Translocation of proteins across the endoplasmic reticulum

Sanford Simon

Rockefeller University, New York, USA

The past year has seen significant advances in the field of protein translocation: the roles of the signal recognition particle and its receptor have been understood in greater detail; many membrane components responsible for translocation have been identified; and insight has been gained into how proteins cross membranes.

Introduction

Eukaryotic cells contain numerous organelles, each enveloped by its own membrane. A membrane must form an effective sea wall of sorts, separating organelle from cytosol. But as no organelle is entirely self-sufficient (or, to strain the metaphor, an island), the membrane surrounding it must also be permeable, allowing the entry and exit of various ions, sugars, nucleotides and proteins. Every organelle, for instance, must import proteins that are synthesized in the cytosol. Import is a two-step process. First, proteins must be targeted to their destination. Then, they must cross the membrane (or, more specifically, the membrane permeability must be modified to allow the selective vectorial transport of the proteins). Those proteins that will become integral to the membrane must, further, become stitched into the bilayer in their proper topography.

Over the past 20 years we have learned a great deal about the first step, protein targeting [1]. A signal in the primary amino acid structure of the protein (the signal sequence) is known to be both necessary and sufficient to target the protein. Once this signal is synthesized, a cytosolic factor called signal recognition particle (SRP) binds to it, as well as to the ribosome. Protein translation is inhibited until the SRP interacts with a receptor (SRP receptor) on the endoplasmic reticulum (ER). The signal sequence is then displaced from the SRP in steps requiring GTP hydrolysis, protein synthesis proceeds and the nascent polypeptide translocates across the membrane (though not necessarily in that order).

Our knowledge of the second step, how secretory proteins cross the membrane and how integral membrane proteins are stitched into the bilayer, is considerably more limited. For instance, three different techniques (genetics, biochemical fractionation and chemical cross-linking) identified three disparate, non-overlapping sets of molecules considered key to translocation. In addition, no overlap was found between the molecular machinery identified in prokaryotes, yeast or mammals, even though there is conservation of function between them. During the past 2 years, however, investigators have added new techniques to their arsenal, such as membrane solubilization and reconstitution, electrophysiology and fluorescence. This article reviews some of the consequences of this work. Among the significant advances have been a greater appreciation for the ubiquitous role of SRP and the membrane proteins involved in translocation as well as new insights into the mechanisms by which proteins cross membranes.

Targeting proteins to the membrane

SRP was originally fractionated from mammalian cytosol as a complex of six proteins [2] and a 7S RNA [3] that is essential to translocation [4]. SRP fulfilled three functions *in vitro*: binding signal sequences [5,6]; inhibiting the translation of nascent polypeptides containing signal sequences [4]; and interacting with a cognate receptor (the SRP receptor) on the membrane of the ER, which released the block of protein synthesis [7,8]. Each of these functions has been mapped to a separate discrete region of the SRP using biochemical fractionation [9]. Most of the current experimental questions can be divided into specific categories. Does translation arrest occur *in vivo*? How does SRP (or, more specifically, the 54 kDa subunit of SRP) recognize signal sequences (whose structures are extremely diverse)? How does SRP interact with its receptor? Is there an SRP in yeast or bacteria? What is the role of GTP?

Our knowledge about SRP has recently increased thanks to evidence for its role in yeast, *Escherichia coli* and *Bacillus subtilis* and detailed analyses of the functional roles for its different subunits and membrane-bound re-

---

Abbreviations

ER—endoplasmic reticulum; **ffh**—*E. coli* homologue of SRP54; **SRP**—signal recognition particle; **SSR**—signal sequence receptor; **TRAM**—translocating chain associated membrane protein.

ceptor. Signal sequences specifically bind to the 54 kDa subunit of SRP, which has been functionally and structurally divided into two regions [10,11]: SRP54G, with a GTP-binding site, and SRP54M, a methionine-rich domain that is responsible for binding to the 7S RNA and can be crosslinked to signal sequences [12,13]. In the absence of the GTP-binding domain, the SRP54M is sufficient for binding signal sequences [14•] and for reconstitution into a SRP(−54G) which is competent for binding signal sequences and arrest of protein synthesis [15•]. Although SRP(−54G) bound signal sequences [15•], chemical modifications of the SRP54G [9,14•] blocked binding, suggesting a role in regulation of the binding of signal sequences [14•]. Significantly, this SRP(−54G) did not promote protein translocation and its block of protein synthesis was not reversed by SRP receptor [15•]. This result is particularly significant in light of the observation that SRP54G is highly homologous to the GTP-binding domains of the SRP receptor [10,11]. The role of GTP in translocation [16–18] has yet to be fully elucidated. However, by combining site-directed mutations in the GTP-binding domain with biochemical reconstitution in a mammalian system, it has become possible to dissect and identify discrete steps in the process of SRP–SRP receptor binding and signal sequence displacement [19•]. This approach holds great promise for identifying the role(s) of GTP in translocation.

Although SRP was readily identified in biochemical assays in mammalian systems, it was undetectable in yeast or bacterial transport or genetic assays. However, sequence analysis revealed homologues of the 7S RNA, the 54 kDa subunit and the SRP receptor in yeast [20–25], E. coli [11,26,27] and B. subtilis [28•]. The role of SRP in protein translocation in these systems has now been substantiated. In E. coli, the homologue of SRP54 (ffh) was specifically crosslinked to signal sequences [29•] and shown to be essential for efficient protein translocation in vivo [30•]. The E. coli ffh was substituted for mammalian SRP54 in re-assembling a reconstituted SRP [31•]. This hybrid complex bound signal sequences and arrested translation as efficiently as native SRP. Elongation arrest, however, was not relieved by SRP receptor. (It remains to be seen if the E. coli homologue of the SRP receptor or other proteins in the E. coli plasma membrane can serve this role.)

