Protein targeting to and translocation across the membrane of the endoplasmic reticulum

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Several approaches are currently being taken to elucidate the mechanisms and the molecular components responsible for protein targeting to and translocation across the membrane of the endoplasmic reticulum. Two experimental systems dominate the field: a biochemical system derived from mammalian exocrine pancreas, and a combined genetic and biochemical system employing the yeast, *Saccharomyces cerevisiae*. Results obtained in each of these systems have contributed novel, mostly non-overlapping information. Recently, much effort in the field has been dedicated to identifying membrane proteins that comprise the translocon. Membrane proteins involved in translocation have been identified both in the mammalian system, using a combination of crosslinking and reconstitution approaches, and in *S. cerevisiae*, by selecting for mutants in the translocation pathway. None of the membrane proteins isolated, however, appears to be homologous between the two experimental systems. In the case of the signal recognition particle, the two systems have converged, which has led to a better understanding of how proteins are targeted to the endoplasmic reticulum membrane.

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Introduction

In eukaryotic cells, the first step in the biogenesis of proteins destined to be secreted and lumenal proteins that are residents of the secretory pathway is the targeting and translocation of these proteins across the membrane of the endoplasmic reticulum (ER). The ER is also the site for the integration of membrane proteins that comprise the plasma membrane and other intracellular membranes of the secretory and endocytic pathways. These proteins are initially synthesized on ribosomes in the cytosol of the cell and are selectively targeted to the ER. Targeting to the ER is specified by a signal sequence contained within the polypeptide chain, usually found at the amino terminus of the protein.

In higher eukaryotes, the vast majority of proteins are targeted to the ER in a obligatory cotranslational, ribosome-dependent manner. A cytoplasmic ribonucleoprotein, termed signal recognition particle (SRP), binds to the signal sequence as it emerges from the ribosome causing an arrest or pause in the elongation of the nascent polypeptide. This pause may extend the time in which the nascent chain can be productively targeted to the ER membrane. Targeting of the ribosome–nascent chain–SRP complex to the ER membrane is mediated by the specific interaction of SRP with the ER membrane heterodimeric protein complex, the SRP receptor (SR, comprised SRα and SRβ subunits). Once SRP interacts with its receptor, the signal sequence dissociates from SRP and elongation arrest is released. Upon release from SRP, the nascent chain inserts into and becomes tightly associated with the ER membrane via interactions with components of the machinery that mediate the translocation of the polypeptide across the membrane, collectively referred to as the translocon.

Following nascent chain insertion, translocation of the nascent chain proceeds through a protein conducting channel across the ER membrane and into the lumen. Interaction of nascent proteins with BiP, a member of the heat-shock protein 70 family that resides in the lumen of the ER, facilitates the native folding and assembly of proteins. During co-translational translocation of nascent polypeptides, enzymes present in the membrane and lumen of the ER modify the polypeptide chain. ER-specific signal sequences are cleaved by signal peptidase, oligosaccharides are covalently attached to the nascent chain by oligosaccharyl transferase and disulfide isomerase catalyzes disulfide bond formation. These modifications to the polypeptide chain are important for the proper folding of the protein and the enzymes that catalyze these modifications may possibly be involved in the process of nascent chain translocation.

Abbreviations

ER—endoplasmic reticulum; **SR**—signal recognition particle receptor; **SRP**—signal recognition particle; **SSR**—signal sequence receptor; **TRAM**—translocating-chain-associating membrane protein.

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This review encompasses the recent advances made in understanding the mechanisms and components responsible for the specific targeting and translocation of proteins across the ER membrane.

Signal recognition, targeting and insertion of pre-proteins into the ER membrane

Mammalian SRP is composed of six polypeptides (72, 68, 54, 19, 14 and 9 kD) and a 7SL RNA molecule. SRP mediates three distinct functional activities: signal recognition, elongation arrest and translocation promotion [1]. These activities are contained within three separate structural domains of the particle. Elongation arrest requires the presence of the 9 and 14 kD proteins, which form a heterodimer that binds to the Alu domain of 7SL RNA [2•]. The 68 and 72 kD proteins also form a heterodimeric RNA-binding complex and are necessary for the interaction of SRP with the ER membrane and the SR [2•]. Investigation of the mechanism of assembly of SRP *in vitro* has shown that the binding of the 9/14 kD and the 68/72 kD heterodimers to 7SL RNA is non-cooperative in the absence of the 54 and 19 kD subunits of SRP [3•]. The 19 kD protein binds to 7SL RNA and is required for the binding of the 54 kD subunit (SRP54) to SRP [4,5•]. SRP54 probably binds directly to a region in 7SL RNA that has been identified as a phylogenetically conserved motif characteristic of SRP RNAs [6]. Photocrosslinking studies have demonstrated that SRP54 specifically binds to the signal sequence of secretory proteins and signal-anchor sequences in nascent integral membrane proteins [7,8,9•].

