytosolic FtsY binds to the SRP which is associated with RNCs in a process that does not depend on nucleotides. Release of the SRP from the nascent chain occurs at the membrane and requires the binding of GTP to at least FtsY.

Following release of SRP, the nascent chain enters the translocon

Thus far, we have shown that nascent polypeptides exposing particularly hydrophobic targeting sequences interact with SRP in the cytosol and with the translocon components SecA and SecY in the membrane. Together with the observation that RNCs are only released from the SRP in the presence of GTP, FtsY and INVs, this suggests that the SRP and FtsY target RNCs to the membrane where the SRP is dissociated from the RNC, allowing the latter to interact with the translocon.

To study the putative transfer of the nascent chain from the SRP to the translocon in more detail, we investigated whether the requirements for release of the SRP and association with the translocon are related. SRNCs were incubated with INVs in the presence or absence of FtsY

and nucleotides, cross-linked and subjected to Na_2CO_3 extraction. In the absence of both FtsY and nucleotides, strong cross-linking is observed to P48 in the supernatant (Figure 3A, lane 1) and pellet fractions (Figure 3B, lane 9), whereas cross-linking to SecA (Figure 3B, lane 5) and SecY (Figure 3B, lane 13) is weak, indicating inefficient dissociation of SRP from 108FtsQ and minimal association with the translocon. At the other end of the spectrum, efficient release of the SRP in the presence of FtsY and GTP (Figure 3A, lanes 3 and 4, and B, lanes 11 and 12) is accompanied by an increase in cross-linking to both SecA (Figure 3B, lanes 7 and 8) and SecY (Figure 3B, lanes 15 and 16), indicating transfer of the released nascent chains to the translocon. The intermediate release of SRP observed in the absence of added FtsY (Figure 3A, lane 2 and see above) results in intermediate association with SecA and SecY (Figure 3B, lanes 6 and 14). These data were also quantified and are presented graphically in Figure 3C. Consistent with these observations, flotation gradient analysis revealed that conditions resulting in dissociation of the SRP–RNC complex (presence of FtsY and GTP) induced efficient co-localization of 108FtsQ with the membrane (data not shown).

These results strongly suggest that the nascent 108FtsQ is transferred to the membrane-embedded translocon upon GTP-dependent release from the SRP by FtsY.

Discussion

Recent evidence indicates that the *E.coli* SRP and its putative receptor FtsY fulfil essential functions in the targeting and membrane assembly of inner membrane proteins (reviewed in De Gier *et al.*, 1997). Overproduction of several inner membrane proteins reduced the cell viability of a strain in which the SRP level is depressed artificially, indicating titration of the essential SRP (Ulbrandt *et al.*, 1997). Moreover, depletion of the SRP components, 4.5S RNA and P48, resulted in impaired integration of several membrane proteins (Mac Farlane and Müller, 1995; De Gier *et al.*, 1996; Ulbrandt *et al.*, 1997). Finally, we have demonstrated previously by *in vitro* cross-linking that the *E.coli* SRP interacts with short nascent polypeptides that carry particularly hydrophobic targeting sequences like inner membrane proteins usually do. Until now, the mechanism by which these SRP-dependent proteins are delivered at the inner membrane has remained obscure.