Protein secretion in B. subtilis was blocked when the 7S RNA homologue was deleted, and secretion was restored with the expression of either human SRP 7S RNA or the E. coli homologue [28•]. In Saccharomyces cerevisiae, protein translocation was compromised for some but not all proteins when SRP is deleted [24,25,32]. Recently, homologues of SRP19 [33•,34•] and SRP receptor [35•] were studied in yeast. Mutations in all these proteins produced similar phenotypes, suggesting that they work together.

SRP causes complete translation arrest in a heterologous system of wheat germ cytosol and mammalian membranes [4]. It mediates transient pausing in a homologous mammalian system [36]. Examination of its role in translation arrest in vivo has been significantly facilitated by the identification of homologues for SRP in yeast.

Mutations of the 7S RNA of Yarrowia lipolytica selectively inhibited the synthesis of only secretory proteins. As untranslocated precursors were not observed in the cytosol, this mutated SRP may still function to arrest translation of secreted proteins, but fail to allow the proteins to translocate [37•,38•]. The size of nascent proteins affects the ability of SRP to induce translation arrest. Small proteins (up to 85 amino acids) translocate without the assistance of SRP [39•]. Translation of larger proteins (up to 17 kDa), was arrested by SRP, suggesting that SRP binds to numerous sites on the nascent chain [40•]. Progress has also been made in identifying the minimal peptide length for insertion in the membrane, which should help identify the steps in peptide dissociation from SRP and translocation across the membrane [41•].

Membrane components

It was originally thought that many different proteins made up the 'translocon', or were involved in the membrane components that mediate translocation. Ribophorins [42], signal sequence receptor (SSR) [43], translocating chain associated membrane protein (TRAM) [44•] and a 205 kDa protein [45•] have all been identified by chemical cross-linking. Alternatively, in E. coli Sec Y [46] and in S. cerevisiae Sec61, 62 and 63 [47,48] and Sec70, 71 and 72 [49•] have been identified with genetic selection schemes. Biochemical assays have determined that the signal peptidase complex [50] and oligosaccharyltransferase [51] act on translocating chains, suggesting that they are intimately involved in translocation. Finally, the observation that proteins translocate across the ER co-translationally [52,53] has inspired the hunt for a ribosome receptor.

Fortunately, there has been considerable consolidation of candidate proteins during the past year. Some of the progress was the consequence of biochemistry. For example, the ribophorins were demonstrated to be the oligosaccharyltransferase [54•–57•]. Other advances came from the hybridization of approaches. The previously identified yeast proteins Sec61p, Sec62p and BiP were chemically crosslinked to nascent translocating chains [58•,59•]. Mutations in either SEC62 or SEC63 decreased the crosslinking of translocating chains to Sec61p. A mammalian homologue of Sec61p was recently identified crosslinked to translocating chains [60•]. The consolidation of the results from mammals and yeast may be further extended based on the observation that this protein also has moderate homology to the E. coli protein, Sec Y [60•,61•].

The role of cytosolic factors in translocation was established because of the availability of a biochemical assay for fractionating and reconstituting translocation [62]. The list of potential membrane components has grown over the past few years in the absence of a similar technique for fractionating membrane proteins. The ability to solubilize, fractionate and reconstitute membranes that still translocate [63–65] is a powerful tool for test-

ing proteins [66•]. ER membranes were solubilized and a potential component, SSR, was depleted before reconstitution of membrane proteins. These membranes were still fully competent to translocate. Translocation was totally blocked when SRP receptor was depleted from the membranes, demonstrating the power of this technique [66•]. It has been used to demonstrate that TRAM is required for protein translocation [44•].

The roles of other proteins were illuminated during the past year. The signal peptidase was demonstrated to have most of its mass and putative active sites on the lumenal side of the ER [67•]. This suggests that signal peptide cleavage occurs in the lumen. The identification of the ribosome-binding protein(s) has yet to be clarified. After the report of a 180 kDa ER protein [68], other candidates have been suggested [69,70,71•,72]. It remains to be seen what role any or all of them serve in protein translocation.

How do proteins cross the membrane?

Membranes are impermeable to most charged molecules. Ions cross membranes through transmembrane aqueous pores, known as ion channels. Recent evidence supports the idea [62] that proteins cross membranes by a similar mechanism. Large channels were observed in the ER when nascent translocating chains were released from the membrane-bound ribosomes; these channels remained open until the ribosomes dissociated from the membrane with high salt concentrations [73]. Thus, the channels are open while a chain is translocating, but relatively impermeable to ions until the nascent chain is released. Similarly, it has been observed that the mammalian homologue of SEC61p associates tightly with ribosomes during translocation and remains after the release of the nascent chains until ribosomes are dissociated with high salt concentrations [60•]. These electrophysiological results parallel observations using a probe incorporated into nascent translocating chains whose fluorescence is affected by the hydrophobicity of their environment [74•]. While translocating across the membrane, the probe reports an aqueous environment. However, the fluorescence cannot be quenched by iodide, again indicating that while a chain is translocating, the channel is relatively impermeable to ions. This approach was used to demonstrate further that the signal sequence, when in the context of a translocating protein, does not partition into the lipid bilayer. Moreover, there is a tight ion-impermeant junction between the ribosome and the membrane when a chain is translocating.

