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MicroReview

Mammalian and *Escherichia coli* signal recognition particles

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Summary

Recent evidence from both biochemical and genetic studies indicates that protein targeting to the prokaryotic cytoplasmic membrane and the eukaryotic endoplasmic reticulum membrane may have more in common than previously thought. A ribonucleoprotein particle was identified in *Escherichia coli* that consists of at least one protein (P48 or Ffh) and one RNA molecule (4.5S RNA), both of which exhibit strong sequence similarity with constituents of the mammalian signal recognition particle (SRP). Like the mammalian SRP, the *E. coli* SRP binds specifically to the signal sequence of presecretory proteins. Depletion of either P48 or 4.5S RNA affects translation and results in the accumulation of precursors of several secreted proteins. This review discusses these recent studies and speculates on the position of the SRP in the complex network of protein interactions involved in translation and membrane targeting in *E. coli*.

Introduction

In both prokaryotic and eukaryotic cells, many proteins are targeted to, inserted into and translocated across biological membranes. The cytoplasmic membrane (CM) of *Escherichia coli* and the endoplasmic reticulum (ER) membrane of the canine pancreas have been especially popular for studies of protein targeting and translocation. In both systems the secretory proteins face the same problems. They have to maintain a translocation-competent conformation in the cytosol, contact the membrane, traverse the membrane and then be released at the *trans* side of the membrane. Most proteins destined to insert into or traverse the CM and ER carry part of their targeting information in an *N*-terminal signal sequence of 15–30 amino acid residues, which contains an essential hydrophobic core region of approximately 10 residues (von Heijne, 1988). These signal sequences are structurally similar and often functionally interchangeable between prokaryotes and eukaryotes, implying conserved underlying mechanisms of signal sequence-mediated targeting and translocation (von Heijne, 1988). However, until recently, little homology has been observed between components of both export systems.

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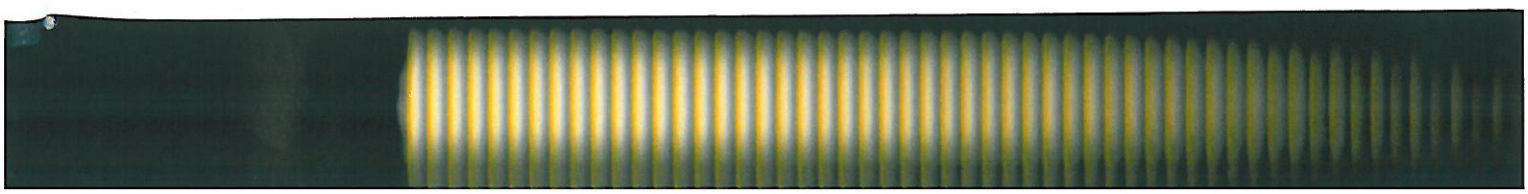
E. coli: the general secretory pathway

In *E. coli* several soluble and membrane proteins have been identified (inititially in genetic studies) that are required at different stages of the general secretory pathway (for an excellent recent review, see Pugsley, 1993). Pre-proteins interact with molecular chaperones like SecB, DnaK/DnaJ and GroEL/GroES to maintain their translocation-competent conformation in the cytosol (Kumamoto, 1991). Little is known about the molecular basis of the pre-protein–chaperone interaction. Recent evidence indicates that SecB binds cotranslationally to only a limited subset of presecretory proteins (Kumamoto and Francetic, 1993). Determination of SecB-binding sites in precursor molecules has met with conflicting results, but most of the available data indicate that SecB binds to multiple sites in the mature portion of the pre-protein (for discussions on this issue, see Pugsley, 1993; Kumamoto, 1991). GroEL was shown to interact with completed pre-β-lactamase by photocross-linking (Bochkareva *et al.*, 1988). Different pre-proteins seem to prefer different chaperones but they can be quite promiscuous when circumstances change. For instance, increased levels of GroEL and DnaJ/DnaK can compensate for the loss of SecB (Altman *et al.*, 1991; Wild *et al.*, 1992). Among these chaperones, SecB seems the most specific for exported proteins. This conclusion is supported by the fact that SecB also fulfils a 'pilot' function by binding to the membrane-associated SecA protein (Hartl *et al.*, 1990). SecA has binding affinity not only for SecB but also for the signal sequence and mature domain of the pre-protein (Akita *et al.*, 1990; Joly