In this report, we describe the use of a cross-linking approach to elucidate late stages in SRP-mediated protein targeting in *E.coli*. As substrate proteins we used FtsQ and Lep, *E.coli* inner membrane proteins that both interact efficiently with the SRP *in vitro* (Valent *et al.*, 1995, 1997) but differ in the membrane orientation of their first transmembrane segments (Wolfe *et al.*, 1983; Carson *et al.*, 1991). Furthermore, Lep has been shown to depend on the SRP for efficient membrane assembly *in vivo* (De Gier *et al.*, 1996). Nascent N-terminal FtsQ and Lep fragments of ~100 amino acids were prepared by *in vitro* translation of truncated mRNA in a cell-free *E.coli* extract and supplemented with INVs. As a result, efficient membrane association of the nascent polypeptides was observed. Interestingly, upon release from the SRP–FtsY complex, the targeted nascent chains were found to be exclusively
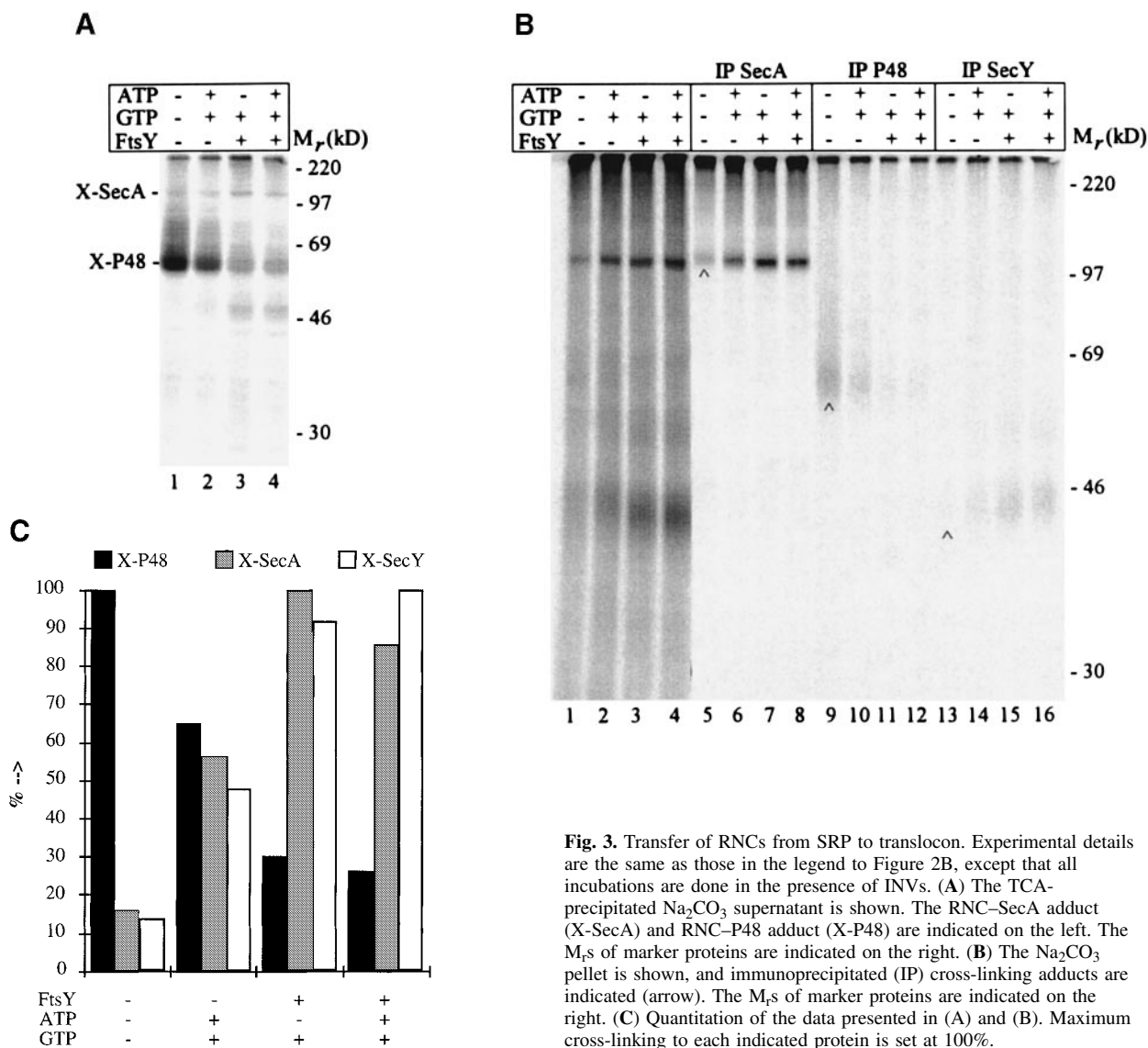
A

C

B

Fig. 3. Transfer of RNCs from SRP to translocon. Experimental details are the same as those in the legend to Figure 2B, except that all incubations are done in the presence of INVs. (**A**) The TCA-precipitated Na$_2$CO$_3$ supernatant is shown. The RNC–SecA adduct (X-SecA) and RNC–P48 adduct (X-P48) are indicated on the left. The M$_r$s of marker proteins are indicated on the right. (**B**) The Na$_2$CO$_3$ pellet is shown, and immunoprecipitated (IP) cross-linking adducts are indicated (arrow). The M$_r$s of marker proteins are indicated on the right. (**C**) Quantitation of the data presented in (A) and (B). Maximum cross-linking to each indicated protein is set at 100%.

in close proximity to the integral translocon components SecA, SecY and SecG. This is the first time that a link between the SRP and Sec targeting pathways has been demonstrated.

