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Assembly strategies and GTPase regulation of the eukaryotic and *Escherichia coli* translocons

Kyle R. Legate and David W. Andrews

Abstract: The translocation of most proteins across the endoplasmic reticulum or bacterial inner membrane occurs through an aqueous pore that spans the membrane. Substrates that are translocated co-translationally across the membrane are directed to the translocation pore via an interaction between the cytosolic signal recognition particle and its membrane-bound receptor. Together the translocation pore and the receptor are referred to as a translocon. By studying the biogenesis of the translocon a number of alternate targeting and membrane-integration pathways have been discovered that operate independently of the signal recognition particle (SRP) pathway. The novel assembly strategies of the translocon and the ways in which these components interact to ensure the fidelity and unidirectionality of the targeting and translocation process are reviewed here.

Key words: protein translocation, translocon, SRP receptor, GTPases.

Résumé : La translocation de la plupart des protéines au travers du réticulum endoplasmique ou de la membrane interne des bactéries se fait par un pore transmembranaire. Les protéines qui passent au travers de la membrane au cours de leur traduction sont dirigées vers le pore de translocation grâce à l'interaction entre la particule de reconnaissance du signal (SRP) dans le cytosol et son récepteur membranaire. Le pore de translocation et le récepteur forment le translocon. En étudiant la biogenèse du translocon, plusieurs voies alternatives de ciblage et d'intégration à la membrane, fonctionnant indépendamment de la voie de la SRP, ont été découvertes. Dans cet article, les nouvelles stratégies d'assemblage du translocon sont passées en revue, ainsi que les mécanismes par lesquels ces constituants interagissent pour assurer la fidélité et le caractère unidirectionnel du processus de ciblage et de translocation.

Mots clés : translocation de protéines, translocon, récepteur de la SRP, GTPases.

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Introduction

Most proteins translocated across or integrated into the eukaryotic endoplasmic reticulum (ER) or integrated co-translationally into the bacterial inner membrane are bound by signal recognition particle (SRP) early during translation (Luirink et al. 1992; Ogg and Walter 1995). Once the amino terminal signal sequence within the growing nascent chain is recognized and bound by SRP, the ribosome–nascent chain–SRP complex is targeted to the membrane via the affinity of SRP for its cognate receptor. In eukaryotes the SRP receptor is composed of α and β subunits (Figs. 1A, 1B) (Tajima et al. 1986; Lauffer et al. 1985), whereas in *Escherichia coli* the receptor consists of a single polypeptide, FtsY (Fig. 1C) (Romisch et al. 1989). The function of the SRP receptor is to target the ribosom–nascent chain to the translocation pore embedded in the membrane. The minimal translocation pore in mammals consists of Sec61α, Sec61β, and Sec61γ (Fig. 1A) (Gorlich and Rapoport 1993). The SRP receptor and the translocation pore together constitute the minimum components necessary to transport polypeptides into or across the membrane. Together these two components are referred to as the translocon.

The mechanism(s) of assembly of the translocon are not entirely clear, since some components of the translocon apparently require the SRP pathway for their own membrane integration. This raises an interesting "chicken and egg" question: How can a translocon assemble if it requires a translocon for assembly? By examining this question we have discovered that several proteins are assembled by SRP-independent mechanisms. It has also become clear that other components must be processed by the SRP-dependent machinery and therefore, in the development of an organism, must be inherited. Here we review the assembly pathways of the subunits of mammalian, yeast, and *E. coli* translocons and speculate on how these assembly strategies might impact translocon function.