These observations are consistent with results from other systems. First, channels very similar to those observed in the ER [73] were observed in the plasma membrane of E. coli upon addition of very low (< nM) concentrations of signal sequences [75•]. This suggests the signal sequence is a ligand that opens the protein-conducting channel. Likewise, a general increase of conductance was observed when pre-proteins were added to E. coli plasma membrane vesicles [76•]. Second, the conductance of channels in the mitochondrial membrane was

substantially reduced, or blocked, in the presence of a peptide resembling a mitochondrial targeting signal, suggesting that the peptide is entering the channel and slowing the flow of ions [77•]. Third, when crosslinkers were incorporated into nascent chains, they could be linked to other transmembrane proteins but not to lipids, indicating that proteins are shielded from the hydrocarbon core of the membrane [78•]. However, these results raise two immediate problems. First, if proteins are in an aqueous pore, what moves them across the membrane? Second, how do transmembrane proteins get out of the channel and into the bilayer?

A Brownian ratchet has been proposed as the mechanism that moves proteins across membranes [79•]. The model assumes that proteins are in a protein-conducting channel and the translocating chains see different chemical environments on the two different sides of the membrane. A protein in a channel should reptate back and forth from thermal energy. But, if a protein is modified in the lumen of the ER (by addition of sugar groups or binding by lumenal contents) the protein will not be able to move out of the lumen. It would reptate back and forth until it finally crosses the membrane. Quantification of this model demonstrated that it can account for the kinetics of protein translocation [79•]. It has also withstood a number of experimental tests: the lumenal protein BiP is one of the main proteins that can be cross-linked to a translocating chain [58•]; BIP is needed in reconstituted yeast ER vesicles for translocation [80•] and lumenal ER proteins are required in reconstituted mammalian microsomes for unidirectional translocation [81•]; addition of a glycosylation site to a short protein ensures the vectoriality of transport [82•]; once a chain is partially across the membrane, it will fully translocate in the absence of any additional energy source [83•]. It has been suggested that chaperone proteins may pull the nascent chain through the membrane [83•,84•]. The Brownian ratchet is a mechanism for transducing chaperone binding to protein movement.

Integration of membrane proteins

Little is known of the biogenesis of membrane proteins. One approach has been to screen for mutations in yeast that selectively block translocation of membrane proteins [49•,61•]. Alternatively, genetically engineered proteins were used to identify the regions that define a transmembrane domain [85•,86•]. Statistical studies of the distribution of charges on either side of the transmembrane segment have supported the idea that an excess of positive charge determines the cytoplasmic side [87]. Experimental results have been ambiguous with some constructs consistent with the rule [88•]. However, other constructs indicate that the charged residues do not affect transmembrane orientation. This suggests that other features determine membrane topology [89•]. A different construct has been used to show that transmembrane segments insert independently with an innate transmembrane orientation [90•,91•]. The observation that some

domains of a protein may not always be on the same side of the membrane has elicited the suggestion that there may be regulation of insertion of membrane segments [92•].

Conclusion

This year has seen great advances in the study of protein translocation. The functions of each of the subunits of SRP and the SRP receptor were further elucidated. The combination of genetics and chemical labeling provided critical evidence for identifying the key membrane components. These can now be tested with a biochemical technique for solubilization, fractionation and reconstitution of membrane components that has proven its ability as a discriminating functional assay. Despite the identification of many new components, the overall picture of protein translocation has been simplified: functions have been identified for proteins; homologies between systems have been recognized. Growing evidence has supported the hypothesis that proteins translocate through protein-conducting channels that shield them from the hydrocarbon core of the bilayer.

These advances, however, beg further questions. How are transmembrane proteins integrated into the bilayer? What determines that a sequence should be a transmembrane domain? Are transmembrane domains translocated with the same machinery as secretory proteins? Are transmembrane segments sequentially inserted into the membrane, or are larger domains inserted? How do transmembrane segments partition from the translocation apparatus into the lipid bilayer? Are protein-conducting channels a general mechanism for protein movement across membranes, for example, in the transport of a-factor by STE-6 [93] or hemolysin by HlyB,D [94]? For that matter, are transmembrane aqueous pores a general mechanism for moving all hydrophilic molecules (sugars, amino acids and nucleotides) across membranes? While substantial progress has been made and many new issues are being raised, it is perhaps significant to remember that even some of the most fundamental issues in protein translocation, such as identifying the essential physical characteristics that define a signal sequence, have yet to be resolved.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

1. WALTER P, LINGAPPA VR: Mechanism of Protein Translocation Across the Endoplasmic Reticulum Membrane. Annu Rev Cell Biol 1986, 2:499–516.

2. WALTER P, BLOBEL G: Purification of a Membrane-Associated Protein Complex Required for Protein Translocation Across the Endoplasmic Reticulum. Proc Natl Acad Sci USA 1980, 77:7112–7116.

3. WALTER P, BLOBEL G: Signal Recognition Particle Contains a 7S RNA Essential for Protein Translocation Across the Endoplasmic Reticulum. Nature 1982, 299:691–698.

4. 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 1981, 91:557–561.

5. 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 1986, 83:8604–8608.

6. KURZCHALIA TV, WIEDMANN M, GIRSHOVICH AS, BOCHKAREVA ES, BIELKA H, RAPOPORT TA: The Signal Sequence of Nascent Preprolactin Interacts with the 54K Polypeptide of the Signal Recognition Particle. Nature 1986, 320:634–636.

7. 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 1982, 95:470–477.

8. MEYER DI, KRAUSE E, DOBBERSTEIN B: Secretory Protein Translocation Across Membranes — the Role of 'Docking Protein'. Nature 1982, 297:647–650.

