Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

1

Transport of Proteins into and across the Endoplasmic Reticulum Membrane

EVE PERARA AND VISHWANATH R. LINGAPPA

I. Introduction
II. Historical Background
III. Targeting
  A. Signal Sequences
  B. Signal Recognition Particle
  C. Signal Recognition Particle Receptor
  D. Summary of Targeting Events
IV. Mechanism of Translocation
  A. Altered Substrates for Translocation
  B. Putative Components of Translocation Machinery
V. Membrane Assembly of Integral Transmembrane Proteins
  A. "Stop Transfer" Sequences
  B. Combination Signal–Stop Transfer Sequences
  C. Polytopic Transmembrane Proteins
  D. Reevaluation of Topogenic Sequences
  E. Summary of Transmembrane Protein Biogenesis
VI. Overview
  References

I. INTRODUCTION

The specific localization of proteins to specialized membrane-bound compartments allows the segregation of various biochemical functions which characterizes eukaryotic cells. Thus, the mitochondria are the exclusive site of oxidative phosphorylation because F_1 ATPase, cytochrome oxidase, and other components are exclusively localized to the mitochondrial membranes. Likewise, oxidative detoxification occurs in the endo-

Copyright © 1988 by Academic Press, Inc.
All rights of reproduction in any form reserved.

plasmic reticulum, degradation of endocytosed proteins in the lysosomes, and so on, as a consequence of protein targeting and localization. While the delimiting lipid bilayer(s) of subcellular compartments serves to maintain specific proteins within the organelle, it poses a distinct barrier to the initial correct segregation of these proteins. Since the synthesis of all but a few proteins (those encoded by mitochondrial or chloroplast DNA) occurs in the cytoplasm, mechanisms must exist by which proteins are efficiently and accurately sequestered into their specific membranous compartments. The subject of this chapter is the transport of proteins across the endoplasmic reticulum (ER) membrane. This is the first step in the sorting of proteins destined for the plasma membrane, Golgi complex, lysosome, or the exterior of the cell. The problem of transport of proteins across the ER membrane is essentially 2-fold: (1) How do the proteins recognize specifically the ER membrane from all the other cellular membrane systems? and (2) What is the mechanism by which these proteins are unidirectionally translocated across the hydrophobic lipid bilayer?

II. HISTORICAL BACKGROUND

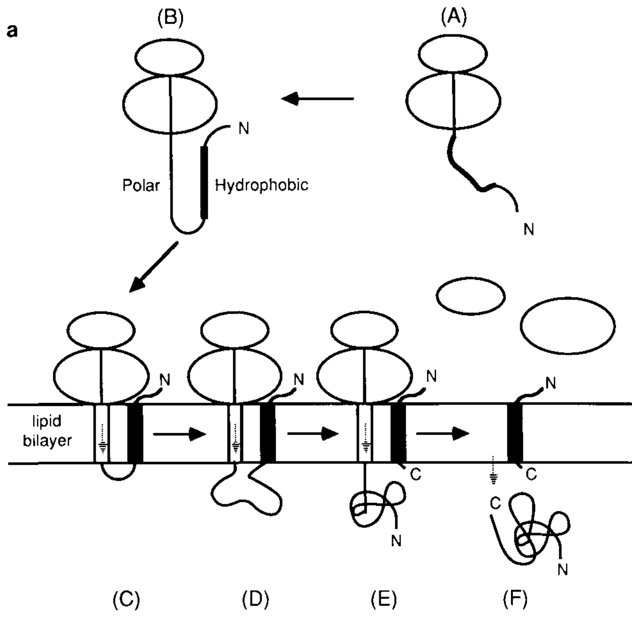
In pioneering work on the secretory pathway, Palade and co-workers found that nascent secretory proteins were associated with the ER membrane via the large ribosomal subunit of the synthesizing polysomes (Sabatini *et al.,* 1966; Palade, 1975), and that the mature polypeptides were released to the lumen of the ER (Redman *et al.,* 1966). These observations raised the question of how mRNAs encoding secretory proteins could be specifically selected to be translated on ER-bound ribosomes. Very early hypotheses included models in which the specificity was proposed to reside in the translating ribosome, in untranslated regions of mRNA, or in the nascent chain itself.

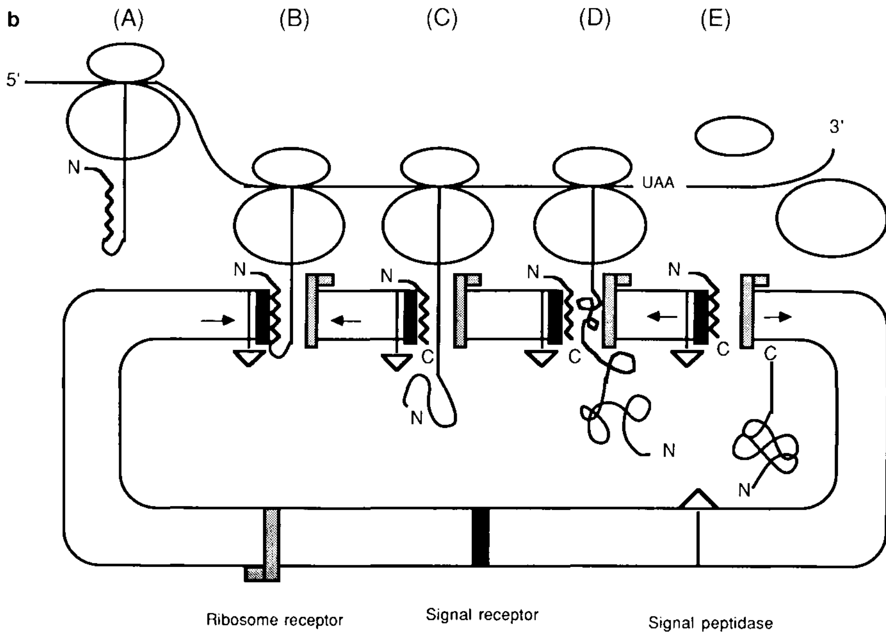
An answer to this question was obtained following the development of heterologous cell-free translation systems which could be programmed with purified mRNAs encoding secretory proteins. It was found that the primary cell-free translation products of immunoglobulin light chain differed from authentic light chain by an amino-terminal extension that was not observed in translation products of nonsecretory proteins. This additional peptide segment was proposed to be involved in the segregation of secretory proteins to the ER lumen (Milstein *et al.,* 1972). This idea received additional support from experiments in which cell-free translation reactions were supplemented with microsomal membranes derived from the rough ER. It was found that secretory proteins were synthesized with amino-terminal sequences not present either in secretory products *in*

vivo or *in vitro* products localized to the microsomal lumen, suggesting that the precursor protein was processed to the mature form by the membranes (Blobel and Dobberstein, 1975a,b). These findings led to the formulation of the signal hypothesis (Blobel and Dobberstein, 1975a,b) in which it was postulated that the emergence of a transient "signal sequence" of amino acid residues as part of the nascent chain directs polysomes synthesizing secretory proteins to, and facilitates their transport across, the ER membrane (see below). Indeed, nearly all eukaryotic secretory proteins examined so far have been found to be synthesized as precursors with amino-terminal, cleaved signal sequences.

The concept of "vectorial discharge" of the nascent polypeptide across the membrane was established by the work of Redman and Sabatini (1966), which demonstrated that puromycin-released nascent (incomplete) polypeptide chains were localized to the lumen of vesicles isolated from the rough ER. This idea was confirmed by the finding that nascent polypeptides emerging from the ribosome were protected from proteases by the microsomal membrane (Sabatini and Blobel, 1970). In addition, a number of posttranslational modifications of proteins known to occur in the luminal space of the ER, such as cleavage of signal sequences by signal peptidase (Blobel and Dobberstein, 1975a), transfer of core oligosaccharides from lipid-linked intermediates to asparagine residues (Lingappa *et al.*, 1978a; Glabe *et al.*, 1980), and intrachain disulfide bond formation, have been shown to be carried out on nascent polypeptides as they traverse the ER membrane. Completed secretory protein precursors are incapable of being translocated (Blobel and Dobberstein, 1975b), and, in fact, there seems to exist a brief period early in a protein's elongation during which translocation can be initiated (Rothman and Lodish, 1977). Thus a picture emerged of obligate coupling of the transport of secretory proteins to their biosynthesis, i.e., translocation occurs concomitant with translation. This feature of protein translocation across the ER membrane distinguishes it from the transport of mitochondrial proteins (Schatz and Butow, 1983) and from the export of bacterial proteins (Randall, 1983).

Two general hypotheses have been advanced regarding the mechanism by which proteins may be transported across, or assembled into, the ER membrane. One type of model (von Heijne and Blomberg, 1979; Engelman and Steitz, 1981) suggests that protein transport across the membrane is dictated solely by the thermodynamics of interactions between the protein and the lipid bilayer without the participation of specific receptors or transport proteins in the membrane aside from "targeting" proteins (see below) and signal peptidase. These "spontaneous insertion" models postulate that the signal sequence approaches the membrane as the hydrophobic limb of a helical hairpin (see Fig. 1a). The free energy


a
(B)
(A)
N
Polar
Hydrophobic
N
lipid
bilayer
N
N
N
N
C
C
C
N
N
(C)
(D)
(E)
(F)


b
(A)
(B)
(C)
(D)
(E)
5'
3'
UAA
N
N
N
N
N
C
C
C
C
N
N
N
Ribosome receptor
Signal receptor
Signal peptidase

gained through the insertion of the hydrophobic signal sequence directly into the lipid bilayer is proposed to exceed the free energy "cost" of transiently burying charged residues in the hydrophilic limb of the helical hairpin. These hydrophilic sequences pulled into the bilayer would nevertheless be thermodynamically unstable and hence would move spontaneously across the bilayer by Brownian motion as protein synthesis continued. The nascent chain would thus be transported unless or until another hydrophobic sequence was encountered which would be thermodynamically stable in the lipid bilayer and thus serve to anchor the polypeptide in the membrane. Removal of signal sequences by signal peptidase is proposed to release secretory proteins to the ER lumen. Much of the appeal of this model has derived from its simplicity. Its early drawback was its difficulty in explaining the translocation of secretory proteins, such as ovalbumin, which lack cleaved signal sequences (Palmiter *et al.*, 1978). Recently, additional evidence has emerged that is difficult to reconcile with the spontaneous insertion models (see below).

The signal hypothesis envisions a distinctly different mechanism for chain translocation (see Fig. 1b; Blobel and Dobberstein, 1975a,b; Blobel, 1980). Key features of a current version of this hypothesis (for review, see Walter and Lingappa, 1986) are as follows: (1) the information for the localization of proteins to the ER lumen is contained within a discrete portion of the nascent polypeptide, itself—the signal sequence; (2) the signal sequence facilitates translocation of the nascent chain through in-

---

Fig. 1. (a)Steps in the process of translocation according to spontaneous insertion models. Translation begins on free cytoplasmic ribosomes (A); on emergence of a sufficient length of amino acid residues, marginally stable folding begins (B). The hydrophobic limb of the hairpin inserts spontaneously into the membrane (C), pulling in the polar limb, as described in the text. As synthesis continues, the growing nascent polypeptide constitutes the relatively polar, thermodynamically unstable, limb of the hairpin and therefore passes through the membrane, folding in the extracytoplasmic space (D). Cleavage of the signal sequence occurs, releasing the amino terminus of the polypeptide into the lumen (E). On completion of translation, the C terminus passes through the membrane and is released into the ER lumen, and the cleaved signal peptide is the left in the membrane (F). (b) Steps in the process of translocation (subsequent to targeting) according to the signal hypothesis. Synthesis begins on cytoplasmic ribosomes (A). Receptor-mediated targeting of the signal sequence-bearing ribosome to the ER membrane is described in the text and depicted in Fig. 2. Once targeted correctly, the signal sequence and the ribosome interact with their respective receptors in the ER membrane, resulting in the assembly of an aqueous, proteinaceous tunnel across the membrane (B). As protein synthesis continues, the chain passes through the tunnel to the lumen of the ER, and the signal sequence is removed by signal peptidase (C). Translocation (possibly of folded polypeptide domains) continues concomitant with protein synthesis (D). On termination of protein synthesis, the ribosomal subunits dissociate, the carboxy terminus passes through the tunnel, and the tunnel components disassemble (E), restoring the integrity of the lipid bilayer.

teractions with a series of receptors both in the cytoplasm and in the ER membrane; (3) transit of the nascent chain across the membrane occurs via a proteinaceous pore or tunnel in the bilayer whose activation and assembly is catalyzed by the signal sequence; (4) the information for termination of translocation before termination of synthesis (e.g., in the case of transmembrane proteins) is encoded also in a discrete segment of the nascent chain termed the "stop transfer sequence" which also acts via particular receptors in the membrane. The appeal of the signal hypothesis has largely derived from experimental verification of its tenets. Its major weakness is that it is difficult to imagine the initiation of specific receptor-mediated events by signal sequences which have such variable primary structures (see below).

III. TARGETING

A fundamental problem for the transport of proteins across a specific intracellular membrane is the selection of the correct membrane by the protein to be transported. From the use of cell-free translation/translocation systems for the fractionation and reconstitution of translocation-associated events, a view of the molecular mechanisms of targeting of nascent secretory polypeptides to the membrane of the rough ER has emerged.

A. Signal Sequences

Most secretory proteins and many transmembrane proteins are synthesized as precursors with transient amino-terminal signal sequences as are bacterial exported proteins. In contrast to the amphipathic signal sequences of mitochondrial proteins, these signal sequences are characterized by their extreme hydrophobicity.

The most reliable definition of a signal sequence if a functional one: The ability of a sequence of amino acids within a protein to direct its translocation. Over a hundred amino-terminal signal sequences have been cataloged (Watson, 1984; von Heijne, 1985), and yet no overall homology in primary structure has been observed. Signal sequences range from 15 to 30 amino acid residues in length. They consist of a very hydrophobic core of variable length (at least six amino acids) flanked on either side by regions containing polar or hydrophilic residues. Often an amino acid with a small side chain, such as glycine, valine, or alanine, occurs at the cleavage site (von Heijne and Blomberg, 1979; von Heijne, 1984). Since no clear sequence homologies exist, the recognition features of signal

sequences have been proposed to reside in their secondary structure. Bacterial and eukaryotic signal sequences are virtually indistinguishable from one another (von Heijne, 1985). In fact, eukaryotic proteins can be secreted and processed by bacteria (Talmadge *et al.*, 1980a,b), and, likewise, prokaryotic proteins are correctly segregated and processed in eukaryotic cell-free systems (Muller *et al.*, 1982).