The ability of nascent FtsQ and Lep to interact efficiently with INVs (Figure 1A) suggests that membrane association of these proteins can occur during translation *in vivo*. Membrane association appears to depend on the context of the ribosome: when the nascent chains were released from the ribosome by puromycin prior to the addition of INVs, membrane association did not occur (data not shown), most likely because the interaction with the SRP is lost (Valent *et al.*, 1997). These data are consistent with the compulsory co-translational mode of protein targeting to dog pancreas microsomes by *E.coli* SRP and FtsY in a heterologous *in vitro* targeting assay (Powers and Walter, 1997). However, they do not necessarily imply that translation and translocation are tightly coupled for these proteins. So far, there is no indication that the SRP affects translation upon its interaction with nascent chains. In fact, such a role seems unlikely for the *E.coli* SRP given its lack of SRP9- and SRP14-like protein subunits that are essential for the translation arrest function of its eukaryotic counter-

part (Siegel and Walter, 1988). It should be noted that in prokaryotes, translation arrest may not be required for SRP functioning due to the short traffic distances and fast translocation rates (Pugsley, 1993). It is conceivable that the *E.coli* SRP pathway just accelerates membrane association of nascent chains and their delivery at the translocon. It remains to be established whether *E.coli* ribosomes also contribute to the association with the membrane in a similar way to eukaryotic ribosomes that have affinity for the Sec61 complex (Kalies *et al.*, 1994; Jungnickel and Rapoport, 1995).

It is of interest that SecA is in close proximity to nascent FtsQ and Lep after SRP-mediated targeting. SecA is considered the molecular motor of post-translational translocation in *E.coli* that drives the stepwise transfer of the pre-protein by ATP-dependent cycles of membrane insertion and de-insertion (Driessen *et al.*, 1998). SecA and SecY have been found juxtaposed to a translocation intermediate of pro-OmpA that depends on SecB for efficient post-translational translocation (Joly and Wickner, 1993). It remains to be established whether the interaction of nascent FtsQ and Lep with SecA that we observe is functional in the sense that SecA generates the energy for

membrane insertion (in the absence of a tight coupling between translation and translocation; see above) or plays a role in the transfer of the nascent chain to the core translocon component SecY. At present, we cannot exclude the possibility that SecA is close to the nascent chain only as a result of its association with the translocon. However, we consider this possibility unlikely given the affinity of SecA for the signal sequence, an interaction that depends on both the N-terminal charged region and the hydrophobic core region (Akita *et al.*, 1990; Hikita and Mizushima, 1992). The elucidation of the exact requirements for membrane insertion awaits *in vitro* reconstitution of the entire SRP pathway.

Nascent membrane-inserted FtsQ and Lep were also found juxtaposed to the core translocon component SecY, reminiscent of the interaction of nascent type I and II ER membrane proteins with Sec61α (High *et al.*, 1991b; High and Stirling, 1993; Do *et al.*, 1996; Laird and High, 1997). A direct interaction between SecY and the targeting signal would be consistent with the proposed signal sequence proof-reading activity of SecY (Driessen *et al.*, 1998). In contrast, *in vivo* studies concerning the Sec dependency of the translocation of the N-terminus of Lep indicated that this event can occur independently of SecA and SecY (Lee *et al.*, 1992). However, in these studies, conditional Sec strains were used in which the Sec function cannot be eliminated completely. It is possible that a low level of SecA or SecY is sufficient for the translocation of the N-terminal domain, which is shorter and uses a more hydrophobic targeting signal than the Sec-dependent C-terminal domain (Wolfe *et al.*, 1985).

Finally, SecG was found to be in the vicinity of the targeted nascent chains. This is probably due to its interaction with SecY (Homma *et al.*, 1997), since a direct interaction with neither FtsQ nor Lep nascent chains could be demonstrated. Interestingly, Sec61β, which is similar in size and hydrophobicity to SecG (Rapoport *et al.*, 1996), has been cross-linked directly to nascent ER membrane proteins (Laird and High, 1997).