Insight into the mechanism of signal sequence recognition was gained when the gene encoding SRP54 was cloned [10,11]. From the deduced amino acid sequence, SRP54 is predicted to contain three domains: an amino-terminal domain of unknown function, followed by a GTPase domain, which contains the consensus sequence motifs for GTP binding. These two domains will be collectively referred to as the G-domain. SRP54 also contains a methionine-rich domain, termed the M-domain. The M-domain is proposed to contain a signal-sequence-binding pocket lined with methionine residues that accommodates diverse signal sequences because of their flexibility [11]. Limited proteolysis confirmed the domain boundary between the G- and M-domains [12]. Photocrosslinking studies demonstrated that the signal sequence binding site of SRP54 is contained in the M-domain which, in addition, contains an RNA-binding site [12–14]. As a free protein, SRP54 can bind signal sequences [15••]. Biochemical dissection of free SRP54 demonstrated that the M-domain of SRP54 alone is sufficient to recognize and bind signal sequences. Upon cell fractionation, all SRP54 is found complexed in SRP (D Zopf and PW, unpublished data). Thus, it is unlikely that free SRP54 plays any role in targeting or translocation.

Experimental evidence suggests that the M- and G-domains of SRP54 physically interact [15••]. Alkylation of the G-domain inhibits binding of the signal sequence to the M-domain, which can be reversed by proteolytic removal of the alkylated G-domain. These findings also suggest that, although the GTPase domain does not bind signal sequences, it may modulate the binding of signal sequences to the M-domain. This prediction is consistent with the fact that GTP is required in the targeting and translocation pathway.

To date, three components involved in protein translocation, SRP54, SRα and SRβ, are members of the GTPase superfamily and have been shown to bind GTP ([10,11,16,17]; J Miller, P Walter, unpublished data). SRP54 and SRα form a unique subfamily of GTPases. The sequence similarities between SRP54 and SRα suggest that these proteins were derived from a common ancestor [10,11]. SRβ is not closely related to other GTPases by sequence and is also unique among GTPases as it contains an amino-terminal transmembrane domain (J Miller, P Walter, unpublished data).

Following the targeting of the ribosome–nascent chain–SRP complex to the ER membrane, the signal sequence dissociates from SRP, and the nascent chain inserts into and becomes tightly associated with the ER membrane via interactions with components of the translocon [18]. In this step, GTP is required for the release of the nascent chain from SRP [19]. The non-hydrolyzable analog, Gpp(NH)p, promotes the release of the nascent chain from SRP and its insertion into the ER membrane, but prevents the subsequent release of SRP from the SR [20••]. Thus, GTP hydrolysis is required for recycling of both SRP and SR for subsequent rounds of targeting and nascent chain insertion. Mutations in the GTP-binding consensus sequences of SRα reduce the efficiency of GTP-dependent nascent chain insertion and prevent the formation of a stable SRP–SR complex in the presence of Gpp(NH)p [21•]. These observations indicate that the GTPase activity of SRα plays a role in targeting and translocation. The specific contribution of each of the three GTPases, SRP54, SRα and SRβ, in targeting and nascent chain insertion in the ER membrane, however, remains to be determined. In general, GTPases function to assemble macromolecular complexes in temporal succession. Thus, one might envision that these GTPases function to assemble accurately components of the translocon, so that the signal sequence can be specifically inserted into the ER membrane [22•].

From *in vitro* studies, the mammalian system has revealed great insight into the mechanism of SRP-dependent signal recognition and targeting. It is very likely, however, that other pathways for ER targeting exist. In *S. cerevisiae*, post-translational ER targeting and translocation have been observed both *in vitro* and *in vivo* [23–25]. In yeast, other cytosolic factors, the hsp 70s, associate with pre-proteins and facilitate post-translational translocation [26,27]. The presence of an SRP-independent post-translational targeting and translocation pathway has been demonstrated *in vitro* for a small set of substrates in the mammalian system as well [28].

The genes encoding the *S. cerevisiae* homologs of SRP54 and SRα were recently cloned [29,30,31••]. Evaluation of the *in vivo* role of the SRP-dependent targeting pathway was facilitated by the molecular genetics techniques available in *S. cerevisiae*. Deletion of the genes encoding either SRP54 or SRα, or both, results in viable, but poorly growing cells, suggesting that the SRP-dependent pathway can be partially by-passed *in vivo* [31••,32••]. Upon depletion of SRP54 or SRα in yeast cells, precursors to both secretory and membrane proteins accumulate in the cytosol [31••,32••,33•]. The degree to which different proteins are affected, however, varies greatly. The translocation of carboxypeptidase Y, a vacuolar protein, for example, is unaffected in SRP-depleted cells, whereas the translocation of Kar2p, a lumenal ER protein, and dipeptidyl aminopeptidase B, a vacuolar membrane protein, are severely diminished. Cytosolic precursor to Kar2p accumulates in SRP-depleted cells, but a portion of newly synthesized Kar2p is still translocated. As accumulated Kar2p precursor cannot be translocated post-translationally, pre-Kar2p is probably targeted co-translationally to the ER membrane in an SRP-independent manner. Thus, there appear to be several possible pathways to the ER membrane: an SRP-dependent co-translational pathway, a post-translational pathway and a possible SRP-independent co-translational pathway. It is likely that in wild-type cells the bulk of protein targeting occurs via the SRP-dependent pathway, and that alternative routes provide a scavenger pathway only in SRP or SR-deficient cells. Future research will focus on the molecular nature of the alternative pathways. It will be interesting to discover whether the pathways utilize the same translocon that is used for SRP-dependent translocation.