and Wickner, 1993). It also binds ATP (Lill *et al.*, 1989) and has been implicated in the generation of energy for the translocation process (Schiebel *et al.*, 1991). SecA interacts with the membrane-embedded SecY/SecE complex (Hartl *et al.*, 1990) which is a constituent of the so-called translocon, the putative protein pore in the CM. The eukaryotic homologue of SecY is the Sec61 protein identified first by a genetic screen in yeast (Deshaies and Schekman, 1987) and most recently by a biochemical approach in mammalian cells (Görlich *et al.*, 1992).

Mammalian cells: SRP-mediated transport

In eukaryotic cells, the signal recognition particle (SRP) recognizes the signal peptide when it protrudes from the ribosome in the cytosol (at 60–90 total amino acid chain length). SRP may be prebound to the ribosome allowing passing sequences to be screened (Siegel and Walter, 1988). The SRP is a large ribonucleoprotein complex consisting of a 7S RNA and six different polypeptides of 9, 14, 19, 54, 68 and 72 kDa. The association between the SRP and the signal peptide lowers the rate of protein synthesis and thereby increases the time span in which the complex can contact the membrane with the nascent chain in a translocation competent conformation (Siegel and Walter, 1988). The ribosome/nascent chain/SRP complex then binds to the ER membrane via an interaction between SRP and SRP receptor (also called the 'docking protein'). Upon binding to the SRP receptor, the signal peptide is displaced from the SRP in a GTP-dependent process and becomes available for interactions with components of the putative translocon (Rapoport, 1992). Translation resumes and the nascent chain inserts co-translationally into the ER membrane. Finally, the SRP is released from the membrane-bound complex in a step that requires GTP hydrolysis (Connolly and Gilmore, 1989). Thus, the SRP is a versatile adaptor which functions as a 'pilot' and as a molecular chaperone to guide the nascent secretory protein to the membrane in a translocation-competent form. The different functions have been ascribed to the individual protein components in the SRP in which the 7S RNA probably plays a scaffolding role. SRP9 and SRP14 form a heterodimer which is necessary for the translation arrest function. SRP68 and SRP72 also form a heterodimer which has been implicated in the mechanism of 'docking' to the ER membrane (Siegel and Walter, 1988). SRP19 assists in the binding of SRP54 to the 7S RNA whereas SRP54 is responsible for binding to the signal sequence (Römisch *et al.*, 1990; Zopf *et al.*, 1990).

SRP54 has a modular structure consisting of an *N*-terminal G-domain, which contains a conserved GTP-binding motif, and a *C*-terminal M-domain, which is rich in methionine residues. Several independent studies have shown that the M-domain is responsible for both binding to the 7S RNA and to the signal sequence of the nascent presecretory protein (Zopf *et al.*, 1990; High and Dobberstein, 1991). Reconstituted SRP containing only the M-domain of SRP54 was shown to be able to recognize the signal sequence albeit with lower efficiency than intact SRP, but it was unable to target the ribosome/nascent chain complex to the ER membrane (Zopf *et al.*, 1993). The M-domain contains four predicted amphipathic α-helices (Bernstein *et al.*, 1989). The methionine residues are found at evolutionary conserved positions and thought to line one side of each α-helix. An attractive model has been put forward in which the helices are juxtaposed with the flexible methionine side chains forming a groove which accommodates the large variety of hydrophobic signal sequences (Bernstein *et al.*, 1989). The G-domain increases the efficiency of signal sequence binding and is probably also involved in the binding of the SRP to the α-subunit of the SRP receptor (Zopf *et al.*, 1993).