Our cross-linking data unambiguously show that release of nascent polypeptides from the SRP and their association with the translocon are linked. The SRα homologue FtsY is found to be essential for the release of the SRP from the nascent chain in a process that depends on binding of GTP to FtsY, analogous to the SRα-dependent dissociation of the eukaryotic SRP from the targeting signal (Connolly and Gilmore, 1989). The present data form the first biochemical definition of the function of FtsY in the SRP pathway and once again underline the strong conservation of the basic mechanism of SRP-mediated protein targeting throughout evolution (Althoff *et al.*, 1994; Wolin, 1994). On the basis of *in vitro* binding studies (Powers and Walter, 1995) and analogy with the eukaryotic system (Connolly *et al.*, 1991), hydrolysis of GTP at both P48 and FtsY is assumed to be required for the dissociation of the SRP–FtsY complex.

FtsY is located in both the cytoplasm and inner membrane of *E.coli* (Luirink *et al.*, 1994). Release of the SRP from the nascent chains occurred at the membrane but, surprisingly, soluble FtsY was able to associate with RNC–SRP complexes in the absence of membranes and nucleotides. Possibly, in these complexes, FtsY has a direct targeting function. This may increase the efficiency
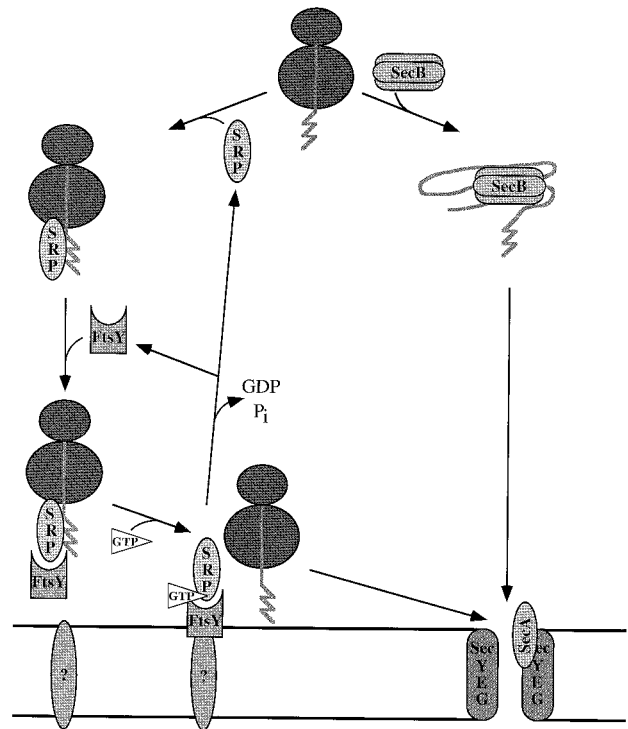

Fig. 4. Model for protein targeting to the *E.coli* cytoplasmic membrane. SecB interacts with a subset of polypeptides at a post-translational or late co-translational stage (right-hand side). The SecB–precursor complex is targeted to SecA at the membrane which initiates translocation through the SecYEG translocon. Particularly hydrophobic targeting signals are recognized and bound by the SRP as soon as they emerge from the ribosome (left-hand side). The RNC-bound SRP is picked up by FtsY in the cytosol. The growing RNC is released from the SRP after the docking of FtsY at an unidentified site at the membrane, an event preceded or accompanied by the binding of GTP to probably both FtsY and SRP. The released RNC inserts into the SecYEG translocon, possibly at the SecA–SecY interface. GTP hydrolysis at both SRP and FtsY serves to dissociate and recycle the targeting components.

or fidelity of the targeting reaction. However, it is not needed *per se*, since a hybrid FtsY that is permanently anchored in the inner membrane via a hydrophobic anchor sequence is able to complement the loss of FtsY *in vivo* (Zelazny *et al.*, 1997). Association of soluble FtsY with the membrane may involve different distinct binding sites, but components involved have not yet been identified (De Leeuw *et al.*, 1997).

In conclusion, based on the results of this study and the published data discussed, we propose that in *E.coli* the SRP and Sec targeting pathways function in parallel and probably converge at the same translocon in the membrane (Figure 4). However, it remains possible that the SRP and Sec pathways deliver proteins at translocons that differ in their exact composition but share common core elements as has been observed in *S.cerevisiae* (Rapoport *et al.*, 1996; Wilkinson *et al.*, 1997). For specific nascent chains, the choice between the targeting routes is determined primarily by the hydrophobicity of the N-terminal targeting sequence (Valent *et al.*, 1995, 1997). Thus, most inner membrane proteins follow the SRP route which offers the advantage of co-translational membrane association to avoid the exposure of aggregation-prone hydrophobic transmembrane segments in the cytosol. In addition, certain secreted proteins with relatively hydro-

phobic signal sequences, such as β-lactamase, may use this pathway preferentially (Phillips and Silhavy, 1992; Luirink *et al.*, 1994). Other pre-proteins interact with SecB which binds to the mature protein domain and delivers it to SecA via a direct, probably electrostatic, interaction (Driessen *et al.*, 1998). Partial overlap in substrate specificity of the two pathways may offer flexibility to the targeting process.