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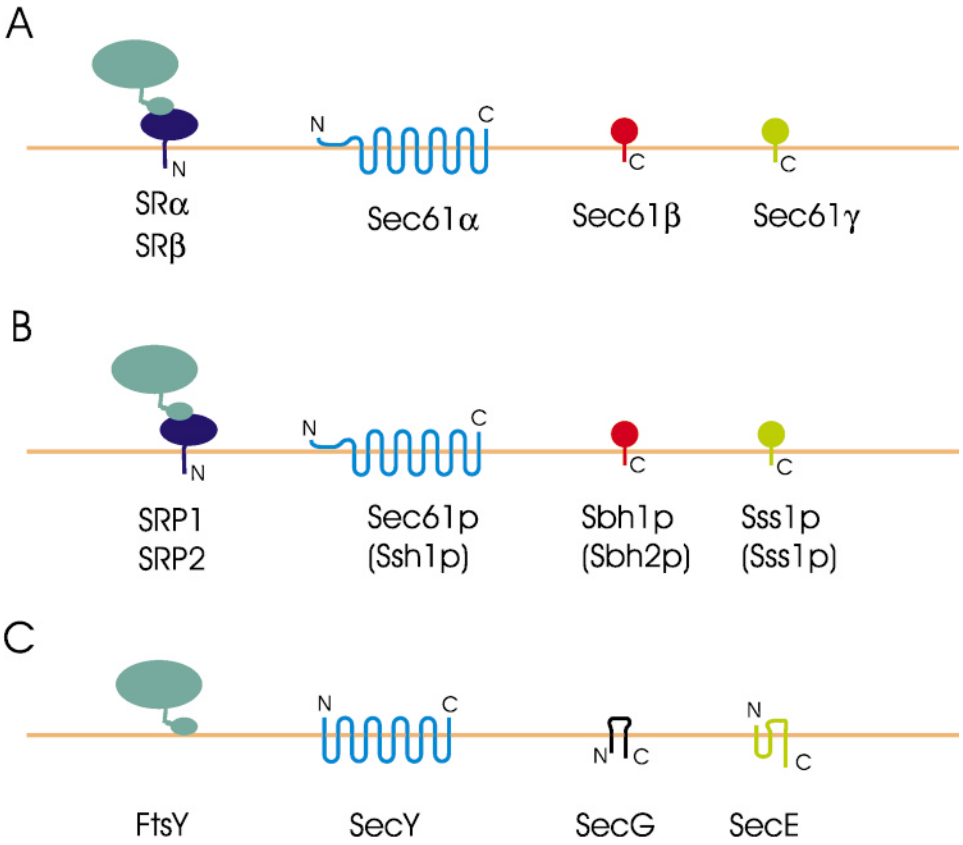
Fig. 1. Conservation of the core membrane-bound translocation apparatus. The conserved subunits of the SRP receptor and translocation pore are shown for (A) mammals, (B) *Saccharomyces cerevisiae*, and (C) *Escherichia coli*. Conserved subunits are coded according to colour, with the non-conserved SecG protein shown in black. N- and C-termini are indicated.



Architecture and assembly of the eukaryotic translocation pore

Sec61α is a multispanning membrane protein that binds the ribosome and contacts the nascent chain as it traverses the membrane (Gorlich et al. 1992; High et al. 1991, 1993*a*, 1993*b*; Kalies et al. 1994; Laird and High 1997; Nicchitta et al. 1995; Prinz et al. 2000; Raden et al. 2000). Nascent membrane proteins and some secreted proteins have also been cross-linked to another transmembrane protein called TRAM that may have a role in discriminating hydrophobic sequences in transmembrane domains from those in secretory signal sequences (Do et al. 1996). However, conflicting evidence exists for the importance and precise role in translocation for TRAM. Nevertheless, Sec61α forms all or part of the central pore of the engaged translocation complex, a hypothesis supported by electron microscopic images of pores engaged in translocation (Beckmann et al. 1997; Menetret et al. 2000). Electron microscopic studies on purified reconstituted Sec61 complex reveal an oligomer containing three or four copies of the trimeric complex, in a pentagonal configuration (Hanein et al. 1996). The central pore of these oligomers has been measured at ~20 Å but may increase to 40–60 Å during a translocation event, demonstrating that the architecture of the complex may be significantly influenced by the ribosome (Hamman et al. 1997).

Sec61β and Sec61γ were identified as subunits of the Sec61 complex because they fractionate biochemically with Sec61α after solubilization in non-ionic detergent (Gorlich and Rapoport 1993). Cross-links between Sec61β and Sec6α have been observed, suggesting that these subunits are tightly associated (Kalies et al. 1998; Meyer et al. 2000). The functions of Sec61β and Sec61γ are largely unknown, but they may have regulatory roles in the recruitment of peripheral components and gating of the translocon (Kalies et al. 1998). Sec61β is indispensable at physiologic temperature, as biochemical removal of this component has been shown to abrogate translocation activity in a system reconstituted from detergent extract. Slowing down the ribosome-binding step by chilling the reaction to 0°C partially restores translocation, suggesting that Sec61β is involved in, but not essential for, the translocation process, possibly by recruiting signal peptidase to the translocon (Kalies et al. 1998). Data on the Sec61γ subunit are lacking, and any insight into its role in translocation is purely speculative at this time.