9. SIEGEL V, WALTER P: Each of the Activities of the Signal Recognition Particle (SRP) is Contained within a Distinct Domain: Analysis of Biochemical Mutants of SRP. Cell 1988, 52:39–49.

10. 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 1989, 340:482–486.

11. ROMISCH 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 1989, 340:478–482.

12. 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 1990, 9:4511–4517.

13. HIGH S, DOBBERSTEIN B: The Signal Sequence Interacts with the Methionine-Rich Domain of the 54-kD Protein of Signal Recognition Particle. J Cell Biol 1991, 113:229–233.

14. LÜTCKE H, HIGH S, ROMISCH K, ASHFORD AJ, DOBBERSTEIN B: The Methionine-Rich Domain of the 54 kDa Subunit of Signal Recognition Particle is Sufficient for the Interaction with Signal Sequences. EMBO J 1992, 11:1543–1551.
Demonstrates that the methionine-rich domain of SRP54 is sufficient to bind signal sequences, although modification of the GTP-binding domain can modify the interaction.

15. KOPF D, BERNSTEIN HD, WALTER P: GTPase Domain of the 54-kD Subunit of the Mammalian Signal Recognition Particle is Required for Protein Translocation but not for Signal Sequence Binding. J Cell Biol 1993, 120:1113–1121.
An SRP molecule was reconstituted missing the GTP-binding site of the 54 kDa subunit. This mutated SRP still bound signal sequences and arrested translation, but did not effect protein translocation.

16. CONNOLLY T, GILMORE R: Formation of a Functional Ribosome–Membrane Junction During Protein Translocation Requires the Participation of a GTP-Binding Protein. J Cell Biol 1986, 103:2253–2261.

17. WILSON C, CONNOLLY T, MORRISON T, GILMORE R: Integration of Membrane Proteins into the Endoplasmic Reticulum Requires GTP. J Cell Biol 1988, 107:69–77.

18. HOFFMAN KE, GILMORE R; Guanosine Triphosphate Promotes the Post-Translational Integration of Opsin into

the Endoplasmic Reticulum Membrane. *J Biol Chem* 1988, 263:4381–4385.

19. RAPIEJKO PJ, GILMORE R: Protein Translocation Across the ER
• Requires a Functional GTP Binding Site in the α Subunit of the Signal Recognition Particle Receptor. *J Cell Biol* 1992, 117:493–503.
Site-directed mutations were made in the GTP-binding domain of the SRP receptor. These mutated molecules were used to re-populate microsomes from which the native receptor was deleted. This approach has been used to map the GTP-dependent steps in the translocation process.

20. PORITZ MA, SIEGEL V, HANSEN W, WALTER P: Small Ribonucleoproteins in *Schizosaccharomyces pombe* and *Yarrowia lipolytica* Homologous to Signal Recognition Particle. *Proc Natl Acad Sci USA* 1988, 85:4315–4319.

21. BRENNWALD P, LIAO X, HOLM K, PORTER G, WISE JA: Identification of an Essential *Schizosaccharomyces pombe* RNA Homologous to the 7SL Component of Signal Recognition Particle. *Mol Cell Biol* 1988, 8:1580–1590.

22. AMAYA Y, NAKANO A, ITO K, MORI M: Isolation of a Yeast Gene, *SRH1*, that Encodes a Homologue of the 54K Subunit of Mammalian Signal Recognition Particle. *J Biochem (Tokyo)* 1990, 107:457–463.

23. HE F, YAVER D, BECKERICH J-M, OGRYDZIAK D, GAILLARDIN C: The Yeast *Yarrowia lipolytica* has Two, Functional, Signal Recognition Particle 7S RNA Genes. *Curr Genet* 1990, 17:289–292.

24. HANN BC, WALTER P; The Signal Recognition Particle in *S. cerevisiae. Cell* 1991, 67:131–144.

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

26. PORITZ MA, STRUB K, WALTER P: Human SRP RNA and *E. coli* 4.5S RNA Contain a Highly Homologous Structural Domain. *Cell* 1988, 55:4–6.

27. PORITZ MA, BERNSTEIN HD, STRUB K, ZOPF D, WILHELM H, WALTER P: An *E. coli* Ribonucleoprotein Containing 4.5S RNA Resembles Mammalian Signal Recognition Particle. *Science* 1990, 250:1111–1117.

28. NAKAMURA K, IMAI Y, NAKAMURA A, YAMANE K: Small Cytoplas-
• mic RNA of *Bacillus subtilis*: Functional Relationship with Human Signal Recognition Particle 7S RNA and *Escherichia coli* 4.5S RNA. *J Bacteriol* 1992, 174:2185–2192.
Protein translocation was blocked when the small cytoplasmic RNA was deleted. However, the defect could be complemented with either the 7S RNA of human SRP or the 4.5S RNA of *E. coli.*

29. LUIRINK J, HIGH S, WOOD H, GINER A, TOLLERVEY D,
• DOBBERSTEIN B: Signal-Sequence Recognition by an *Escherichia coli* Ribonucleoprotein Complex. *Nature* 1992, 359:741–743.
The incorporation of a photoactivatable cross-linker into the signal sequence of pre-prolactin was used to demonstrate that signal sequences bind the *E. coli* ribonucleoprotein complex which resembles mammalian SRP (see [27,28•]).

30. PHILLIPS GJ, SILHAVY TJ: The *E. coli ffh* Gene is Neces-
• sary for Viability and Efficient Protein Export. *Nature* 1992, 359:744–746.
Expression of the *E. coli* 48 kDa protein, which shares homology with the 54 kDa subunit of SRP (see [27,28•]) was made dependent upon arabinose. Depletion of this protein blocked translocation of a spectrum of proteins.