Materials and methods

Materials
Restriction enzymes and RNasin were from Boehringer Mannheim GmbH (Mannheim, Germany). Megashortscript T7 transcription kit was from Ambion Inc. (Austin, TX). Puromycin and nucleotides were supplied by Sigma Chemical Co. (St. Louis, MO). [^{35}S]Methionine was from Amersham International (Buckinghamshire, UK). OptiPrep (60% solution) was from Nycomed Pharma AS (Oslo, Norway). DSS was from Pierce (Rockford, IL). Oligonucleotides were purchased from Isogen Bioscience BV (Maarssen, The Netherlands).

Strains and plasmid constructs
Strain MC4100 was used to obtain translation lysates and INVs (both prepared as described in De Vrije *et al.*, 1987). Strain Top10F′ was used to maintain the plasmid constructs pC4Meth94Lep, pC4Meth101FtsQ and pC4Meth84PhoA-WT and mutant derivatives (Valent *et al.*, 1997).

In vitro transcription, translation, targeting and cross-linking
Plasmids were linearized with *Hin*dIII and transcribed using T7 polymerase. The resulting truncated mRNAs coding for the N-terminal region of the proteins were translated for 20 min at 25°C in an *E.coli in vitro* translation system (Valent *et al.*, 1997).

When using mRNAs encoding FtsQ truncates, 80 μg/ml ribosome-binding site (RBS) block primer (5′-ATTAGAATTCTCCTTCTTA-3′) complementary to the RBS sequence (underlined) and its flanking regions was added 2 min after initiation of translation. RBS block primer inhibits initiation of translation, thus minimizing polysome formation which often results in RNCs of heterogeneous lengths (Valent *et al.*, 1997).

To allow targeting, RNCs were incubated with INVs (1.25 mg/ml protein) for 5 min at 25°C and subsequently incubated on ice for 5 min. Cross-linking was induced with 2 mM DSS for 10 min at 25°C and quenched at 0°C by adding 1/10 volume of quench buffer (1 M glycine, 100 mM NaHCO$_3$ pH 8.5).

To separate integral membrane from soluble and peripheral cross-linked complexes, samples were treated with 0.18 M Na$_2$CO$_3$ (pH 11.3) for 15 min on ice. The membrane fractions containing integral membrane proteins were collected by ultracentrifugation (10 min, 115 000 *g*) and resuspended in RN buffer [100 mM KOAc, 5 mM Mg(OAc)$_2$, 50 mM HEPES–KOH pH 7.9]. Both pellet and supernatant fractions were either trichloroacetic acid (TCA) precipitated or immunoprecipitation as described (Luirink *et al.*, 1992). The material used for immunoprecipitation was 10-fold the amount used for TCA precipitation.

Flotation gradient analysis
Translation reactions (50 μl) were incubated with INVs (1.8 mg/ml protein) for 5 min at 37°C. Samples were chilled for 5 min on ice, and RNCs and INVs were collected after centrifugation through a high salt sucrose cushion (High *et al.*, 1991a) in 15 μl of buffer I [0.5 M KOAc, 5 mM Mg(OAc)$_2$, 50 mM HEPES–KOH pH 7.6] and mixed with 105 μl of buffer III [0.5 M KOAc, 5 mM Mg(OAc)$_2$, 250 mM sucrose, 50% OptiPrep, 50 mM HEPES–KOH pH 7.6]. The samples were transferred to 1 ml ultracentrifuge tubes and overlaid with 580 μl of buffer II [0.5 M KOAc, 5 mM Mg(OAc)$_2$, 125 mM sucrose, 30% OptiPrep, 50 mM HEPES–KOH pH 7.6] and 300 μl of buffer I. After ultracentrifugation for 3 h at 166 000 *g* in a swing-out rotor, the flotation gradient was collected as four fractions (350, 200, 200 and 250 μl) from the top. The fractions were TCA precipitated and analysed by SDS–PAGE and phosphor imaging. The quality of the fractionation was confirmed by monitoring the distribution of soluble and integral membrane proteins by immunoblotting (data not shown). Flotation efficiencies were quantified using the Imagequant quantification software from Molecular Dynamics.