Two protein systems have been identified with which signal sequences may interact: the targeting proteins (signal recognition particle and its receptor, see below) on the cytoplasmic side of the ER membrane and signal peptidase on the luminal aspect. Whether additional systems are involved, or whether proteins which actually span the bilayer are recognized, remains to be demonstrated. These two recognition systems may interact with distinct regions of the signal sequence (Blobel and Dobberstein, 1975b). Analysis of signal sequence mutants in bacterial export systems demonstrates that the proposed two sites are at least functionally distinguishable since mutants are observed which can be translocated but are not processed by signal peptidase (Lin *et al.*, 1978; Koshland *et al.*, 1982; Kadonaga *et al.*, 1985). Not surprisingly, the hydrophobic core which characterizes signal sequences appears to be critical for translocation since disruption of the integrity of this core, either by the introduction of charged residues or by small deletions in this hydrophobic stretch, abolishes export in bacteria (Emr and Silhavy, 1982; Bedouelle *et al.*, 1980). However, the hydrophobic core is not sufficient for translocation since deletion of the coding region for the six carboxy-terminal amino acids of the α-amylase signal sequence, leaving the hydrophobic stretch intact, abolishes secretion from *Escherichia coli* (Palva *et al.*, 1982).

While a signal sequence can be sufficient to translocate some proteins both *in vitro* and *in vivo* in eukaryotic systems (Lingappa *et al.*, 1984; Simon *et al.*, 1987), the same has not been demonstrated for export of proteins from bacteria (Moreno *et al.*, 1980; Kadonaga *et al.*, 1984). Whether this reflects differences in the passenger proteins used or differences in the mechanism of translocation between prokaryotes and eukaryotes remains to be determined. While translocation in eukaryotic cell-free systems appears to be cotranslational, translocation in *E. coli in vivo* and *in vitro* seems to be posttranslational (Randall, 1983; Randall and Hardy, 1986; Muller and Blobel, 1984). Thus, while the mechanism for translocation of proteins across cellular membranes appears to be highly conserved, critical differences do exist. It is important to note that fine structure analyses of signal sequences have been carried out exclusively on prokaryotic proteins in bacterial cells. A systematic analysis of signal sequence structure and function in eukaryotic cell-free systems has yet to be done.

Signal sequences usually occur at the extreme amino terminus of a protein and are cleaved from the nascent polypeptide before translation is complete. There are at least two exceptions to this rule: (1) Internal and uncleaved signal sequences have recently been described for a number of transmembrane proteins (Bos *et al.*, 1984; Spiess and Lodish, 1985; Friedlander and Blobel, 1985; Eble *et al.*, 1986). (2) The secretory protein, ovalbumin, does not have a cleaved signal sequence (Palmiter *et al.*, 1978) but has the functional equivalent (Lingappa *et al.*, 1978b), and location of which remains controversial (Lingappa *et al.*, 1979; Meek *et al.*, 1982; Braell and Lodish, 1982; Tabe *et al.*, 1984).

B. Signal Recognition Particle

Signal recognition particle (SRP) activity was first recognized by the ability of a microsomal membrane high salt wash to restore translocation activity to rough microsomes whose ability to translocate in the wheat germ cell-free translation system had been abolished by high salt extraction (Warren and Dobberstein, 1978). The SRP molecule has been purified to homogeneity from a high salt extract of canine pancreas rough microsomes (Walter and Blobel, 1980), and its role in translocation has been studied in detail (Walter *et al.*, 1981; Walter and Blobel, 1981a,b). Subcellular fractionation demonstrates a roughly equal distribution of SRP between a membrane-associated and cytoplasmic (ribosome-associated or free) state (Walter and Blobel, 1983b). SRP appears to act as a cytoplasmic "adaptor" for signal-bearing polysomes, targeting them to the ER membrane.

1. Structure of Signal Recognition Particle

SRP is a ribonucleoprotein complex composed of six nonidentical polypeptides: a 19 kDa and a 54 kDa monomer, and two heterodimers, one consisting of the 9 kDa and the 14 kDa polypeptides and the other composed of 68 kDa and 72 kDa polypeptides (Siegel and Walter, 1985) and one molecule of the small cytoplasmic 7SL RNA, 300 nucleotides in length (Walter and Blobel, 1982). When disassembled, neither the protein nor RNA fraction alone is active. Reconstitution of the active molecule is possible, however, when polypeptides and RNA are reassembled together (Walter and Blobel, 1983a; Siegel and Walter, 1985). The 7SL RNA seems to act as scaffolding, around which the polypeptide subunits assemble; the subunits have little or no affinity for one another in the absence of the RNA. Systematic reconstitution experiments reassembling SRP molecules lacking defined polypeptide or RNA domains have allowed different assayable functions of SRP to be assigned to specific

structural domains of the molecule (see below; Siegel and Walter, 1985, 1986). Recent evidence demonstrates that the SRP molecule is oblong in shape (Andrews *et al.*, 1985) and that the RNA runs the length of the molecule (Andrews *et al.*, in press).

2. *Signal Sequence Recognition*

The purification of SRP has allowed detailed analysis of its role in translation and translocation of secretory proteins and its binding properties to other identified components in the translation/translocation system. While SRP was shown to bind translationally inactive ribosomes, the emergence of a signal sequence from the large ribosomal subunit results in an increase in the affinity of SRP for the ribosome by as much as four to five orders of magnitude (Walter *et al.*, 1981).

SRP binds to the signal sequence directly. This was suggested first by the finding that incorporation of the amino acid analog, β-hydroxyleucine, into leucine-rich signal sequences abolished SRP–signal sequence interactions (Walter *et al.*, 1981). Incorporation of a photoactivatable cross-linking amino acid analog into the signal sequence of nascent preprolactin has allowed cross-linking to the 54 kDa subunit of SRP, providing direct evidence for signal sequence–SRP binding (Kurzchalia *et al.*, 1986; Krieg *et al.*, 1986).

3. *Elongation Arrest*

When purified SRP is added to wheat germ cell-free translation reactions in the absence of microsomal membranes it specifically blocks elongation of signal sequence-bearing nascent chains soon after the signal emerges from the ribosome, concomitant with the increased affinity of SRP for the ribosome–nascent chain complex (Walter and Blobel, 1981b). On subsequent addition of salt-washed microsomal membranes, SRP is released from the nascent chain, translation resumes, and translocation occurs cotranslationally.

The elongation arrest function of SRP has been mapped to the 9/14 kDa protein and to 7SL RNA sequences that are homologous to repetitive Alu RNA (Siegel and Walter, 1985, 1986). Preparation of SRPs lacking either one of these domains yields a particle which can recognize signal sequences and facilitate translocation across salt-treated (SRP-free) microsomal membranes but lacks the elongation arrest activity. These partially reconstituted particles are active in promoting protein translocation, but only during a brief window of time early in nascent chain growth. Thus it appears that the role of SRP arrest may be to extend the window of time during which the nascent polypeptide is in a translocation competent state.

The mechanism by which SRP arrests translation is currently unclear.

Analysis of the structure and domain function of the SRP molecule presents an interesting possibility. The dimensions of the SRP molecule are such that the particle could span from the site at which the signal sequence emerges from the large ribosomal subunit to the elongation site between the two ribosomal subunits (Andrews *et al.*, 1985). The 54 kDa subunit binds to signal sequences directly (Kurzchalia *et al.*, 1986; Krieg, *et al.*, 1986); perhaps the 9/14 kDa subunit or 7SL RNA of SRP also bind the ribosome, hindering subsequent binding of aminoacyl-tRNAs and continued protein synthesis.

The physiological significance of the elongation arrest activity of SRP is controversial since not all signal sequence-bearing proteins experience a tight elongation arrest (Anderson *et al.*, 1983), nor is strict elongation arrest by canine SRP observed in some mammalian cell-free systems (Meyer, 1985). However, a kinetic delay in chain elongation is observed specifically for signal sequence-bearing proteins in a fractionated mammalian translation system supplemented with purified canine SRP (P. Walter, personal communication). This is consistent with the notion that an important role of SRP may be to extend the window of time during which the nascent secretory protein is in a "translocation competent state."

C. Signal Recognition Particle Receptor

The SRP receptor (also termed docking protein, Meyer *et al.*, 1982a) is an ER membrane protein localized to the cytoplasmic face of the membrane (Meyer *et al.*, 1982b) and has been purified from dog pancreas rough microsomes using SRP affinity chromatography (Gilmore *et al.*,1982a,b). A 60 kDa cytoplasmic domain of SRP receptor can be cleaved from the membrane by proteases and added back to reconstitute translocation activity (Walter *et al.*, 1979; Meyer and Dobberstein, 1980a,b), but apart from the membrane it is inactive (Gilmore *et al.*, 1982). SRP receptor has recently been shown to consist of two subunits, the previously identified 69 kDa polypeptide (now termed α subunit) and a 30 kDa β subunit (Tajima *et al.*, 1986).

The release of SRP-induced elongation arrest of secretory proteins by microsomal membranes is a function of the SRP receptor (Gilmore *et al.*, 1982a). Since SRP receptor was isolated by virtue of its affinity for SRP (Gilmore *et al.*, 1982b), it seems likely that the release of arrest may occur via a direct interaction between SRP and SRP receptor. This idea is further supported by data demonstrating that purified, detergent-solubilized SRP receptor causes SRP to lose its high affinity for signal sequence-bearing polysomes concomitant with the release of SRP-induced elongation arrest (Gilmore and Blobel, 1983).

Recently the amino acid sequence for the 69 kDa α subunit of SRP receptor has been determined from a cloned cDNA (Lauffer *et al.*, 1985). A domain consisting of clusters of predominantly basic mixed charge residues and which resembles nucleic acid binding proteins has been suggested to bind to SRP, possibly via the 7SL RNA.

The SRP receptor plays a critical role beyond releasing SRP-induced elongation arrest. Partially reconstituted SRPs which do not arrest nascent chain elongation but are capable of facilitating translocation require SRP receptor (Siegel and Walter, 1985). The requirement for SRP receptor in the absence of elongation arrest may reflect an essential role in targeting the ribosome–nascent chain to the microsomal membrane.

Quantitation of SRP and SRP receptor in pancreatic cells indicates that both are present in substoichiometric amounts relative to membrane-bound ribosomes (Gilmore *et al.*, 1982b; Walter and Blobel, 1980) and, thus, that SRP receptor is not involved directly in the process of translocation. Rather, it appears that the nascent chain–ribosome–SRP–SRP receptor interaction is transient and that the role of SRP and its receptor is primarily to target the nascent secretory polypeptide to the appropriate membrane system.

D. Summary of Targeting Events

In summary, the initial targeting events as they are currently understood have led to the model of the SRP cycle depicted in Fig. 2 (for review, see Walter *et al.*, 1984). On emergence of a signal sequence from ribosomes synthesizing secretory proteins in the cytoplasm, SRP binds the signal sequence directly, interrupting chain elongation and perhaps maintaining the nascent chain–ribosome complex in some "translocation competent state." The affinity of SRP for its receptor on the cytoplasmic face of the ER membrane targets the SRP–ribosome–nascent chain complex to that membrane system. Following interaction with its receptor in the ER membrane, SRP loses its affinity for the signal sequence-bearing ribosome and releases the complex, perhaps to another series of receptors in the membrane. Translation resumes and translocation across the membrane occurs. It is not known whether SRP initiates the translocation event or whether its role is merely to target the nascent chain to the correct location.[1]

[1] It should be noted that some small proteins (<10 kDa) such as M13 procoat protein (Watts, et al., 1983) and honeybee prepromelittin (Zimmermann and Mollay, 1986; Muller and Zimmermann, 1987) appear to bypass this well-characterized targeting mechanism. How these proteins are targeted to the ER membrane remains to be determined.

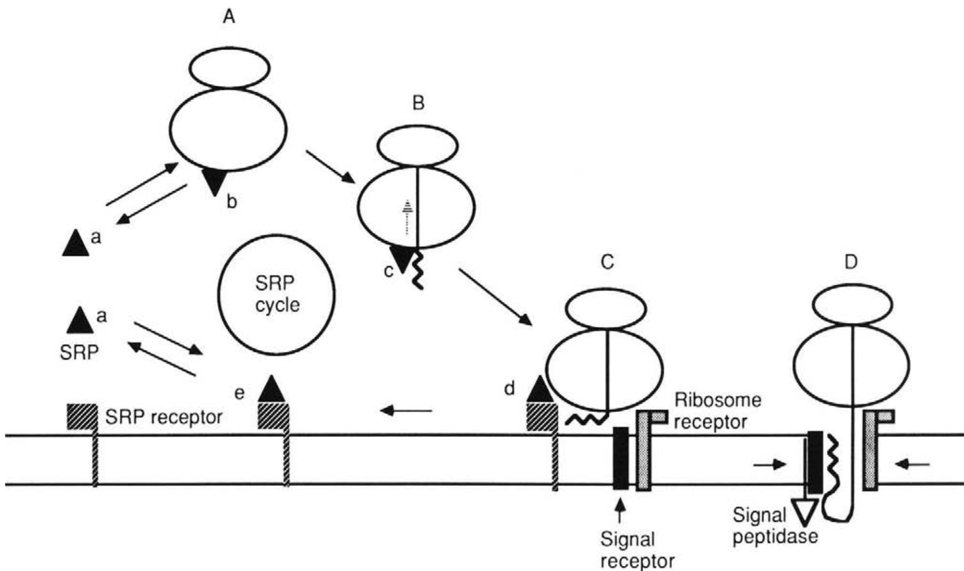


Fig. 2. Model of signal recognition particle (SRP) cycle for targeting nascent secretory and transmembrane proteins to the ER membrane. Soluble SRP (a) exists in equilibrium with a membrane-bound form, presumably bound to SRP receptor (e), and a ribosome-bound form (b). On translation of mRNA encoding a signal sequence for targeting to the ER membrane (zigzag lines), the affinity of SRP for the translating ribosome is enhanced (represented by dashed arrow, B) and SRP binds to the signal sequence directly (c), effecting elongation arrest (B–C). On interaction with ER membranes, elongation arrest is released and SRP and SRP–receptor are free to be recycled (SRP cycle, a–e), the synthesizing ribosome interacts with other transmembrane proteins, leading to formation of a functional ribosome–membrane junction, translation resumes, and translocation across the membrane occurs (D). Models for translocation are depicted in Fig. 1.

IV. MECHANISM OF TRANSLOCATION

The use of heterologous cell-free translation/translocation systems has provided a very powerful approach for the isolation of molecular components and the fractionation and reconstitution of specific activities involved in targeting of nascent chains to the ER membrane. However, the mechanism(s) by which these targeted nascent chains cross the lipid bilayer remains a mystery. This is due in large part to the obligate coupling of translocation to translation. In cell-free systems, and presumably *in vivo,* translocation can occur only during the limited time and under the fastidious conditions required for protein synthesis.

A. Altered Substrates for Translocation

One important concept regarding translocation is that information for transport across the membrane resides within the protein itself. With the availability of cloned genes and recombinant DNA technology has come the ability to manipulate this information by creating artificial substrates for translocation and thereby probe its mechanism. The analysis of altered substrates *in vitro* and *in vivo* has yielded a number of insights into the mechanism of translocation.