Yeast possesses two different translocons that operate on substrates translocated either co- or post-translationally. The pore involved in co-translational translocation consists of Sec61p, Sbh1p, and Sss1p, homologous to Sec61α, Sec61β, and Sec61γ, respectively (Fig. 1B) (Gorlich et al. 1992; Hartmann et al. 1994; Panzner et al. 1995). These three components also participate in post-translational translocation in concert with the tetrameric Sec62–Sec63 complex (Panzner et al. 1995). A combination of biochemical and genetic approaches identified a second complex consisting of Ssh1p (a homologue of Sec61p), Sbh2p (a homologue of

Sbh1p), and Sss1p which is dedicated to co-translational translocation (Finke et al. 1996). This redundancy in translocation pathways means that in yeast some components are essential for growth and some are dispensable. Sec61p is essential, as it forms the translocation pore of the post-translational translocon and one of the two co-translational complexes (Deshaies and Schekman 1987; Panzner et al. 1995). Likewise, Sss1p is essential, as it is present in all translocons (Esnault et al. 1993; Panzner et al. 1995). Ssh1p can be deleted, since its function is redundant, but cells containing a Ssh1p deletion grow slowly (Finke et al. 1996). Either Sbh1p or Sbh2p can be deleted with no detrimental effect, but deletion of both components causes impaired growth at 37°C, and an accumulation of secretory precursors, in agreement with results obtained in the mammalian system (Finke et al. 1996; Kalies et al. 1998).

From an evolutionary perspective the biogenesis of the translocation pore in the ER membrane is puzzling because the main component of the translocon spans the membrane 10 times and assembles into the membrane in a translocon-dependent manner (Wilkinson et al. 1996). It is likely therefore that a small amount of Sec61α or its homologues in non-mammalian cells must be inherited. In contrast, Sec61β and Sec61γ (and the yeast homologue of Sec61γ, Sss1p) belong to a class of proteins that are characterized by the absence of an amino terminal signal sequence and the presence of a single carboxyl-terminal transmembrane domain called an insertion sequence. The information required to appropriately target proteins with insertion sequences has been found in the amino acid sequences flanking the transmembrane domain, or within the transmembrane domain itself (reviewed in Wattenberg and Lithgow 2001). Since this information resides within the carboxyl-terminus of the protein and will not be exposed to the cytosol until translation has completed and the ribosome releases the protein, targeting and membrane insertion must proceed post-translationally.

Two different mechanisms for targeting insertion sequence proteins have been elucidated. In one, typified by cytochrome b5, membrane insertion is spontaneous and non-saturable (Kim et al. 1997). The second, utilized by vesicle associated membrane proteins (VAMPs), requires both ATP and a trypsin-sensitive, saturable receptor on the target membrane (Kim et al. 1997, 1999). Mutational analysis of VAMP2 and VAMP8 revealed the importance of a positively charged amphipathic helix amino terminal to the transmembrane domain as the relevant targeting sequence. Replacing the positively charged amino acids with uncharged hydrophilic amino acids decreased the efficiency of membrane insertion incrementally.

Sec61γ contains a putative amphipathic helix with several positively charged amino acids amino-terminal of the transmembrane domain, suggesting that it may target to and insert into the ER membrane in a fashion similar to that of the VAMPs. Consistent with this hypothesis, Sec61γ insertion is dependent on the inclusion of ATP in the reaction mixture, and treatment of canine microsomes with trypsin abolishes insertion, implying the involvement of a membrane receptor (P.K. Kim and D.W. Andrews, unpublished data). Sss1p (the yeast Sec61γ homologue) also contains an amphipathic helix amino-terminal to the transmembrane domain. Whether this feature is important for the targeting and insertion of Sss1p into the yeast ER is currently being tested. Although Sec61β does not contain a sequence as positively charged as Sec61γ or the VAMP proteins, it also requires ATP and a trypsin-sensitive membrane component, suggesting that both Sec61β and Sec61γ insert into the ER membrane using a similar mechanism.

Thus at least two of the polypeptides associated with the translocation pore (Sec61γ and Sec61β) can be assembled into the membrane independent of the SRP and SRP receptor. Furthermore, the study of these proteins suggests that the mechanism of assembly of VAMPs is a bona fide targeting pathway used by a wide variety of proteins accessing the membrane of the ER. A prediction of these results is that common components integrate VAMPs and Sec61γ and Sec61β into the ER membrane. It remains to be determined whether or not this pathway overlaps that of proteins such as cytochrome b5 which do not require a membrane-bound receptor but are nevertheless targeted to specific membranes with high fidelity. From an evolutionary standpoint it is likely that the spontaneous insertion mechanism arose first, as its only requirement is a preexisting lipid bilayer. The "membrane protein-assisted" mechanism may have arisen as one solution to the problem of a cell containing multiple membranes and (or) may be only facilitative. A prediction of the latter hypothesis is that proteins like Sec61γ and Sec61β may insert with reduced efficiency into liposomes or may insert into liposomes depending on the lipid composition.