Reconstitution of SRP and SRNC complex
Equimolar amounts of purified 4.5S RNA and P48His6 (Lentzen *et al.*, 1994) were incubated in reconstitution buffer (125 mM NH$_4$Cl, 12.5 mM MgCl$_2$, 25 μM EDTA, 0.5 M KOAc, 25% glycerol, 25 mM HEPES–KOH pH 7.5) for 10 min at room temperature to allow complex formation. Samples were chilled on ice and applied to a 1.5 ml discontinuous 5–20% sucrose gradient in 20 mM HEPES–KOH (pH 7.5), 100 mM NH$_4$Cl, 10 mM MgCl$_2$ and 250 mM KOAc, and centrifuged for 5 h at 200 000 *g*. Fractions (100 μl) were taken from the top, and 5 μl of each fraction was examined by SDS–PAGE. The gel was first stained in RNA staining buffer [90 mM boric acid, 2.5 mM EDTA, 150 μg/l ethidium bromide, 90 mM Tris (uncalibrated) pH 8.3] to visualize the 4.5S RNA, and subsequently with Coomassie Brilliant Blue to visualize P48His6. The 2–4 fractions containing the peak amounts of both components were pooled. The concentration of SRP was determined by measuring the absorbance at 260 nm (1 A_{260} corresponds to 1.1 μM 4.5S RNA).

To allow SRP–RNC complex formation, 108FtsQ was produced and incubated for 5 min at 25°C with 350 nM of the reconstituted SRP which was the minimal saturating concentration in our assay as established by quantitative immunoblotting (data not shown). Samples were chilled on ice, and SRP–RNC complexes were purified from the translation mixture by centrifugation through a high salt sucrose cushion (High *et al.*, 1991a). These purified complexes were designated SRNCs.

Sample analysis and quantification
All samples were analysed on 12 or 15% SDS–polyacrylamide gels. Radiolabelled proteins were visualized by phosphor imaging using a Molecular Dynamics PhosphorImager 473 and quantified using the Imagequant quantification software from Molecular Dynamics.

References

Akita,M., Sasaki,S., Matsuyami,S.-I. 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.

Akiyama,Y. and Ito,K. (1986) Overproduction, isolation and determination of the amino-terminal sequence of the SecY protein, a membrane protein involved in protein export in *Escherichia coli*. *Eur. J. Biochem.*, **159**, 263–266.

Althoff,S., Selinger,D. and Wise,J.A. (1994) Molecular evolution of SRP cycle components: functional implications. *Nucleic Acids Res.*, **22**, 1933–1947.

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.

Bukau,B., Hesterkamp,T. and Luirink,J. (1996) Growing up in a dangerous environment: a network of multiple targeting and folding pathways for nascent polypeptides in the cytosol. *Trends Cell Biol.*, **6**, 480–486.

Carson,M.J., Barondess,J. and Beckwith,J. (1991) The FtsQ protein of *Escherichia coli*: membrane topology, abundance and cell division phenotypes due to overproduction and insertion mutations. *J. Bacteriol.*, **173**, 2187–2195.

Connolly,T. and Gilmore,R. (1989) The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell*, **57**, 599–610.

Connolly,T., Rapiejko,P.J. and Gilmore,R. (1991) Requirement of GTP hydrolysis for dissociation of the signal recognition particle from its receptor. *Science*, **252**, 1171–1173.

Dalbey,R.E. (1991) Leader peptidase. *Mol. Microbiol.*, **5**, 2855–2860.

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.

De Gier,J.-W.L., Valent,Q.A., von Heijne,G. and Luirink,J. (1997) The *E.coli* SRP: preferences of a targeting factor. *FEBS Lett.*, **408**, 1–4.

De Leeuw,E., Poland,D., Mol,O., Sinning,I., ten Hagen-Jongman,C.M., Oudega,B. and Luirink,J. (1997) Membrane association of FtsY, the *E.coli* SRP receptor. *FEBS Lett.*, **416**, 225–229.