1. Signal Sequence Function

Initial studies involving the fusion protein consisting of the β-lactamase signal sequence fused to the cytoplasmic protein, globin, demonstrated that a signal sequence alone was sufficient to permit translocation across the ER membrane both *in vitro* (Lingappa *et al.*, 1984) and *in vivo* (Simon *et al.*, 1987). Thus, the information for translocation was contained within the signal sequence and not the protein being transported.

Expression of a fusion protein in which a cleaved amino-terminal signal sequence was engineered to an internal position raised interesting questions as to the mechanism of translocation (Perara and Lingappa, 1985). The cDNA for the normally cytoplasmic protein, globin, was engineered 5′ to that of preprolactin such that the initial 110 codons of globin were followed immediately by the entire coding region of preprolactin (see Fig. 3). The encoded hybrid protein consisted of the signal sequence of prolactin flanked at its amino terminus by globin and at its carboxy terminus by native prolactin (see Fig. 3). When the cDNA was expressed in cell-free systems by *in vitro* transcription and translation, not only was this signal sequence (formerly amino terminal, now internal) recognized by the translocation machinery of the membrane and cleaved by signal peptidase, but both the carboxy flanking domain and the amino domain (still attached to the cleaved signal at its carboxy end) were translocated to the lumen of microsomal vesicles (see Fig. 3). Extraction of the vesicles with carbonate demonstrated that neither the prolactin domain nor the globin domain with the signal attached at its carboxy terminus were integrated into the microsomal membrane. This result suggested that signal sequences do not bury into the lipid bilayer directly, as suggested by some (von Heijne and Blomberg, 1979; Engelman and Steitz, 1981; Briggs *et al.*, 1986), but rather that they facilitate translocation via associations with integral membrane proteins (see also Gilmore and Blobel, 1985). Another important implication of these findings concerned the ability of the signal sequence to facilitate translocation of an amino-terminal protein domain: Since synthesis of the amino-terminal domain proceeds before

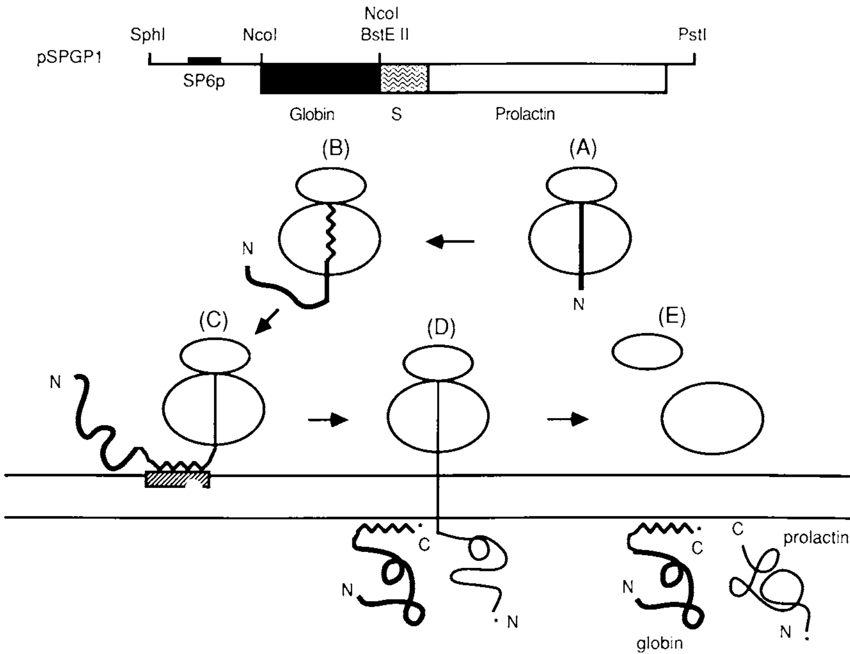


Fig. 3. Restriction map of relevant region of expression plasmid, pSPGP1, and schematic representation of translation and translocation of globin-prolactin fusion protein (GP), encoded by pSPGP1 (for details, see Perara and Lingappa, 1985). The SP6 promoter is represented by the small black bar denoted SP6p. The initial 109 codons of globin are represented by the solid black bar, the 30 codon preprolactin signal sequence coding region by the zigzag-patterned bar, and the 199 codons of mature prolactin by the open bar. In the translocation scheme, mRNA and protein components have been omitted for simplicity. The globin domain is represented by the heavy black line, the signal sequence by the zigzag, and prolactin by the thin black line. The globin domain is synthesized before the signal sequence emerges from the ribosome (A and B). The signal sequence is shown binding to a putative signal receptor on the cytoplasmic face of the ER membrane (C); the signal sequence facilitates translocation of both the globin and prolactin domains and is cleaved accurately (D). Thus the globin domain, with the prolactin signal peptide now at its carboxy terminus, and mature prolactin are localized to the microsomal lumen (E). Note: It is not known which domain is translocated first, globin or prolactin, nor is it understood how the signal sequence facilitates translocation of the globin (amino-terminal) domain (i.e., does it cross in an NH_2 to COOH fashion as does prolactin, COOH to NH_2, or as a folded domain?). In addition, apparent translocation efficiency of the amino-terminal globin domain is reduced relative to that of the processed prolactin (Perara and Lingappa, 1985).

the signal sequence emerges from the ribosome, its translocation could not be driven by the energy of its own synthesis. Thus the possibility was raised that machinery in the membrane, rather than the driving force of the synthesizing ribosome, performed work to achieve translocation.

2. *Dissociation of Translocation from Translation*

The use of engineered translocation substrates has allowed the processes of translation and translocation to be uncoupled for proteins whose translocation is normally strictly cotranslational (Perara *et al.*, 1986). By synthesizing truncated mRNA for a secretory protein which lacked a termination codon, arrested nascent polypeptides were generated which remained associated with the synthesizing ribosomes (see Fig. 4). When protein synthesis was blocked, such arrested chains were translocated only in the presence of nucleoside triphosphates and an ATP-regenerating system. Interestingly, the release of the "arrested" nascent polypeptide chains from the ribosome of synthesis by puromycin abolished translocation. Therefore, translocation across the ER membrane is not dependent on ongoing protein synthesis. The synthesizing ribosome does not "push" the nascent chain across the bilayer (von Heijne and Blomberg, 1979; Wickner and Lodish, 1985), nor does translocation occur "spontaneously" (Engelman and Steitz, 1981). Rather, the transport process consumes energy generated by nucleoside triphosphate hydrolysis (see also Chen and Tai, 1987; Schlenstedt and Zimmermann, 1987). In addition, it appears that the coupling of translation to translocation may reflect a role of the synthesizing ribosome in translocation independent of its role in protein synthesis. Similar findings have been demonstrated for integral membrane proteins (Perara *et al.*, 1986; Mueckler and Lodish, 1986a,b). Posttranslational translocation of human placental lactogen has also been shown to require an association of the precursor polypeptide with the ribosome (Caufield *et al.*, 1986).

It appears that this newly recognized role of the ribosome in translocation is for some aspect of targeting and not for translocation of the polypeptide per se, since ribosome-independent translocation has been observed in several instances (Hansen *et al.*, 1986; Schlenstedt and Zimmermann, 1987; Perara and Lingappa, in preparation). A small fusion protein consisting of the 23 amino acid signal sequence of β-lactamase followed by the initial 70 amino acids of chimpanzee α-globin is capable of posttranslational translocation and requires nucleoside triphosphate hydrolysis whether associated with the ribosome or not (E. Perara and V. R. Lingappa, in preparation). In most cases, however, translocation of truncated secretory and transmembrane proteins is dependent on an associa-

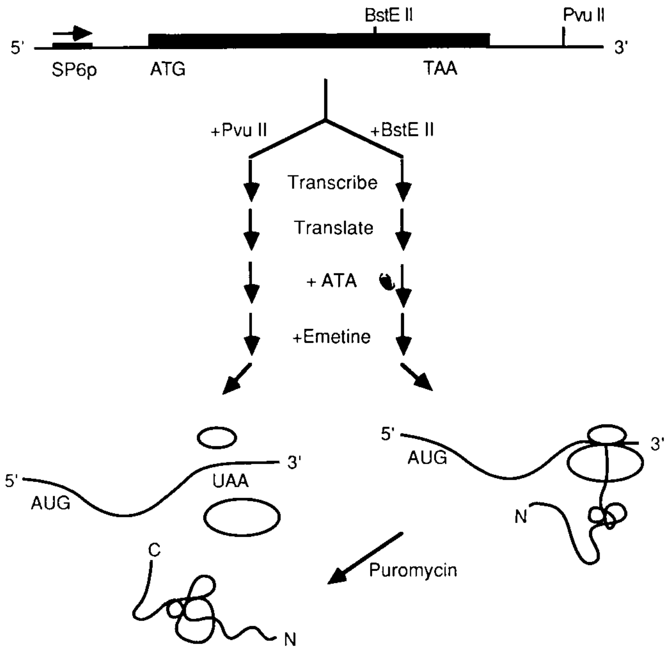


Fig. 4. Generation of substrates for dissociation of translocation from protein synthesis (see Perara *et al.*, 1986). SP6 expression plasmids encoding secretory proteins were digested with a restriction endonuclease which cut specifically either within the coding region, 5′ to the termination codon (*Bst*EII), or in the 3′noncoding region (*Pvu*II). On *in vitro* transcription, the latter treatment yields a full-length mRNA while the former treatment yields a truncated transcript lacking a termination codon. Ribosomes are able to translate the truncated transcript but do not dissociate for lack of a termination codon, thus translation yields intact polysomes with emergent nascent chains. In contrast, translation of full-length transcripts yields completed, free polypeptide chains. By treating translation reactions with aurintricarboxylic acid (ATA) and emetine, polypeptide chains were presented to microsomal membranes in the absence of protein synthesis. Ribosome-associated translation products of truncated RNAs were released from the ribosome by treatment with puromycin.

tion of the nascent chain with the ribosome of synthesis (Perara and Lingappa, unpublished observations).

Ribosome-independent translocation has also been observed for the yeast protein, prepro-α-factor (~18.5 kDa), in the recently developed homologous cell-free translation/translocation system derived from yeast (Waters and Blobel, 1986; Rothblatt and Meyer, 1986a; Hansen *et al.*, 1986). This transport was insensitive to uncouplers and ionophores but was dependent on nucleoside triphosphate hydrolysis (Hansen *et al.*, 1986; Waters and Blobel, 1986; Rothblatt and Meyer, 1986b) and required

proteinaceous factors in the membrane (as demonstrated by sensitivity to the alkylating agent, *N*-ethylmaleimide; Hansen *et al.*, 1986). It is not clear whether the posttranslational translocation of α-factor in this system represents a peculiarity of this particular molecule or is a general characteristic of the yeast *in vitro* translocation system and/or of this organism. The tight coupling between translocation and translation has been described only for higher eukaryotic cell-free systems.

These recent findings demonstrate that the ER membrane is capable of translocating completed signal-bearing proteins and protein domains via an energy-requiring mechanism. Why, then, must some chains remain associated with the ribosome in order for translocation to occur, while others need not, and why is efficient translocation normally coupled to protein synthesis? One possible explanation is that the translocation competence of a given protein or polypeptide may reflect the accessibility of its signal sequence to signal receptors in the ER membrane and/or cytoplasmic receptors such as SRP. Thus, the signal sequence of a very short polypeptide or a protein that lacks rigid secondary structure may be readily accessible to these receptors, while longer proteins may require an association with the synthesizing ribosome, possibly in conjunction with SRP to present the signal sequence to the membrane. It appears that the ribosome serves to extend the window of time during which a given nascent polypeptide is translocation competent. The translocation competence of nascent chains varies from one protein to another, thus some nascent secretory proteins are translocation competent only during a narrow window of time early in their synthesis (Rothman and Lodish, 1977; Braell and Lodish, 1982; Siegel and Walter, 1985), while translocation of others can occur later in elongation (Ainger and Meyer, 1986) and still others, such as prepro-α-factor, may translocate even as completed polypeptides (Hansen *et al.*, 1986; Schlenstedt and Zimmermann, 1987).

A requirement for high-energy phosphate bond hydrolysis has been demonstrated for translocation (recent work suggests that ATP hydrolysis is required for co-translational translation [Chen and Tai, 1987], however, it remains to be determined how hydrolysis facilitates protein transport across the membrane. Energy may be expended to arrange and/or maintain the nascent chain in a translocation-competent state, to assemble or ''activate'' a protein tunnel in the membrane, or directly for movement of the polypeptide to the lumen of the ER.

B. Putative Components of Translocation Machinery

The capacity of the ER membrane to translocate already synthesized proteins (Hansen *et al.*, 1986; Mueckler and Lodish, 1986a; Müller and

Zimmermann, 1986; Schlenstedt and Zimmerman, 1987) or protein domains (Perara and Lingappa, 1985; Perara *et al.*, 1986) can be explained either by the existence of a large tunnel or pore in the membrane (through which such folded domains could diffuse or be transported), or, alternatively, by a membrane "denaturase" to effect unfolding of these domains prior to their transport. The signal hypothesis proposes that translocation occurs via an aqueous channel formed by integral membrane proteins, the assembly of which is directed by the signal sequence. Recently some evidence for this idea has emerged. Partially translocated nascent chains were generated by oligonucleotide-mediated hybrid arrest (Gilmore and Blobel, 1985). These polypeptides, spanning the membrane, with their amino termini in the lumen and their carboxy termini in the cytoplasmic space were extractable from the microsomes with protein denaturants such as urea, suggesting that the translocation process occurs in an environment accessible to aqueous perturbants and that it involves interactions of the nascent chain with proteins in the ER membrane.

It is predicted that such a putative tunnel would consist of a complex assembly of a number of integral membrane proteins essential for translocation and related processes such as signal sequence cleavage, N-linked oligosaccharide transfer, and other posttranslational modifications known to occur in the ER lumen. Candidates for components of a translocon include a ribosome receptor, signal sequence receptor, and signal peptidase.

1. *Ribosome Receptor*

Binding of polysomes synthesizing secretory proteins to the ER membrane has long been thought to play an important role in the vectorial transport of nascent chains across the membrane (Redman and Sabatini, 1966). Such ribosomes bind to the ER membrane both via their nascent chains (Gilmore and Blobel, 1985) and directly via their large subunits by a salt-labile interaction (Adelman *et al.*, 1973). This binding is saturable and sensitive to proteases (Hortsch *et al.*, 1986).