Examining the *in vivo* role of SRP revealed that pre-proteins can utilize alternative targeting pathways with varying efficiencies. This may explain why previous genetic screens in *S. cerevisiae* failed to detect SRP. A new selection has been used to isolate a translocation-defective mutant in a novel gene, *Sec65* [34••]. This gene encodes a homolog to the 19 kD subunit of mammalian SRP [35••,36••]. The translocation defect present in cells harboring the mutant allele, *sec65-1*, confirms the role of SRP in targeting and translocation *in vivo*. Biochemical and genetic studies demonstrate that Sec65p, SRP54p and a small cytoplasmic RNA, scR1, are part of a 16S ribonucleoprotein particle. Sec65p is required for the integrity of the yeast SRP and promotes, as in the case of mammalian SRP, the binding of SRP54 [36••].

The *in vivo* role of SRP has also been studied in other eukaryotic organisms. Mutations in the gene encoding an SRP-RNA of *Yarrowia lipolytica* exhibit a temperature-dependent growth phenotype [37•,38•]. At non-permissive temperatures, the synthesis of a major secreted protein, alkaline extracellular protease, is dramatically reduced, whereas overall protein synthesis is unaffected. This observation suggests that the mutated SRP is deficient in membrane targeting, but still functions in its ability to arrest translation of pre-proteins.

Translocation of proteins across the endoplasmic reticulum membrane

Subsequent to the targeting of a nascent protein, the ribosome–nascent chain complex associates with the ER membrane and translocation of the nascent chain across the membrane proceeds. It has long been proposed that protein translocation occurs through a proteinaceous channel. It has been shown that large ion conducting channels are present in ER membranes [39,40••]. Conductance through these channels is dependent on the release of nascent chains from ribosome–nascent chain complexes engaged in the process of translocation, suggesting that translocation proceeds through these channels [40••]. These findings also suggest that the ribosome may play a role in keeping the channels open during protein translocation.

The protein-conducting channel is likely to be a dynamic structure. Its subunit composition may vary at different sequential translocation stages, such as initiation of translocation, steady-state translocation and termination of translocation. Different pre-proteins may require the function of different translocation components. This may result from the specific targeting pathway that they utilize, or from topogenic determinants, e.g. stop transfer sequences. One major goal in the field is to identify, biochemically isolate and determine the function of the components that play a role in the process of nascent chain translocation and membrane protein integration.

A major advance in the study of protein translocation was the development of a reconstitution method by which translocation competent vesicles can be prepared from a detergent extract of ER membranes [41]. With this assay, membrane components involved in translocation can be identified directly. This reconstitution system has been utilized successfully to fractionate detergent-solubilized ER membrane components required for translocation [42•]. It has also been utilized to analyze whether components, identified by other approaches, contribute to the translocation process [43••]. Immunodepletion of SR from the detergent extract, for example, results in a complete loss of translocation activity. This *in vitro* assay, however, may not readily reveal components required for the regulation of translocation or components that are not rate-limiting for translocation.

Crosslinking of nascent chains to membrane components has been performed to identify components potentially involved in translocation. Two groups independently identified a 35–39 kD ER glycoprotein by photocrosslinking, and termed the crosslinked product signal sequence receptor (SSRα) and mp39, respectively [44,45]. The 35-39 kD glycoprotein does not, however, appear to be a signal sequence receptor, because mature portions of secretory proteins can also be crosslinked to it [45]. Using the deduced size of the glycoprotein crosslinking target, a polypeptide was purified and designated the SSRα protein [46]. This polypeptide is part of a heterotetrameric membrane protein complex

[43••]. Antibodies raised against SSRα immunoprecipitate crosslinked nascent chains, and Fab fragments block translocation, consistent with the idea that SSR is close to the site of translocation [46,47]. To test directly whether SSR is required for translocation, detergent-solubilized extracts were immunodepleted of the complex and SSR-depleted extracts were reconstituted into artificial vesicles [43••]. Depletion of SSR does not affect nascent chain targeting, secretory protein translocation or membrane protein integration [43••]. A number of explanations could account for this apparent discrepancy. SSR could function in translocation in a manner that is not detected by the *in vitro* translocation assay. Alternatively, SSR may not be required for translocation and may only fortuitously be found in proximity to nascent chains. It is certain, however, that the polypeptide identified as SSR does not function as a signal sequence receptor, as its name implies, and that it is not required for an essential rate-limiting step in translocation.