A peptide-binding motif similar to the one suggested for the interaction between signal sequences and the SRP54 protein has been identified for the major histocompatibility complex (MHC) class I and II molecules (Bjorkman *et al.*, 1987; Brown *et al.*, 1993). In this case, peptides of nine amino acid residues were found to bind in a groove formed by two α-helices. The α-helices are arranged side by side on a platform built by β-pleated sheets. In the case of the signal sequence-binding domain of SRP54 the platform would be formed by the 7S RNA.

Evidence for an *E. coli* SRP

The search for an SRP-like particle in *E. coli* has long been discouraged by the inability to identify SRP-like components in genetic screenings for export mutants. These screens identified very successfully several of the *sec* genes described above. Recently, this search gained new impetus when sequence comparisons revealed the existence of *E. coli* homologues of SRP54 and SRP7S RNA, P48 (also called Ffh for fifty-four homologue) and 4.5S RNA, respectively (Römisch *et al.*, 1989; Bernstein *et al.*, 1989; Poritz *et al.*, 1988).

The P48 gene was initially identified as an open reading frame upstream of the *trmD* operon at 56 min of the *E. coli* chromosome (Byström *et al.*, 1983). P48 is very similar over its entire length to SRP54 and seems to have the same modular structure (Römisch *et al.*, 1989; Bernstein *et al.*, 1989). The M-domain of P48 lacks one of the predicted *C*-terminal amphipathic helices of SRP54. This could explain some of the differences between the prokaryotic and eukaryotic membrane targeting system.

E. coli 4.5S RNA is one of the smallest members of the family of SRP7S-like RNAs found in mammalian cells, plants, yeast, archaebacteria and eubacteria (Larsen and Zwieb, 1991). It forms an extended stem-loop structure,

which is homologous to the most highly conserved domain of SRP7S RNA.

Two independent studies gave the first hints that P48 and 4.5S RNA are part of an SRP-like complex in *E. coli* (Ribes *et al.*, 1990; Poritz *et al.*, 1990). 4.5S RNA was found to be in a complex with P48 in a wild-type *E. coli* extract (Ribes *et al.*, 1990; Poritz *et al.*, 1990) and to bind to SRP54 *in vitro* (Poritz *et al.*, 1990). To investigate the function of 4.5S RNA, a strain was constructed which allowed conditional expression of 4.5S RNA. Depletion of 4.5S RNA (or overexpression of a dominant lethal 4.5S RNA allele) showed pleiotropic effects, including an early induction of the heat-shock response, a relatively late inhibition of cell growth and protein synthesis, and finally cell death (Ribes *et al.*, 1990; Poritz *et al.*, 1990). Expression of SRP7S RNA could partially complement for the loss of 4.5S RNA in this strain (Ribes *et al.*, 1990). Effects on secretion were limited to a decreased processing of pre-β-lactamase at late time points after depletion (Ribes et al., 1990; Poritz *et al.*, 1990). At the time it was not clear whether this was a consequence of the heat-shock response or of a genuine secretion defect.

Support for the latter possibility came from three recent studies. In an elegant genetic approach similar to the one described above, Phillips and Silhavy (1992) investigated the effects of cellular depletion of the protein component of the RNP, P48. Rather surprisingly, precursor forms of all tested secretory proteins accumulated, which is indicative of a general effect on protein secretion. It must be noted that P48-depleted cells exhibited an elongated cell shape suggesting impaired cell division.

