

*Review-Hypothesis***Ribosome – SRP – signal sequence interactions****The relay helix hypothesis**

Gunnar von Heijne

*Research Group for Theoretical Biophysics, Department of Theoretical Physics, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

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The role of the signal sequence in protein export is reviewed, and some difficulties inherent in the conventional picture of how it interacts with other components of the export machinery are pointed out. An alternative model is suggested, which seems to account better for some of the critical experimental findings made so far.

*Protein export      Signal sequence      Signal recognition particle      Docking protein*

**1. INTRODUCTION**

The signal sequence has a pivotal role in the process of protein export across the eukaryotic endoplasmic reticulum (ER) membrane and the prokaryotic inner membrane. This transient N-terminal extension on the secreted protein is now known to induce the binding of the signal recognition particle (SRP) to the translating ribosome (at least in eukaryotes, but possibly also in prokaryotes [1,2]), thus targeting the ribosome to export sites on the membrane. Furthermore, once it binds to the ribosome, SRP seems to bring about a translational arrest or slowing down of chain elongation, that is lifted only upon a subsequent interaction between the SRP and the so-called docking protein present in the ER membrane [3,4]. There are some indications that the signal sequence may influence this latter interaction as well [5,6].

In spite of these very specific events brought about by the presence of a signal sequence, these sequences are remarkably variable in terms of amino acid composition. On a gross level, a signal sequence displays three distinct regions: an N-

terminal positively charged region (n-region) between 1 and 15 residues long; a central hydrophobic region (h-region) some 7 to 17 residues long; and a C-terminal more polar region (c-region) where specific patterns of amino acids have been found near the site of cleavage between the signal sequence and the mature protein [7].

An intact h-region with a minimum length of about 7 residues has been shown to be an absolute requirement for triggering the export process [8], and a positive net charge in the n-region seems to be required for proper release of the SRP-induced translational block [5,6]. As long as these two requirements are met, however, almost any variation in amino acid sequence seems to be allowed in the signal sequence [7].

In this paper, recent findings with a bearing on the initial steps in the secretion of a protein are reviewed, and a new hypothesis concerning the mechanism through which the signal sequence mediates these events is put forward. It is hypothesized that the signal sequence does *not* bind directly to the SRP, but rather induces a conformational transition in the growing nascent

chain that propagates to the translation-site on the ribosome and allows SRP to bind and block further elongation. In the face of recently reported experimental results, the proposed mechanism is still compatible with a co-translational export mechanism, at least in eukaryotes.

## 2. RELEVANT EXPERIMENTAL FINDINGS

The main facts concerning the principal components involved in the initial targeting of a protein for the export pathway can be summarized as follows:

(i) The signal recognition particle, SRP, is a rod-shaped particle composed of 6 distinct polypeptides and a small RNA molecule. It is some 50–60 Å wide and 230–240 Å long [9].

(ii) In the eukaryotic ribosome, the nascent chain emerges from some kind of protective 'channel' through the large subunit at an exit site nearly  $160 \pm 25$  Å from the peptidyl transfer site [10].

(iii) The docking protein interacts directly with the SRP, and releases the arrest of elongation even in membrane-free preparations [11,12].

(iv) In vitro, the presence of SRP seems to be an obligatory requirement for proper export, and SRP-induced translational arrest has been demonstrated in most studies. A distinct 'arrest peptide' of some 7 kDa in molecular mass has been successfully isolated in only two cases, however [4].

(v) Export-competent membranes can be added to a synchronized in vitro protein synthesizing system up to a time when the nascent chain is some 130 residues long, and still support export [13].

Recently, it has also been found that SRP can be added as late as when some 120 residues have been incorporated into a growing immunoglobulin  $\lambda$ -light chain, and still induce elongation-arrest and subsequent export in vitro (D. Meyer, personal communication).

(vi) Mutations that substitute charged or polar residues for hydrophobic ones in the h-region of the signal sequence block export and seem to render the protein insensitive to SRP (or rather its putative prokaryotic counterpart) [14,15]. Moreover, incorporation of a polar leucine-analog into a leucine-rich signal sequence also results in a non-exported protein whose translation is not arrested by SRP [16].