The ribophorins (I and II), two integral membrane glycoproteins which are present in rough microsomes but absent from smooth membranes (Kreibich *et al.*, 1978a), have been suggested as ribosome receptor(s). Several indirect lines of evidence support this idea, including cofractionation of ribosomes and ribophorins following detergent solubilization (Kreibich *et al.*, 1978a) or protein cross-linking treatment (Kreibich *et al.*, 1978b) of rough microsomes and a good stoichiometry between the number of ribophorins and the ribosome binding capacity of rough microsomes (Marcantonio *et al.*, 1984). However, controlled proteolysis of rough microsomes suggests that ribophorins do not mediate functional

ribosome binding directly since ribosome binding activity of rough microsomes is lost following protease treatment to which ribophorins appear resistant (Hortsch *et al.*, 1986). In addition, translocation of some secretory proteins can occur *in vitro* across smooth microsomes which lack ribophorins (Bielinska *et al.*, 1979). Therefore, it appears that the ribophorins do not play an essential role in translocation nor in functional binding of ribosomes to the ER membrane. The role of ribophorins or other ribosome binding proteins in translocation remains to be determined.

2. *Signal Sequence Receptor*

In addition to ribosome binding sites, an independent signal sequence receptor in the ER membrane has also been suggested (Prehn *et al.*, 1980, 1981; Gilmore and Blobel, 1985; Hortsch *et al.*, 1986). Posttranslational binding of signal-bearing proteins is specific, saturable, and protease sensitive, as well as specific for rough microsomes (Prehn *et al.*, 1980, 1981). A nascent, SRP-arrested polypeptide binds to microsomal membranes in an SRP–receptor-dependent manner and remains associated with the membrane even after extraction of the ribosome with puromycin and high salt (Gilmore and Globel, 1985) but is extractable with protein denaturants such as urea or alkaline pH. Through a series of elegant cross-linking experiments, Wiedmann *et al.* (1987) have recently identified a signal sequence receptor that is an integral membrane glycoprotein of the ER.

3. *Signal Peptidase*

Signal peptidase is an integral membrane protein presumed to act on the luminal side of the ER membrane because cleavage of signal sequences takes place only on translocated secretory proteins unless the membrane is solubilized (Jackson and Blobel, 1977). Since signal peptidase removes signal sequences from nascent polypeptides as they cross the ER membrane (Blobel and Dobberstein, 1975b) it is postulated to be associated with a complex of proteins in the membrane that are involved in other aspects of translocation (e.g., tunnel proteins). Signal peptidase has recently been purified from canine pancreas rough microsomes as a relatively abundant complex of four to six polypeptides (Evans *et al.*, 1986). Bacterial leader peptidase I, which can accurately cleave eukaryotic signal sequences (Watts *et al.*, 1983), exists as a single polypeptide (Wolfe *et al.*, 1982). It is believed that eukaryotic signal peptidase is very similar to this bacterial enzyme since bacterial secretory proteins can be accurately processed by canine rough microsomes (Muller *et al.*, 1982). By analogy, it is thought that eukaryotic signal peptidase also exists as one polypeptide and that the additional copurifying proteins may be involved in other

translocation-related processes. The finding that this complex exists in roughly stoichiometric amounts relative to membrane-bound ribosomes has lead to speculation that it may form a core around which still other membrane proteins assemble to form a translocation apparatus in the membrane (Evans *et al.*, 1986).

V. MEMBRANE ASSEMBLY OF INTEGRAL TRANSMEMBRANE PROTEINS

The biogenesis of integral transmembrane proteins (ITMPs) adds an additional degree of complexity to the problem of transport of proteins across the ER membrane (Rothman and Lenard, 1977). Not only must certain polypeptide domains be translocated across the lipid bilayer but specific domains must span the membrane while others are left in the cytoplasmic space. Integral membrane proteins can be considered in two broad categories (see Fig. 5): (1) bitopic ITMPs which span the membrane only once, with their amino and carboxy termini on opposite sides of the membrane, and (2) polytopic membrane proteins which span the membrane multiple times. These can be further classified according to the disposition of the termini relative to the membrane: proteins whose amino and carboxy termini are on opposite sides of the membrane can be referred to as "trans" ITMPs, with the terminus residing in the extracytoplasmic space specified [NH_2-trans, e.g., vesicular stomatitis virus (VSV) glycoprotein or bovine rhodopsin; or COOH-trans, e.g., influenza neuraminidase]; those which span the membrane with both termini on the same side can be considered "cis" ITMPs, with the side specified (C-cis, cytoplasmic, e.g., erythrocyte Band III protein; E-cis, extracytoplasmic, e.g., hepatitis B surface antigen; see Fig. 5).

Several lines of evidence suggest that the assembly of proteins in the ER membrane is an event similar to the process of transport of secretory proteins across the membrane: both secretory and integral transmembrane proteins appear to be synthesized on membrane-bound polysomes (Morrison and Lodish, 1975), both have signal sequences that interact with SRP (Anderson *et al.*, 1982; Bos *et al.*, 1984), both compete with one another for membrane-associated components required for translocation (Lingappa *et al.*, 1978a), and both require hydrolysis of nucleoside triphosphates for translocation into or across the membrane (Waters and Blobel, 1986; Hansen *et al.*, 1986; Mueckler and Lodish, 1986b; Perara *et al.*, 1986). For a secretory protein these events result in complete translocation across the ER membrane, while in the case of ITMPs protein domains are only partially translocated. The mechanism by which pro-

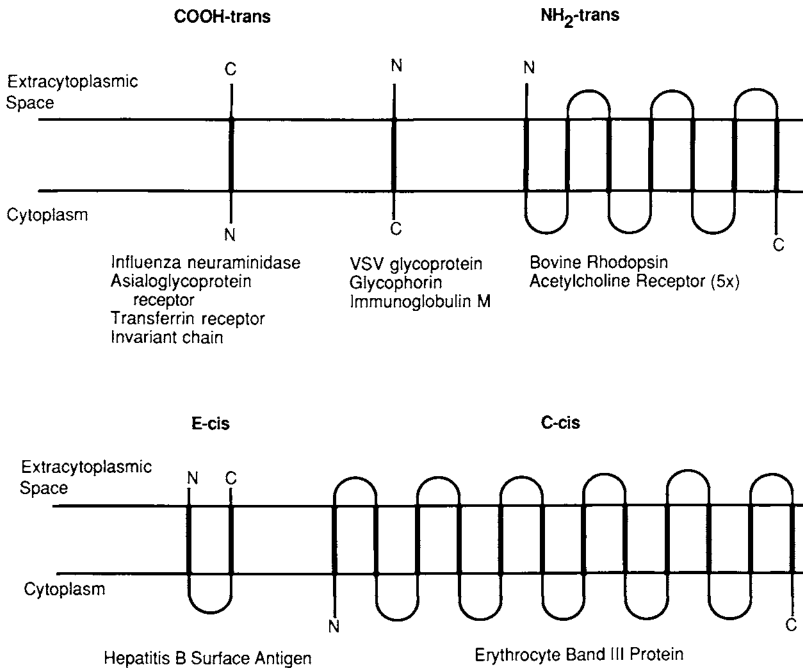


Fig. 5. Examples of orientations of integral transmembrane proteins. For definition of terminology, see text. Membrane-spanning segments are represented by heavy black lines. References for sequence and/or experimental orientation data are as follows: influenza neuraminidase (Bos *et al.*, 1984), asialoglycoprotein receptor (Spiess and Lodish, 1985, 1986), transferrin receptor (Schneider *et al.*, 1984; McClelland *et al.*, 1984; Zerial *et al.*, 1986), invariant chain (Lipp and Dobberstein, 1986), VSV glycoprotein (Katz *et al.*, 1977), glycophorin (Bretscher, 1971, 1975), immunoglobulin M (McCune *et al.*, 1980), bovine rhodopsin (Nathans and Hogness, 1983), acetylcholine receptor (Young *et al.*, 1985), hepatitis B surface antigen (Eble *et al.*, 1986), and erythrocyte Band III protein (Kopito and Lodish, 1985; Wickner and Lodish, 1985).

teins are oriented asymmetrically in the membrane operates with high fidelity—the orientation appears to be essentially identical for all copies of a given ITMP (Katz and Lodish, 1979). The orientation achieved in the ER membrane is the same as that in the final destination, e.g., the plasma membrane (Katz and Lodish, 1979). The process by which ITMPs are transported intracellularly from their site of synthesis at the ER membrane to their ultimate destination (e.g., Golgi membrane or plasma membrane) is poorly understood (for review, see Kelly, 1985).

As described for secretory proteins, two general hypotheses have been advanced to explain how the assembly of proteins in the membrane is achieved. One is a variant of the helical hairpin/spontaneous insertion

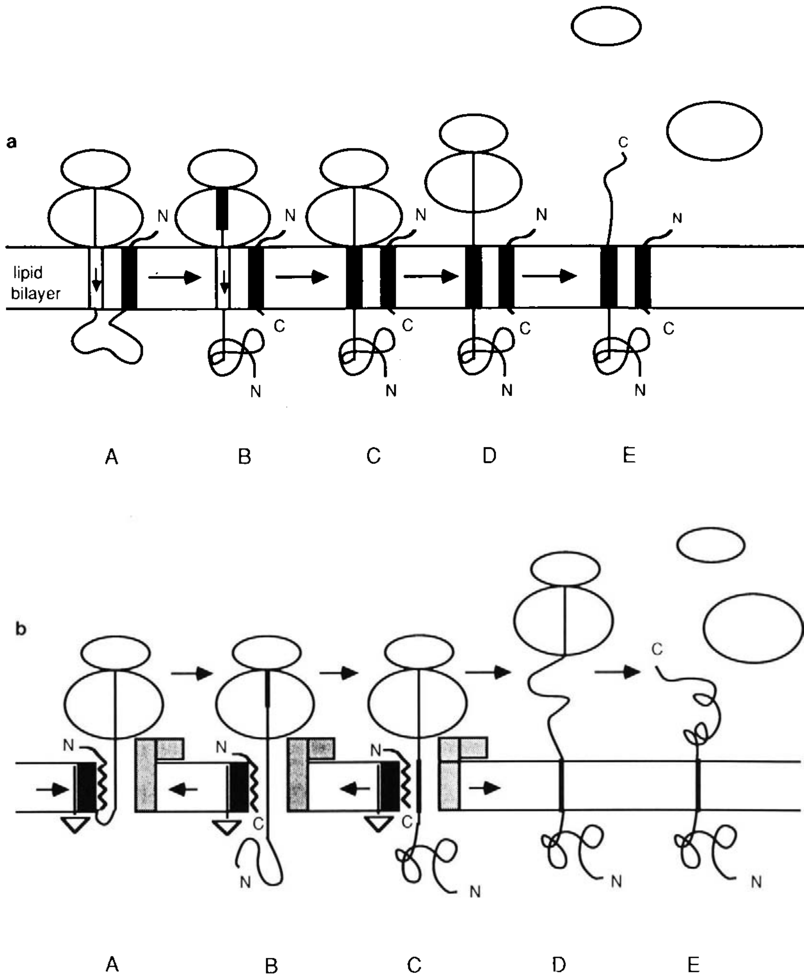


Fig. 6. (a) Integral transmembrane assembly of proteins according to spontaneous insertion/helical hairpin hypotheses. Translocation occurs as described in the text and Fig. 1a (A), until a sufficiently hydrophobic domain (stop transfer, represent by solid black bar) emerges from the ribosome (B). This region is thermodynamically stable in the lipid core of the bilayer and thus serves to halt the transfer of the nascent chain (C). The remainder of the polypeptide is synthesized in the cytoplasmic space (D, E). (b) Integral transmembrane protein assembly according to the signal hypothesis. Targeting of the nascent chain occurs as depicted in Fig. 2, and the signal sequence directs assembly of a tunnel for translocation across the membrane (A). Translocation occurs, and the signal sequence is cleaved as depicted in Fig. 1b (B). On emergence of a stop transfer sequence, components of the translocation machinery are disassembled (C) and the ribosome–membrane junction is disrupted, causing the remainder of the polypeptide to be synthesized in the cytoplasmic space (D, E).

hypothesis (Engelman and Steitz, 1981) and the direct transfer model (von Heijne and Blomberg, 1979), according to which the thermodynamics of protein–lipid interactions between the nascent chain and the ER membrane govern the protein's transport across, and integration into, the membrane. In this view, if a hydrophobic rather than a hydrophilic domain emerges as the second transmembrane region of the helical hairpin, the stability of this domain in the hydrophobic environment of the lipid bilayer will prevent further translocation and integrate the chain into the membrane (see Fig. 6a). Alternatively, according to the signal hypothesis, "topogenic" sequences in the nascent chain serve to "stitch" the nascent polypeptide in the membrane in the correct transmembrane orientation largely via interactions with receptor proteins which initiate or terminate translocation (Blobel, 1980; see Fig. 6b). According to both hypotheses the information for translocation and membrane orientation resides in particular segments of the protein such as signal and "stop transfer" sequences. Signal sequences, as described earlier, act to initiate transport of the nascent chain across the membrane, while stop transfer sequences terminate the transport process such that the subsequently synthesized polypeptide domain is maintained in the cytoplasmic space. Varying arrangements of these two types of topogenic sequences within a protein can conceivably account for any particular membrane orientation.

A. "Stop Transfer" Sequences

Early studies on transmembrane protein biogenesis carried out on VSV glycoprotein in cell-free translation systems demonstrated that this simple bitopic transmembrane protein possesses a transient amino-terminal signal sequence (Lingappa *et al.*, 1978a) and spans the membrane with a topology indistinguishable from that observed *in vivo*, i.e., with a large amino-terminal extracytoplasmic domain and a small carboxy-terminal cytoplasmic domain (Katz *et al.*, 1977; Katz and Lodish, 1979). Similar to translocation of secretory proteins *in vitro*, integration of VSV glycoprotein into microsomal membranes was found to occur only when microsomes were present during protein synthesis (Katz *et al.*, 1977; Toneguzzo and Ghosh, 1977). Moreover, competition experiments revealed that nascent VSV glycoprotein competed with a nascent secretory protein for a membrane component(s) involved in transfer across the membrane (Lingappa *et al.*, 1978a). Thus it was proposed that the biogenesis of secretory proteins and integral transmembrane proteins share early events in common. What causes some proteins to be only partially translocated?

A clue to the answer to this question is found in the immunoglobulin M (IgM) heavy chain which exists in two forms—one on the cell surface and the other secreted—which differ only in the presence on the former of a carboxy-terminal transmembrane segment and small cytoplasmic domain (Vasalli *et al.*, 1979; Kehry *et al.*, 1980; Singer and Williamson, 1980; McCune *et al.*, 1980). Apparently this carboxy-terminal extension serves to anchor the protein in the membrane. Indeed, other bitopic NH_2-trans ITMPs, which are synthesized with amino-terminal cleaved signal sequences and carboxy-terminal transmembrane segments, can be experimentally converted to secretory proteins by deletion of the transmembrane region (Boeke and Model, 1982; Gething and Sambrook, 1982; Rose and Bergmann, 1982), demonstrating that such segments are necessary for halting translocation.

