

The protein translocation machinery of the endoplasmic reticulum

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The rough endoplasmic reticulum (r.e.r.) has been postulated to possess a single translation-coupled translocation system (in multiple copies) that effects signal sequence-mediated translocation of all secretory and lysosomal proteins and integration of all integral membrane proteins whose port of entry is the rough endoplasmic reticulum (G. Blobel 1980 *Proc. natn. Acad. Sci. U.S.A.* **77**, 1496–1500). Two proteins have been isolated that are components of the r.e.r. translocation system. Their properties and function in protein translocation across and integration into membranes are discussed.

Substantial experimental data have recently been provided on the co-translational translocation of proteins across and integration into the endoplasmic reticulum. So far, two components have been purified from dog pancreas and shown to be required for this translocation process.

One of these is the so-called signal recognition particle (SRP), an 11S ribonucleoprotein (Walter & Blobel 1982*a*). SRP consists of six non-identical polypeptide chains (molecular masses 72, 68, 54, 19, 14 and 9 kDa) (Walter & Blobel 1980) and one molecule of 7S RNA (Walter & Blobel 1982*a*). The RNA has been identified by partial sequence analysis (Walter & Blobel 1982*a*) to be the previously described (Zieve & Penman 1976) and recently sequenced (Ullu *et al.* 1982; Li *et al.* 1982) small cytoplasmic 7S RNA (7S RNA, ScL). Both RNA and protein are required for SRP's activity. In dog pancreas at physiological salt concentration (150 mM potassium ions) the bulk of SRP appears to be about equally distributed between a membrane-bound and a free or ribosome/polysome-associated form (Walter & Blobel 1982*b*).

The other component, termed SRP receptor (Gilmore *et al.* 1982*a*), is a protein of molecular mass 72 kDa (Gilmore *et al.* 1982*b*; Meyer *et al.* 1982*b*) that has been purified from detergent-solubilized microsomal membranes by SRP-affinity chromatography (Gilmore 1982*b*). The SRP receptor is an integral membrane protein of the endoplasmic reticulum. It consists of a large cytoplasmic domain of molecular mass 60 kDa (Meyer & Dobberstein 1980*b*) that can be severed from the membrane in an intact form by treatment with a variety of proteases and can be added back to the proteolysed membranes to reconstitute activity (Gilmore *et al.* 1982*a*; Walter *et al.* 1979; Meyer & Dobberstein 1980*a*).

The function of these components in the protein translocation process was deduced from assay systems reconstituted *in vitro*. By using such assays, SRP was found to function in decoding the information contained in the signal peptide of nascent secretory (Walter *et al.* 1981; Stoffel *et al.* 1981; Muller *et al.* 1982), lysosomal (Erickson *et al.* 1982) and membrane (Anderson *et al.* 1982) proteins to the effect that it mediates the specific attachment of the translating ribosome to the microsomal membrane (Walter & Blobel 1981*a*). In the absence of microsomal membranes SRP specifically arrests the elongation of secretory protein synthesis *in vitro* (Walter *et al.* 1981) just after the signal peptide has emerged from the ribosome, thereby preventing the completion

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of pre-secretory proteins (many of which may be potentially harmful to the cell) (Walter & Blobel 1981*b*) in the cytoplasmic compartment. Upon interaction of these arrested ribosomes with a specific integral membrane protein, the SRP receptor (Gilmore *et al.* 1982*a*; Meyer *et al.* 1982*a*), on the microsomal membrane, this elongation arrest is released and the nascent chain is translocated across (Walter & Blobel 1981*b*) or – as in integral membrane proteins – integrated into (Anderson *et al.* 1982) the lipid bilayer.

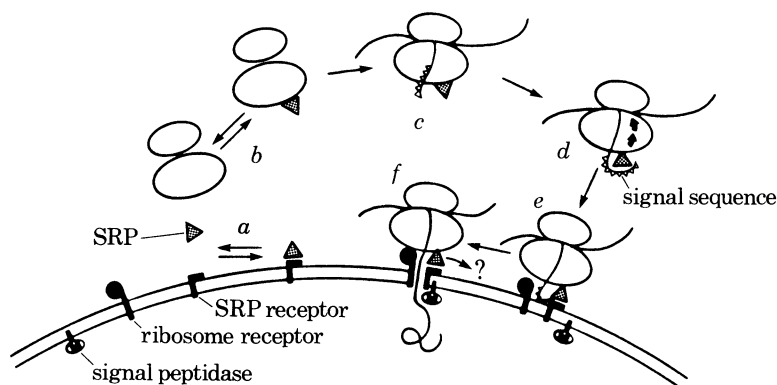


FIGURE 1. Model for co-translational protein translocation across the rough endoplasmic reticulum membrane. For details see text.