(vii) Mutations in the n-region that result in a negative net charge in this part of the signal sequence seem to interfere with both export and synthesis of the protein, possibly because the elongation-arrest is not as effectively released by the docking protein in these cases [5,6].

(viii) A synthetic signal sequence, when added to an in vitro system, can inhibit the export of other proteins, presumably by binding to a receptor present on the cytosolic face of stripped rough ER membranes [17,18].

(ix) As noted above, so far as is known, the n- and h-regions of the signal sequence are only selected for net charge and overall hydrophobicity, respectively [7]. Beyond this, no conserved patterns of amino acids that could be responsible for a specific, direct interaction between the SRP and the signal sequence have been identified, and, indeed, such an interaction has not yet been reported in the literature.

## 3. MODELS OF SIGNAL SEQUENCE FUNCTION

In the 'classical' model of the initial events leading to protein export, the SRP is thought to bind to the signal sequence as soon as it emerges at the ribosomal exit site. The elongated shape of the SRP makes it possible that it may itself bridge the distance between the exit site and the peptidyl transfer site on the ribosome, thus relaying the information that elongation should be arrested. Alternatively, this could conceivably come about via some conformational changes relayed through the ribosomal constituents proper [9]. In any case, the interaction between the SRP and the signal sequence would have to generate a conformational signal that could be relayed through elaborate protein-RNA structures over considerable distances.

The experimental information reviewed above implies two major difficulties with this kind of scheme, however: points (vi) and (ix) require that a rather unspecific hydrophobic interaction between the SRP and the signal sequence should be enough to trigger the subsequent events, and point (v) makes it hard to uphold the idea that protein export in eukaryotic cells is a co-translational event where the protein is threaded through the membrane in a linear fashion: cytosolic ribosomes pro-

tect no more than about 40 residues in the nascent chain from proteolytic attack [19], and if SRP is added only when the chain is some 130 residues long, at least 70 residues in addition to the signal sequence would already have emerged from the ribosome. In prokaryotes, post-translational export seems to be a possible, but perhaps minor, pathway for export [20], but in eukaryotes no indications of post-translational export (in the sense of export of whole domains en bloc) have ever been reported.

In addition, a perhaps naive problem is to picture how the signal peptide can interfere with the SRP-docking protein interaction if it is somehow sequestered inside the SRP.

If we take these difficulties at face value, can we devise some other model that might more easily account for the experimental results? In particular, can we do without the assumption that SRP binds directly to the signal peptide? In the following, I would like to suggest such a scheme, which also circumvents the implication of post-translational export in eukaryotic cells already alluded to.

Let us assume that the hydrophobic h-region in the signal sequence actually has two functions: to interact with the hydrophobic interior of the membrane, and to act as a plug or stopper that would, because of its hydrophobic character, rather remain inside the ribosome than be exposed to the aqueous cytosol. Thus, when the h-region reaches

the ribosomal exit site it effectively 'plugs' the ribosomal channel, forcing the subsequent N-terminal region of the mature protein to adopt a more compact conformation. The 40-odd residues normally protected inside the ribosome must be in a fully extended conformation to span the 160 Å-channel (3.6 Å per residue); if the chain is forced into an  $\alpha$ -helical conformation (1.5 Å per residue) the channel will hold some  $105 \pm 15$  residues.

Such a helix not only might explain point (v) and still leave co-translational export a viable possibility, it also provides a natural way for relaying the information that the protein being made is destined for export to the vicinity of the peptidyl transfer site. Once this has happened, SRP may exert its effect through a *local* interaction at the transferase site alone. If protein synthesis is carried out in the absence of SRP, the signal sequence may be extruded from the ribosome once the 'relay helix' has been fully formed, removing the plug and making it possible for the chain to once more adopt an extended conformation in the ribosomal channel. Adding SRP after this point will thus have no effect.

Finally, in a model along these lines, the signal sequence is free to interact with the docking protein or the membrane when the ribosome has docked properly to an export site, fig.1.