That stop transfer sequences are sufficient to terminate translocation and to direct integration into the membrane was demonstrated by Yost *et al.* (1983), who engineered the IgM transmembrane segment to an internal position within a chimeric secretory protein, converting it to an integral transmembrane protein with predicted topology relative to the ER membrane (see Fig. 7). Moreover, the stop transfer sequence was able to act at other positions within the polypeptide beside the extreme carboxy terminus.

1. *Structure of "Stop Transfer" Sequences*

As is true for signal sequences, stop transfers are essentially functionally defined and exhibit little homology in primary sequence. Analysis of many transmembrane domains reveals that they consist of 20–30 hydrophobic and neutral amino acid residues often flanked by one or more positively charged residues on the cytoplasmic side (Sabatini *et al.*, 1982). It has been postulated that the hydrophobic stretch spans the membrane—a length of 20 amino acids is believed to be sufficient to span the 3-nm thickness of the lipid bilayer as an α-helix (Tanford, 1978). The basic residues are thought to play a role in membrane association by interactions with the negatively charged phospholipid head groups at the membrane surface (Sabatini *et al.*, 1982).

2. *Structure/Function Analyses of Stop Transfer Sequences*

The basic residues at the cytoplasmic boundaries of some membrane-spanning domains do not appear critical for the stop transfer function (Davis *et al.*, 1985; Cutler and Garoff, 1986; Zuniga and Hood, 1986) although they may play a role in stablizing the protein in the membrane

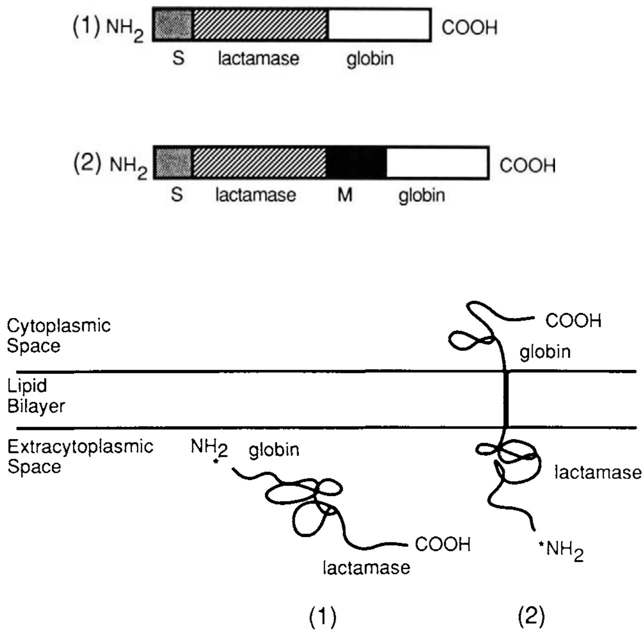


Fig. 7. A stop transfer sequence confers predictable transmembrane orientation on a previously secretory protein (see Yost *et al.*, 1983). Hybrid proteins consisting of lactamase and globin or lactamase, stop transfer, and globin are depicted as (1) and (2), respectively. The signal sequence of lactamase is denoted by S and the IgM transmembrane segment as M. The transmembrane orientation of fusion proteins 1 and 2 are depicted below. Cleavage of the amino-terminal lactamase signal sequence is indicated by an asterisk, and the IgM transmembrane segment by the bold black line spanning the bilayer. Fusion protein 1 is completely translocated scross the microsomal membrane. Insertion of the M segment between the lactamase and globin domains in fusion protein 2 serves to terminate translocation at that point, leaving the lactamase domain in the lumen and the globin domain in the cytoplasmic space.

(Cutler *et al.*, 1986). Likewise, introduction of a single charged residue into the membrane-spanning domain of Semliki Forest virus protein E2 has no effect on membrane orientation or physiological function of the protein (Cutler and Garoff, 1986) but destabilizes the membrane association as determined by carbonate extraction (Cutler *et al.*, 1986), a procedure which strips membranes of all polypeptides save those integrated directly into the hydrophobic core of the lipid bilayer (Fujiki *et al.*, 1982).

Several groups have attempted to determine experimentally the minimum length of a membrane-spanning domain by carrying out gradual deletions in defined membrane-spanning regions (Adams and Rose, 1985;

Davis *et al.*, 1985). Such experiments suggest that as few as 14–17 amino acids of a stop transfer domain are sufficient for full function. When reduced to 8–12 amino acids these domains still appear able to direct proper membrane orientation, but their stability in the membrane is impaired (Davis *et al.*, 1985). A problem with these types of experiments is that the polypeptide context in which the stop transfer domains is assayed is critical, making it difficult to draw broad conclusions from a single example. For example, positioning of the potential stop transfer domains close to the carboxy terminus improves the ability of such a domain to anchor the polypeptide in the membrane (Davis and Model, 1985; Davis *et al.*, 1985; Davis and Hsu, 1986). Also, the existence of hydrophobic domains adjacent to the membrane-spanning regions being deleted [e.g., in VSV glycoprotein (Adams and Rose, 1985)] is likely to confound the interpretation of such experiments since they may compensate for the deleted hydrophobic region. Nevertheless, it appears on the basis of some of these experiments (Davis *et al.*, 1985) that stop transfer domains may have two functionally distinct roles: one to stop the translocation process and the other to engage in a stable membrane integration.

3. *Role of Hydrophobicity in Stop Transfer Function*

It has been suggested that topology of transmembrane proteins occurs as a result of spontaneous physical partitioning of hydrophobic protein regions into the hydrophobic core of the lipid bilayer (von Heijne and Blomberg, 1979; Engelman and Steitz, 1981). Indeed, insertion of a synthetic repetitive domain of 16 or more hydrophobic amino acid residues has been shown to be adequate to stop the translocation of a secretory protein and to confer predictable membrane topology to the resultant transmembrane protein in bacteria (Davis and Model, 1985). On the other hand, the fusion-related hydrophobic domain of the myxovirus, Sendai F protein, which consists of some 26 consecutive uncharged or hydrophobic residues is completely translocated both in the natural eukaryotic context and in a bacterial assay system (Davis and Hsu, 1986). Moreover, severely truncated membrane-spanning regions consisting of only 12–16 hydrophobic residues can direct topology similar to wild-type transmembrane sequences (Davis *et al.*, 1985).

Thus, while a "sufficiently" hydrophobic region of a translocated protein can act to halt translocation, it is not clear whether hydrophobicity is the sole feature that determines the function of authentic stop transfer sequences. Nor is it known whether critical hydrophobic interactions are between the nascent chain and the lipid bilayer or between the nascent chain and hydrophobic membrane proteins.

B. Combination Signal–Stop Transfer Sequences

Several bitopic COOH-trans ITMPs such as influenza neuraminidase (Bos *et al.*, 1984), asialoglycoprotein receptor (Spiess and Lodish, 1986), invariant chain (Lipp and Dobberstein, 1986), and transferrin receptor (Zerial *et al.*, 1986) have been identified which have internal, uncleaved signal sequences which also serve to anchor the protein in the membrane. These proteins are situated with their amino termini in the cytoplasm and their carboxy domains in the extracytoplasmic space (see Fig. 5). The hydrophobic membrane-spanning regions facilitate the transport of only the carboxy domains in an SRP-dependent fashion (Spiess and Lodish, 1986; Lipp and Dobberstein, 1986). These membrane-spanning regions may be considered simply as internal, uncleaved signal sequences. However, it is important to distinguish them from signal sequences of secretory proteins—the uncleaved signal of ovalbumin is completely translocated and secreted; moreover, when engineered to an internal position, a normally amino-terminal signal sequence can facilitate translocation of both flanking protein domains and does not integrate directly into the bilayer (Perara and Lingappa, 1985).

Signal–stop topogenic elements are not confined to this particular orientation. In the first transmembrane segment of the polytopic ITMP, bovine rhodopsin, a combined signal–stop transfer sequence has been identified which translocates the amino terminus and leaves the carboxy flanking domain in the cytoplasm (Friedlander and Blobel, 1985; Perara *et al.*, 1986). As in the case of COOH-trans ITMPs, what accounts for domain translocation specificity (i.e., whether the amino or carboxy flanking domain is translocated) of NH_2 is not clear. One possibility is that lack of translocation of a particular domain may depend on its folding in a translocation-incompetent manner. Alternatively, in the case of polytopic transmembrane proteins, adjacent topogenic domains of a protein may also influence the domain translocation specificity of a given topogenic sequence (Eble *et al.*, 1987).

We shall refer to domains which are able to facilitate both translocation and integration into the bilayer as signal–stop transfer sequences. Whether separate signal and stop transfer functions can be assigned to distinct regions of these domains remains to be determined. Until that issue is resolved it remains unclear whether such domains should be considered a special class of topogenic sequence, whether they represent two distinct elements in tandem, or whether they are distinguished from simple signal sequences merely by the inability of the cytoplasmically disposed domain to be translocated.

C. Polytopic Integral Transmembrane Proteins

Those ITMPs which span the membrane more than once are referred to as polytopic membrane proteins. The most detailed analysis of polytopic ITMPs has been done on the polytopic subunits in the photosynthetic reaction center of the bacterium, *Rhodopseudomonas viridis*. The determination of the tertiary structure by X-ray crystallography (Deisenhofer *et al.*, 1985) in conjunction with the primary amino acid sequences (Michel *et al.*, 1986) has allowed structural analysis of these proteins at nearly atomic resolution. However, the topologies of most polytopic ITMPs are predicted from their primary sequences, with very hydrophobic regions proposed to span the membrane.

Initial events in the assembly of polytopic ITMPs into the ER membrane appear to be similar to those involved in the translocation of secretory and bitopic transmembrane proteins. Some have amino-terminal cleaved signal sequences (Anderson *et al.*, 1982), while others possess uncleaved signal sequences (Anderson *et al.*, 1983; Rottier *et al.*, 1984; Friedlander and Blobel, 1985; Eble *et al.*, 1986). Their integration is dependent on SRP (Anderson *et al.*, 1982, 1983; Friedlander and Blobel, 1985; Rottier *et al.*, 1985; Mueckler and Lodish, 1986a; Eble *et al.*, 1987) although some do not experience elongation arrest (Anderson *et al.*, 1983). Thus, polytopic ITMPs utilize the same targeting system as simple secretory and bitopic transmembrane proteins. But is each transmembrane domain established by specific signal and stop transfer sequences (Blobel, 1980), or, once targeted to the ER via a signal sequence and SRP, is subsequent membrane assembly "spontaneous" (Wickner and Lodish, 1985; Mueckler and Lodish, 1986a)?

The biogenesis of only a few polytopic transmembrane proteins has been studied in any detail. The analysis of biogenesis has relied heavily on molecular genetics techniques which allow deletion of putative topogenic domains and/or analysis of their independent function in defined polypeptide contexts.

1. *Multiple Signal Sequences*

Bovine rhodopsin is believed to span the bilayer seven times with the amino terminus in the extracytoplasmic space and the carboxy terminus in the cytoplasm (Nathans and Hogness, 1983). Deletions of the cDNA encoding bovine opsin revealed that this protein has at least two SRP-dependent signal sequences, one of which is located in the first transmembrane segment and the other in the sixth (Friedlander and Blobel, 1985). Both signals facilitate translocation of at least the amino flanking domain and integrate into the lipid bilayer directly, thus classifying them as sig-

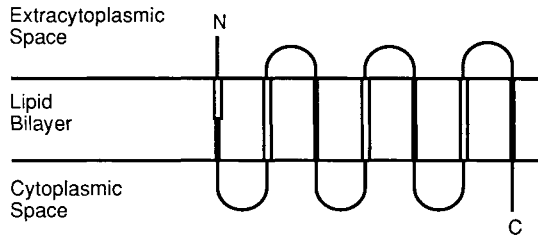


Fig. 8. Predicted arrangement of signal and stop transfer sequences to account for transmembrane orientation of bovine rhodopsin (see Friedlander and Blobel, 1985). Open rectangles represent signal sequences and solid black rectangles denote stop transfer sequences.

nal–stop transfers. The existence of two more signal sequences and four stop transfer sequences in alternating transmembrane domains has been proposed to account for the complex topology of opsin (Friedlander and Blobel, 1985; see Fig. 8). These observations must be interpreted with caution, however, in light of more recent findings (described in Section V,D) which suggest common features between signal and stop transfer sequences. Nevertheless, this study demonstrates that multiple topogenic elements, which recognize known receptors for translocation, exist in polytopic membrane proteins and suggests that the translocation of multiple protein domains of a polytopic membrane protein may occur via the same mechanism as that of a secretory or simple bitopic membrane protein.

The membrane biogenesis of hepatitis B surface antigen is somewhat more clearly understood. This is a simpler polytopic membrane protein which spans the membrane two times (Eble *et al.*, 1985, 1987). Analysis of each of the two known membrane-spanning regions in fusion proteins *in vitro* reveal that hepatitis B surface antigen contains two uncleaved signal sequences, both of which interact with SRP (Eble *et al.*, 1987). The first is located within the initial 32 amino acids of the protein and is capable of translocating both amino and carboxy flanking domains, as well as itself, completely across the membrane, although in native hepatitis B surface antigen it resides in the membrane (see Fig. 9). The second topogenic element appears to be a signal–stop transfer sequence (see Section V,B) which facilitates translocation of only the carboxy-flanking domain and integrates into the lipid bilayer directly, leaving the amino terminus in the cytoplasmic space (see Fig. 9). It is unclear exactly how these two topogenic sequences act together to achieve the ultimate transmembrane orientation, i.e., do the two signals act sequentially—first one, then the other—or do they interact with one another to specify the correct topol-

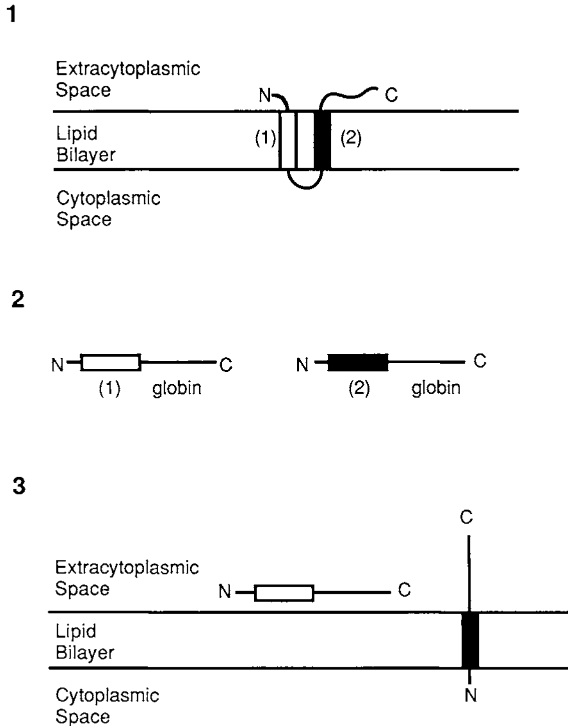


Fig. 9. Hepatitis B surface antigen possesses two types of signal sequences (see Eble *et al.*, 1986, 1987). The transmembrane orientation of hepatitis B surface antigen is represented schematically in Part 1. The two transmembrane domains are designated by open and solid black bars [(1) and (2), respectively]. Transmembrane domains (1) and (2) were analyzed independently in globin fusion proteins (shown in Part 2), with globin flanking regions (1) or (2) at their carboxy termini. Transmembrane orientations of globin fusion proteins are depicted in Part 3. Transmembrane domain 1 facilitated translocation of the flanking globin domain and was translocated itself. Transmembrane segment 2 also facilitated translocation of the globin domain but remained integrated in the membrane with a small, cytoplasmically disposed amino terminus.

ogy? It appears from this study that all "information" for residing in the bilayer need not necessarily be specified exclusively by the membrane-spanning domain itself.

