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Roles of Membrane Proteins in the Synthesis of Secretory Proteins

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Summary. **Secretory proteins are synthesized on membrane-bound ribosomes and translocated from the cytoplasmic to the luminal side of the rough endoplasmic reticulum membrane. A number of membrane proteins are essential for this protein translocation. In the present article, the molecular properties and functional roles of these membrane proteins are reviewed. On the basis of the results, an updated version of the scheme describing the translocating process is presented. The process consists of four steps: 1) elongation arrest; 2) targeting; 3) landing; and 4) translocation and processing.**

Introduction

Proteins destined to be secreted from a cell are synthesized on membrane-bound ribosomes and are vectorially translocated across the endoplasmic reticulum (ER) membrane.[1,2] This fact raises the question as to how the cell can distinguish mRNAs for such proteins from those for intracellular proteins such as cytoplasmic or mitochondrial proteins, and then selectively translate the former on membrane-bound ribosomes. The signal hypothesis[3] was proposed to account for this phenomenon. Over the last 20 years much evidence in favor of this model has been accumulated from numerous experimental systems. The essential doctrines of an updated version of this hypothesis (Fig. 1) include signal sequence-mediated targeting, cotranslational translocation and the processing of nascent polypeptide chains.[4-6] This review deals with the membrane proteins that play important roles in these processes.

Targeting process

Two components, the signal recognition particle (SRP) and the SRP receptor, have been shown to function in the targeting events preceding the actual translocation event. SRP receptor is an integral membrane protein, while SRP is a cytosolic ribonucleoprotein complex which is about equally distributed between membrane-associated (38%) and free (15%) or ribosome-associated (47%) states.[7]

Signal recognition particles

The process of targeting is initiated in the cytosol when SRP binds to a signal sequence in the emerging nascent polypeptide.[8,9] SRP was first recognized by its ability to restore the translocation activity of salt-extracted microsomes in the wheat germ cell-free translation system.[10] It was purified to homogeneity from a salt extract of canine pancreas microsomes using this activity as an assay.[11] SRP is composed of six distinct polypeptides (72, 68, 54, 19, 14 and 9 kDa, respectively) and one 7S RNA molecule (about 300 nucleotides), and has a sedimentation coefficient of 11S.[12] The six polypeptides are organized into four proteins,[13,14] two of which are monomeric (referred to as p54 and p19) and two of which are heterodimeric (referred to as p68/72 and p9/14). When SRP is disassembled under nondenaturing conditions, the RNA and the protein fractions are inactive by themselves, but together they can readily be reconstituted into an active particle.[13,14] One function of the RNA is to form a structural lattice.[14]

SRP interacts with the signal sequence after emerging from the ribosome, and arrests their further elongation.[15] The interaction of SRP with its receptor on the ER membrane then causes SRP displacement and a concomitant release of the elongation arrest.[9,16,17] These functions of SRP can be assigned to specific structural domains of the particle. The p9/14 is essential for elongation arrest[13] and

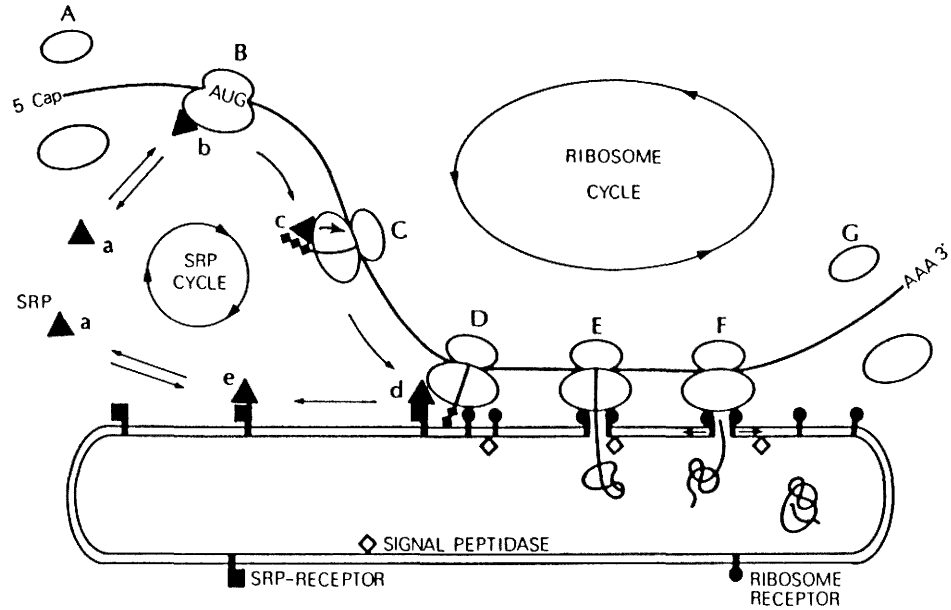

Fig 1. A modified version of the signal hypothesis by Walter et al.[5] on the translation-translocation of secretory proteins. SRP binds with relatively low affinity to inactive ribosomes (Fig. B). Upon the emergence of a signal sequence as part of the nascent polypeptide chain, SRP recognizes the signal sequence and arrests elongation within a certain range of the chain length (Fig. C). The SRP-ribosome-nascent chain complex is then targeted to the membrane of the ER via the direct interaction of SRP with the SRP receptor. SRP and its receptor detach from the ribosome and can reenter the cycle, and the elongation arrest is released (Fig. D). The ribosome-nascent chain complex attaches to the putative ribosome receptors, leading to the formation of a functional ribosome-membrane junction (Fig. D-E). The protein translocation occurs via the formation of a "pore-like" structure (Fig. E). The signal sequence is removed from the elongating chain by signal peptidase localized on the luminal side of the ER membrane. Upon termination of protein synthesis, the completed polypeptide is released into lumen of the ER (Fig. F).