Further investigation revealed that translocating nascent chains can be crosslinked to another glycoprotein in the same molecular weight range as SSRα [48••]. Using crosslinking and reconstitution approaches, the crosslink target, a membrane glycoprotein termed the translocating chain associating membrane protein (TRAM), was purified. The deduced amino acid sequence indicates that TRAM is a multispanning membrane protein. In a reconstitution assay, TRAM is either stimulatory or required for the efficient translocation of several secretory substrates.

Other ER proteins in the vicinity of translocating nascent secretory and membrane proteins have been identified by crosslinking [9•,49•,50••]. A 34 kD non-glycosylated membrane protein that is distinct from SSRα and TRAM crosslinks to both nascent secretory and membrane protein polypeptides [49•]. Similarly, several glycosylated and non-glycosylated ER membrane proteins, which are in close proximity to membrane proteins containing stop-transfer or signal-anchor sequences, have been identified [9•,50••]. Some of these crosslinks may be specific to nascent membrane-spanning proteins and, thus, may function solely in the integration of membrane proteins (see High and Dobberstein, this issue, pp 581–586). Synthetic signal peptides have also been photocrosslinked to specific integral ER membrane proteins [51•]. Much effort in the years to come will be directed at purifying these proteins identified by crosslinking and determining their roles in protein translocation and integration.

Ribosome-binding sites present in the ER membrane are thought to be involved in steady state translocation of nascent chains. Initially, ribophorin I and II were thought to mediate ribosome binding to the ER, but were subsequently shown not to be involved [52, 53, 54]. Ribophorin I antibodies, however, block protein translocation, consistent with their being in close proximity to ribosomes and translocation sites [55]. Recently, it was observed that a membrane protein complex comprised of both ribophorin I and II and a 48 kD protein is associated with oligosaccharyltransferase activity [56••]. This suggests that the ribophorins are required to catalyze the attachment of oligosaccharides to proteins, thus ending the search for the function of ribophorins. Other ribosome receptor candidates, a 180 kD rough ER membrane protein and a 35 kD membrane protein, have been identified [57,58•]. Ribosome-binding activity solubilized and reconstituted from ER membranes, however, does not cofractionate with the 180 kD protein, indicating that another, as yet unidentified, protein(s) may function as the ribosome receptor [59•,60•]. Additional experimental evidence suggests that the 180 kD protein may not be required for *in vitro* translocation [61•]. A definitive demonstration of a ribosome receptor in the future requires the candidate protein to bind stoichiometrically to ribosomes.

In *S. cerevisiae*, mutations that disrupt translocation of pre-proteins across the ER membrane were selected using pre-protein–enzyme fusions. Mutations in three genes, *SEC61*, *SEC62* and *SEC63*, which encode ER membrane-spanning proteins, impair protein translocation [25, 62]. It is unclear at the present time whether all pre-proteins require the products of these genes for translocation *in vivo*. Recently, a new mutant allele, *sec61-3*, has been isolated and appears to affect the translocation of a wide spectrum of secretory proteins as well as the integration of membrane proteins [34••]. Mutations in *Sec62p* and *Sec63p*, however, only appear to affect the translocation a subset of pre-proteins [25]. Immunoprecipitation and crosslinking experiments indicate that Sec61p, Sec62p and Sec63p are present in a multisubunit complex with two other proteins of molecular weights 31.5 and 23 kD , respectively [63]. The yeast *KAR2* gene, which encodes a homolog of the mammalian BiP, is also necessary for translocation [64, 65•]. Mammalian BiP, however, does not appear to be required for translocation *in vitro* [66,67].

Preproteins in the process of translocation can be crosslinked to Sec61p and Kar2p [68••,69••]. Crosslinking of pre-proteins to Sec61p is dependent on functional Sec62p and Sec63p [69••]. With short nascent chains, crosslinks to Sec62p are also observed [68••]. These observations suggest that Sec62p/Sec63p may act prior to Sec61p. Although *kar2* mutants exhibit translocation defects *in vitro*, they do not inhibit crosslinking of pre-protein to Sec61p as severely [69••]. Thus, Kar2p may act after Sec61p in translocation. Crosslinking of nascent chains to Sec61p requires ATP hydrolysis [68••,69••]. The translocation factor responsible for the ATP-dependent interaction is unknown. Evidence from the mammalian system also suggests that a membrane ATPase is required for translocation [70•, 61•].

In yeast, additional mutations, termed *sec70*, *sec71* and *sec72*, have been isolated and shown to cause defects in protein translocation and membrane protein integration [71••]. Future work will focus on cloning these genes and determining their role in the process of translocation and membrane integration. The selection for genes involved in targeting and translocation has not been exhaustive. Thus, it is likely that the development of new selection schemes for mutations will yield additional genes involved in the process.