2. *Charged Domains May Span the Membrane*

It is generally believed that membrane-spanning regions are composed of extremely hydrophobic regions of a protein, and the topology of many polytopic membrane proteins has been predicted by their amino acid se-

quences (Nathans and Hogness, 1983; Noda *et al.*, 1983; Devillers-Thiery *et al.*, 1983; Mueckler *et al.*, 1985). Such predictions, however, are not always correct. For example, the subunits of the acetylcholine receptor have been predicted by their primary sequence to span the membrane four times (Noda *et al.*, 1983; Devillers-Thiery *et al.*, 1983). However, immunoprecipitation and immunocytochemistry using antibodies to specific segments of the protein have provided strong evidence for a model in which the homologous receptor subunits each cross the membrane five times (Young *et al.*, 1985). Four of the predicted membrane-spanning regions are rich in hydrophobic and nonpolar residues while one (the fourth) is amphipathic and can form an α helix that is hydrophobic on one side and highly charged on the other. The homologous amphipathic helices of the five subunits are predicted to assemble in the membrane such that the charged side of each subunit faces the center, stabilizing this conformation in the lipid bilayer and forming an ion channel (Young *et al.*, 1985). It is unlikely that such amphipathic membrane-spanning segments provide "information" for membrane insertion; their localization spanning the membrane may be determined by adjacent topogenic elements in the protein.

Polytopic membrane assembly does not appear to be a simple partitioning of hydrophobic sequences into the hydrophobic core of the bilayer, since polar polypeptide domains have been shown to span the membrane (Young *et al.*, 1985) and hydrophobic regions are sometimes completely translocated (Davis and Hsu, 1986). Moreover, recent reports suggest that cytoplasmic or membrane factors, or both, may determine the orientation of a given protein relative to the membrane. A bacterial polytopic transmembrane protein integrates into *E. coli* inverted vesicles in a cell-free translation system derived from *E. coli*, but it is completely translocated across canine rough microsomes when expressed in the wheat germ system (Watanabe *et al.*, 1986). It is suggested that differences in the two types of membranes may account for the different topologies of the same protein, though differences between the *E. coli* and wheat germ extracts may also account for these observations. In fact, a similar finding has been observed in the expression of a protein in a wheat germ versus rabbit reticulocyte cell-free system, in both cases using dog pancreas rough microsomes. Expression in the wheat germ system results in a polytopic transmembrane orientation, while in reticulocyte lysate approximately equal amounts of the E-cis ITMP and a completely translocated form are observed. On expression in *Xenopus* oocytes the major product is a secreted soluble monomer (Hay *et al.*, 1987).

It is not known whether assembly of polytopic proteins into the ER membrane is receptor mediated aside from the requirement for SRP to

target the nascent chain to the ER. While at least two proteins have been demonstrated to contain more than one sequence which is recognized by SRP it is not known whether SRP mediates the interactions of each sequence with the membrane or whether it is required only once to target the nascent chain to the ER membrane and that, once there, subsequent topogenic sequences in the nascent chain are able to interact directly with the membrane or membrane proteins.

While the assembly of most polytopic ITMPs appears to be "cotranslational" (Rottier *et al.*, 1984; Eble *et al.*, 1986), it is not clear whether multiple transmembrane segments insert into the membrane sequentially (i.e., as they emerge from the ribosome) or not. The observation that a fragment of the glucose transporter molecule can integrate into rough microsomes posttranslationally with an orientation similar to that observed for cotranslational integration (Mueckler and Lodish, 1986a) suggests that proper orientation need not be achieved via sequential insertion or translocation events.

D. Reevaluation of Topogenic Sequences

Information for membrane assembly appears to reside in the membrane protein itself, encoded in discrete "topogenic" sequences (Blobel, 1980; Lingappa *et al.*, 1984; Yost *et al.*, 1983). From the work discussed thus far topogenic domains can be considered as one of three basic types:

1. Conventional signal sequence. These are usually located at the amino termini of most secretory and many transmembrane proteins. They facilitate translocation of flanking protein domains in an SRP-dependent fashion and are usually (but not always) cleaved by signal peptidase in the ER membrane. These signal sequences do not appear to integrate into the bilayer directly and may themselves be translocated.
2. Stop transfer sequence. Conventional stop transfer sequences abort the translocation process initiated by a distant, preceding signal sequence and often integrate into the lipid bilayer.
3. Combined signal–stop transfer sequence. These signal–stop sequences require SRP to initiate translocation of one or the other flanking protein domain and are not themselves translocated but integrate directly into the membrane.

Recent findings suggest that the distinctions between these topogenic sequences may not be absolute. Analysis of stop transfer sequences in proteins which lack amino-terminal signal peptides reveals a number of unexpected features of these topogenic elements.

1. *Domain Translocation Activity*

The epidermal growth factor (EGF) receptor is a typical bitopic NH_2-trans transmembrane protein with large extracytoplasmic and cytoplasmic domains (Ullrich *et al.*, 1984). It is synthesized with an amino-terminal cleaved signal sequence which presumably facilitates the translocation of the extracytoplasmic domain. The membrane-spanning domain is believed to function as a stop transfer sequence, terminating translocation and resulting in the integration of the protein into the bilayer, with the remainder of the protein maintained in the cytoplasmic space. The retroviral oncogene, v-*erbB,* encodes a truncated version of EGF receptor which lacks the first 550 amino acids of the amino-terminal (extracytoplasmic) domain, including the signal sequence, as well as a small portion of the extreme carboxy terminus. Nevertheless, the v-*erbB* gene product spans the plasma membrane *in vivo,* as determined by immunocytochemistry, in an orientation similar to that of the EGF receptor, i.e., with an extracytoplasmic amino terminus and the carboxy terminus in the cytoplasm (Schatzman *et al.*, 1986). Thus, it appears that the membrane-spanning region of EGF receptor may possess an intrinsic capacity to direct the translocation of the truncated amino-terminal domain and to integrate itself correctly into the membrane.

In vitro analyses of a classic stop transfer sequence in chimeric proteins lacking a signal sequence also suggest that bona fide "stop transfer" sequences possess domain translocation activity (Mize *et al.*, 1986; Zerial *et al.*, 1987). The transmembrane segment at the extreme carboxy terminus of IgM (the M segment) has been shown to have intrinsic domain translocating activity in certain polypeptide contexts *in vitro* (Mize *et al.*, 1986). When an amino-terminal signal sequence was present, either in engineered proteins or in the native IgM molecule, only the domain between the signal and stop transfer sequences was translocated (Yost *et al.*, 1983; Mize *et al.*, 1986); the remainder (the domain carboxy terminal to the M segment) remained in the cytoplasmic space (see Fig. 10). However, when engineered between two antigenically distinct cytoplasmic protein domains, lacking any defined signal sequence, the M segment facilitated the transport of either the amino and carboxy flanking domains across microsomal membranes (Mize *et al.*, 1986; see Fig. 9). A normally amino-terminal signal sequence in a similar polypeptide context facilitates translocation of both flanking protein domains and is translocated itself into the lumen (Perara and Lingappa, 1985; Mize *et al.*, 1986, see Fig. 9).

2. *Functional Recognition by Signal Recognition Particle*

The translocation activity of "stop transfer sequences" is SRP mediated, and SRP can interact with them to arrest elongation of the nascent

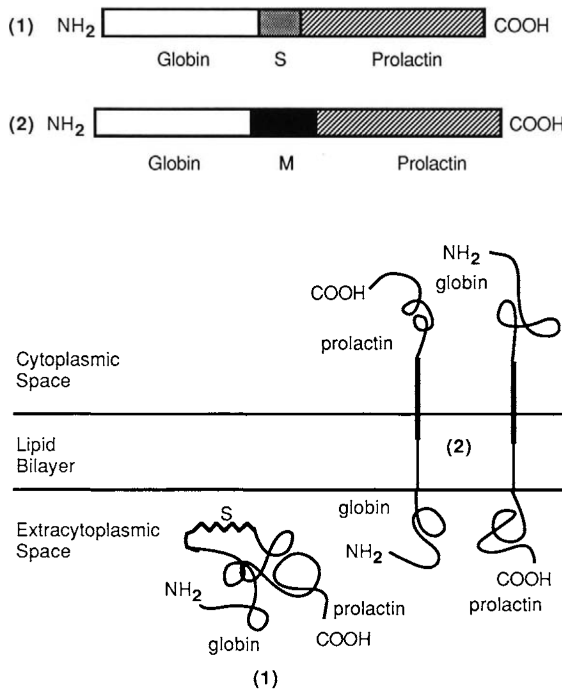


Fig. 10. Comparison of translocation activities of the M segment of immunoglobulin M and the prolactin signal sequence in similar polypeptide contexts (see Mize *et al.*, 1986). Fusion proteins 1 and 2 consisting of globin and prolactin domains flanking either a signal sequence or a transmembrane region, respectively, are shown. The dispositions of the fusion proteins are represented below. The signal sequence is represented by the zigzag line, labeled S. The stop transfer domain is represented by the heavy black line and labeled M. See text for details.

chain (Mize *et al.*, 1986; Zerial *et al.*, 1987). In this respect stop transfer sequences are qualitatively indistinguishable from classic amino-terminal signal sequences in a similar context. Thus, it appears that stop transfer and signal sequences may share at least some structural features which direct functional interactions with the same receptor, SRP, as well as with putative receptor elements on the cytoplasmic, but not the luminal (e.g., signal peptidase), aspect of the ER membrane.

Why should a transmembrane segment, such as that of IgM, possess recognition features for SRP and for initiation of translocation? Since it is located at the extreme carboxy terminus of IgM and emerges from the ribosome only after termination of protein synthesis and dissociation of the ribosomal subunits, this cotranslational translocation activity seems

unlikely to play any role in its native stop transfer function. While the possibility exists that SRP may mediate normal stop transfer function, another possible explanation may be that signal and stop transfer functions are mediated by common membrane receptors which share recognition features with SRP. The observation that an artificial stretch of 23 hydrophobic amino acids interacts with SRP (Zerial *et al.*, 1987) suggests that hydrophobicity is a key recognition feature for SRP.

E. Summary of Transmembrane Protein Biogenesis

Information for translocation and membrane assembly appears to reside in the membrane protein, itself, encoded in discrete "topogenic" sequences (Lingappa *et al.*, 1984; Yost *et al.*, 1983), their position relative to one another (Coleman *et al.*, 1985; Mize *et al.*, 1986; Eble *et al.*, 1987) and their immediate polypeptide environment or context (Davis and Model, 1985; Davis and Hsu, 1986). Signals for the initiation and termination of translocation appear to share structural features for recognition by common receptors, at least for SRP. While both signals and stops may possess intrinsic domain translocation activity, they are distinguishable in that signal sequences may be translocated themselves while stop transfers are not (Perara and Lingappa, 1985; Mize *et al.*, 1986). A systematic analysis of various arrangements of identified signal and stop transfer sequences in identical polypeptide and protein synthesis contexts should allow clarification of the functions of these sequences.

Our understanding of how the process of translocation is interrupted and how complex polytopic membrane proteins are assembled is limited by our lack of understanding as to how translocation occurs in the first place. For example, is topogenic "information" in a complex polytopic membrane protein "read" in a linear order from amino to carboxy terminus as the growing polypeptide emerges from the ribosome, or do subdomains interact with cytoplasmic and ER membrane translocation components?

VI. OVERVIEW

We have reviewed here past and recent progress in the understanding of the localization of proteins to the endoplasmic reticulum. Rapid advances have been made due to the combined efforts to identify molecular components and the use of molecular genetic manipulation to create altered translocation substrates. While some questions have been an-

swered, new ones have been raised and other long-standing issues remain unresolved.

Studies of translocation across the ER membrane initially focused on the membrane-bound ribosomes of the rough ER and the characteristic vectorial discharge (or cotranslational translocation) of the nascent chain. In the last decade the molecular mechanisms by which particular proteins are selected to be synthesized by ER-bound polysomes have been clarified, at least to one level of resolution. More recently it has been found that translocation need not be coupled to protein synthesis, rather, the characteristic coupling of translocation to translation may reflect the requirement for an association of the nascent chain with the ribosome of synthesis. Thus attention has returned to the membrane-bound ribosomes of the rough ER. What is the nature and importance of the ribosome–membrane interaction? What role does the ribosome play in the initiation and termination of translocation?

While the molecular events of targeting have been well characterized, the mechanism by which selected proteins cross the ER membrane remains a mystery. An increasing body of evidence is accumulating for the participation of membrane proteins in this process. The finding that completed protein domains can be accommodated by the translocation mechanism suggests the participation of additional proteins in the translocation process, either as components of a pore or tunnel which could accommodate folded polypeptide domains or as enzymes to unfold folded domains. In addition, translocation of completed proteins is dependent on nucleoside triphosphate hydrolysis. Thus translocation is not a "spontaneous" process as has been predicted previously (Wickner, 1979; von Heijne and Blomberg, 1979; Engelman and Steitz, 1981). While it is clear that translocation is not simply governed by thermodynamically favorable protein–lipid interactions between the nascent chain and the membrane, the molecular environment traversed by the polypeptide as it enters the ER lumen remains to be determined.

The approach of expressing engineered translocation substrates in cell-free translation/translocation systems has provided many insights into the mechanism of translocation and transmembrane integration and will continue to do so. In addition, future research will undoubtedly focus on the identification, purification, reconstitution, and mechanistic analysis of additional molecular components of the targeting and translocation apparatus. Finally, the importance of the events and components which have been defined and established in cell-free systems remains to be determined *in vivo*. Additional components may be necessary to orchestrate the complex series of molecular interactions resulting in translocation in a living cell.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health, Grant GM 31626. We thank Russell Hirsch for helpful criticism of the manuscript.

REFERENCES

Adams, G. A., and Rose, J. K. (1985). Structural requirements of a membrane-spanning domain for protein anchoring and cell surface transport. *Cell* **41,** 1007–1015.