The drawing in figure 1 represents a model (taken from Walter & Blobel 1981*b*) illustrating schematically both facts and speculations about protein translocation across the rough endoplasmic reticulum (r.e.r.). It was proposed (Walter & Blobel 1981*b*, 1982*b*) that an equilibrium exists between a free, soluble form of SRP, SRP bound to ribosomes and SRP bound to the SRP receptor (figure 1*a*, *b*). Upon translation of an mRNA coding for a signal sequence (figure 1*c*) that is addressed to the r.e.r. translocation system and that is present in all secretory proteins, all lysosomal proteins and all those integral membrane proteins whose exclusive site of integration is the r.e.r., there is an enhancement of the apparent affinity of SRP for the translating ribosomes by several orders of magnitude (figure 1*d*). Concomitantly, and presumably through the ribosome, SRP arrests the elongation of the initiated polypeptide chain, preventing its completion in the cytoplasm. Translation arrest is released only upon interaction of the SRP arrested ribosome with the SRP receptor (figure 1*e*).

We have estimated (Gilmore *et al.* 1982*b*; Walter *et al.* 1981) that one equivalent of dog pancreas microsomal membranes contains approximately 500 fmol of bound ribosomes, approximately 20 fmol of SRP, and about 100 fmol of SRP receptor. Thus the content of both SRP and SRP receptor is less than that of bound ribosomes. This suggested (Walter & Blobel 1981*b*) that the ribosome–SRP–SRP-receptor interaction might be a transient one, merely targeting the SRP-arrested ribosome to a specific membrane site that is represented in part by the SRP receptor and in part by other integral membrane proteins. The latter could be represented by ribophorins I and II, which have been characterized by Kreibich & Sabatini and their coworkers (Kreibich *et al.* 1978*a*, *b*; Marcantonio *et al.* 1982). Once targeting has occurred, the ribosome–SRP–SRP-receptor interaction might then be replaced (figure 1*f*) by a direct interaction of the ribosomes with ribophorins I and II, an interaction that might persist for the entire chain translocation event. Ribophorins I and II have been reported each to be present

in microsomal membranes in stoichiometric amounts to those of membrane-bound ribosomes (Kreibich *et al.* 1978*a, b*). It should be noted, however, that Bielinska *et al.* (1979) have argued against the involvement of ribophorins in chain translocation. In any case it should be emphasized that these proposals suggesting a possible cascade in the formation of a productive ribosome-membrane junction and involving several integral membrane proteins are at this moment entirely speculative.

The ability of SRP to arrest elongation and the capacity of the SRP receptor to release the arrest might be of important regulatory significance. Modulation of the arrest-releasing activity either by other, as yet unidentified, components or by direct modification of SRP or the SRP receptor, or both, may provide the cell with an on-off switch for translocation-coupled protein synthesis and thereby provide a mechanism for a fast and regulatable response to a variety of physiological stimuli.

Both SRP and the mode of co-translational protein translocation seem to be highly conserved through evolution (Müller *et al.* 1982; Talmadge *et al.* 1980). SRP therefore appears to be an integral and indispensable component of the protein synthesis machinery of living cells assuring the correct topogenesis of a specific subset of proteins (Blobel 1980). Considering its structural features and its intimate (although most likely transient) functional association with ribosomes, it could almost be regarded as a 'third ribosomal subunit' functioning as the adaptor between the cytoplasmic translation and the membrane-bound protein translocation machinery.

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Discussion

P. N. CAMPBELL (*Courtauld Institute, The Middlesex Hospital, London, U.K.*). I should like to ask Professor Blobel's views on the significance of the hydrophobic property of the signal peptide. I realize that it interacts with a hydrophobic SRP but this is not of course an essential feature for the interaction of two proteins.

G. BLOBEL. At this moment we do not know what the precise requirements for the interaction between signal peptide and SRP are. In fact, a direct interaction between the signal peptide and SRP still remains to be demonstrated. This is why we have used the term 'recognition' rather than 'receptor' in naming SRP.