Regulation of protein translocation

Translocation of pre-proteins across the ER membrane is modulated in several ways. Determinants contained within the protein, such as stop-transfer sequences, must signal the translocation apparatus via some mechanism. Recently, another topogenic determinant has been discovered [72••]: signals contained within apolipoprotein B can mediate a pause in translocation. Whether a specific translocon component mediates this pause in translocation remains to be determined.

There is increasing evidence that the ribosome also plays a role in the regulation of translocation. Ionic conductance through the putative protein translocation channels in the ER membrane depends on the presence of a ribosome engaged in translocation of a nascent chain [40••]. Consistent with these findings, it was shown by crosslinking that membrane proteins in the process of integration remain in the vicinity of specific ER proteins until termination of translation occurs [50••]. Upon termination, crosslinks to these ER proteins no longer form. Even after the cytoplasmic tail of a nascent membrane protein has been lengthened by nearly 100 amino acids, the stop-transfer signal remains in the vicinity of specific ER membrane proteins. This suggests that the ribosome, upon termination of translation, transduces a signal to the translocon to complete membrane protein integration [50••].

Conclusion

The mechanisms employed for targeting and translocation of pre-proteins across the ER membrane have only begun to be resolved. Three distinct GTPases are known to interact during protein targeting, and the functional importance of the individual GTP-binding sites is still a mystery. In addition to SRP-mediated targeting, there appear to be other targeting pathways to the ER. Future goals will be to identify components in other targeting pathways and to determine their relative importance in pre-protein targeting *in vivo*.

A number of putative components of the translocon have been identified. Surprisingly however, at present there is no correspondence between the components identified in yeast and mammalian cells. Much of the effort in the field will be devoted to obtain additional membrane components and to decipher their mechanistic function in translocation. In pursuing this goal, we will gain insight into whether different pre-proteins may require a different subset of components for translocation as a result of the specific targeting pathway that they use or as a result of specific topogenic determinants contained within them. Insights will also be gained into the regulatory mechanisms that govern the assembly and disassembly of the translocon and the ribosome during the translocation of pre-proteins across the ER membrane.

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Deletion of the gene encoding the *S. cerevisiae* homolog of the SR α-subunit resulted in viable, but poorly growing cells. Depletion of the SR α-subunit caused precursors of secretory and membrane proteins to accumulate in the cytosol; different pre-proteins are affected to different degrees.

32. HANN BC, WALTER P: **The Signal Recognition Particle in *S.***
•• ***cerevisiae*.** *Cell* 1991, 67:131–143.

Deletion of the *SRP54* gene of *S. cerevisiae* resulted in viable, but poorly growing cells. The depletion caused precursors of secretory and membrane proteins to accumulate in the cytosol, but different pre-proteins are affected to a different degree.

33. AMAYA Y, NAKANO A: **SRH1 Protein, the Yeast Homolog of**
• **the 54 kDa Subunit of Signal Recognition Particle, is Involved in ER Translocation of Secretory Proteins.** *FEBS Lett* 1991, 283:325–328.

Depletion of SRP54 homolog of *S. cerevisiae* resulted in the accumulation of precursors of secretory proteins.

34. STIRLING CJ, ROTHBLATT J, HOSOBUCHI M, DESHAIES R, SCHEKMAN
•• R: **Protein Translocation Mutants Defective in the Insertion of Integral Membrane Proteins into the Endoplasmic Reticulum.** *Mol Biol Cell* 1992, 3:129–142.

Mutants that were defective in the insertion of membrane proteins were obtained by a selection that used a gene fusion of histidinol dehydrogenase and an integral membrane protein, hydroxylmethylglutaryl CoA reductase, as the targeting domain. Two temperature-sensitive lethal mutants in two complementation groups were isolated: a new allele of *sec61* and a mutation in a new gene *sec65*.

35. STIRLING CJ, HEWITT EW: **The *Saccharomyces cerevisiae***
•• **SEC65 Gene Encodes a Component of the Yeast Signal Recognition Particle with Homology to Human SRP19.** *Nature* 1992, 356:534–537.

The DNA sequence of the *SEC65* gene suggests that its protein is an *S. cerevisiae* homolog of SRP19. Deletion of the *SEC65* gene resulted in viable but poorly growing cells that exhibit translocation defects. An extrageneic suppressor of *sec65-1* was cloned and is identified as the *SRP54* gene, consistent with the observations of Hann *et al.* [36••].

36. HANN B, STIRLING CJ, WALTER P: **SEC65 Gene Product is a Subunit of the Yeast Signal Recognition Particle Required for Its Integrity.** *Nature* 1992, 356:532–533.
••
The *Sec65p* gene product is shown to be a subunit of *S. cerevisiae* SRP. A mutation in *Sec65P* disrupted the integrity of SRP, specifically causing SRP54 to dissociate from the particle. Overproduction of SRP54p suppressed both the *ts* lethal and the translocation defect phenotypes of *sec65-1* mutant cells.

