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Minireview

The *E. coli* SRP: preferences of a targeting factor

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Abstract Research on the targeting of proteins to the cytoplasmic membrane of *E. coli* has mainly focused on the so-called 'general secretory pathway' (GSP) which involves the Sec-proteins. Recently, evidence has been obtained for an alternative targeting pathway in *E. coli* which involves the signal recognition particle (SRP). The constituents of this SRP pathway in *E. coli* are homologous to those of the well-characterized eukaryotic SRP pathway, which is the main targeting pathway for both proteins translocated across and inserted into the endoplasmic reticulum membrane. However, until recently, no clear function could be assigned to the SRP in *E. coli*. New studies point to an important role of the *E. coli* SRP in the assembly of inner membrane proteins.

Key words: *Escherichia coli*; Protein-targeting; Signal recognition particle; Inner membrane protein

1. Introduction

The targeting and insertion of proteins into the membrane of the rough endoplasmic reticulum (ER) of eukaryotic cells or the inner membrane of prokaryotic cells is an essential step in the biosynthesis of both secreted and membrane proteins. These processes require an apolar signal sequence in the polypeptide chain and a machinery of cytoplasmic and membrane components that recognizes these signals and promotes their insertion and partial translocation [1,2].

E. coli studies on the requirements for biosynthesis of both secreted and inner membrane proteins have historically been focused on components of the so-called 'general secretory pathway' (GSP) which involves the Sec-proteins [1–5]. The GSP is used by most proteins that are completely translocated across the inner membrane and end up in the periplasm or outer membrane. In this pathway, SecA is a peripheral subunit of the membrane-embedded translocation machinery (consisting of SecY, E, G, D, F, yaiC and possibly additional subunits) and plays a central role in both targeting and translocation. SecA functions as the receptor for the complex of pre-protein and the cytosolic chaperone SecB.

Whereas almost all translocated proteins depend on the Sec-machinery for translocation into the periplasm, it has been proposed that the assembly of inner membrane proteins, except for those containing long periplasmic loops, may be Sec-independent. It should be noted, however, that this conclusion was based on negative results and that Sec-function may not have been completely eliminated in the conditional *sec*-strains used in these studies [6,7].

While components necessary for the assembly of inner membrane proteins have, thus, been difficult to identify, a flurry of papers published in the past few months indicate that the *E. coli* SRP plays an important role in the assembly of inner membrane proteins.

2. SRP-targeting pathway in eukaryotes

In eukaryotic systems, the biosynthesis of membrane proteins and translocated proteins seem to proceed by similar mechanisms [1,2]. The whole process of targeting starts with the signal recognition particle (SRP)-binding to the N-terminal signal sequence when the nascent chain has reached a critical length of $\approx$60 amino acids, i.e. when the signal sequence is exposed just outside the ribosome. There seems to be a correlation between the hydrophobicity of the signal sequence core region and the affinity of the signal sequence for the SRP [8,9] though it has been suggested that features other than hydrophobicity might also play a role in SRP-binding [10]. Translation is inhibited by the SRP-signal sequence interaction until the SRP contacts its receptor at the membrane and dissociates from the nascent chain. The ribosome then makes a tight seal with the translocon, translation is resumed and the nascent chain inserts co-translationally into the aqueous translocation channel. Hydrophylic polypeptide chains are translocated across the ER membrane through the translocon whereas hydrophobic transmembrane segments halt in the translocon and subsequently move out laterally into the lipid bilayer [11,12].

The targeting of most proteins to the ER membrane is mediated by the SRP-targeting pathway but alternative targeting routes and bypasses have been described [1]. This is probably most strinkingly illustrated by the observation that a SRP-knock out mutant of *Saccharomyces cerevisiae* is still viable and seems to adapt to the absence of SRP [13].

3. *E. coli* SRP: a targeting factor in search of a substrate (Fig. 1)

3.1. Targeting of secretory proteins

In *E. coli*, in addition to the Sec-proteins, an SRP has been identified that is relatively simple and contains a 4.5S RNA and P48 (or Ffh for Fifty-four homologue) that are homologous to the eukaryotic SRP 7S RNA and SRP 54-kDA subunit, respectively [14–16]. In addition, an SRP-receptor, FtsY, has been identified on basis of its sequence homology with the

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Abbreviations: GSP, general secretory pathway; SRP, signal recognition particle; ER, endoplasmic reticulum; Ffh, Fifty-four homolog; LacY, lactose permease; Lep, leader peptidase