Architecture and assembly of the prokaryotic translocation pore

In *E. coli* the pore involved in co-translational translocation consists of the SecYEG complex, with SecY showing homology to Sec61α–Sec61p and SecE showing homology to Sec61γ–Sss1p (Fig. 1C) (Gorlich et al. 1992; Hartmann et al. 1994). SecG shows no sequence homology to any translocon components in either yeast or mammals. SecG is a nonessential gene and, although it increases the efficiency of translocation in vivo (Nishiyama et al. 1994), it does not appear to be required for co-translational translocation in vitro (Koch and Muller 2000). In vivo, deletion of SecG is compensated for by overexpression of the SecYE complex (Duong and Wickner 1997).

SecE is an essential gene (Schatz et al. 1989) but whether it is involved in the translocation of SRP-dependent precursors is a matter of some dispute. One study has shown that SecE is not required for the translocation of SRP-independent precursors proOmpA and proLamB (Yang et al. 1997), whereas other data suggest that SecE is required for translocation of proOmpA but not an SRP-dependent fusion protein containing the amino terminus and first transmembrane domain of the *E. coli* inner membrane protein ProW (Cristobal et al. 1999). Another study showed a requirement for SecE in the translocation of both SRP-dependent and SRP-independent precursors (Neumann-Haefelin et al. 2000). Clearly, more research into the role of SecE in translocation is required to settle these discrepancies in the experimental data.

The structural arrangement of the bacterial translocation pore is also controversial. Electron microscopic studies of purified SecYE reveal a quasi-pentagonal arrangement of three or four dimers resembling the eukaryotic complex (Meyer et al. 1999). Other data suggest that addition of the ATPase SecA assembles SecYEG into a tetrameric arrangement resembling the eukaryotic translocation pore in both shape and size (Manting et al. 2000). SecA associates with the post-translational translocon and facilitates translocation by binding substrate proteins and forcing them through the translocon by repeated cycles of peptide binding, translocation, and release (reviewed in Manting and Driessen 2000). It is not clear whether SecA is a component of the co-translational translocation pore in vivo, but a subset of SRP-dependent proteins appear to require SecA to translocate large periplasmic domains across the membrane (Valent et al. 1998; Qi and Bernstein 1999; Neumann-Haefelin et al. 2000).

A biochemical cross-linking approach employing epitope-tagged versions of SecE and SecY in a solubilized reconstituted system of SecYEG suggests that the pore operates as a monomer (Yahr and Wickner 2000). Upon reconstituting translocation pores containing equal amounts of tagged and untagged subunits, Yahr and Wickner (2000) were unable to detect cross-link products that would suggest an oligomeric arrangement. However, their data cannot rule out the possibility that cross-links between subunits of the SecYEG trimer are favoured over cross-links to neighbouring complexes. Also, the immunoprecipitation conditions used to detect an interaction between a proOmpA precursor and components of the translocation pore may have resulted in the disassembly of the pore while maintaining the interaction of proOmpA with one of the SecYEG trimers. In this case, mixed complexes might not be detected, leading to the conclusion that SecYEG functions as a monomer.

Little is known of the biogenesis of the *E. coli* translocation pore. SecY, as a multispanning membrane protein, requires the SRP pathway to integrate into the bacterial inner membrane (Koch et al. 1999). SecE and SecG both span the membrane more than once, so they are unlikely to insert into the membrane like Sec61β–Sec61γ or Sss1p (Nishiyama et al. 1996; Schatz et al. 1989). Therefore they probably insert into the membrane via preexisting SecY containing translocons. Since the co- and post-translational translocation pathways in *E. coli* appear to converge on the same SecYEG translocons, it is likely that translocons must be inherited by daughter cells to translocate all three polypeptides and assemble more translocons.

