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Translocation of proteins across
the endoplasmic reticulum

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

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Introduction

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

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

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

Targeting proteins to the membrane

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

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

Abbreviations

ER—endoplasmic reticulum; fh—E. coli homologue of SRP54; SRP—signal recognition particle; SSR—signal sequence receptor; TRAM—translocating chain associated membrane protein.
gene. Signal sequences specifically bind to the 54 kDa subunit of SRP, which has been functionally and structurally divided into two regions [10,11]: SRP54G, with a GTP-binding site, and SRP54M, a methionine-rich domain that is responsible for binding to the 7S RNA and can be crosslinked to signal sequences [12,13]. In the absence of the GTP-binding domain, the SRP54G is sufficient for binding signal sequences [14*] and for reconstitution into a SRP(−54G) which is competent for binding signal sequences and arrest of protein synthesis [15*]. Although SRP(−54G) bound signal sequences [15*], chemical modifications of the SRP54G [9,14*] blocked binding, suggesting a role in regulation of the binding of signal sequences [14*]. Significantly, this SRP(−54G) did not promote protein translocation and its block of protein synthesis was not reversed by SRP receptor [15*]. This result is particularly significant in light of the observation that SRP54G is highly homologous to the GTP-binding domains of the SRP receptor [10,11]. The role of GTP in translocation [16-18] has yet to be fully elucidated. However, by combining site-directed mutations in the GTP-binding domain with biochemical reconstitution in a mammalian system, it has become possible to dissect and identify discrete steps in the process of SRP-SRP receptor binding and signal sequence displacement [19*]. This approach holds great promise for identifying the role(s) of GTP in translocation.

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

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

SRP causes complete translation arrest in a heterologous system of wheat germ cytosol and mammalian membranes [4]. It mediates transient pausing in a homologous mammalian system [36]. Examination of its role in translation arrest in vivo has been significantly facilitated by the identification of homologues for SRP in yeast. Mutations of the 7S RNA of Yarrowia lipolytica selectively inhibited the synthesis of only secretory proteins. As untranslocated precursors were not observed in the cytosol, this mutated SRP may still function to arrest translation of secreted proteins, but fail to allow the proteins to translocate [37,38*]. The size of nascent proteins affects the ability of SRP to induce translation arrest: Small proteins (up to 85 amino acids) translocate without the assistance of SRP [39*]. Translation of larger proteins (up to 17 kDa), was arrested by SRP, suggesting that SRP binds to numerous sites on the nascent chain [40*]. Progress has also been made in identifying the minimal peptide length for insertion in the membrane, which should help identify the steps in peptide dissociation from SRP and translocation across the membrane [41*].

Membrane components

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

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

The role of cytosolic factors in translocation was established because of the availability of a biochemical assay for fractionating and reconstituting translocation [62]. The list of potential membrane components has grown over the past few years in the absence of a similar technique for fractionating membrane proteins. The ability to solubilize, fractionate and reconstitute membranes that still translocate [63-65] is a powerful tool for test-
ing proteins [66∗]. ER membranes were solubilized and a potential component, SSR, was depleted before reconstitution of membrane proteins. These membranes were still fully competent to translocate. Translocation was totally blocked when SRP receptor was depleted from the membranes, demonstrating the power of this technique [66∗]. It has been used to demonstrate that TRAM is required for protein translocation [44∗].

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

How do proteins cross the membrane?

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

These observations are consistent with results from other systems. First, channels very similar to those observed in the ER [73] were observed in the plasma membrane of E. coli upon addition of very low (< nM) concentrations of signal sequences [75∗]. This suggests the signal sequence is a ligand that opens the protein-conducting channel. Likewise, a general increase of conductance was observed when pre-proteins were added to E. coli plasma membrane vesicles [76∗]. Second, the conductance of channels in the mitochondrial membrane was substantially reduced, or blocked, in the presence of a peptide resembling a mitochondrial targeting signal, suggesting that the peptide is entering the channel and slowing the flow of ions [77∗]. Third, when crosslinkers were incorporated into nascent chains, they could be linked to other transmembrane proteins but not to lipids, indicating that proteins are shielded from the hydrocarbon core of the membrane [78∗]. However, these results raise two immediate problems. First, if proteins are in an aqueous pore, what moves them across the membrane? Second, how do transmembrane proteins get out of the channel and into the bilayer?

A Brownian ratchet has been proposed as the mechanism that moves proteins across membranes [79∗]. The model assumes that proteins are in a protein-conducting channel and the translocating chains see different chemical environments on the two different sides of the membrane. A protein in a channel should reattach back and forth from thermal energy. But, if a protein is modified in the lumen of the ER (by addition of sugar groups or binding by luminal contents), the protein will not be able to move out of the lumen. It would reattach back and forth until it finally crosses the membrane. Quantification of this model demonstrated that it can account for the kinetics of protein translocation [79∗]. It has also withstood a number of experimental tests: the luminal protein BIP is one of the main proteins that can be cross-linked to a translocating chain [56∗]; BIP is needed in reconstituted yeast ER vesicles for translocation [80∗] and luminal ER proteins are required in reconstituted mammalian microsomes for unidirectional translocation [81∗]; addition of a glycosylation site to a short protein ensures the vectoriality of transport [82∗]; once a chain is partially across the membrane, it will fully translocate in the absence of any additional energy source [83∗]. It has been suggested that chaperone proteins may pull the nascent chain through the membrane [83∗,84∗]. The Brownian ratchet is a mechanism for transducing chaperone binding to protein movement.