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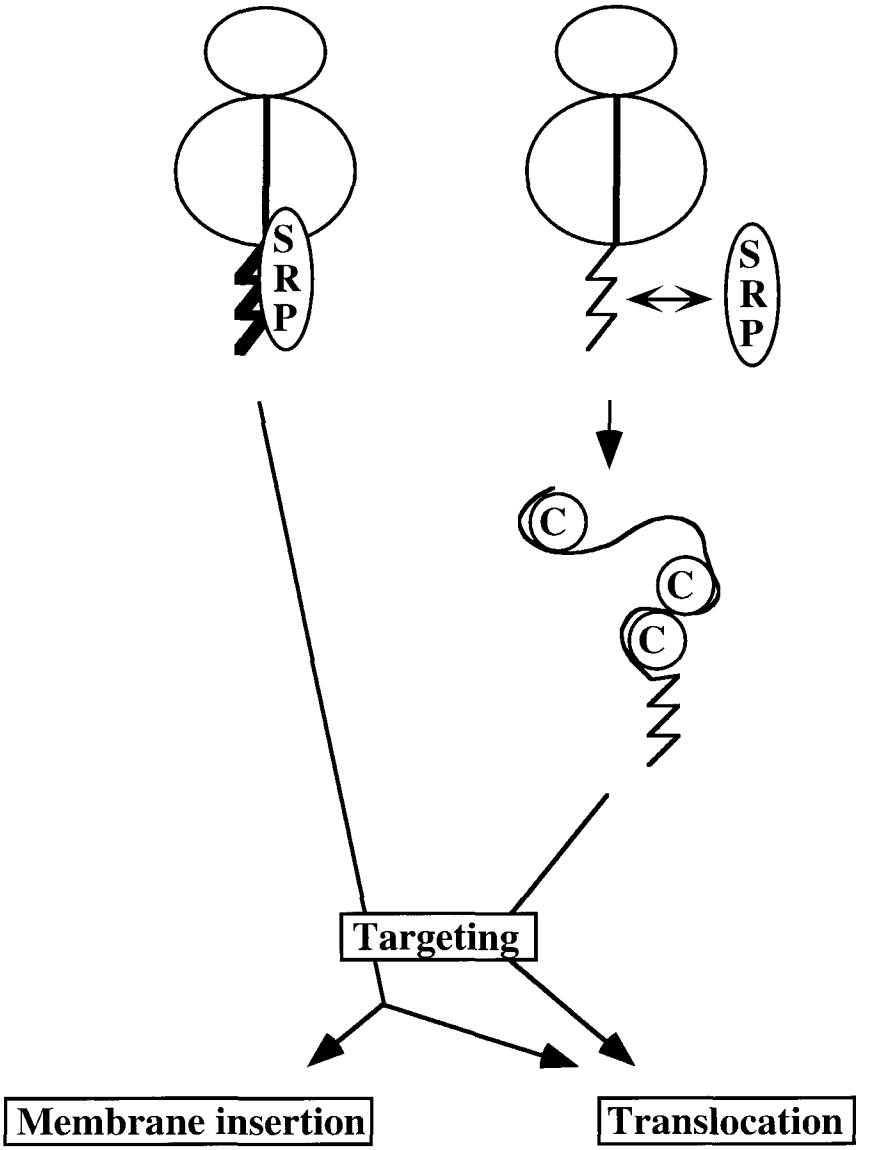

Fig. 1. Model for the function of the *E. coli* SRP. The SRP has a high affinity for hydrophobic targeting signals (thick crenated line) and promotes targeting of predominantly inner membrane proteins. The SRP has a low affinity for less hydrophobic targeting signals (thin crenated line) at the N-terminus of secreted proteins. Maintenance of translocation competence and targeting is mediated by chaperones (C), like secB.

α-subunit of the eukaryotic SRP-receptor and has been found to have affinity for the SRP in vitro [14,16,17]. The SRP subunits 4.5S RNA and P48 as well as their receptor FtsY are essential for viability. However, disruption of the SRP pathway by depletion of 4.5S RNA, P48 or FtsY has a relatively mild effect on the processing of most secreted proteins, with the notable exception of β-lactamase which carries an unusually hydrophobic signal peptide and shows strong precursor accumulation [18–21]. Like the 54-kDa subunit of the eukaryotic SRP, P48 binds to the signal sequence of nascent, ribosome-associated polypeptides as was demonstrated using an in vitro photo cross-linking approach. As in eukaryotes, a correlation has been found between the hydrophobicity of the core region of a signal sequence and its affinity for the *E. coli* SRP [22,23].