Assembly of the SRP receptor

The SRP receptor provides the link between the cytosolic ribosome–nascent chain complex (RNC) and the translocation pore. Since a direct interaction between the SRP receptor and the translocon has not been detected, it may be that the SRP receptor serves to enhance the efficiency of targeting to the translocation pore by reducing the three-dimensional movement of the SRP-bound RNC to the two-dimensional surface of the membrane. The simplest SRP receptors are found in prokaryotes where they are composed of a single polypeptide. In *E. coli* the SRP receptor is the protein FtsY, the gene for which was originally discovered as necessary for proper cell division (Gill et al. 1986). Consistent with a role in cell division, the gene for FtsY is located within an operon containing two other genes suggested to be involved in cell division. A direct role in cell division has not been forthcoming and it is now widely thought that the cell-division defect associated with inactivation of FtsY is an indirect result of its role in protein targeting.

Targeting of FtsY to the *E. coli* plasma membrane occurs independent of the SRP-mediated targeting pathway. Instead, FtsY has been shown to bind to the cell membrane through a direct interaction with phospholipids (de Leeuw et al. 2000). The lipid-binding domain has been mapped to the amino-terminus of FtsY and is composed of a region rich in acidic amino acids (the A region) and a small independently folded domain (the N region) previously thought to be associated with the GTPase domain of FtsY (Millman and Andrews 1999). Recently, the membrane-binding domain of FtsY has been shown to bind to phosphatidylethanolamine, a major component of the *E. coli* inner membrane (Millman et al. 2001). However, experiments with phosphatidylethanolamine-depleted strains of *E. coli* suggest that FtsY also binds to a trypsin-sensitive component on the inner membrane. Until this component is identified and characterized it cannot be concluded that the SRP receptor can be functionally assembled onto membranes in the absence of the SRP-mediated targeting system.

Surprisingly, the lipid-binding domain of FtsY is not conserved in other prokaryotes. Some species such as rickettsia lack this region entirely, whereas others such as streptomycetes appear to contain a conventional (presumably SRP dependent) amino-terminal transmembrane domain. Therefore, there is still much to be learned about the assembly of SRP receptors in prokaryotes.

In eukaryotes, the SRP receptor is composed of two subunits. The β subunit (SRβ) appears to be a conventional type I transmembrane protein (Miller et al. 1995). Since it inserts via the SRP pathway, daughter cells must inherit SRβ, similar to Sec61α. In contrast, SRα is a peripheral membrane protein associated with the ER membrane via an unusually strong interaction with SRβ (Andrews et al. 1989; Young et al. 1995). Through a series of gel filtration and immunoprecipitation assays and using a variety of deletion mutants of SRα, the region of SRα that binds to SRβ was shown to constitute an independently folded amino-terminal domain, termed SRX2 (Young et al. 1995). The demonstration that SRα is composed of two independently folded domains (one involved in membrane assembly and the other in GTPase activity and binding of SRP) led to the identification in vitro of a translation pause site in the mRNA encoding the linker region between these domains (Young and Andrews 1996).

The sequence in the SRα mRNA that mediates ribosome pausing resembles a class of viral frameshift sequences. Upon emergence of SRX2 from the ribosome, the ribosome encounters the pause site, whereupon the SRX2 domain folds and binds to SRβ on the ER membrane. Thus the GTPase domain of SRα is probably translated after SRX2 is already bound to SRβ. This may allow the two domains of SRα to fold independently and may also increase efficiency by preventing the GTPase domain from interfering with

binding of the SRX2 portion of the protein to SRβ. The concomitant localization of the mRNA to the ER membrane also ensures that SRα molecules synthesized by subsequent ribosomes are in close juxtaposition to the membrane, presumably enhancing targeting efficiency (Young and Andrews 1996). Consistent with SRα assembly during synthesis on preexisting SRβ, the latter is known to be maintained on membranes in an excess of 10% over SRα (Tajima et al. 1986).

In a complementary approach, immunoprecipitation assays employing deletion mutants of SRβ have shown that the nucleotide-bound form of the GTPase domain of SRβ is necessary and sufficient for binding SRα (Legate et al. 2000). This implies that the SRX2 domain of SRα constitutes a novel GTPase-binding domain.