In a second biochemical study we demonstrated that P48 binds specifically to the signal peptide of nascent pre-secretory proteins (Luirink *et al.*, 1992). Truncated pre-prolactin mRNA (coding for 86 amino acid residues) was translated in a cell-free sytem in the presence of Lys-tRNA carrying a photoactivatable crosslinking group in its side chain. After purification, the ribosome/nascent chain complexes were incubated with *E. coli* cell extracts and crosslinking was induced. P48 was found to be cross-linked to the pre-prolactin signal sequence, but not to a mutated, non-functional signal sequence. Evidence was obtained that the interaction of P48 with the signal peptide is similar to that of SRP54: (i) SRP54 competes with P48 for binding to the signal peptide (ii) P48 binds as part of an RNP containing 4.5S RNA (iii) P48 binds only to nascent polypeptides, not to polypeptides which have been released from the ribosome. A striking difference, however, is the dependence of P48 binding on the presence of 4.5S RNA in the cell extract used for crosslinking, whereas SRP54 binds very efficiently in the absence of SRP7S RNA. The binding region of P48 on the 4.5S RNA was recently mapped by site-directed mutagenesis (Wood *et al.*, 1992). P48 binds to two loop structures in the central portion of the 4.5S RNA. Interestingly, a correlation was found between the inability of mutant 4.5S RNA molecules to bind P48 and to restore growth *in vivo* in 4.5S RNA depleted cells. This indicated that the *E. coli* SRP indeed functions as a ribonucleoprotein particle.

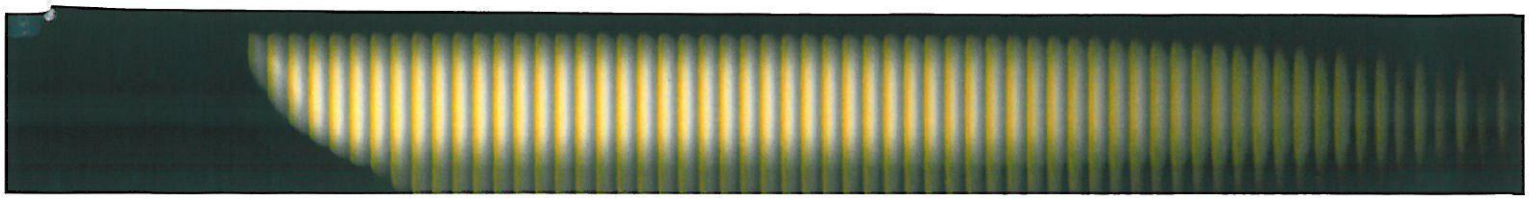
In a third study, Bernstein and coworkers (1993) reconstituted mammalian SRP in which SRP54 had been replaced by P48. This chimeric SRP was still capable of signal peptide binding and imposing translational arrest, but could not promote translocation of pre-prolactin into the ER. Possibly, P48 does not interact with the eukaryotic SRP receptor.

4.5S RNA and translational regulation

From physiological and genetic studies of the functions of 4.5S RNA in *E. coli* a role for this molecule related to translation has been suggested (an excellent discussion of this aspect can be found in a review article by Brown, 1991). Synthesis of 4.5S RNA was found to be co-ordinately regulated with that of ribosomal RNA and transfer RNA (Ikemura and Dahlberg, 1973). Selection of suppressors that reduce the 4.5S RNA requirement were found in genes coding for components of the translational apparatus, the elongation factor G (EF-G) (Brown, 1987) and in the binding site for 4.5S RNA on 23S ribosomal RNA or resulted in the increased concentration of some uncharged tRNAs (Brown, 1989). Both the 4.5S RNA and the P48 have been found to interact with ribosomes, individually and as an RNP particle. Both were shown to be released from ribosomes by treatment with puromycin (Brown, 1987; 1989; Luirink *et al.*, 1992). The finding that P48 affects the 4.5S RNA requirement and the fact that 4.5S RNA can be found free and complexed to P48 make it likely that free 4.5S RNA and the RNP complex function in different pathways (Brown, 1991). The free form has been implicated in modulating the rate of translation to allow proteins to fold properly (Brown, 1991). Such a function would explain the finding that a reduction in the amount of 4.5S RNA leads to misfolding of proteins. We envisage that the P48/4.5S RNA complex has a more selective function: as P48 contacts signal sequences in nascent polypeptides this function would be limited to secretory and membrane proteins. Given the function of 4.5S RNA in general translation regulation we would assume a similar, although more selective function for the P48/4.5S RNA complex. It relays information from the nascent chain via the RNA to the translating ribosome and via its GTP-binding domain to a component yet to be characterized.