31. BERNSTEIN HD, ZOPF D, FREYMAN DM, WALTER P: Functional
• Substitution of the Signal Recognition Particle 54 kD Subunit by its *E. coli* Homolog. *Proc Natl Acad Sci USA* 1993, 90:5229–5233.
The *E. coli* homolog of SRP54 was substituted for its mammalian counterpart in a reconstituted SRP. This hybrid bound signal sequences

and arrested translation as efficiently as native mammalian SRP. However, it could not effect translocation and the SRP receptor could not relieve translation arrest.

32. HANN BC, PORITZ MA, WALTER P: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* Contain a Homologue to the 54-kD Subunit of the Signal Recognition Particle that in *S. cerevisiae* is Essential for Growth. *J Cell Biol* 1989, 109:3223–3230.

33. HANN BC, 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.
SEC65 was demonstrated to co-sediment and co-immunoprecipitate with yeast SRP54.

34. STIRLING CJ, HEWITT EW: The *S. cerevisiae SEC65* Gene En-
• codes a Component of Yeast Signal Recognition Particle with Homology to Human SRP19. *Nature* 1992, 356:534–537.
Cloning and sequencing of the *SEC65* gene demonstrated its homology to human *SRP19.* Disruption of the gene resulted in slowly growing cells and accumulation of translocation precursors of pre-pro-α-factor.

35. OGG SC, PORITZ MA, WALTER P: Signal Recognition Particle
• Receptor is Important for Cell Growth and Protein Secretion in *Saccharomyces cerevisiae. Mol Biol Cell* 1992, 3:895–911.
The SRP receptor in *S. cerevisiae* was cloned and sequenced and had a GTP-binding domain similar to its mammalian counterpart. Disruption of the gene produced a phenotype similar to disruption of the yeast genes for SRP54.

36. WOLIN SL, WALTER P: Signal Recognition Particle Mediates a Transient Elongation Arrest of Preprolactin in Reticulocyte Lysate. *J Cell Biol* 1989, 109:2617–2622.

37. YAVER DS, MATOBA S, OGRYDZIAK DM: A Mutation in the
• Signal Recognition Particle 7S RNA of the Yeast *Yarrowia lipolytica* Preferentially Affects Synthesis of the Alkaline Extracellular Protease: *In Vivo* Evidence for Translocational Arrest. *J Cell Biol* 1992, 116:605–616.
See [38•].

38. HE F, BECKERICH J-M, GAILLARDIN C: A Mutant of 7SL RNA in
• *Yarrowia lipolytica* Affecting the Synthesis of a Secreted Protein. *J Biol Chem* 1992, 267:1932–1937.
This paper and [37•] used a temperature-sensitive mutation in the 7S RNA of the SRP in the yeast *Y. lipolytica.* The mutation caused a conditional block of growth and selective inhibition of synthesis of secreted proteins, suggesting that SRP-induced translation arrest was still occurring in the mutation.

39. SCHLENSTEDT G, GUDMUNDSSON GH, BOMAN HG, ZIMMERMANN
• R: Structural Requirements for Transport of Preprocecropin A and Related Presecretory Proteins into Mammalian Microsomes. *J Biol Chem* 1992, 267:24328–24332.
Proteins of up to 85 amino acids could be translocated by two parallel pathways: an SRP-independent pathway, or an SRP-dependent path that occurred co-translationally. Both pathways were dependent on the signal sequence.

40. OKUN MM, SHIELDS D: Translocation of Preproinsulin Across
• the Endoplasmic Reticulum Membrane. The Relationship between Nascent Polypeptide Size and Extent of Signal Recognition Particle-Mediated Inhibition of Protein Synthesis. *J Biol Chem* 1992, 267:11476–11482.
The SRP-induced arrest of translation was studied for pre-proinsulin mRNAs that were truncated at various lengths. Proteins of up to 17 kDa could be arrested.

41. WOLIN SL, WALTER P: Discrete Nascent Chain Lengths are
• Required for the Insertion of Presecretory Proteins into Microsomal Membranes. *J Cell Biol* 1993, 121:1211–1219.
A study of the relationship between length of the nascent chain and attachment of ribosomes to the membrane.

42. KREIBICH G, ULRICH BL, SABATINI DD: Proteins of Rough Microsomal Membranes Related to Ribosome Binding. I. Identification of Ribophorins I and II, Membrane Pro-

teins Characteristic of Rough Microsomes. *J Cell Biol* 1978, 77:464–487.

43. WIEDMANN M, KURZCHALIA TV, HARTMANN E, RAPOPORT TA: A Signal Sequence Receptor in the Endoplasmic Reticulum Membrane. *Nature* 1987, 328:830–833.

44. GÖRLICH D, HARTMANN E, PREHN S, RAPOPORT TA: A Protein of
• the Endoplasmic Reticulum Involved Early in Polypeptide Translocation. *Nature* 1992, 357:47–52.
Describes the identification of TRAM, which crosslinked to nascent secretory chains near the initiation of translocation. When membranes were solubilized, immunodepleted of TRAM and reconstituted, they no longer translocated proteins. Re-addition of TRAM before reconstitution restored translocation.

45. ANDERSON L, DENNY JB: Protein Translocation in the En-
• doplasmic Reticulum. Ultraviolet Light Induces the Non-covalent Association of Nascent Peptides with Translocon Proteins. *J Biol Chem* 1992, 267:23916–23921.
A cell-free translation system was photolyzed while synthesizing β-lactamase. A 205 kDa protein crosslinked to nascent chains in the presence of rough microsomes. This linkage was blocked by N-ethylmaleimide (which blocks translocation).