Role of translocon GTPases in protein targeting and translocation

The fact that assembly of the SRP receptor in mammalian cells requires the SRβ subunit to be nucleotide bound strongly suggests that regulation of heterodimerization via nucleotide binding and (or) hydrolysis has some regulatory activity. One model for the regulation of translocation suggests that the SRP receptor binds to assembled but unoccupied Sec61 complexes. The specificity of this interaction may be mediated by SRβ. According to this model, SRα anchors the ribosome–nascent chain complex to SRβ, which in turn interacts with the translocon. It was a surprise then to discover that in yeast the SRP receptor could still function upon genetic deletion of the transmembrane domain of SRβ (srp102-ΔTMD) (Ogg et al. 1998). Fractionation of this strain showed that srp102p-ΔTMD was predominantly soluble. Thus, SRβ may be able to interact with the translocon without binding tightly to membranes. However, a small amount of srp102-ΔTMD still localized to the membrane fraction. Since the SRP receptor acts catalytically, it is also possible that the small membrane-bound fraction maintains the SRP pathway sufficiently in these cells so they grow indistinguishably from wild-type cells. Consistent with the hypothesis that some SRβ was correctly targeted to the ER membrane in the absence of the amino-terminal transmembrane domain, it was later discovered, in a mammalian cell free system, that SRβ contains a cryptic signal sequence that results in a significant fraction of SRβ continuing to target to the ER membrane after deletion of the transmembrane domain (Legate et al. 2000).

Nascent chain targeting and translocation is essentially unidirectional. In eukaryotes directionality may be achieved by the coordinated action of the three translocation GTPases: the 54-kDa subunit of the SRP (SRP54), SRα, and SRβ. In *E. coli* two GTPases are required: the bacterial SRP (Ffh) and the SRP receptor (FtsY).

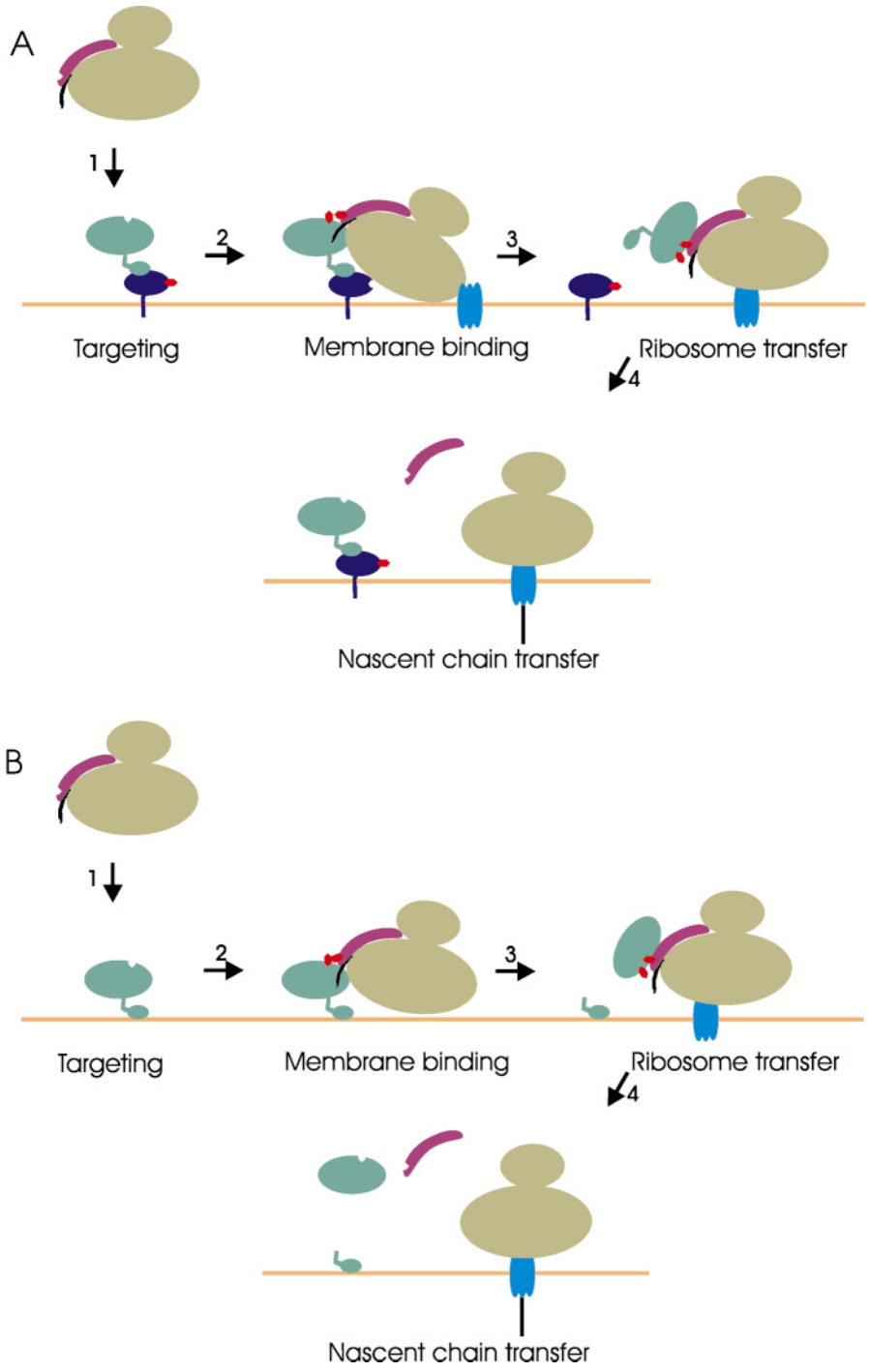
Upon recognition of the signal sequence by the SRP, the affinity of SRP54 for GTP is increased, but bound GTP remains exchangeable with cytosolic GTP (Bacher et al. 1996; Rapiejko and Gilmore 1997). The SRP–nascent chain complex is targeted to the membrane via the affinity of the SRP for its receptor (Fig. 2A, step 1). Once SRP54 interacts with SRα, the affinity of both molecules for GTP greatly increases, and both proteins become tightly associated with each other (Fig. 2A, step 2) (Connolly and Gilmore 1993; Connolly et al. 1991; Rapiejko and Gilmore 1997). In vitro, the half-life of this interaction is >6 h, therefore this step alone is sufficient to make targeting unidirectional (Rapiejko and Gilmore 1992). Coincubation of SRP54 and SRα in vitro results in the hydrolysis of GTP, demonstrating the reciprocal role of these proteins as GTPase-activating proteins (GAPs) (Connolly and Gilmore 1993; Miller et al. 1993). The presence of a nascent chain bound to SRP54 inhibits the GTPase activity of both proteins (Miller et al. 1993), ensuring that the molecules remain bound to each other until after the signal peptide is transferred to the translocon.