37. HE F, BECKERICH J-M, GAILLARDIN G: **A Mutant of 7SL in *Yarrowia lipolytica* Affecting the Synthesis of a Secreted Protein.** *J Biol Chem* 1992, 267:1932–1937.
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38. YAVER DS, MATOBA S, ORGRYDZIAK DM: **A Mutation in the Signal Recognition Particle 7S RNA of the Yeast Yarrowia Lypolytica Prefers Affects Synthesis of the Alkaline Extracellular Protease: *in Vivo* Evidence for Translational Arrest.** *J Cell Biol* 1992, 116:605–616.
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[37•] and [38•] demonstrate that mutations in one of the two genes encoding the 7SL SRP-RNA result in *ts* lethal phenotypes and non-permissive temperatures cause a specific decrease in the synthesis of a secreted protein. These findings suggest that the mutant SRP can function in translational arrest but not translocation.

39. SIMON SM, BLOBEL G, ZIMMERBERG J: **Large Aqueous Channels in Membrane Vesicles Derived from the Rough Endoplasmic Reticulum of Canine Pancreas or the Plasma Membrane of *Escherichia coli*.** *Proc Natl Acad Sci USA* 1989, 86:6176–80.

40. SIMON SM, BLOBEL G: **A Protein-conducting Channel in the Endoplasmic Reticulum.** *Cell* 1991, 65:371–380.
••
Rough ER vesicles were fused to planar lipid bilayers and conductance across the membrane was measured. Vectorial discharge of translocating nascent chains using puromycin led to a large increase in membrane conductance. Dissociation of the ribosome from the membrane with the addition of salt abolished conductance, indicating that observed conductance was dependent on the interaction of the ribosome with the membrane.

41. NICCHITTA CV, BLOBEL G: **Assembly of Translocation competent Proteoliposomes from Detergent Solubilized Rough Microsomes.** *Cell* 1990, 60:259–269.

42. NICCHITTA C, MIGLIACCIO G, BLOBEL G: **Biochemical Fractionation and Assembly of the Membrane Components that Mediate Nascent Chain Targeting and Translocation.** *Cell* 1991, 65:587–598.
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Total ER detergent extracts were fractionated; the resulting fractions had no translocation activity when assayed separately but translocation restored when the fractions were combined.

43. MIGLIACCIO G, NICCHITTA C, BLOBEL G: **The signal sequence receptor, unlike the signal recognition particle receptor, is not essential for protein translocation.** *J Cell Biol* 1992, 117:15–25.
••
Detergent extracts were immunodepleted of either SR or SSR and used in a reconstitution assay. Vesicles depleted of SR were unable to target, translocate or integrate nascent secretory and membrane proteins. In contrast, these activities were unaffected in vesicles derived from SSR-depleted extracts. These findings indicate that SSR does not function in a rate-limiting step in protein translocation.

44. WIEDMANN M, KURZCHALIA TV, HARTMANN E, RAPOPORT TA: **A Signal Sequence Receptor in the Endoplasmic Reticulum Membrane.** *Nature* 1987, 328:830–832.

45. KRIEG UC, JOHNSON AE, WALTER P: **Protein Translocation across the Endoplasmic Reticulum Membrane: Identification by Photo-crosslinking of a 39-kD Integral Membrane Glycoprotein as Part of a Putative Translocation Tunnel.** *J Cell Biol* 1989, 109:2033–2043.

46. GÖRLICH D, PREHN S, HARTMANN E, HERZ J, OTTO A, KRAFT R, WEIDMANN M, KNESPEL S, DOBBERSTEIN B, RAPOPORT TA, *ET AL*: **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* 1990, 111:2283–2294.

47. HARTMANN E, WEIDMANN M, RAPOPORT TA: **A Membrane Component of the Endoplasmic Reticulum that May be Essential for Protein Translocation.** *EMBO J* 1989, 8:2225–2229.

48. GÖRLICH D, HARTMANN E, PREHN S, RAPOPORT TA: **A Protein of the Endoplasmic Reticulum Involved Early in Polypeptide Translocation.** *Nature* 1992, in press.
••
Crosslinking studies revealed a membrane glycoprotein in the vicinity of translocating nascent chains. Using crosslinking and reconstitution approaches, this protein, termed TRAM, was purified and shown to be stimulatory or required for the translocation of several secretory proteins.

49. KELLARIS KV, BOWEN S, GILMORE R: **ER Translocation Intermediates Are Adjacent to a Non-glycosylated 34-kD Integral Membrane Protein.** *J Cell Biol* 1991, 114:21–23.
•
Nascent secretory and membrane proteins in the process of translocation and integration are shown to crosslink to a 34 kD non-glycoprotein ER membrane protein.