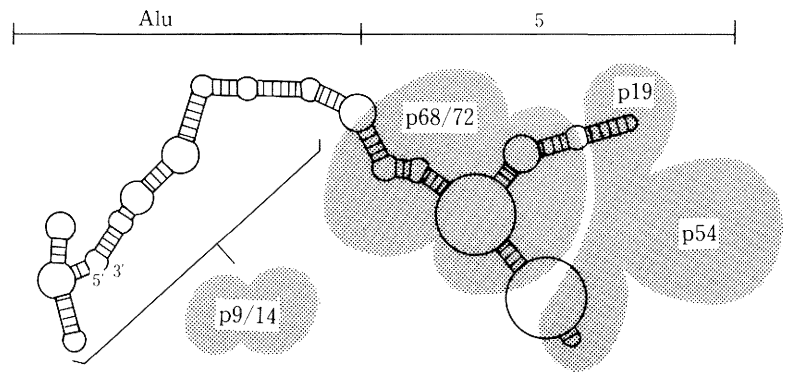

Fig 2. Model of the relative geometry of the protein domains in signal recognition particle (SRP) with respect to the RNA secondary structure.[24] The relative positions of p68/72 and p19 on the SRP secondary structure as determined by footprinting analysis are indicated. Binding of p54 is shown to occur through p19. The precise binding site of p9/14 has not been determined; however, it is clear from nucleolytic dissection studies of SRP that p9/14 binds to and largely protects the Alu end of SRP RNA. Activities assigned to the protein domains are indicated.

the p68/72 is required for docking to the ER membrane.[18] The association of p54 with SRP requires the presence of p19 that binds to 7S RNA directly.[18] The p19 exposes a binding site for p54 on the RNA. The p54 is also shown to bind to the signal sequence of nascent polypeptide chains by crosslinking experiments.[19-21] The methionine-rich carboxyl terminus (M-domain) of p54 contains an RNA binding site and can be crosslinked to a signal sequence.[22,23] These protein domains in SRP are schematically indicated in Fig. 2, as superimposed on the secondary structure of SRP RNA.[24] This model places the elongation arrest domain and the signal recognition domain at opposite ends of the particle. Since SRP is shown to be 24 nm long,[25] it is possible to block elongation by the entrance of p9/14 into the aminoacyl tRNA site 16 nm away from the nascent chain exit site.[24,26]

SRP receptor (docking protein)

Targeting occurs through the docking of the SRP-translation complex to a rough ER-specific SRP receptor-the docking protein.[16,27] The discovery and purification of the SRP receptor are based on observations that the protein translocation activity of microsomal membranes completely disappears by their limited proteolysis, but that the activity can be restored by the addition of an extract prepared by limited proteolysis of the original microsomal membrane.[28,29] This proteolytic dissection and functional reconstitution provided the assay for the purification of the protease-solubilized SRP receptor. The activity was purified as a basic 52-kDa protein (apparent mobility on SDS-PAGE is 60 kDa).[30] When assayed in the absence of microsomal membranes, SRP caused an elongation arrest in the synthesis of presecretory protein. The elongation arrest was released by the addition of microsomal membranes to the system. The SRP receptor was functionally defined as an activity residing in a microsomal membrane protein that would release the elongation arrest.[15] This activity was purified by SRP-Sepharose affinity chromatography and attributed to a 69-kDa ER membrane protein (apparent mobility is 72 kDa).[16]

The SRP receptor is composed of two subunits, termed SRα and SRβ. The primary structure of the SRα was determined from its cognate cloned cDNA.[31,32] SRα has a large cytoplasmic disposed fragment (52 kDa) that can be cleaved from the membrane by low concentrations of either trypsin or elastase.[16,30] This fragment was shown to begin with residue 152 of the intact protein.[31] The amino-terminal 151 amino acids (17.5 kDa) comprise the membrane-anchoring fragment of the SRα and the remaining 487 amino acids (52 kDa) comprise the cytoplasmic fragment of the SRα.[31] At the amino terminus of the molecule are two hydrophobic sequences, amino acids 1-22 and 64-79, believed to anchor the SRα in the lipid bilayer of the ER membrane. SRβ is a 30-kDa ER membrane protein that is copurified with SRα or isolated by affinity techniques, and forms a tight complex with SRα in detergent solution that is stable to high salt and can be immunoprecipitated with antibodies to either SRα or SRβ.[33] Both subunits are present in the ER membrane in equimolar amounts.[33] Thus the SRP receptor appears to be a heterodimeric protein that contains a second 30-kDa subunit (SRβ) in addition to the 69-kDa subunit (SRα). SRα does not assemble into the ER membrane via either a SRP-dependent process or hydrophobic insertion sequence. Instead, membrane assembly of SRα is receptor-mediated: requiring the presence in the membrane of another integral protein (probably SRβ) that mediates its targeting and insertion.[34,35]

Immunochemical studies have shown that the SRP receptor is restricted in its subcellular localization to the rough membrane of ER, and that there is one molecule of SRP receptor for roughly 10 to 20 bound ribosomes.[36,37] Thus it is suggested that the SRP receptor functions catalytically and is recycled once correct targeting of the ribosome has been achieved.[17] Another 30-kDa ER membrane protein (mp30) was copurified with SRα.[33] mp30 is present in the ER membrane in a several-fold molar excess of SRα. The affinity of mp30 to SRP-Sepharose suggests that it serves a yet unknown function in protein translocation.