Once tethered to the ER via an association between SRP54 and SRα, the ribosome must interact with the Sec61 complex. However, an interaction between the SRP receptor and the Sec61 complex has not been observed. Nevertheless it is possible that SRβ plays a role in the transfer of the ribosome–nascent chain to Sec61. Coincubation of ribosome–nascent chains with the SRP receptor has been demonstrated to increase the GTPase activity of SRβ, simultaneously reducing its affinity for nucleotide (Bacher et al. 1999). These results have been interpreted to suggest that the ribosome is both a GAP and a guanine nucleotide unloading factor for SRβ. Due to its high affinity for GTP (20 nM, in agreement with other members of the ras superfamily of GTPases (Hattori et al. 1985; Manne et al. 1984)), SRβ is normally GTP bound (Bacher et al. 1999). However, an interaction with the ribosome in the manner described by Bacher et al. (1999) could then promote and stabilize the empty state of SRβ.

Immunoprecipitation experiments employing a mutant version of SRβ depleted of nucleotide by gel filtration demonstrated that the empty state of SRβ was unable to interact with SRα (Legate et al. 2000). Therefore the interaction between the ribosome and SRβ may influence heterodimerization of SRα and SRβ. Dissociation of SRα and SRβ may serve to transfer the ribosome-nascent chain from the SRP receptor to the translocon. Two possible models for regulating this transfer step can be envisioned. In one model the relatively high affinity of the ribosome for Sec61α and the proximity of the two that results from binding of the SRP–ribosome–nascent chain complex to the SRP receptor are sufficient to ensure that release of SRα from SRβ results in transfer of the ribosome to the Sec61 complex (Fig. 2A, step 3). In the other model an association between the ribosome and Sec61 could be required to activate the GAP property of the ribosome, thereby preventing premature GTP hydrolysis by SRβ and concomitant release of SRα–SRP–ribosome–nascent chain complexes to the cytoplasm. In either case, once the ribosome has bound to the Sec61 complex, SRP and SRα are released from the nascent chain, concomitant with the nucleotide hydrolysis by SRP54 and SRα which releases these proteins from each other (Fig. 2A, step 4) (Connolly et al. 1991). The SRP is then free to mediate another round of targeting, and SRα rebinds to a vacant SRβ on the ER membrane.