50. THRIFT RN, ANDREWS DW, WALTER P, JOHNSON AE: **A Nascent Membrane Protein is Located Adjacent to ER Membrane Protein throughout Its Integration and Translation,** *J Cell Biol* 1991, 112:809–821.
••
This study demonstrates that membrane proteins in the process of integration are photocrosslinked to several newly identified ER membrane proteins until protein synthesis is terminated. These findings suggest that the ribosome is involved in completing membrane protein integration.

51. ROBINSON A, WESTWOOD OMR, AUSTEN BM: **Interactions of Signal Peptides with Signal Recognition Particle.** *Biochem J* 1990, 266:149–156.
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Addition of synthetic signal sequence reversed SRP-dependent elongation arrest and photocrosslinked to both SRP54 and SRP68. A 45 kD ER membrane-associated protein is also observed to crosslink to the synthetic signal sequence.

52. KREIBICH G, ULRICH B, SABATINI D: **Proteins of Rough Microsomal Membranes Related to Ribosome Binding 1. Identification of Ribophorins I and II, Membrane Proteins Characteristic of Rough Microsomes.** *J Cell Biol* 1978, 77:464–487.

53. HORTSCH M, AVOSSA D, MEYER D: **Characterization of Secretory Protein Translocation: Ribosome–Membrane Interaction in Endoplasmic Reticulum.** *J Cell Biol* 1986, 103:241–253.

54. 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* 1987, 245:811–819.

55. YU Y, SABATINI D, KREIBICH G: **Antiribophorin Antibodies Inhibit the Targeting to the ER Membrane of Ribosomes Containing Secretory Polypeptides.** *J Cell Biol* 1990, 111:1335–1342.

56. KELLEHER D, KREIBICH G, GILMORE R: **Oligosaccharyltransferase Activity is Associated with a Protein Complex Composed of Ribophorins I and II and a 48 kD Protein.** *Cell* 1992, 69:1–11.
••
Oligosaccharyltransferase activity from canine pancreas ER co-purifies with a protein complex composed of 66, 63 and 48 kD subunits. The 66 and 63 kD subunits are identical to ribophorin I and II, which are found in the vicinity of the translocon. Ribophorin I contains a dolichol recognition consensus sequence.

57. SAVITZ AJ, MEYER DI: **Identification of a Ribosome Receptor in the Rough Endoplasmic Reticulum.** *Nature* 1990, 346:540–544.

58. TAZAWA S, UNUMA M, TONDOKORO J, ASANO Y, OHSUMI T, ICHIMURA T, SUGANO H: **Identification of a Membrane Protein Responsible for Ribosome Binding in Rough Microsomal Membranes.** *J Biochem* 1991, 109:89–98.
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A 36 kD ER membrane protein was identified as a candidate for the ribosome receptor by crosslinking ribosomes to vesicles reconstituted with fractionated ER proteins that demonstrated ribosome-binding activity.

59. Nunnari J, L. ZD, Ogg SC, Walter P: **Characterization of**
• **Rough Endoplasmic Reticulum Ribosome Binding Activity.** *Nature* 1991, 352:638–640.

Ribosome-binding activity quantitatively solubilized does not co-fractionate with the 180kD protein proposed to be the ribosome receptor [57]. In contrast to the 180 kD, the ribosome-binding activity co-fractionates with a significantly smaller, positively charged protein. These observations indicate that the 180kD protein is not responsible for ribosome-binding activity. This conclusion is supported by the findings of Collins and Gilmore [60•].

60. Collins P, Gilmore R: **Ribosome Binding to the Endoplasmic**
• **Reticulum: a 180-kD Protein Identified by Crosslinking to Membrane-bound Ribosomes is not Required for Ribosome Binding Activity.** *J Cell Biol* 1991, 114:639–649.

Ribosomes in the process of translocation crosslink to several ER membrane proteins, including SR, ribophorin I and SSRα. The predominant crosslink product was a 180kD protein that was proposed to be a ribosome receptor [57]. Ribosome-binding activity, however, could be fractionated from the 180kD protein, indicating that the 180kD protein does not function as a ribosome receptor. This conclusion is in agreement with the suggestions of Nunnari *et al.* [59•].

61. Zimmerman D, Walter P: **An ATP-binding Membrane Protein**
• **Is Required for Protein Translocation Across the Endoplasmic Reticulum Membrane.** *Cell Regul* 1991, 2:851–859.

Photocrosslinking of ER membranes with 8-N_3-ATP resulted in inhibition of translocation activity and could be correlated with the labeling of several ER proteins, indicating that a membrane-associated ATP-binding protein is involved in translocation. The 180kD protein proposed to be a ribosome receptor was also shown to crosslink to 8-N_3-ATP, but crosslinking of the 180kD protein had no effect on translocation.