Targeting

SRP is thought to bind in a signal-sequence-independent manner with relatively low affinity to biosynthetically inactive ribosomes.[9] Upon the emergence of a signal sequence as part of the nascent polypeptide chain, the affinity of SRP for the ribosome increases.[15] The p54 subunit of SRP binds to the signal sequence,[19,20] and elongation of the nascent chain is arrested by the binding of a SRP domain consisting of the p9/14 heterodimer and the Alu-like portion of the 7S RNA to the aminoacyl tRNA site on the ribosome.[6,25] The SRP-ribosome-nascent chain complex is then targeted to the membrane of the ER via the direct interaction of the p68/72 of SRP with the SRP receptor on the membrane.[18] The SRP lacking the elongation-arrest domain is still active in signal recognition and targeting.[38] There-

fore, elongation arrest cannot be a prerequisite for protein translocation across the membrane.[13] In the absence of elongation arrest, however, most signal-bearing nascent proteins lose their ability to be translocated if elongation proceeds beyond a critical point in the absence of membranes. Thus elongation arrest seems to maintain the nascent chain in a translocation-competent state by preventing (or delaying) its further elongation into the cytoplasmic space.[6]

On interaction with the SRP receptor, SRP is released from both the ribosome[17] and the signal sequence.[21] The SRP receptor-mediated displacement of SRP from the ribosome-bound nascent chain was shown to be GTP-dependent both with intact membrane and with the purified SRP receptor.[39] The SRα was shown to contain a version of the tripartite GTP-binding consensus sequence found in other GTP-binding proteins such as Tu, Gs and *ras* p21, and to be able to bind GTP by photoaffinity labelling and a GTP-binding assay to membrane-blotted SRα.[39] Cloning and sequencing of the p54 subunit of SRP revealed that SRP itself also contains a GTP-binding domain closely homologous to that in the SRα, suggesting that the GTP molecule is involved in signal recognition and decoding.[40,41] It is possible that the signal sequence, upon interaction of SRP with the SRP receptor, is translocated from the GTP-binding domain of the p54 subunit of SRP to the homologous domain of the SRα.[41] Experimental evidence argues against this hypothesis because no crosslinking of the signal sequence to SRα was observed.[21] This negative result, however, could still be explained by the transient nature of the interaction.[40]

Landing process

Two integral membrane proteins, the signal sequence receptor (SSR) and the ribosome receptor (RR), are involved in the landing event which includes interaction of a signal sequence with SSR and attachment of the ribosome to RR in the ER membrane.

Signal sequence receptor

Attachment of the arrested translation complex to the ER membrane is mediated by the SRP receptor and is accompanied by displacement of SRP from both the ribosome[17] and the signal sequence.[21] The signal sequence is then transferred into the close proximity of an integral, glycosylated 35-kDa membrane protein, called the signal sequence receptor (SSR).[42] Identification of the SSR was based on a photocrosslinking approach whereby a photoreactive lysine derivative was introduced into the signal sequence of nascent preprolactin and crosslinking to SSR was induced by irradiation.[20,42] The SSR was shown to be identical with a 34-kDa protein purified from canine pancreatic microsomes.[43] The SSR appears to be essential for protein translocation across the ER membrane as shown by the inhibitory action of antibodies directed against it and of monovalent Fab-fragments produced from them.[43] The ER membrane contains at least as many molecules of the 34-kDa membrane protein as bound ribosomes. The protein can be detected immunologically in tissues of various organisms, indicating a universal function.[43]

The primary structure of the SSR was deduced from cDNA clones and from direct protein sequencing.[44] The SSR is synthesized with a cleavable amino-terminal signal sequence and contains only one classiclal membrane-spanning segment (between residues 207 and 230). Its insertion into the ER membrane during biosynthesis depends on the function of the SRP. The amino terminus faces the lumen of the ER and the carboxyl terminus the cytosol. SSR can be phosphorylated in its cytoplasmic tail both in intact cells and in a cell-free system, suggesting a regulation of its function.[44] Further evidence revealed that the crosslinking to the SSR is induced not only to the signal sequence but also to the longer fragments of nascent preprolactin.[45] Thus it is possible that SSR is a constituent of a protein environment of the nascent chain as it is transferred through the membrane. Other workers[46] identified a signal sequence binding protein (45 kDa) that has 30-kDa domain integrated into the rough ER membrane and is capable of binding a signal sequence.

Ribosome receptor

Ribosomes are bound to the ER membrane via the nascent polypeptide chain as well as by a salt-labile interaction.[47] Such binding is saturable, sensitive to proteases, and specific for the large (60S) ribosomal subunit.[48,49] Using a recombination system consisting of labelled ribosomes and stripped rough ER membrane, several pieces of evidence have suggested the presence of a specific proteinaceous receptor for ribosomes in the membrane of rough ER.[4] Two integral membrane glycoproteins, ribophorins I (65 kDa) and II (63 kDa) in rat liver rough microsomes, were proposed as ribosome receptors on the basis of several indirect lines of evidence.[50,51] However, ribophorins are not directly involved in ribosome binding since the capacity of ribosome-stripped microsomal membrane to rebind ribosomes in vitro can be abolished by a mild treatment of the microsomes with

proteases, a treatment which does not degrade the ribophorins.[52] Moreover, liposomes containing lecithin and only the nonglycoprotein components of rough microsomes were shown to be capable of binding ribosomes under the same conditions.[53]