De Vrije,T., Tommassen,J. and De Kruijff,B. (1987) Optimal posttranslational translocation of the precursor of PhoE protein across *Escherichia coli* membrane vesicles requires both ATP and the proton motive force. *Biochim. Biophys. Acta*, **900**, 63–72.

Do,H., Falcone,D., Lin,J.L., Andrews,D.W. and Johnson,A.E. (1996) The cotranslational integration of membrane proteins into the phospholipid bilayer is a multistep process. *Cell*, **85**, 369–378.

Doud,S.K., Chou,M.M. and Kendall,D.A. (1993) Titration of protein transport activity by incremental changes in signal peptide hydrophobicity. *Biochemistry*, **32**, 1251–1256.

Driessen,A.J.M., Fekkes,P. and van der Wolk,J.P.W. (1998) The Sec system. *Curr. Opin. Microbiol.*, in press.

Economou,A. and Wickner,W. (1994) SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell*, **78**, 835–843.

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.

Hesterkamp,T., Hauser,S., Lütcke,H. and Bukau,B. (1996) *Escherichia coli* trigger factor is a prolyl isomerase that associates with nascent polypeptide chains. *Proc. Natl Acad. Sci. USA*, **93**, 4437–4441.

High,S. and Stirling,C.J. (1993) Protein translocation across membranes: common themes in divergent organisms. *Trends Cell Biol.*, **3**, 335–339.

High,S., Flint,N. and Dobberstein,B. (1991a) Requirements for the membrane insertion of signal-anchor type proteins. *J. Cell Biol.*, **113**, 25–34.

High,S., Görlich,D., Wiedmann,M., Rapoport,T.A. and Dobberstein,B. (1991b) The identification of proteins in the proximity of signal-anchor sequences during their targeting to and insertion into the membrane of the ER. *J. Cell Biol.*, **113**, 35–44.

High,S. *et al.* (1993) Site-specific photocross-linking reveals that Sec61p and TRAM contact different regions of a membrane-inserted signal sequence. *J. Biol. Chem.*, **268**, 26745–26751.

Hikita,C. and Mizushima,S. (1992) The requirement of a positive charge at the amino terminus can be compensated for by a longer central hydrophobic stretch in the functioning of signal peptides. *J. Biol. Chem.*, **267**, 12375–12379.

Homma,T., Yoshihisa,T. and Ito,K. (1997) Subunit interactions in the *Escherichia coli* protein translocase: SecE and SecG associate independently with SecY. *FEBS Lett.*, **408**, 11–15.

Johnson,A.E. (1997) Protein translocation at the ER membrane: a complex process becomes more so. *Trends Cell Biol.*, **7**, 90–95.

Joly,J.C. and Wickner,W. (1993) The SecA and SecY subunits of translocase are the nearest neighbours of the translocating preprotein, shielding it from phospholipids. *EMBO J.*, **12**, 255–263.

Jungnickel,B. and Rapoport,T.A. (1995) A posttargeting signal sequence recognition event in the endoplasmic reticulum membrane. *Cell*, **82**, 261–270.

Kalies,K.U., Görlich,D. and Rapoport,T.A. (1994) Binding of ribosomes to the rough endoplasmic reticulum mediated by the sec61p-complex. *J. Cell Biol.*, **126**, 925–934.

Kumamoto,C.A. and Francetic,O. (1993) Highly selective binding of nascent polypeptides by an *Escherichia coli* chaperone protein *in vivo*. *J. Bacteriol.*, **175**, 2184–2188.

Kusters,R., Lentzen,G., Eppens,E., van Geel,A., van der Weijden,C.C., Wintermeyer,W. and Luirink,J. (1995) The functioning of the SRP receptor FtsY in protein-targeting in *E.coli* is correlated with its ability to bind and hydrolyse GTP. *FEBS Lett.*, **372**, 253–258.

Laird,V. and High,S. (1997) Discrete cross-linking products identified during membrane protein biosynthesis. *J. Biol. Chem.*, **272**, 1983–1989.

Lee,J.-I., Kuhn,A. and Dalbey,R.E. (1992) Distinct domains of an oligotopic membrane protein are Sec-dependent and Sec-independent for membrane insertion. *J. Biol. Chem.*, **267**, 938–943.

Lentzen,G., Dobberstein,B. and Wintermeyer,W. (1994) Formation of SRP-like particle induces a conformational change in *E.coli* 4.5S RNA. *FEBS Lett.*, **348**, 233–238.