Model for chaperone-mediated protein targeting in *E. coli*

Both cytosolic and proteins destined for secretion have appeared to interact in a hierarchical manner with a



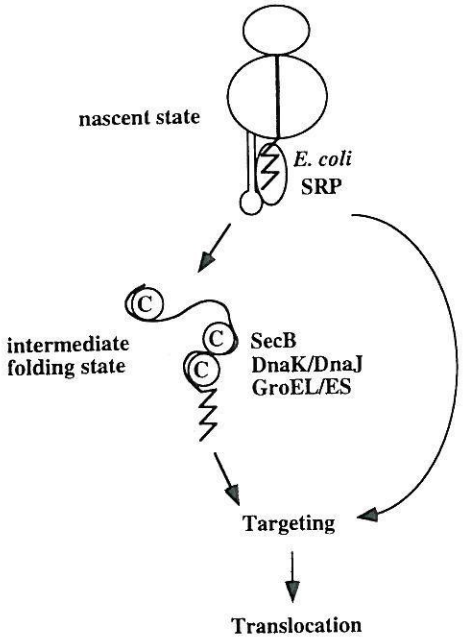


Fig. 1. Model for the function of the *E. coli* SRP. The model is discussed in the text.

complex set of chaperones (Hartl and Wiedmann, 1993). From the results discussed above we propose that the *E. coli* SRP functions as a chaperone specific for signal sequences in nascent pre-proteins and maintains their translocation-competent conformation (Fig. 1). The interaction with the nascent chain would persist until SRP is contacted by another chaperone (like SecB) or a component of the translocation apparatus, SecA or SecY. In this view SRP would be a delivery system for the signal sequence in nascent polypeptides. In this respect it would be interesting to see whether the SRP and other chaperones can bind concomitantly to longer nascent chains or whether their binding is mutually exclusive.

Release of the *E. coli* SRP from nascent chains may also be mediated by direct interaction with a cognate receptor, similar to the interaction between mammalian SRP and its receptor, the docking protein (Meyer *et al.*, 1982). A good candidate for such a receptor is the FtsY protein. FtsY displays sequence similarity to the mammalian docking-protein in its *C*-terminal part which contains conserved GTP-binding motifs (Ribes *et al.*, 1990; Poritz *et al.*, 1990). Interestingly, a putative docking protein homologue has also been found in an archaebacterium (Ramirez and Matheson, 1991). FtsY is essential for cell viability and has been localized in the cytoplasmic membrane (Gill and Salmond, 1987; J. Luirink, unpublished results). Initially, FtsY has been implicated in cell division because its gene is located in an operon together with *ftsE* and *ftsX* (Gill and Salmond, 1986). In this operon several temperature-sensitive mutations have been mapped that cause cell filamentation at the non-permissive temperature. However, *fts* mutations were not mapped in *ftsY* as revealed by localized mutagenesis of the *ftsYEX* operon (Gibbs *et al.*, 1992). The precise function of FtsY awaits further analysis.

Genetic, biochemical and comparative approaches have yielded the initial insight into the possible functions of mammalian and *E. coli* SRP and its constituent components. Not all the pieces of the puzzle can yet be put together. However, it is already clear that studying SRP structure and function in different organisms we will not only learn about translation regulation, protein folding and membrane translocation but also how variable these processes were linked together during evolution.