Recently an integral 34-kDa membrane nonglycoprotein (p34) was identified as a ribosome receptor in the rough ER of rat liver from the following evidence.[54] On mild proteolysis of the liposomes[55] containing a membrane nonglycoprotein fraction, the p34 underwent selective digestion in parallel with the loss of ribosome-binding activity of the liposomes. The direct interaction between the p34 and the 60S ribosomal subunits was indicated by thiol-cleavable photocrosslinking.[55] Furthermore, the liposomes containing only purified p34 were shown to have the ribosome-binding activity. The p34 was restricted to the rough ER and nuclear membranes in the liver cells. The rough ER membrane contained at least as many molecules of the p34 as the bound ribosomes.[56] The p34 can be detected by immunological means in various tissues of different animals, indicating its universal function.[56] The anti p34 antiserum inhibited reassociation of ribosomes to stripped rough microsomes (the author et al., unpublished).

An integral membrane protein of relative molecular mass (180 kDa) has been identified as a ribosome receptor in the rough ER of the canine pancreas.[57] The protein presents itself only in the rough ER membrane, having a large (at least 160 kDa) cytosolic domain that mediates the binding of ribosomes. The liposomes containing the purified 180-kDa protein indicate the capacity of binding ribosomes and have a dissociation constant that is close to those obtained for ribosome binding to microsomal membranes.

Landing

On interaction with the SRP receptor, SRP is released from both the ribosome[17] and the signal sequence.[21] Subsequently, the signal sequence transferres via SRα or directly to SSR. At the same time, the 60S subunit of the ribosome carrying the nascent polypeptide chain attaches to the ribosome receptor on ER membrane. The elongation of the nascent polypeptide chain resumes.[16,27] The interaction between the ribosome and its receptor on the ER membrane is specific and of a salt-labile nature, and it represents the affinity constant of the order of $1\text{-}3\times10^{8}M^{-1}$.[48,53,57] The SSR is reported to be phosphorylated in its cytosolic tail.[44] The significance of the phosphorylation in landing process remains to be determined.

Translocation and processing

Very little is known about the mechanism and components involved in the translocating process of the nascent polypeptide chain across the membrane of ER. The signal hypothesis[58] supposes translocation through complex protein pores in the ER membrane, in combination with the processing of the precursor into its nature form. Other models[59,60] assume protein translocation to be a spontaneous process without the requirement of specific membrane receptors or transport proteins. In these models the structure of the protein (as modulated by the interaction with lipids) plays an important role in the insertion and translocating process. There are some recent data on precursor-lipid interaction which suggest that the lipid component of the membrane plays an essential role in protein translocation.[61] Recent data demonstrate a requirement for energy (derived from hydrolysis of nucleotide triphosphates or from an electrochemical gradient) to drive posttranslational protein translocation across mitochondrial membranes[62,63] and also canine and yeast ER membranes.[64,65] These findings may rule out spontaneous protein translocation across the membrane, although it remains to be established how the energy of hydrolysis is used by the translocating machinery.

On the other hand, alkylating reagent N-ethylmaleimide is shown to be capable of inhibiting translocation (beyond the SRP-docking protein-mediated recognition step), but has no affect on the ability of ribosomes to bind to the ER membrane.[52] Since the signal sequence receptor comprises no cysteinyl residue,[44] it appears that N-ethylmaleimide-sensitive proteins, as yet unidentified, are involved in protein translocation. Moreover, evidence has been reported that the partially translocated nascent chain can be extracted by 4M urea, suggesting that the initial interaction of the signal sequence with the membrane as well as subsequent chain conductance occurs in a microenvironment that is accessible to aqueous reagents.[66]

The molecular environment of a nascent polypeptide chain during translocation across the ER membrane was also examined by photocrosslinking.[67] Nascent preprolactin chains of various lengths, synthesized by *in vitro* translation of truncated messenger RNAs in the presence of ANB-Lys-tRNA, were used to position photoreactive probes at various locations within the membrane. Upon photolysis, each nascent chain species was crosslinked to a 39-kDa integral membrane glycoprotein (mp39).[67] Thus, different portions of the nascent preprolactin

chain are in close proximity to the same membrane protein during the course of translocation. Therefore, the mp39 appears to be part of a putative translocation tunnel. Furthermore, the similarity of the molecular and crosslinking properties of mp39 and the 34-kDa glycoprotein that was previously identified as a signal sequence receptor[43] suggests that these two proteins may be identical.[67]

Recently, bifunctional crosslinking reagents were used to prove the protein environment in the ER membrane of the 34-kDa SSR. A 22-kDa glycoprotein was identified as a second subunit tightly bound to the 34-kDa SSR even after membrane solubilization.[68] The 34-kDa polypeptide, termed αSSR, and the 22-kDa polypeptide, the βSSR, represent a heterodimer.[68] Nascent chains of preprolactin and α-lactamase at different stages of their translocation through the membrane produced crosslinked products with the αSSR in high yields, indicating that it is a major membrane protein in the neighborhood of translocating nascent chains of secretory proteins.[68]

Signal peptidase catalyzes the endoproteolytic cleavage of a signal sequence from a nascent polypeptide chain during the process of translocation.[3,69] The purified enzyme consists of a complex of six polypeptides with an apparent molecular mass of 25, 23, 22, 21, 18, and 12 kDa and, in contrast to SSR, is lumenally oriented.[70-72] The 22- and 23-kDa subunits are shown to be glycoproteins. It appears likely that only one subunit of this complex carries out signal peptide cleavage. The structural association of the other subunits in stoichiometric amounts may reflect requirements besides signal cleavage in their chain translocation across the ER membrane.[71]