Adelman, M. R., Sabatini, D. D., and Blobel, G. (1973). Ribosome-membrane interaction. Nondestructive disassembly of rat liver rough microsomes into ribosomal and membranous components. *J. Cell Biol.* **56:** 206–229.

Ainger, K. I., and Meyer, D. I. (1986). Translocation of nascent secretory proteins across membranes can occur late in translation. *EMBO J.* **5,** 951–955.

Anderson, D. J., Walter, P., and Blobel, W. (1982). Signal recognition protein is required for the integration of acetylcholine receptor δ subunit, a transmembrane glycoprotein, into the endoplasmic reticulum membrane. *J. Cell Biol.* **92,** 501–506.

Anderson, D. J., Mostov, K. E., and Blobel, G. (1983). Mechanisms of integration of de novo-synthesized polypeptides into membranes: Signal-recognition particle is required for integration into microsomal membranes of calcium ATPase and of lens MP26 but not of cytochrome b_5. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 7249–7253.

Andrews, D. W., Walter, P., and Ottensmeyer, F. P. (1985). Structure of the signal recognition particle by electron microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 785–789.

Andrews, D. W., Walter, P., and Ottensmeyer, F. P. Evidence for an extended 75L RNA in the signal recognition particle. *EMBO J.* (in press).

Budouelle, H., Bassford, Jr., P. J., Fowler, A. V., Sabin, I., Beckwith, J., and Hofnung, M. (1980). Mutations which alter the function of the signal sequence of the maltose binding protein of *Escherichia coli*. *Nature* (*London*) **285,** 78–81.

Bergman, L. W., and Kuehl, W. M. (1979). Formation of an intrachain disulfide bond on nascent immunoglobulin light chains. *J. Biol. Chem.* **254,** 8869–8876.

Bielinska, M., Rogers, F., Rucinsky, T., and Boime, I. (1979). Processing *in vitro* of placental peptide hormones by smooth microsomes. *Proc. Natl. Acad. Sci. U.S.A.* **76,** 6152–6156.

Blobel, G. (1980). Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 1496–1500.

Blobel, G., and Dobberstein, B. (1975a). Transfer of proteins across membranes I. Presence of proteolytically processed and unprocessed nascent immunoglubulin murine myeloma. *J. Cell Biol.* **67,** 835–851.

Blobel, G., and Dobberstein, B. (1975b). Transfer of proteins across membranes II. Reconstitution of functional rough microsomes from heterologous components. *J. Cell Biol.* **67,** 852–862.

Boeke, J. D., and Model, P. (1982). A procaryotic membrane anchor sequence: carboxyl terminus of bacteriophage fl gene III protein retains it in the membrane. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5200–5204.

Bos, T. J., Davis, A. R., and Nayak, D. P. (1984). NH_2-terminal hydrophobic region of influenza virus neuraminidase provides the signal function in translocation. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 2327–2331.

Braell, W. A., and Lodish, H. F. (1982). Ovalbumin utilizes an amino-terminal signal sequence. *J. Biol. Chem.* **257,** 4578–4582.

Bretscher, M. S. (1971). Major human erythrocyte glycoprotein spans the cell membrane. *Nature New Biol.* **231,** 229–232.

Bretscher, M. S. (1975). C-Terminal region of the major erythrocyte sialoglycoprotein is on the cytoplasmic side of the membrane. *J. Mol. Biol.* **98,** 831–833.

Briggs, M. S., Cornell, D. G., Dluhy, R. A., and Gierasch, L. M. (1986). Conformations of signal peptides induced by lipids suggest initial steps in protein export. *Science* **233,** 206–208.

Chen, L., and Tai, P. C. (1987). Evidence for the involvement of ATP in co-translational translocation. *Nature (London)* **328,** 164–166.

Coleman, J., Inukai, M., and Inouye, M. (1985). Dual functions of the signal peptide in protein transfer across the membrane. *Cell* **43,** 351–360.

Cutler, D. F., and Garoff, H. (1986). Mutants of the membrane-binding region of Semliki Forest virus E2 protein. I. Cell surface transport and fusogenic activity. *J. Cell Biol.* **102,** 889–901.

Cutler, D. F., Malancon, P., and Garoff, H. (1986). Mutants of the membrane-binding region of Semliki Forest virus E2 protein. II. Topology and membrane binding. *J. Cell Biol.* **102,** 902–910.

Davis, N. G., and Hsu, M. C. (1986). The fusion-related hydrophobic domain of Sendai F protein can be moved through the cytoplasmic membrane of *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A.* **83,** 5091–5095.

Davis, N. G., and Model, P. (1985). An artificial anchor domain: hydrophobicity suffices to stop transfer. *Cell* **47,** 607–614.

Davis, N. G., Boeke, J. D., and Model, P. (1985). Fine structure of a membrane anchor domain. *J. Mol. Biol.* **181,** 111–121.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985). Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature (London)* **318,** 618–624.

Devillers-Thiery, A., Giraudat, J., Bentaboulet, M., and Changeux, J.-P. (1983). Complete mRNA coding sequence of the acetylcholine binding α-subunit of *Torpedo marmorata* acetylcholine receptor: A model for the transmembrane organization of the polypeptide chain. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 2067–2071.

Eble, B., Lingappa, V., and Ganem, D. (1986). Hepatitis B surface antigen: An unusual secreted protein initially synthesized as a transmembrane polypeptide. *Mol. Cell. Biol.* **6,** 1454–1463.

Eble, B. E., MacRae, D. R., Lingappa, V. R., and Ganem, D. (1987). Multiple topogenic sequences determine the transmembrane orientation of hepatitis B surface antigen. *Mol. Cell. Biol.* (in press).

Emr, S. D., and Silhavy, T. J. (1982). Molecular components of the signal sequence that function in the initiation of protein export. *J. Cell Biol.* **95,** 689–696.

Engelman, D. M., and Steitz, T. A. (1981). The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. *Cell* **23,** 411–422.

Evans, E. A., Gilmore, R., and Blobel, G. (1986). Purification of microsomal signal peptidase as a complex. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 581–585.

Friedlander, M., and Blobel, G. (1985). Bovine opsin has more than one signal sequence. *Nature (London)* **318,** 338–343.

Fujiki, Y., Hubbard, A. L., Fowler, S., and Lazarow, P. B. (1982). Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. *J. Cell Biol.* **93,** *97–102.*

Gething, M. J., and Sambrook, J. (1982). Construction of influenza hemagluttinin genes that code for intracellular and secreted forms of the protein. *Nature (London)* **300,** 598–603.

Gilmore, R., and Blobel, G., (1983). Transient involvement of signal recognition particle and its receptor in the microsomal membrane prior to protein translocation. *Cell* **35,** 677–685.

Gilmore, R., and Blobel, G. (1985). Translocation of secretory proteins across the microsomal membrane occurs through an environment accessible to aqueous perturbants. *Cell* **46,** 497–505.

Gilmore, R., Blobel, G., and Walter, P. (1982a). Protein translocation across the endoplasmic reticulum. I. Detection in the microsomal membrane of a receptor for the signal recognition particle. *J. Cell Biol.* **95,** 463–469.

Gilmore, R., Walter, P., and Blobel, G. (1982b). Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J. Cell Biol.* **95,** 470–477.

Glabe, C. G., Hanover, J. A., and Lennarz, W. J. (1980). Glycosylation of ovalbumin nascent chains. *J. Biol. Chem.* **255,** 9236–9242.

Hansen, W., Garcia, P., and Walter, P. (1986). *In vitro* protein translocation across the yeast endoplasmic reticulum: ATP-dependent posttranslational translocation of prepro-α-factor. *Cell* **45,** 397–406.

Hay, B., Barry, R. A., Lieberburg, I., Prusiner, S. B., and Lingappa, V. R. (1987). Biogenesis and transmembrane orientation of the cellular isoform of the scrapie prion protein. *Mol. Cell. Biol.* **7,** 914–919.

Hay, B., Prusiner, S. B., and Lingappa, V. R. Evidence for a secretory form of the cellular prion protein. *Biochemistry* (in press).

Hortsch, M., Avossa, D., and Meyer, D. I. (1986). Characterization of secretory protein translocation: Ribosome–membrane interaction in endoplasmic reticulum. *J. Cell Biol.* **103,** 241–253.

Jackson, R. C., and Blobel, G. (1977). Posttranslational cleavage of prescretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity. *Proc. Natl. Acad. Sci. U.S.A.* **74,** 5598–5602.

Kadonaga, J. T., Gautier, A. E., Straus, D. R., Charles, A. D., Edge, M. D., and Knowles, J. R. (1984). The role of β-lactamase signal sequence in the secretion of proteins by *E. coli. J. Biol. Chem.* **259,** 2149–2154.

Kadonaga, J. T., Pluckthun, A., and Knowles, J. R. (1985). Signal sequence mutants of β-lactamase. *J. Biol. Chem.* **260,** 16192–16199.

Katz, F. N., and Lodish, H. F. (1979). Transmembrane biogenesis of the vesicular stomatitis virus glycoprotein. *J. Cell Biol.* **80,** 416–426.

Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G., and Lodish, H. F. (1977). Membrane assembly *in vitro:* Synthesis, glycosylation, and asymmetric insertion of a transmembrane protein. *Proc. Natl. Acad. Sci. U.S.A.* **74,** 3278–3282.

Kehry, M., Ewald, S., Douglas, R., Sibley, C., Raschke, W., Fambrough, D., and Hood, L. (1980). The immunoglobulin μ chains of membrane-bound and secreted IgM molecules differ in their C-terminal segments. *Cell* **21,** 393–406.

Kelly, R. B. (1985). Pathways of protein secretion in eukaryotes. *Science* **230,** 25–32.

Kopito, R. R., and Lodish, H. F. (1985). Primary structure and transmembrane orientation of the murine anion exchange protein. *Nature (London)* **316,** 234–238.

Koshland, D., Sauer, R. T., and Botstein, D. (1982). Diverse effects of mutations in the signal sequence on the secretion of β-lactamase in *Salmonella typhimurium. Cell* **30,** 903–914.

Kreibich, G., Ulrich, B. L., and Sabatini, D. D. (1978a). 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.

Kreibich, G., Freienstein, C. M., Pereyra, B. N., Ulrich, B. L., and Sabatini, D. D. (1978b).

Proteins of rough microsomal membranes related to ribosome binding. II. Crosslinking of bound ribosomes to specific membrane proteins exposed at the binding sites. *J. Cell Biol.* **77,** 488–506.

Krieg, U. C., Walter, P., and Johnson, A. E. (1986). Photocross-linking of the signal sequence of nascent preprolactin to the 54 kDa polypeptide of the signal recognition particle. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 8604–8608.

Kurzchalia, T. V., Wiedmann, M., Girshovich, A. S., Bochkareva, E. S., Bielka, H., and Rapoport, T. A. (1986). The signal sequence of nascent preprolactin interacts with the 54K polypeptide of the signal recognition particle. *Nature (London)* **320,** 634–636.

Lauffer, L., Garcia, P. D., Harkins, R. N., Coussens, L., Ullrich, A., and Walter, P. (1985). Topology of signal recognition particle receptor in endoplasmic reticulum membrane. *Nature (London)* **318,** 334–338.

Lin, J. J. C., Kanazawa, H., Ozols, J., and Wu, H. C. (1978). An *Escherichia coli* mutant with an amino acid alteration within the signal sequence of outer membrane prolipoprotein. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 4891–4895.

Lingappa, V. R., Katz, F. N., Lodish, H. F., and Blobel, G. (1978a). A signal sequence for the insertion of a transmembrane glycoprotein. *J. Biol. Chem.* **253,** 8667–8670.

Lingappa, V. R., Shields, D., Woo, S. L. C., and Blobel, G. (1978b). Nascent chicken ovalbumin contains the functional equivalent of a signal sequence. *J. Cell Biol.* **79,** 567–572.

Lingappa, V. R., Lingappa, J. R., and Blobel, G. (1979). Chicken ovalbumin contains an internal signal sequence. *Nature (London)* **281,** 117–121.

Lingappa, V. R., Chaidez, J., Yost, C. S., and Hedgpeth, J. (1984). Determinants for protein localization: β-Lactamase signal sequence directs globin across microsomal membranes. *Proc. Natl. Acad. Aci. U.S.A.* **81,** 456–460.

Lipp, J., and Dobberstein, B. (1986). Signal recognition particle-dependent membrane insertion of mouse invariant chain: A membrane-spanning protein with a cytoplasmically exposed amino terminus. *J. Cell Biol.* **102,** 2169–2175.

McClelland, A., Kuhn, L. C., and Ruddle, F. H. (1984). The human transferrin receptor gene: Genomic organization, and the complete primary structure of the receptor deduced from a cDNA sequence. *Cell* **39,** 267–274.

McCune, J. M., Lingappa, V. R., Fu, S. M., Blobel, G., and Kunkel, H. G. (1980). Biogenesis of membrane-bound and secreted immunoglobulins. I. Two distinct translation products of human μ-chain, with identical N-termini and different C-termini. *J. Exp. Med.* **152,** 463–468.

Marcantonio, E. E., Amar-Costesec, A., and Kreibich, G. (1984). Segregation of the polypeptide translocation apparatus to regions of the endoplasmic reticulum containing ribophorins and ribosomes II. Rat liver microsomal subfractions contain equimolar amount of ribophorins and ribosomes. *J. Cell Biol.* **99,** 2254–2259.

Meek, R. L., Walsh, K. A., and Palmiter, R. D. (1982). The signal sequence of ovalbumin is located near the NH_2-terminus. *J. Biol. Chem.* **257,** 12245–12251.

Meyer, D. I. (1985). Signal recognition particle (SRP) does not mediate a translational arrest of nascent secretory proteins in mammalian cell-free systems. *EMBO J.* **4,** 2031–2033.

Meyer, D. I., and Dobberstein, B. (1980a). A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: requirements for its extraction and reassociation with the membrane. *J. Cell Biol.* **87,** 498–502.

Meyer, D. I., and Dobberstein, B. (1980b). Identification and characterization of a membrane component essential for the translocation of nascent proteins across the membrane of the endoplasmic reticulum. *J. Cell Biol.* **87,** 503–508.

Meyer, D. I., Krause, E., and Dobberstein, B. (1982a). Secretory protein translocation across membranes: The role of 'docking protein.' *Nature* (*London*) **297,** 647–650.

Meyer, D. I., Louvard, D., and Dobberstein, B. (1982b). Characterization of molecules involved in protein translocation using a specific antibody. *J. Cell Biol.* **92,** 579–583.

Michel, H., Weyer, K. A., Gruenberg, H., Dunger, I., Oesterhelt, D., and Lottspeich, F. (1986). The 'light' and 'medium' subunits of the photosynthetic reaction centre from *Rhodopseudomonas viridis:* isolation of the genes, nucleotide and amino acid sequence. *EMBO J.* **5,** 1149–1158.