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References

Akita, M. *et al.* (1990) *J Biol Chem* **265:** 8164–8169.
Altman, E. *et al.* (1991) *EMBO J* **10:** 239–245.
Bernstein, H.D. *et al.* (1989) *Nature* **340:** 482–486.
Bernstein, H.D. *et al.* (1993) *Proc Natl Acad Sci USA* **90:** 5229–5233.
Bjorkman, P.J. *et al.* (1987) *Nature* **329:** 512–518.
Bochkareva, E.S. *et al.* (1988) *Nature* **336:** 254–257.
Brown, J.H. *et al.* (1993) *Nature* **364:** 33–39.
Brown, S. (1987) *Cell* **49:** 825–833.
Brown, S. (1989) *J Mol Biol* **209:** 79–90.
Brown, S. (1991) *The New Biologist* **3:** 430–438.
Byström, A.S. *et al.* (1983) *EMBO J* **2:** 899–905.
Connolly, T., and Gilmore, R. (1989) *Cell* **57:** 599–610.
Deshaies, R., and Schekman, R. (1987) *J Cell Biol* **105:** 633–645.
Gibbs, T.W. *et al.* (1992) *Mol Gen Genet* **234:** 121–128.
Gill, D.R. *et al.* (1986) *Mol Gen Genet* **205:** 134–145.
Gill, F., and Salmond, G.P.C. (1987) *Mol Gen Genet* **210:** 504–508.
Görlich, D. *et al.* (1992) *Cell* **71:** 489–503.
Hartl, F.-U., and Wiedmann, M. (1993) *Current Biology* **3**: 86–89.
Hartl, F.-U. *et al.* (1990) *Cell* **63:** 269–279.
von Heijne, G. (1988) *Biochim Biophys Acta* **947:** 307–333.
High, S., and Dobberstein, B. (1991) *J Cell Biol* **113:** 229–233.
Ikemura, T., and Dahlberg, J.E. (1973) *J Biol Chem* **248**: 5033–5041.
Joly, J.C., and Wickner, W. (1993) *EMBO J* **12:** 255–263.
Kumamoto, C.A. (1991) *Mol Microbiol* **5:** 19–22.
Kumamoto, C.A., and Francetic, O. (1993) *J Bacteriol* **175:** 2184–2188.
Larsen, N., and Zwieb, (1991) C. *Nucl Acids Res* **19:** 209–213.
Lill, R. *et al.* (1989) *EMBO J* **8:** 961–966.
Luirink, J. *et al.* (1992) *Nature* **359:** 741–743.
Meyer, D.I. *et al.* (1982) *Nature* **297:** 647–650.

Phillips, G.J., and Silhavy, T.J. (1992) *Nature* **359:** 744–746.
Poritz, M.A. *et al.* (1988) *Cell* **55:** 4–6.
Poritz, M.A. *et al.* (1990) *Science* **250:** 1111–1117.
Pugsley, A.P. (1993) *Microbiol Rev* **57:** 50–108.
Ramirez, C., and Matheson, A.T. (1991) *Mol Microbiol* **5:** 1687–1693.
Rapoport, T. (1992) *Science* **258:** 931–936.
Ribes, V. *et al.* (1990) *Cell* **63:** 591–600.
Römisch, K. *et al.* (1989) *Nature* **340:** 478–482.
Römisch, K. *et al.* (1990) *J Cell Biol* **111:** 1793–1802.
Schiebel, E. *et al.* (1991) *Cell* **64:** 927–939.
Siegel, V., and Walter, P. (1988) *TIBS* **13:** 314–315.
Wild, J. *et al.* (1992) *Genes Dev* **6:** 1165–1172.
Wood, H. *et al. Nucl Acids Res* **20:** 5919–5925.
Zopf, D. *et al.* (1990) *EMBO J* **9:** 4511–4517.
Zopf, D. *et al.* (1993) *J Cell Biol* **120:** 1113–1121.