Final discussion

Membrane protein components related to the translocation of a nascent polypeptide chain across the ER membrane are listed in Table 1. On the basis of the results reviewed here, the total process of the translocation of nascent polypeptide chain across the ER membrane is schematically indicated in Fig. 3. A signal sequence after emerging from the ribosome is recognized by SRP through its p54 subunit and when

Table 1. Membrane protein components related to the translocation of newly synthesized proteins across the ER membrane

Component		Molecular size	Function	Reference
Signal recognition particle[a] (SRP)		p68/72(dimer)	Targeting to SRP receptor	(18)
		p54	Signal sequence recognition	(19, 23)
		p19	Assisting p54 in RNA binding	(18)
		p9/14(dimer)	Elongation arrest (ribosome binding)	(13)
		7SL RNA	Structural lattice	(14)
SRP receptor (docking protein)	SRα	69 kDa	Receptor for p68/72 of SRP	(15, 27)
	SRβ	30 kDa	Receptor for SRα	(33, 35)
Mp30		30 kDa	Unknown (showing affinity to SRP)	(33)
Signal sequence receptor (SSR)		34 kDa[g]	Signal sequence binding & translocation tunnel	(43, 45)
		45 kDa	Signal sequence binding	(46)
Mp39 (probably identical to 34-kDa SSR)		39 kDa[g]	Signal sequence binding & translocation tunnel	(67)
Ribosome receptor (RR)		34 kDa	Ribosome binding (rat liver)	(54)
		180 kDa	Ribosome binding (canine pancreas)	(57)
Signal peptidase		25, 23[g], 22[g], 21, 18, 12 kDa (comlex)	Cleavage of signal sequence & translocation tunnel (?)	(71)
Ribophorins	I	65 kDa[g]	Unknown	(50, 73)
	II	63 kDa[g]	Unknown	

a: non-membranous component g: glycoprotein

elongation of the nascent polypeptide chain is arrested (Fig. 3-1). On interaction of SRP with its receptor SRα (Fig. 3-2), the SRP is released both from the ribosome and from the signal sequence (Fig. 3-3). The signal sequence is then transferred to the SSR (Fig. 3-3). It seems that GTP plays an essential role in this process. The ribosome attaches to the membrane via a direct interaction of the ribosome with the RR (Fig. 3-3). Elongation of the nascent polypeptide chain resumes, and the elongating polypeptide chain traverses the membrane (Fig. 3-4). The signal sequence is removed from the elongating chain by signal peptidase, and the elongating chain is extruded into the lumen of the ER to form a competent conformation (Fig. 3-4). Since it is likely that SSR and RR are present in stoichiometric amounts with bound ribosomes, they may be assured to play a role during the actural translocating process. It is possible that αSSR, βSSR, RR and signal peptidase complex are constituents of the translocating machinery, "translocon".[6]

Concluding remarks

The major conclusion which can be drawn from this review is that a significant lack exists in our understanding of those proteins which play essential roles in the translocation of newly synthesized proteins