62. Deshaies RJ, Schekman R: **A Yeast Mutant Defective at an Early Stage in Import of Secretory Protein Precursors into the Endoplasmic Reticulum.** *J Cell Biol* 1987, 105:633–45.

63. Deshaies RJ, Sanders SL, Feldheim DA, Schekman R: **Assembly of Yeast Sec Proteins Involved in Translocation into the Endoplasmic Reticulum into a Membrane-bound Multisubunit Complex.** *Nature* 1991, 349:806–808.

64. Vogel JP, L.M. M, Rose MD: **Loss of BiP/GRP78 Function Blocks Translocation of Secretory Proteins in Yeast.** *J Cell Biol* 1990, 110:1885–1895.

65. Nguyen T, Law D, Williams D: **Binding Protein BiP Is Re-**
• **quired for Translocation of Secretory Proteins into the Endoplasmic Reticulum in *Saccharomyces cerevisiae*.** *Proc Natl Acad Sci USA* 1991, 88:1565–1569.

BiP was depleted to 10% of the wild type level resulting in a block in prepro-α-factor and invertase secretion. Invertase accumulated in the cytosol of BiP-depleted cells in a signal sequence cleaved form, whereas prepro-α-factor was found associated with membranes in a protease-resistant form that was oriented towards the cytosol. These results indicate that BiP is involved in translocation, in agreement with Vogel *et al.* [64], and suggest that BiP may be acting at a step subsequent to the targeting of precursor to the ER membrane

66. Yu Y, Zhang Y, Sabatini DD, Kreibich G: **Reconstitution of Translocation-competent Membrane Vesicles from Detergent-solubilized Dog Pancreas Rough Microsomes.** *Proc Natl Acad Sci USA* 1989, 86:9931–9935.

67. Zimmerman DL, Walter P: **Reconstitution of Protein Translocation Activity from Partially Solubilized Microsomal Vesicles.** *J Biol Chem* 1990, 265:4048–4053.

68. Musch A, Wiedmann M, Rapoport T: **Yeast Sec Proteins In-**
•• **teract with Polypeptides Traversing the Endoplasmic Reticulum Membrane.** *Cell* 1992, 69:343–352.

Photocrosslinking of translocation intermediates, formed using ribosome-associated truncated nascent chains, indicate that Sec61p is in the vicinity of the translocating nascent chain. When shorter nascent chain intermediates are employed, crosslinking to Sec62p is also observed. Crosslinks to Sec62p are abolished by addition of ATP, indicating that Sec62p acts before Sec61p.

69. Sanders S, Whitfield K, Vogel J, Rose M, Schekman R: **Sec61p**
•• **and BiP Directly Facilitate Polypeptide Translocation into the ER.** *Cell* 1992, 69:353–365.

Sec61p crosslinked in an ATP-dependent manner to a translocation intermediate formed using a prepro-α-factor-avidin chimeric protein. Mutations in *Sec62p* and *Sec63p* inhibit crosslinking to *Sec61p*, whereas mutations in *Kar2p* do not affect crosslinking of the translocation intermediate to *Sec61p* to such an extent, suggesting that the Sec62p and Sec63p function before, and Kar2p protein functions after, Sec61p in translocation.

70. Zimmermann R, Zimmermann M, Mayinger P, Klappa P: **Pho-**
• **toaffinity Labeling of Dog Pancreas Microsomes with 8-azido-ATP Inhibits Association of Nascent Preprolactin with the Signal Sequence Receptor Complex.** *FEBS Lett* 1991, 286:95–99.

Photocrosslinking with azido-ATP inacitivated an ER membrane protein(s), distinct from SR α-subunit, required for translocation. Treatment inhibited the insertion of a nascent secretory protein into the ER membrane, suggesting that the azido-ATP sensitive membrane-associated protein(s) acts at or before signal sequence insertion in the process of translocation.

71. Green N, Fang H, Walter P: **Mutants in Three Novel Com-**
•• **plementation Groups Inhibit Membrane Protein Insertion into and Soluble Protein Translocation across the Endoplasmic Reticulum Membrane of Saccharomyces cerevisiae.** *J Cell Biol* 1992, 116:597–604.

Mutations that inhibit membrane protein integration in three novel complementation groups, *SEC70*, *SEC71* and *SEC72*, were selected using a gene fusion of histidinol dehydrogenase and an integral membrane protein, arginine permease, as the targeting domain. The mutants also inhibit the translocation of soluble proteins into the ER. The integration and translocation of soluble and membrane proteins are impaired to different extends in *SEC70*, *SEC71* and *SEC72*.

72. Chuck S, Lingappa V: **Pause Transfer: A Topogenic Sequence**
•• **in Apolipoprotein B Mediates Stopping and Restarting of Translocation.** *Cell* 1992, 68:9–21.

Identification of two regions, the pause transfer sequences, within apolipoprotein B. The regions mediate both the stopping and restarting of translocation and are responsible for the observed transmembrane translocation intermediates of apolipoprotein B.

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