3.2. Targeting of inner membrane proteins

Several lines of evidence indicate that in *E. coli* the SRP and its receptor FtsY play an important role in synthesis and assembly of inner membrane proteins. Using a direct in vivo approach, we have demonstrated that the membrane insertion of both the inner membrane protein leader peptidase (Lep) and the insertion of a Lep-derivative that inserts with an inverted topology (Lep-inv) compared to the wild-type Lep are both strongly inhibited upon depletion of P48 or 4.5S RNA [24]. The latter observation is particularly interesting, as previous studies have failed to demonstrate a role for the translocon-components SecA and SecY in the assembly of Lep-inv [25]. A role for the SRP in the assembly of the inner membrane protein lactose permease (LacY) has also been suggested using an in vivo approach [26].

In a recent study, Ulbrandt et al. used an elegant genetic approach to identify potential SRP substrates in *E. coli* [27]. An *E. coli* genomic library was transformed into a strain that is conditional for P48 expression and clones were selected that confer synthetic lethality upon low-level expression of P48.

Surprisingly, a subset of polytopic inner membrane proteins was selected, suggesting that they are natural substrates of the SRP. It should be noted that the strength of the phenotype seemed correlated with the expression level of the inner membrane protein. Thus, it remains possible that the selection is biased by expression and number of potential SRP-binding sites. Consistent with this possibility, high level expression of Lep and periplasmic β-lactamase (neither protein was selected in the screening) titrates SRP and subsequently reduces the amount of free SRP [23].

The preference of SRP for inner membrane proteins is corroborated by in vitro cross-linking studies [22,23]. As mentioned before, it was demonstrated that the efficiency of cross-linking of SRP to stalled nascent chains is correlated with the hydrophobicity of the signal sequence. Since transmembrane domains are more hydrophobic than signal sequences, they may have a higher affinity for SRP. The correlation between hydrophobicity and SRP affinity seems to be a conserved property since it was also observed for mammalian, yeast and chloroplast SRP [8,22,28] and membrane proteins may, thus, be a generally preferred substrate for SRP. On the other hand, cross-linking of P48 to less hydrophobic signal sequences has also been observed, albeit with lower efficiencies [22,23]. It is, thus, not unlikely that SRP is used by secreted proteins as well under certain circumstances, e.g. when the Sec-targeting pathway is disrupted or overloaded. Also, properties of the nascent chain that are not related to the signal sequence may influence SRP-binding, like the rate of synthesis, the presence of natural translation pause sites, folding and interactions with other chaperones.

Not only membrane insertion but also synthesis of inner membrane proteins can be inhibited by disruption of the SRP-targeting pathway. Depletion of FtsY was found to inhibit expression of the polytopic inner membrane proteins LacY and SecY in a reversible manner whereas the expression of one cytosolic and one periplasmic protein was hardly affected [29]. By analogy with the eukaryotic system, it is possible that the SRP causes a translation arrest which cannot be relieved in the absence of FtsY. A translation arrest would presumably be mechanistically different, since the *E. coli* SRP lacks homologues of the eukaryotic 9- and 14-kDa SRP subunits which are essential for the eukaryotic translation arrest [30]. An alternative explanation is rapid degradation of proteins that are not properly inserted in the inner membrane upon the depletion of FtsY. Either way, this study confirms the involvement of the SRP-targeting pathway in the assembly of inner membrane proteins.

4. Concluding remarks

Recent studies on the role of the SRP in *E. coli* stress the importance of this factor in the targeting and insertion of inner membrane proteins although a role in targeting of secreted proteins can definitely not be ruled out at this stage.

In eukaryotes, the SRP-targeting pathway delivers proteins at the translocon. The translocon is required both for the assembly of secreted polypeptide chains and transmembrane domains. It appears that the role of the Sec-machinery in the assembly of inner membrane proteins in *E. coli* is restricted to the translocation of long periplasmic loops. This raises the question where and how (co- or post-translationally) the SRP-targeting pathway in *E. coli* delivers the inner membrane protein at the inner membrane. The eukaryotic and *E. coli* translocons are related [31] and it is tempting to speculate that the SRP-targeting pathway, just like the Sec-targeting pathway, delivers the protein at the translocon and that the choice between the Sec- and SRP-targeting pathways is mainly determined by the hydrophobicity of the signal sequence. Consistently, there is a correlation between a decrease in hydrophobicity of a signal sequence and an increase in SecB dependency [32]

The SecB pathway appears to be involved in a mostly post-translational mode of targeting [5,33] and it may be that the SRP pathway in *E. coli*, just like in eukaryotes, is mainly involved in a co-translational mode of targeting. For inner membrane proteins, probably the main SRP substrates in *E coli*, co-translational mode of protein-targeting would be favourable since this would minimize the risk of exposing hydrophobic domains to the cytoplasm. For secreted proteins, the advantage of co-translational targeting could be that partially folded structures that could hamper translocation cannot be formed.

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