Milstein, C., Brownlee, G. G., Harrison, T. M., and Mathews, M. B. (1972). A possible precursor of immunoglobulin light chains. *Nature New Biol.* **239,** 117–120.

Mize, N. K., Andrews, D. W., and Lingappa, V. R. (1986). A stop transfer sequence recognizes receptors for nascent chain translocation across the endoplasmic reticulum membrane. *Cell* **47,** 711–719.

Moreno, F., Fowler, A. V., Hall, M., Silhavy, T. J., Zabin, I., and Schwartz, M. (1980). A signal sequence is not sufficient to lead β-galactosidase out of the cytoplasm. *Nature* (*London*) **286,** 356–359.

Morrison, T. G., and Lodish, H. F. (1975). The site of synthesis of membrane and non-membrane proteins of vesicular stomatitis virus. *J. Biol. Chem.* **250,** 6955–6962.

Mueckler, M., and Lodish, H. F. (1986a). The human glucose transporter can insert post-translationally into microsomes. *Cell* **44,** 629–637.

Mueckler, M., and Lodish, H. F. (1986b). Posttranslational insertion of a fragment of the glucose transporter into microsomes requires phosphoanhydride bond cleavage. *Nature* (*London*) **322,** 459–552.

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhard, G. E., and Lodish, H. F. (1985). Sequence and structure of a human glucose transporter. *Science* **229,** 941–945.

Muller, M., and Blobel, G. (1984). *In vitro* translocation of bacterial proteins across the plasma membrane of *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A.* **81,** 7241–7425.

Muller, G., and Zimmermann, R. (1987). Import of honeybee prepromelittin into the endoplasmic reticulum: structural basis for independence of SRP and docking protein. *EMBO J.* **6,** 2099–2107.

Muller, M., Ibrahimi, I., Chang, C. N., Walter, P., and Blobel, G. (1982). A bacterial secretory protein requires SRP for translocation across the endoplasmic reticulum. *J. Biol. Chem.* **257,** 11860–11863.

Nathans, J., and Hogness, D. S. (1983). Isolation, sequence analysis, and intron–exon arrangement of the gene encoding bovine rhodopsin. *Cell* **34,** 807–814.

Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Furutani, Y., Hirose, T., Takashima, H., Inayama, S., Miyata, T., and Numa, S. (1983). Structural homology of *Torpedo californica* acetylcholine receptor subunits. *Nature* (*London*) **302,** 528–532.

Palade, G. (1975). Intracellular aspects of the process of protein synthesis. *Science* **189,** 347–358.

Palmiter, R. D., Gagnon, J., and Walsh, K. A. (1978). Ovalbumin: A secreted protein without a transient hydrophobic leader sequence. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 94–98.

Palva, I., Sarvas, M., Lehtovaara, P., Sibakov, M., and Kaarianen, L. (1982). Secretion of *Escherichia coli* β-lactamase from *Bacillus subtilis* by the aid of α-amylase signal sequence. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5582–5586.

Perara, E., and Lingappa, V. R. (1985). A former amino terminal signal sequence engineered to an internal location directs translocation of both flanking protein domains. *J. Cell Biol.* **101,** 2292–2301.

Perara, E., Rothman, R. E., and Lingappa, V. R. (1986). Uncoupling translocation from translation: Implications for transport of proteins across membranes. *Science* **232,** 348–352.

Prehn, S., Tsamaloukas, A., and Rapaport, T. A. (1980). Demonstration of specific receptors of the rough endoplasmic membrane for the signal sequence of carp preproinsulin. *Eur. J. Biochem.* **107,** 185–195.

Prehn, S., Nurnberg, P., and Rapoport, T. A. (1981). A receptor for signal segments of secretory proteins in rough endoplasmic reticulum membranes. *FEBS Lett.* **123,** 79–84.

Randall, L. (1983). Translocation of domains of nascent periplasmic proteins across the cytoplasmic membrane is independent of elongation. *Cell* **33,** 231–240.

Randall, L. L., and Hardy, S. J. S. (1986). Correlation of competence for export with lack of the tertiary structure of the mature species: A study *in vivo* of maltose-binding protein in *E. coli. Cell* **46,** 921–928.

Redman, C. M., and Sabatini, D. D. (1966). Vectorial discharge of peptides released by puromycin from attached ribosomes. *Proc. Natl. Acad. Sci. U.S.A.* **56,** 608–615.

Redman, C. M., Siekevitz, P., and Palade, G. (1966). Synthesis and transfer of amylase in pigeon pancreatic microsomes. *J. Biol. Chem.* **241,** 1150–1158.

Rose, J. K., and Bergmann, J. E. (1982). Expression from cloned cDNA of cell-surface secreted forms of the glycoprotein of vesicular stomatitis virus in eurcaryotic cells. *Cell* **30,** 753–762.

Rothblatt, J. A., and Meyer, D. I. (1986a). Secretion in yeast: Reconstitution of the translocation and glycosylation of α-factor and invertase in a homologous cell-free system. *Cell* **44,** 619–628.

Rothblatt, J. A., and Meyer, D. I. (1986b). Secretion in yeast: Translocation and glycosylation of prepro-α-factor *in vitro* can occur via an ATP-dependent posttranslational mechanism. *EMBO J.* **5,** 1031–1036.

Rothman, J. E., and Lodish, H. F. (1977). Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. *Nature* (*London*) **269,** 775–780.

Rothman, J. E., and Lenard, J. (1977). Membrane asymmetry. *Science* **195,** 743–753.

Rottier, P., Brandenburg, D., Armstrong, J., van der Zeijst, B., and Warren, G. (1984). Assembly *in vitro* of a spanning membrane protein of the endoplasmic reticulum: The E1 glycoprotein of coronavirus mouse hepatitis virus A59. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 1421–1425.

Rottier, P., Armstrong, J., and Meyer, D. I. (1985). Signal recognition particle-dependent insertion of coronavirus E1, an intracellular membrane glycoprotein. *J. Cell Biol.* **260,** 4648–4652.

Sabatini, D. D., and Blobel, G. (1970). Controlled proteolysis of nascent polypeptides in rat liver cell fractions II. Location of the polypeptides in rough microsomes. *J. Cell Biol.* **45,** 146–157.

Sabatini, D. D., Tashiro, Y., and Palade, G. E. (1966). On the attachment of ribosomes to microsomal membranes. *J. Mol. Biol.* **19,** 503–524.

Sabatini, D. D., Kreibich, G., Morimoto, T., and Adesnik, M. (1982). Mechanisms for the incorporation of proteins in membranes and organelles. *J. Cell Biol.* **92,** 1–22.

Schatz, G., and Butow, R. A. (1983). How are proteins imported into mitochondria? *Cell* **32,** 316–318.

Schatzman, R. C., Ivan, G. I., Privalsky, M. L., and Bishop, M. J. (1986). Orientation of the v-*erbB* gene product in the plasma membrane. *Mol. Cell. Biol.* **6,** 1329–1333.

Schlenstedt, G., and Zimmermann, R. (1987). Import of frog prepropeptide GLa into microsomes requires ATP but does not involve docking protein or ribosomes. *EMBO J.* **6,** 699–703.

Schneider, C., Owen, M. J., Banville, D., and Williams, J. G. (1984). Primary structure of human transferrin receptor deduced from the mRNA sequence. *Nature (London)* **311,** 675–678.

Siegel, V., and Walter, P. (1985). Elongation arrest is not a prerequisite for secretory protein translocation across the microsomal membrane. *J. Cell Biol.* **100,** 1913–1921.

Siegel, V., and Walter, P. (1986). Removal of the Alu structural domain from signal recognition particle leaves its protein translocation activity intact. *Nature (London)* **320,** 81–84.

Simon, K., Perara, E., and Lingappa, V. R. (1987). Translocation of globin fusion proteins across the endoplasmic reticulum membrane in *Xenopus laevis* oocytes. *J. Cell Biol.* **104,** 1165–1172.

Singer, P. A., and Williamson, A. R. (1980) Different species of messenger RNA encode receptor and secretory IgM chains differing at their carboxyl termini. *Nature (London)* **285,** 297–299.

Spiess, M., and Lodish, H. F. (1985). Sequences of a second human asialoglycoprotein receptor: Conservation of two receptor genes during evolution. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 6465–6469.

Spiess, M., and Lodish, H. F. (1986). An internal signal sequence: The asialoglycoprotein receptor membrane anchor. *Cell* **44,** 177–185.

Tabe, L., Krieg, P., Strachan, R., Jackson, D., Wallis, E., and Colman, A. (1984). Segregation of mutant ovalbumins and ovalbumin–globin fusion proteins in *Xenopus* oocytes: identification of an ovalbumin signal sequence. *J. Mol. Biol.* **180,** 645–666.

Tajima, S., Lauffer, L., Rath, V. L., and Walter, P. (1986). The signal recognition particle (SRP) receptor is a complex containing two distinct polypeptide chains: Identification of the SRP receptor β-subunit. *J. Cell Biol.* **103,** 1167–1178.

Talmadge, K., Kaufman, J., and Gilbert, W. (1980a). Bacteria mature preproinsulin to proinsulin. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 3988–3992.

Talmadge, K., Stahl, S., and Gilbert, W. (1980b). Eukaryotic signal sequence transports insulin antigen in *E. coli*. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 3369–3373.

Tanford, C. (1978). The hydrophobic effect and the organization of living matter. *Science* **200,** 1012–1018.

Toneguzzo, F., and Ghosh, H. P. (1977). Synthesis and glycosylation *in vitro* of glycoprotein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U.S.A.* **74,** 1516–1520.

Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. H., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D., and Seeburg, P. H. (1984). Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature (London)* **309,** 418–425.

Vasalli, P., Tedghi, R., Lisowska-Berstein, B., Tartakoff, A., and Jaton, J.-C. (1979). Evidence for hydrophobic region within heavy chains of mouse B lymphocyte membrane-bound IgM. *Proc. Natl. Acad. Sci. U.S.A.* **76,** 5515–5519.

von Heijne, G. (1984). How signal sequences maintain cleavage specificity. *J. Mol. Biol.* **173,** 243–251.

von Heijne, G. (1985). Signal sequences: The limits of variation. *J. Mol. Biol.* **184,** 99–105.

von Heijne, G., and Blomberg, C. (1979). Transmembrane translocation of proteins. The direct transfer model. *Eur. J. Biochem.* **97,** 175–181.

Walter, P., and Blobel, G. (1980). Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 7112–7116.

Walter, P., and Blobel, G. (1981a). Translocation of proteins across the endoplasmic reticulum II. Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of *in-vitro*-assembled polysomes synthesizing secretory protein. *J. Cell Biol.* **91,** 551–556.

Walter, P., and Blobel, G. (1981b). 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.

Walter, P., and Blobel, G. (1982). SRP contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature (London)* **299,** 691–698.

Walter, P., and Blobel G. (1983a). Disassembly and reconstitution of SRP. *Cell* **34,** 525–533.

Walter, P., and Blobel, G. (1983b). Subcellular distribution of signal recognition particle and 7SL RNA determined with polypeptide-specific antibodies and complementary DNA probe. *J. Cell Biol.* **97,** 1693–1699.

Walter, P., and Lingappa, V. R. (1986). Mechanism of protein translocation across the endoplasmic reticulum. *Annu. Rev. Cell Biol.* **2,** 499–516.

Walter, P., Jackson, R. C., Marcus, M. M., Lingappa, V. R., and Blobel, G. (1979). Tryptic dissection and reconsitution of translocation activity for nascent presecretory proteins across microsomal membranes. *Proc. Natl. Acad. Sci. U.S.A.* **76,** 1795–1799.

Walter, P., Ibrahimi, I., and Blobel, G. (1981). Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to in-vitro-assembled polysomes synthesizing secretory protein. *J. Cell Biol.* **91,** 545–550.

Walter, P., Gilmore, R., and Blobel, G. (1984). Protein translocation across the endoplasmic reticulum. *Cell* **38,** 5–8.

Warren, G., and Dobberstein, M. (1978). Protein transfer across microsomal membranes reassembled from separated membrane components. *Nature (London)* **273,** 569–571.

Watanabe, M., Hunt, J. F., and Blobel, G. (1986). *In vitro* synthesized bacterial outer membrane protein is integrated into bacterial inner membranes but translocated across microsomal membranes. *Nature (London)* **323,** 71–73.

Waters, M. G., and Blobel, G. (1986). Secretory protein translocation in a yeast cell-free system can occur posttranslationally and requires ATP hydrolysis. *J. Cell Biol.* **102,** 1543–1550.

Watson, M. E. E. (1984). Compilation of published signal sequences. *Nucleic Acids Res.* **12,** 5145–5164.

Watts, C., Wickner, W., and Zimmerman, R. (1983). M13 procoat and a pre-immunoglobulin share processing specificity but use different membrane receptor mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 2809–2813.

Wickner, W. (1979). The assembly of proteins into biological membranes: the membrane trigger hypothesis. *Annu. Rev. Biochem.* **48,** 23–45.

Wickner, W. T., and Lodish, H. F. (1985). Multiple mechanisms of protein insertion into and across membranes. *Science* **230,** 400–407.

Wiedmann, M., Kurzchalia, T., Hartmann, E., and Rapoport, T. A. (1987). A signal sequence receptor in the endoplasmic reticulum membrane. *Nature (London)* (in press).

Wolfe, P. B., Silver, P., and Wickner, W. (1982). The isolation of homogeneous leader

peptidase from a strain of *Escherichia coli* which overproduces the enzyme. *J. Biol. Chem.* **257,** 7898–7902.

Yost, C. S., Hedgpeth, J., and Lingappa, V. R. (1983). A stop transfer confers predictable transmembrane orientation to a previously secreted protein in cell-free systems. *Cell* **34,** 759–766.

Young, E. F., Ralston, E., Blake, J., Ramachandran, J., Hall, Z. W., and Stroud, R. M. (1985). Topological mapping of acetylcholine receptor: Evidence for a model with five transmembrane segments and a cytoplasmic COOH-terminal peptide. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 626–630.

Zerial, M., Melancon, P., Schneider, C., and Garoff, H. (1986). The transmembrane segment of the human transferrin receptor functions as a signal peptide. *EMBO J.* **5,** 1543–1550.

Zerial, M., Huylebroeck, D., and Garoff, H. (1987). Foreign transmembrane peptides replacing the internal signal sequence of transferrin receptor allow its translocation and membrane binding. *Cell* **48,** 147–155.

Zimmermann, R., and Mollay, C. (1986). Import of honeybee prepromelittin into the endoplasmic reticulum. *J. Biol. Chem.* **261,** 12889–12895.

Zuniga, M. C., and Hood, L. E. (1986). Clonal variation in cell surface display of an H-2 protein lacking a cytoplasmic tail. *J. Cell Biol.* **102,** 1–10.