46. EMR SD, HANLEY-WAY S, SILHAVY TJ: Suppressor Mutations that Restore Export of a Protein with a Defective Signal Sequence. *Cell* 1981, 23:79–88.

47. 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, 105:633–645.

48. ROTHBLATT JA, DESHAIES RJ, SANDERS SL, DAUM G, SCHEKMAN R: Multiple Genes are Required for Proper Insertion of Secretory Proteins into the Endoplasmic Reticulum in Yeast. *J Cell Biol* 1989, 109:2641–2652.

49. 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 affect the translocation of membrane proteins were selected using a scheme similar to that in [47,48]. Three new complementation groups were identified, suggesting that many more genes may be involved. Distinct proteins were affected differently by the mutations.

50. EVANS EA, GILMORE R, BLOBEL G: Purification of Microsomal Signal Peptidase as a Complex. *Proc Natl Acad Sci USA* 1986, 83:581–585.

51. KATZ FN, ROTHMAN JE, LINGAPPA VR, BLOBEL G, LODISH HF: Membrane Assembly *in vitro*: Synthesis, Glycosylation and Asymmetric Insertion of a Transmembrane Protein. *Proc Natl Acad Sci USA* 1977, 74:3278–3282.

52. REDMAN CM, SABATINI DD: Vectorial Discharge of Peptides Released by Puromycin from Attached Ribosomes. *Proc Natl Acad Sci USA* 1966, 56:608–615.

53. REDMAN CM, SIEKEVITZ P, PALADE GE: Synthesis and Transfer of Amylase in Pigeon Pancreatic Microsomes. *J Biol Chem* 1966, 241:1150–1158.

54. KELLEHER DJ, KREIBICH G, GILMORE R: Oligosaccharyl-
• transferase Activity is Associated with a Protein Complex Composed of Ribophorins I and II and a 48 kd Protein. *Cell* 1992, 69:55–65.
A protein complex consisting of subunits of 66, 63 and 48 kDa co-purified with the oligosaccharyltransferase activity. The 66 and 63 kDa proteins were shown to be ribophorin I and II.

55. TE HEESEN S, JANETZKY B, LEHLE L, AEBI M: The Yeast *WBP1*
• is Essential for Oligosaccharyltransferase Activity *In Vivo* and *In Vitro*. *EMBO J* 1992, 11:2071–2075.
Depletion of the yeast protein WBP1 correlated with a defect in transferring oligosaccharides to two proteins *in vivo* and reduced glycosylation of a pentapeptide *in vitro*.

56. SILBERSTEIN S, KELLEHER DJ, GILMORE R: The 48-kDa Subunit
• of the Mammalian Oligosaccharyltransferase Complex is

Homologous to the Essential Yeast Protein WBP1. *J Biol Chem* 1992, 267:23658–23663.
The 48 kDa protein, purified as part of the complex described in [54•], was shown to be homologous to a previously identified yeast protein required for the oligosaccharyltransferase activity in [55•].

57. TE HEESEN S, KNAUER R, LEHLE L, AEBI M: Yeast Wbp1p
• and Swp1p Form a Protein Complex Essential for Oligosaccharyltransferase Activity. *EMBO J* 1993, 12:279–284.
SWP1p was identified as a protein that suppresses mutations in Wbp1p (see [55•]). Swp1p is essential for oligosaccharyl transferase activity and it can be chemically crosslinked to Wbp1p *in vivo*.

58. SANDERS SL, WHITFIELD KM, VOGEL JP, ROSE MD, SCHEKMAN
• RW: Sec61p and BiP Directly Facilitate Polypeptide Translocation into the ER. *Cell* 1992, 69:353–365.
Proteins trapped in the process of translocation were crosslinked to Sec61p and BiP. Crosslinking to Sec61 required functional Sec62p and ATP. Two mutations of BiP reduced crosslinking to Sec61p. A third allele affected translocation but not crosslinking of the nascent chain to Sec61p. These results suggest that BiP interacts with proteins while they are translocating.

59. MÜSCH A, WIEDMANN M, RAPOPORT TA: Yeast Sec Proteins
• Interact with Polypeptides Traversing the Endoplasmic Reticulum Membrane. *Cell* 1992, 69:343–352.
Nascent translocating chains were crosslinked to the yeast protein Sec62p early in translocation. Proteins translocating post-translationally were only crosslinked before the addition of ATP and proteins translocating co-translationally were only crosslinked when they were still short chains. All proteins were crosslinked to Sec61p throughout the translocation process.

60. GÖRLICH D, PREHN S, HARTMANN E, KALIES K-U, RAPOPORT TA:
• A Mammalian Homolog of SEC61p and SECYp is Associated with Ribosomes and Nascent Polypeptides During Translocation. *Cell* 1992, 71:489–503.
A mammalian homologue of the yeast Sec61p was identified that also has homology to the *E. coli* Sec Y. The protein was crosslinked to translocating chains and found to associate tightly with ribosomes during protein translocation.

61. 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.
Yeast mutants defective in protein assembly were selected using histidinol dehydrogenase linked to a transmembrane protein using the genetic scheme described in [47,48]. New alleles were identified in *sec61* and a new mutation in *sec65* (see [34•,35•]).

62. BLOBEL G, DOBBERSTEIN B: Transfer of Proteins Across Membranes. II. Reconstitution of Functional Rough Microsomes from Heterologous Components. *J Cell Biol* 1975, 67:852–862.

63. 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.

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

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

66. MIGLIACCIO G, NICCHITTA CV, 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-solubilized membranes were depleted of either SSR or the SRP receptor before membrane reconstitution. Membranes lacking SRP receptor were not translocation-competent. Membranes lacking SSR resembled mock-depleted membranes suggesting that either SSR is not involved in translocation or there is another functionally equivalent protein.