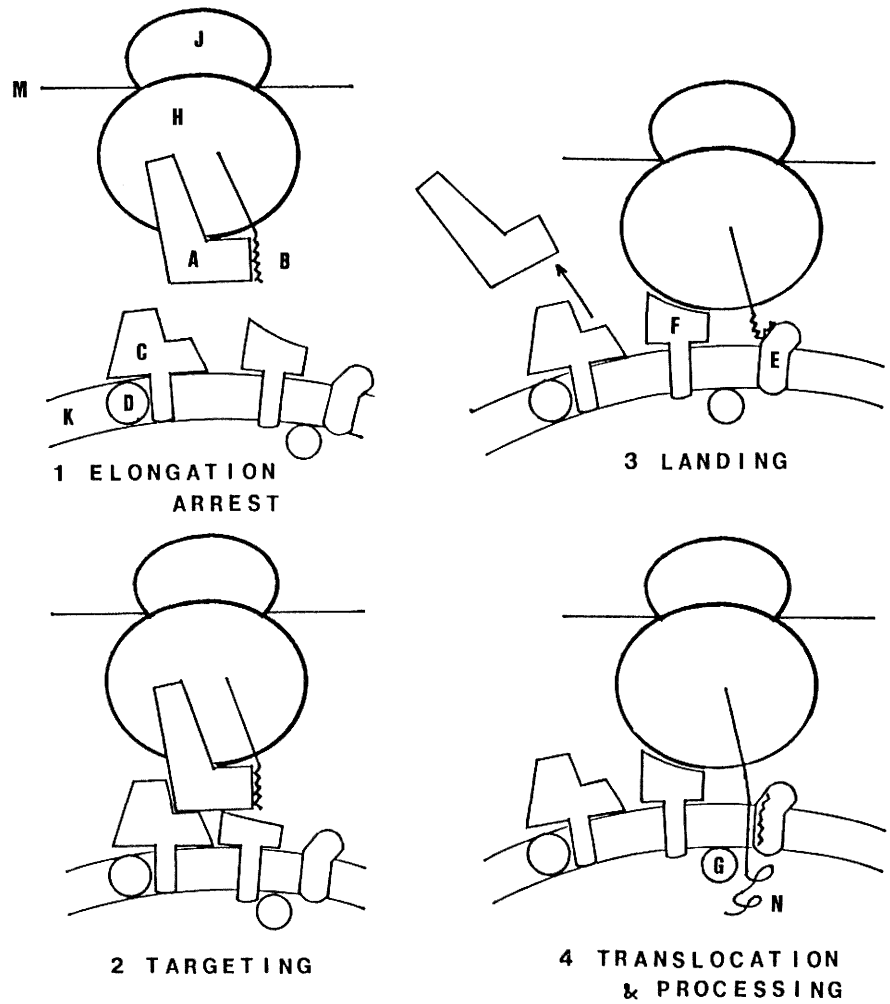

Fig 3. Scheme of translocating process of a nascent polypeptide across the ER membrane. This process, consisting of four steps, is described in detail in the text. A, signal recognition particle (SRP); B, signal sequence emerging from ribosome; C, α-subunit of SRP receptor (SRα); D, β-subunit of SRP receptor (SRβ); E, signal sequence receptor (SSR); F, ribosome receptor (RR); G, signal peptidase complex; H, 60S ribosomal subunit; J, 40S ribosomal subunit; K, membrane of the ER; M, messenger RNA; N, elongating polypeptide chain after cleavage of the signal sequence.

across the ER membrane. There are two fundamental problems to resolve regarding translocation across the ER membrane: 1) How can a ribosome-nascent chain complex recognize the ER membrane? 2) How is a polypeptide chain translocated across lipid bilayer into the lumen of ER membrane? A novel approach for the problem described in (1) has been advanced recently by the concept of signal sequence-mediated process. Our understanding of problem (2) is now very deficient. The most challenging problems include further fractionation and purification of all the essential components involved in the targeting and translocating processes. Our goal must be to reconstitute the essential components into proteoliposomes that have the function of targeting and translocating a newly synthesized protein.

REFERENCES

1) Palade G: Intracellular aspects of the process of protein synthesis. *Science (Wash. DC)* **189**: 347-358, 1975.
2) Sabatini DD, Kreibich G: Functional specialization of membrane-bound ribosomes in eukaryotic cells. In: Martonosi A (ed) the Enzymes of Biological Membranes. vol. 2 Plenum Press, New York, 1976, p 531-579.
3) Blobel G, Dobberstein B: Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J Cell Biol* **67**: 835-851, 1975.
4) Hortsch M, Meyer DI: Transfer of secretory proteins through the membrane of the endoplasmic reticulum. *Int Rev Cytol* **102**: 215-242, 1986.
5) Walter P, Gilmore R, Blobel G: Protein translocation across the endoplasmic reticulum. *Cell* **38**: 5-8, 1984.
6) Walter P, Lingappa VR: Mechanism of protein translocation across the endoplasmic reticulum membrane. *Ann Rev Cell Biol* **2**: 499-516, 1986.
7) Walter P, Blobel G: Subcellular distribution of signal recognition particle and 7SL-RNA determined with polypeptide-specific antibodies and complementary DNA probe. *J Cell Biol* **97**: 1693-1699, 1983.
8) Walter P, Ibrahimi I, Blobel G: Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to *in vitro* assembled polysomes synthesizing secretory proteins. *J Cell Biol* **91**: 545-550, 1981.
9) Walter P, Blobel G: Translocation of proteins across the endoplasmic reticulum. II. Signal recognition protein (SRP) mediates the selecting binding to microsomal membranes of *in vitro* assembled polysomes synthesizing secretory proteins. *J Cell Biol* **91**: 551-556, 1981.
10) Warren G, Dobberstein B: Protein transfer across microsomal membranes reassembled from separated membrane components. *Nature (Lond.)* **273**: 569-571, 1978.
11) Walter P, Blobel G: Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc Natl Acad Sci USA* **77**: 7112-7116, 1980.
12) Walter P, Blobel G: Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature* **299**: 691-698, 1982.
13) Siegel V, Walter P: Elongation arrest is not a prerequisite for secretory protein translocation across the microsomal membrane. *J Cell Biol* **100**: 1913-1921, 1985.
14) Walter P, Blobel G: Disassembly and reconstitution of signal recognition particle. *Cell* **34**: 525-533, 1983.
15) Walter P, Blobel G: Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence dependent and site specific arrest of chain elongation that is released by microsomal membranes. *J Cell Biol* **91**: 557-561, 1981.
16) Gilmore R, Walter P, Blobel G: Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J Cell Biol* **96**: 470-477, 1982.
17) Gilmore R, Blobel G: Transient involvement of signal recognition particle and its receptor in the microsomal membrane prior to protein translocation. *Cell* **35**: 677-685, 1983.
18) Siegel V, Walter P: Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. *Cell* **52**: 39-49, 1988.
19) Krieg UC, Walter P, Johnson AE: Photocrosslinking of the signal sequence of nascent preprolactin to the 54-kilodalton polypeptide of the signal recognition particle. *Proc Natl Acad Sci USA* **83**: 8604-8608, 1986.
20) Kurzchalia TV, Wiedmann M, Girshovich AS, Bochkareva ES, Bielka H, Rapoport TA: The signal sequencce of nascent preprolactin interacts with the 54k polypeptide of the signal recognition particle. *Nature* **320**: 634-636, 1986.
21) Wiedmann M, Kurzchalia TV, Bielka H, Rapoport TA: Direct probing of the interaction between the signal sequence of nascent preprolactin and the signal recognition particle by specific cross-linking. *J Cell Biol* **104**: 201-208, 1987.
22) Roemisch K, Webb J, Lingelbach K, Gausepohl H, Dobberstein B: The 54-kD protein of signal recognition particle contains a methionine-rich RNA binding domain. *J Cell Biol.* **111**: 1793-1802, 1990.
23) Zopf D, Bernstein HD, Johnson AE, Walter P: The methionine-rich domain of the 54-kD protein subunit