Luirink,J. and Dobberstein,B. (1994) Mammalian and *Escherichia coli* signal recognition particles. *Mol. Microbiol.*, **11**, 9–13.

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.

Luirink,J., ten Hagen-Jongman,C.M., Van der Weijden,C.C., Oudega,B., High,S., Dobberstein,B. and Kusters,R. (1994) An alternative protein targeting pathway in *Escherichia coli*: studies on the role of FtsY. *EMBO J.*, **13**, 2289–2296.

Mac Farlane,J. and Müller,M. (1995) Functional integration of a polytopic membrane protein of *E.coli* requires the bacterial signal recognition particle. *Eur. J. Biochem.*, **223**, 766–771.

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.

Mothes,W., Prehn,S. and Rapoport,T.A. (1994) Systematic probing of the environment of a translocating secretory protein during translocation through the ER membrane. *EMBO J.*, **13**, 3973–3982.

Nakai,M., Goto,A., Nohara,T., Sugita,D. and Endo,T. (1994) Identification of the SecA protein homolog in pea chloroplasts and its possible involvement in thylakoidal protein transport. *J. Biol. Chem.*, **269**, 31338–31341.

Phillips,G.J. and Silhavy,T.J. (1992) The *E.coli ffh* gene is necessary for viability and efficient protein export. *Nature*, **359**, 744–746.

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.

Powers,T. and Walter,P. (1995) Reciprocal stimulation of GTP hydrolysis by two directly interacting GTPases. *Science*, **269**, 1422–1424.

Powers,T. and Walter,P. (1997) Co-translational protein targeting catalyzed by the *Escherichia coli* signal recognition particle and its receptor. *EMBO J.*, **16**, 4880–4886.

Pugsley,A.P. (1993) The complete general secretory pathway in gram-negative bacteria. *Microbiol. Rev.*, **57**, 50–108.

Rapiejko,P.J. and Gilmore,R. (1997) Empty site forms of the SRP54 and SRα GTP-ases mediate targeting of ribosome–nascent chain complexes to the endoplasmic reticulum. *Cell*, **89**, 703–713.

Rapoport,T.A., Jungnickel,B. and Kutay,U. (1996) Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. *Annu. Rev. Biochem.*, **65**, 271–303.

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* proteins with putative GTP-binding domains. *Nature*, **340**, 478–482.

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.

Siegel,V. and Walter,P. (1988) Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. *Cell*, **52**, 39–49.

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.

Valent,Q.A., Kendall,D.A., High,S., Kusters,R., Oudega,B. and Luirink,J. (1995) Early events in preprotein recognition in *E.coli*: interactions of SRP and trigger factor with nascent polypeptides. *EMBO J.*, **14**, 5494–5505.

Valent,Q.A., de Gier,J.-W.L., van Heijne,G., Kendall,D.A., ten Hagen-Jongman,C.M., Oudega,B. and Luirink,J. (1997) Nascent membrane and presecretory proteins synthesised in *Escherichia coli* associate with signal recognition particle and trigger factor. *Mol. Microbiol.*, **25**, 53–64.

Wilkinson,B.M., Regnacq,M. and Stirling,C.J. (1997) Protein translocation across the membrane of the endoplasmic reticulum. *J. Membr. Biol.*, **155**, 189–197.

Wolfe,P.B., Wickner,W. and Goodman,J.M. (1983) Sequence of the leader peptidase gene of *Escherichia coli* and the orientation of leader peptidase in the bacterial envelope. *J. Biol. Chem.*, **258**, 12073–12080.

Wolfe,P.B., Rice,M. and Wickner,W. (1985) Effects of two *sec* genes on protein assembly into the plasma membrane of *Escherichia coli*. *J. Biol. Chem.*, **260**, 1836–1841.

Wolin,S.L. (1994) From the elephant to *E.coli*: SRP-dependent protein targeting. *Cell*, **77**, 787–790.

Yuan,J.G., Henry,R., McCaffery,M. and Cline,K. (1994) SecA homolog in protein transport within chloroplasts: evidence for endosymbiont-derived sorting. *Science*, **266**, 796–798.

Zelazny,A., Seluanov,A., Cooper,A. and Bibi,E. (1997) The NG domain of the prokaryotic signal recognition particle receptor, FtsY, is fully functional when fused to an unrelated integral membrane polypeptide. *Proc. Natl Acad. Sci. USA*, **94**, 6025–6029.