67. SHELNESS GS, LIN L, NICCHITTA CV; **Membrane Topology and**
• **Biogenesis of Eukaryotic Signal Peptidase.** *J Biol Chem* 1993, 268:5201–5208.
The membrane topology of the subunits of the signal peptidase complex (see [50]) was mapped. Each protein has a single transmembrane domain with its amino-terminal facing the cytosol. The putative active domains were approximately the same distance from the bilayer as the active domain for the analogous domain in the signal peptidase of *E. coli*. Each of the subunits required SRP to be translocated into the membrane.

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

69. NUNNARI JM, ZIMMERMAN DL, OGG SC, WALTER P: **Characterization of the Rough Endoplasmic Reticulum Ribosome-Binding Activity.** *Nature* 1991, 352:638–640.

70. 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 (Tokyo)* 1991, 109:89–98.

71. ICHIMURA T, OHSUMI T, SHINDO Y, OHWADA T, YAGAME H,
• MOMOSE Y, OMATA S, SUGANO H: **Isolation and Some Properties of a 34-kDa-Membrane Protein that may be Responsible for Ribosome Binding in Rat Liver Rough Microsomes.** *FEBS Lett* 1992, 296:7–10.
Describes the purification of a protein previously shown to bind ribosomes (see [70]). When reconstituted into liposomes, its ribosome-binding properties were similar to those of intact microsomes. Subcellular fractionation was used to localize the protein ribosome-bound membranes in a variety of mammalian tissues.

72. COLLINS PG, 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.

73. SIMON SM, BLOBEL G: **A Protein-Conducting Channel in the Endoplasmic Reticulum.** *Cell* 1991, 65:371–380.

74. CROWLEY KS, REINHART GD, JOHNSON AE: **The Signal Sequence**
• **Moves Through a Ribosomal Tunnel into a Non-Cytoplasmic Aqueous Environment at an Early Stage of Translocation.** *Cell* 1993, in press.
A fluorescent probe was incorporated into nascent secretory chains. While the chain was in the ribosome, or translocating the membrane, the probe indicated it was in an aqueous environment. However, iodide ions did not quench the fluorescence. If the protein was translated in the absence of membranes, the fluorescence was quenched with iodide, even while the protein was in the ribosomal tunnel. This suggests that the seal between the ribosome and the aqueous protein-conducting channel is not freely permeable to iodide.

75. SIMON SM, BLOBEL G: **Signal Peptides Open Protein-Conduct-**
• **ing Channels in *E. coli*.** *Cell* 1992, 69:677–684.
The signal peptide of LamB was shown to cause an increase of membrane conductance when applied to the cytoplasmic side of the *E. coli* plasma membrane. At low concentrations (approximately one signal peptide per *E. coli* volume) individual channels were observed whose size was similar to those channels seen in mammalian ER (see [72]). This indicates that the signal sequence is the ligand that opens the protein-conducting channels.

76. SCHIEBEL E, WICKNER W: **Preprotein Translocation Creates a**
• **Halide Anion Permeability in the *Escherichia coli* Plasma Membrane.** *J Biol Chem* 1992, 267:7505–7510.
Addition of pre-proteins dissipated the membrane potential across inverted plasma membrane vesicles of *E. coli*. The rate was affected by the halide concentration.

77. THIEFFRY M, NEYTON J, PELLESCHI M, FÈVRE F, HENRY J-P:
• **Properties of the Mitochondrial Peptide-Sensitive Cationic Channel Studied in Planar Bilayers and Patches of Giant Liposomes.** *Biophys J* 1992, 63:333–339.
A voltage-dependent cation channel in mitochondrial membranes was blocked when a peptide with some of the properties of a mitochondrial

signal sequence was added. The results indicate that the peptide enters and crosses the channel, which is observed as a blockage of ionic flow.

78. JOLY JC, WICKNER W: **The SecA and SecY Subunits of**
• **Translocase are the Nearest Neighbors of a Translocating Preprotein, Shielding it from Phospholipids.** *EMBO J* 1993, 12:255–263.
A photoactivated cross-linker was attached to the cysteine residues of proOmpA fused to dihydrofolate reductase. Partially translocated polypeptides were crosslinked to SecA and SecY. They were not observed to be linked to SecE, Bank I or the membrane phospholipids. This suggests that a translocating chain is completely shielded from the lipid bilayer by proteins.

79. SIMON SM, PESKIN CS, OSTER GF: **What Drives the Transloca-**
• **tion of Proteins?** *Proc Natl Acad Sci USA* 1992, 89:3770–3774.
Proteins were proposed to translocate by biased random thermal motion. Translocating chains fluctuate back and forth in protein-conducting channels. Chemical asymmetries (binding of sugar groups or chaperones, differences in folding due to ions or pH, disulfide bond formation, signal peptide cleavage) bias the diffusion from the cytosol to the lumen. The model can quantitatively account for observed rates of translocation.

80. BRODSKY JL, HAMAMOTO S, FELDHEIM D, SCHEKMAN R: **Recon-**
• **stitution of Protein Translocation from Solubilized Yeast Membranes Reveals Topologically Distinct Roles for BiP and Cytosolic Hsc70.** *J Cell Biol* 1993, 120:95–102.
Protein translocation was reconstituted in vesicles from wild-type yeast ER or fom sec63 or kar2 (BiP mutants). Translocation was deficient with vesicles from the mutants. However, the vesicles from kar2 translocated if wild-type BiP was included during the reconstitution.