of the signal recognition particle contains an RNA binding site and can be crosslinked to a signal sequence. *EMBO J* **9**: 4511-4517, 1990.
24) Siegel V, Walter P: Binding sites of the 19-kDa and 68/72-kDa signal recognition particle (SRP) proteins on SRP RNA as determined by protein-RNA "footprinting". *Proc Natl Acad Sci USA* **85**: 1801-1805, 1988.
25) Andrews DW, Walter P, Ottensmeyer FP: Structure of the signal recognition particle by electron microscopy. *Proc Natl Acad Sci USA* **82**: 785-789, 1985.
26) Bernabeau C, Tobin EM, Fowler A, Zabin I, Lake JA: Nascent polypeptide chains exit the ribosome in the same relative position in both eucaryotes and procaryotes. *J Cell Biol* **96**: 1471-1474, 1983.
27) Meyer DI, Krause E, Dobberstein B: Secretory protein translocation across membrane: The role of the "docking protein". *Nature* **297**: 647-650, 1982.
28) Meyer DI, Dobberstein B: A membrane component essential for the vectorial translocation of nascent proteins across the endoplasmic reticulum: requirements for its extraction and reassociation with the membrane. *J Cell Biol* **87**: 498-502, 1980.
29) Walter P, Jackson RC, Marcus MM, Lingappa VR, Blobel G: Tryptic dissection and reconstitution of translocation activity for nascent presecretory proteins across microsomal membranes. *Proc Natl Acad Sci USA* **76**: 1795-1799, 1979.
30) Meyer DI, Dobberstein B: Identification and characterization of a membrane component essential for the translocation of nascent secretory proteins across the membrane of the endoplasmic reticulum. *J Cell Biol* **87**: 503-508, 1980.
31) Lauffer L, Garcia PD, Harkins RN, Coussens L, Ulrich A, Walter P: Topology of a signal recognition particle receptor in endoplamic reticulum membrane. *Nature* **318**: 334-338, 1985.
32) Hortsch M, Labeit S, Meyer DI: Complete cDNA sequence coding for human docking protein. *Nucleic Acid Res* **16**: 361-362, 1988.
33) Tajima S, Lauffer L, Rath VL, Walter P: The signal recognition particle receptor is a complex that contains two distinct polypeptide chains. *J Cell Biol* **103**: 1167-1178, 1986.
34) Andrews DW, Lauffer L, Walter P, Lingappa VR: Evidence for a two-step mechanism involved in assembly of functional signal recognition particle receptor. *J Cell Biol* **108**: 797-810, 1989.
35) Hortsch M, Meyer DI: The human docking protein does not associate with the membrane of the rough endoplasmic reticulum via a signal or insertion sequence-mediated mechanism. *Biochem Biophys Res Commun* **150**: 111-117, 1988.
36) Hortsch M, Meyer DI: Immunochemical analysis of rough and smooth microsomes from rat liver: segregation of docking protein in rough membranes. *Eur J Biochem* **150**: 559-564, 1985.
37) Hortsch M, Griffiths G, Meyer DI: Restriction of docking protein to the rough endoplasmic reticulum: immunocytochemical localization in rat liver. *J Cell Biol* **38**: 271-279, 1985.
38) Siegel V, Walter P: Removal of the Alu structure domain from signal recognition particle leaves its protein translocation activity intact. *Nature* **320**: 81-84, 1986.
39) Connolly T, Gilmore R: The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell* **57**: 599-610, 1989.
40) Bernstein HD, Poritz MA, Strub K, Hoben PJ, Brenner S, Walter P: Model for signal sequence recognition from amino-acid sequence of 54k subunit of signal recognition particle. *Nature* **340**: 482-486, 1989.
41) Roemisch K, Webb J, Herz J, Prehn S, Frank R, Vingron M, Dobberstein B: Homology of 54k protein of signal-recognition particle, docking protein and two E. coli proteins with putative GTP-binding domains. *Nature* **340**: 478-482, 1989.
42) Wiedmann M, Kurzchalia TV, Hartmann E, Rapoport TA: A signal sequence receptor in the endoplasmic reticulum membrane. *Nature* **328**: 830-833, 1987.
43) Hartmann E, Wiedmann M, Rapoport TA: A membrane component of the endoplasmic retitulum that may be essential for protein translocation. *EMBO J* **8**: 2225-2229, 1989.
44) Prehn S, Herz J, Hartmann E, Kurzchalia TV, Frank R, Roemisch K, Dobberstein B, Rapoport TA: Structure and biosynthesis of the signal-sequence receptor. *Eur J Biochem* **188**: 439-445, 1990.
45) Wiedmann M, Goerlich D, Hartmann E, Kurzchalia TV, Rapoport TA: Photocrosslinking demonstrates proximity of a 34 kDa membrane protein to different portions of preprolactin during translocation through the endoplasmic reticulum. *FEBS Lett* **257**: 263-268, 1989.
46) Robinson A, Kaderbhai MA, Austen B: Identification of signal sequence binding proteins integrated into the rough endoplasmic reticulum membrane. *Biochem J* **242**: 767-777, 1987.
47) Adelman MR, Sabatini DD, Blobel G: Ribosome-membrane interaction. Nondestructive disassembly of rat liver rough microsomes into ribosomal and membranous components. *J Cell Biol* **56**: 206-229, 1973.
48) Borgese N, Mok W, Kreibich G, Sabatini DD: Ribosome-membrane interaction: *in vitro* binding of ribosomes to microsomal membranes. *J Mol Biol* **88**: 559-580, 1974.
49) Sabatini DD, Tashiro Y, Palade GE: On the attachment of ribosomes to microsomal membranes. *J Mol Biol* **19**: 503-524, 1966.
50) Kreibich G, Ulrich BL, Sabatini DD: Proteins of rough microsomal membranes related to ribosome binding. I. Identification of ribophorins I and II,