Under conditions where translocation pores are saturated by translating ribosomes or digested by protease, additional ribosome–nascent chains continue to be targeted and tethered to the membrane (Murphy et al. 1997; Song et al. 2000). What tethers these post-targeting intermediates to the membrane is

Fig. 2. Schematic diagrams of the putative targeting and nascent-chain transfer steps in (A) eukaryotes and (B) *Escherichia coli*. Components shown are the ribosome (olive green), SRP (purple), nascent chain (black), SRP receptor (dark blue and green), translocon (light blue), GTP (red), and membrane (orange). Refer to the text for details.



currently unknown. The observation that following the targeting reaction SRα can be completely digested by protease without releasing post-targeting intermediates argues against the possibility that SRα is the membrane-bound anchor for these post-targeting intermediates (Murphy et al. 1997). However, upon targeting to the ER through a SRP54–SRα interaction, the ribosome–nascent chains may become tethered to the ER by an interaction between the ribosome and SRβ.

Several pieces of recently published data are consistent with a role for SRβ in tethering these post-targeting intermediates. Treatment of ER microsomes with protease concentrations that digested Sec61α effectively blocked SRP-mediated targeting of ribosome–nascent chains at the level of the post-targeting intermediate (Song et al. 2000). These protease conditions did not affect the integrity of SRβ, although SRα was completely digested. Addition of exogenous SRα to facilitate targeting to these membranes resulted

in the detection of post-targeting intermediates only when the integrity of SRβ was unaffected. SRα cannot functionally interact with membranes containing digested SRβ, and so could not facilitate targeting to these membranes (Legate et al. 2000). However, ribosome–nascent chain complexes have been shown to interact with liposomes containing the SRP receptor from which SRα has been proteolytically removed (Bacher et al. 1999). Taken together with the observation that SRα is dispensable once targeting has occurred (Murphy et al. 1997), these results implicate SRβ as the membrane-bound anchor for post-targeting intermediates.

The bacterial SRP pathway has been shown to closely mimic the eukaryotic pathway in many respects (Fig. 2B). Ffh (SRP54) and FtsY (SRα) both bind and hydrolyze GTP as reciprocal GAPs, an activity that is inhibited by synthetic signal peptides (Powers and Walter 1995; Miller et al. 1994). Furthermore, Ffh can bind signal peptides when it is assembled with its cognate RNA or when assembled into a chimeric mammalian SRP (Luirink et al. 1992; Valent et al. 1995; Bernstein et al. 1993). A major difference between the two pathways is the absence of SRβ in bacteria. In the absence of a homologue of SRβ, is FtsY binding to the *E. coli* cell membrane regulated? The first evidence that it may be regulated is the finding that more than 50% of the FtsY in *E. coli* is recovered in the cytosol after fractionation (Luirink et al. 1994). By using gel filtration chromotography to study the membrane-binding characteristics of *E. coli* FtsY, it was found that some of these molecules underwent a proteolytic cleavage event between the membrane-binding AN domain and the catalytic G domain (Millman and Andrews 1999). It is unknown whether cleavage is coupled to targeting of RNCs or translocation of nascent polypeptides, or whether it occurs independently of these processes. Also unknown is the identity of the protease that cleaves FtsY, although it has been determined that it is a component of the bacterial inner membrane (Millman and Andrews 1999).

Should the cleavage event be coupled to translocation, the GTP-binding site may in part regulate a conformational change in FtsY to facilitate access to the protease. Fluorescence measurements using a tryptophan residue within the effector region of the G domain revealed significant structural changes within the FtsY GTPase upon binding Ffh and GTP (Jagath et al. 2000). Such a structural change may reveal the cleavage site to the protease. Cleavage of FtsY upon binding Ffh–GTP would make targeting unidirectional as in the eukaryotic system, although by a completely different mechanism. A second hypothesis takes into account the large amount of cytosolic FtsY. In this model, binding of Ffh by FtsY occurs in the cytoplasm. The interaction between Ffh and FtsY causes a conformational change leading to exposure of the lipid-binding domain, permitting binding to the membrane and access to the protease. More work is required to differentiate the steps leading to targeting of SRP-dependent substrates to the *E. coli* inner membrane.

By studying the biogenesis and assembly of the translocon, roles for a number of targeting pathways have emerged that operate independently of the SRP and the SRP receptor. These pathways include insertion sequences, lipid-binding proteins, and protein–protein interactions that occur during mRNA-encoded translation pause sequences. In addition, the mechanisms of assembly of translocon proteins such as the SRP receptor suggest new models for the function of these proteins which can now be tested. Together these disparate pathways function together to make the SRP pathway work. Although the future work in our laboratory will focus on the details of these assembly pathways, it is hoped that the novel mechanisms we have uncovered will reveal themselves in the targeting and hetero-oligomerization of other protein complexes involved in a variety of cellular functions.

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