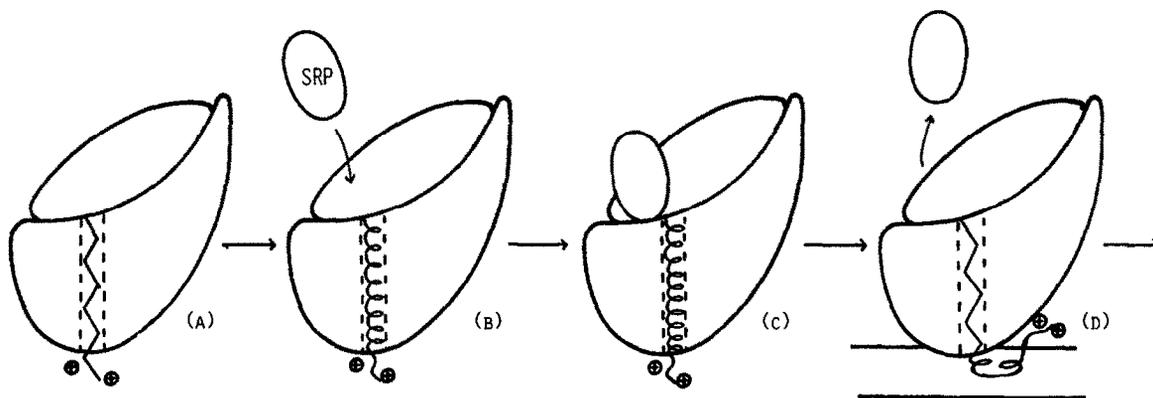


Fig.1. The relay-helix model. The h-region of the signal sequence is assumed to prefer the inside of a hydrophobic channel through the ribosome (A), and will not emerge from the ribosome until forced to do so, i.e. when the whole channel is filled by a compact  $\alpha$ -helix extending all the way up to the transferase site (B). If an SRP binds in the vicinity of the transferase site with a fully formed relay helix it will arrest elongation (C), until the SRP is displaced from the ribosome by interacting with the docking protein. With the ribosome properly positioned over the membrane, the h-region can partition directly into the hydrophobic membrane interior, thus relaxing the relay helix (D). Other integral or peripheral membrane proteins (not shown on the drawing) may be necessary to bind the ribosome to the membrane.

In prokaryotes, the signal sequence does not seem to be cleaved from the mature chain until a 'critical molecular mass' (CMM) of up to 30 kDa (corresponding to ~300 amino acids) has been reached [21], neither is the chain sensitive to externally added proteases until around this point [22]. From the point of view argued here, cleavage of the signal sequence might be as late as when the first few residues have appeared on the periplasmic side of the membrane, i.e. when the chain has reached a length of some 180 residues (120 residues in the ribosome, 25 in the membrane, 10 (say) on the periplasmic side, and another 25 in the signal sequence). This goes some way towards explaining the high CMM values found, but still leaves around 100 residues to account for if one wants to uphold co-translational export as the main export route also in prokaryotes. Perhaps some special structure on the periplasmic side of the *E. coli* inner membrane protects the nascent chain from periplasmic proteases (and inter alia from added proteases) until it is large enough to fold into a reasonably stable, protease-resistant structure.

#### 4. CONCLUSION

The relay-helix model apparently can reconcile the observation that SRP can exert its effect even when added after a substantial length of chain has been made with the widely held assumption that export is co-translational in eukaryotic cells. It also suggests how the positively charged N-terminus and the hydrophobic h-region of the signal sequence can interact freely with membrane phospholipids or membrane receptors without being obstructed by the presence of the SRP. Finally, it provides a possible mechanism whereby the information that the protein being made is destined for export can be relayed from the exit site to the peptidyl transferase site on the ribosome.

The model has some interesting features that could be investigated further. It is conceivable that the primary sequence of the *whole* relay-helix could influence the relay mechanism and thus modulate the interaction between the ribosome and the SRP, and, indeed, it has been shown that, in addition to the signal sequence, some 40–50 N-terminal residues of the *mature* lamB protein are required to initiate export and route a lamB–lacZ fusion protein to its correct destination [23]. It is

also conceivable that signal-sequence mutation suppressors might be found in those ribosomal proteins that line the ribosomal channel. Such suppressor mutations should be allele-specific, allowing a particular hydrophobic → charged amino acid replacement in the h-region to be compensated for by forming an energetically favorable salt-bridge to the ribosomal protein.

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