81. NICCHITTA CV, BLOBEL G; **Lumenal Proteins of the Mam-**
• **malian Endoplasmic Reticulum are Required to Complete Protein Translocation.** *Cell* 1993, 73:989–998.
Depletion of lumenal proteins from reconstituted mammalian microsomes had no effect on the targeting of proteins but blocked subsequent translocation of proteins. The inclusion of lumenal ER proteins restored translocation as assayed by increased protease protection of the nascent polypeptide and co-fractionation of the peptide with the microsomes.

82. OOI CE, WEISS J: **Bidirectional Movement of a Nascent**
• **Polypeptide Across Microsomal Membranes Reveals Requirements for Vectorial Translocation of Proteins.** *Cell* 1992, 71:87–96.
A truncated secretory polypeptide moved bidirectionally across the ER. After signal peptide cleavage it did not fractionate with microsomes. However, addition of a glycosylation site to the translocating chain ratcheted the peptide in the microsome and bidirectional transport was no longer observed.

83. ARKOWITZ RA, JOLY JC, WICKNER W: **Translocation can Drive**
• **the Unfolding of a Preprotein Domain.** *EMBO J* 1993, 12:243–253.
A construct of proOmpA–dihydrofolate reductase stabilized with its ligands NADPH and methotrexate was trapped as a translocation intermediary. Upon removal of the ligands, the dihydrofolate reductase unfolded and translocated. This occurred with neither ATP nor $\Delta_{\mu H+}$.

84. KANG P-J, OSTERMANN J, SHILLING J, NEUPERT W, CRAIG EA,
• PFANNER N: **Requirement for hsp70 in the Mitochondrial Matrix for Translocation and Folding of Precursor Proteins.** *Nature* 1990, 348:137–143.

85. SAKAGUCHI M, TOMIYOSHI R, KUROIWA T, MIHARA K, OMURA T;
• **Functions of Signal and Signal-Anchor Sequences are Determined by the Balance Between the Hydrophobic Segment and the N-Terminal Charge.** *Proc Natl Acad Sci USA* 1992, 89:16–19.
Mutations were made in a signal sequence that affected either the charge at the amino terminus or the length of the hydrophobic segment. Short hydrophobic segments acted as signal sequences. Longer hydrophobic segments formed transmembrane domains unless preceded by positive charges at the amino terminus.

86. GREEN N, WALTER P: **C-Terminal Sequences can Inhibit**
• **the Insertion of Membrane Proteins into the Endoplasmic**

Reticulum of *Saccharomyces cerevisiae. Mol Cell Biol* 1992, 12:276-282.

Segments from histidinol dehydrogenase added immediately on the carboxyl-terminal side of a transmembrane domain of arginine permease blocked its insertion into the membrane. A spacer region (taken from invertase) allowed the subsequent histinol dehydrogenase fragment to translocate.

87.   VON HEIJNE G, GAVEL Y: Topogenic Signals in Internal Membrane Proteins. *Eur J Biochem* 1988, 174:671-678.

88.   ANDERSSON H, BAKKER E, VON HEIJNE G: Different Positively
•   Charged Amino Acids have Similar Effects on the Topology of a Polytopic Transmembrane Protein in *Escherichia coli. J Biol Chem* 1992, 267:1491-1495.

Arginine and lysine were shown to have similar effects on transmembrane orientation when on the cytoplasmic side of a transmembrane segment. The effects of histidine, though weaker, were potentiated by lowering the cytoplasmic pH.

89.   ANDREWS DW, YOUNG JC, MIRELS LF, CZARNOTA GJ: The Role of
•   the N Region in Signal Sequence and Signal-Anchor Function. *J Biol Chem* 1992, 267:7761-7769.

Charged groups were inserted in the signal sequence of preprolactin, which converted it into a transmembrane protein. Net charges of + 2.5 or − 6.0 produced transmembrane topologies that did not conform to the 'positive-inside' rule (see [85•]).

90.   LOCKER JK, ROSE JK, HORZINEK MC, ROTTIER PJM: Membrane
•   Assembly of the Triple-Spanning Coronavirus M Protein. Individual Transmembrane Domains Show Preferred Orientation. *J Biol Chem* 1992, 267:21911-21918.

Each transmembrane domain functioned independently to translocate a protein. Each adopted the transmembrane topography corresponding to its orientation in the intact protein.

91.   CALAMIA J, MANOIL C: Membrane Protein Spanning Segments
•   as Export Signals. *J Mol Biol* 1992, 224:539-543.

Different transmembrane fragments of the lac permease were shown to function independently as signal sequences when fused to alkaline phosphatase (as a receptor gene).

92.   SKACH WR, CALAYAG MC, LINGAPPA VR: Evidence for an
•   Alternate Model of Human P-Glycoprotein Structure and Biogenesis. *J Biol Chem* 1993, 268:6903-6908.

Reporter groups were inserted into the sequence of the MDR-1 protein to analyze transmembrane topography. The observed topography did not conform to predictions from computer models. One reporter region was not consistently on one side of the membrane. The authors suggested there was regulation of the transmembrane topography that could also account for disparate functions attributed to the protein.

93.   KUCHLER K, STERNE RE, THORNER J: *Saccharomyces cerevisiae STE6* Gene Product: a Novel Pathway for Protein Export in Eukaryotic Cells. *EMBO J* 1989, 8:3973-3984.

94.   MACKMAN N, NICAUD JM, GRAY L, HOLLAND IB: Identification of Polypeptides Required for the Export of Haemolysin 2001 from *E. coli. Mol Gen Genet* 1985, 201:529-536.

S Simon, Laboratory of Cellular Biophysics, Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.