membrane proteins characteristic of rough microsomes. *J Cell Biol* **77**: 464-487, 1978.
51) Kreibich G, Freienstein CM, Pereyra BN, Ulrich BL, Sabatini DD: Proteins of rough microsomal membranes related to ribosome binding. II. Cross-linking of bound ribosomes to specific membrane proteins exposed at the binding sites. *J Cell Biol* **77**: 488-506, 1978.
52) Hortsch M, Avossa D, Meyer DI: Characterization of secretory protein translocation: Ribosome-membrane interaction in endoplasmic reticulum. *J Cell Biol* **103**: 241-253, 1986.
53) Yoshida H, Tondokoro N, Asano Y, Mizusawa K, Yamagishi R, Horigome T, Sugano H: Studies on membrane proteins involved in ribosome binding on the rough endoplasmic reticulum. *Biochem J* **245**: 811-819, 1987.
54) Tazawa S, Unuma M, Tondokoro N, Asano Y, Ohsumi T, Ichimura T, Sugano H: Identification of a membrane protein responsible for ribosome binding in rough microsomal membranes. *J Biochem* **109**: 89-98, 1991.
55) Yamaguchi M, Sakai M, Horigome T, Omata S, Sugano H: Reconstitution into liposomes of membrane proteins involved in ribosome binding on rough endoplasmic reticulum: Ribosome-binding capacity. *Biochem J* **194**: 907-913, 1981.
56) Shindo Y, Ohsumi T, Ichimura T, Sugano H: Studies on ribosome binding proteins on the rough ER membrane of rat liver: Isolation of the p34 and its immunochemical localization in cells and in animal tissues. *Seikagaku* **62**: 821, 1990. (in Japanese)
57) Savitz AJ, Meyer DI: Identification of a ribosome receptor in the rough endoplasmic reticulum. *Nature* **346**: 540-544, 1990.
58) Blobel G: Intracellular protein topogenesis. *Proc Natl Acad Sci USA* **77**: 1496-1500, 1980.
59) Engelman DM, Steiz TA: The spontaneous insertion of proteins into and across membranes: The helical hairpin hypothesis. *Cell* **23**: 411-422, 1981.
60) Wickner W: Assembly of proteins into membranes. *Science* **210**: 861-868, 1980.
61) Rietveld A, Kruijff B: Phospholipids as a possible instrument for translocation of nascent proteins across biological membranes. *Bioscience Rep* **6**: 775-782, 1986.
62) Eilers M, Schatz G: Protein unfolding and the energetics of protein translocation across biological membranes. *Cell* **52**: 481-483, 1988.
63) Pfanner N, Neupert W: Transport of proteins into mitochondria: a potassium diffusion potential is able to drive the import of ADP/ATP carrier. *EMBO J* **4**: 2819-2825, 1985.
64) Perara E, Rothman JE, Lingappa VR: Uncoupling translocation from translation: Implication for transport of proteins across membranes. *Science* **232**: 348-352, 1986.
65) Waters MG, Blobel G: Secretory protein translocation in a yeast cell-free system can occur posttranslationally and requires ATP hydrolysis. *J Cell Biol* **102**: 1543-1550, 1986.
66) Gilmore R, Blobel G: Translocation of secretory proteins across the microsomal membrane occurs through an environment accessible to aqueous perturbants. *Cell* **42**: 497-505, 1985.
67) Krieg U, Johnson AE, Walter P: Protein translocation across the endoplasmic reticulum membrane: Identification by photocross-linking of a 39-kD integral membrane glycoprotein as part of a putative translocation tunnel. *J Cell Biol* **109**: 2033-2043, 1989.
68) Goerlich D, Prehn S, Hartmann E, Herz J, Otto A, Kraft R, Wiedmann M, Knespel S, Dobberstein B, Rapoport TA: The signal sequence receptor has a second subunit and is part of a translocation complex in the endoplasmic reticulum as probed by bifunctional reagents. *J Cell Biol* **111**: 2283-2294, 1990.
69) Mollay C, Vilas U, Kreil G: Cleavage of honeybee prepromelittin by an endoproteinase from rat liver microsomes: Identification of intact signal peptide. *Proc Natl Acad Sci USA* **79**: 2260-2263, 1982.
70) Baker RK, Lively MO: Purification and characterization of hen oviduct microsomal signal peptidase. *Biochemistry* **26**: 8561-8567, 1987.
71) Evans EA, Gilmore R, Blobel G: Purification of microsomal signal peptidase as a complex. *Proc Natl Acad Sci USA* **83**: 581-585, 1986.
72) Shelness G, Kanwar Y, Blobel G: cDNA-derived primary structure of the glycoprotein component of canine microsomal signal peptidase complex. *J Biol Chem* **263**: 17063-17070, 1988.
73) Yu Y, Sabatini DD, Kreibich G: Antiribophorin antibodies inhibit the targeting to the ER membrane of ribosomes containing nascent secretory polypeptides. *J Cell Biol* **111**: 1335-1342, 1990.