Integration of membrane proteins

Little is known of the biogenesis of membrane proteins. One approach has been to screen for mutations in yeast that selectively block translocation of membrane proteins [49∗,61∗]. Alternatively, genetically engineered proteins were used to identify the regions that define a transmembrane domain [85∗,86∗]. Statistical studies of the distribution of charges on either side of the transmembrane segment have supported the idea that an excess of positive charge determines the cytoplasmic side [87]. Experimental results have been ambiguous with some constructs consistent with the rule [88∗]. However, other constructs indicate that the charged residues do not affect transmembrane orientation. This suggests that other features determine membrane topology [89∗]. A different construct has been used to show that transmembrane segments insert independently with an innate transmembrane orientation [90∗,91∗]. The observation that some
domains of a protein may not always be on the same side of the membrane has elicited the suggestion that there may be regulation of insertion of membrane segments [92*].

Conclusion

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

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

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60. GORICH D, PREHN S, HARTMANN E, KALIES K-U, RAPPOPORT TA: A mammalian homologue of SEC6lp and SECYlp is associated with ribosomes and nascent polypeptides during protein translocation. Cell 1992, 71:489-503.

A mammalian homologue of the yeast Sec6lp was identified that has homology to the E. coli SepC. The protein was crosslinked to translocating chains and found to associate tightly with ribosomes during protein translocation.

61. STRUBING CJ, ROTHBLATT J, HOSOBUCHI M, DESHAIES R, SCHERMAN R: Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. Mol Biol Cell 1992, 3:129-142.

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

62. REDMAN CM, DOKBERSTEIN R: Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. J Cell Biol 1975, 67:852-862.

63. YU Y, ZHANG Y, SARATIN DD, KREIBICH G: Reconstitution of translocation competent membrane vesicles from detergent-solubilized dog pancreas rough microsomes. Proc Natl Acad Sci USA 1989, 86:9931-9935.

64. NICCHITTA CV, BLOEBEL G: Assembly of translocation-competent proteoliposomes from detergent-solubilized rough microsomes. Cell 1990, 60:259-269.

65. ZIMMERMAN DJ, WALTER P: Reconstitution of protein translocation activity from partially solubilized microsomal vesicles. J Biol Chem 1990, 265:4048-4053.

66. MIGLIOCCIO G, NICCHITTA CV, BLOEBEL G: The signal recognition particle receptor, unlike the signal recognition particle receptor, is not essential for protein translocation. J Cell Biol 1992, 117:15-25.

Detergent-solubilized membranes were depleted of either SSR or the SRP receptor before membrane reconstitution. Membranes lacking SRP receptor were not translocation competent. Membranes lacking SSR resembled mock-depleted membranes suggesting that either SSR is not involved in translocation or there is another functionally equivalent protein.
67. Shellenb GS, Lin L, Nicchita CV: Membrane Topology and the Biogenesis of Eukaryotic Signal Peptidease. J Biol Chem 1993, 268:5201-5208.

The membrane topology of the subunits of the signal peptidase complex (see [50]) was mapped. Each protein has a single transmembrane domain with its amino-terminal facing the cytosol. The putative active domains were approximately the same distance from the bilayer as the active domain for the analogous domain in the signal peptidase of E. coli. Each of the subunits required SRP to be translocated into the membrane.

68. Savitz AJ, Meyer DI: Identification of a Ribosome Receptor in the Rough Endoplasmic Reticulum. Nature 1990, 346:540-544.

69. Nunnari JM, Zimmerman DI, Oog SC, Walter P: Characterization of the Rough Endoplasmic Reticulum Ribosome-Binding Activity. Nature 1991, 352:638-640.

70. Tazawa S, Unuma M, Tondokoro N, Asano Y, Ohsumi T, Ichimura T, Suga H: Identification of a Membrane Protein Responsible for Ribosome Binding in Rough Microsomal Membranes. J Biochem (Tokyo) 1991, 109:89-98.

71. Ichimura T, Ohsumi T, Shindo Y, Ohtsuka T, Yamagishi M, Momose Y, Omatu S, Sugano H: Isolation and Some Properties of a 34-kDa-Membrane Protein that may be Responsible for Ribosome Binding in Rat Liver Rough Microsomes. FEBS Lett 1992, 296:7-10.

This purification of a protein previously shown to bind ribosomes (see [70]). When reconstituted into liposomes, its ribosome-binding properties were similar to those of intact microsomes. Subcellular fractionation was used to localize the protein ribosome-bound membranes in a variety of mammalian tissues.

72. Collins PG, Gilmore R: Ribosome Binding to the Endoplasmic Reticulum: A 180-kD Protein Identified by Crosslinking to Membrane-Bound Ribosomes is not Required for Ribosome Binding Activity. J Biol Chem 1991, 114:639-649.

73. Simon SM,Blobel G: A Protein-Conducting Channel in the Endoplasmic Reticulum. Cell 1991, 65:371-380.

74. Crowley KS, Reinhart GD, Johnson AE: The Signal Sequence Moves Through a Ribosomal Tunnel into a Non-Cytosolic Aqueous Environment at an Early Stage of Translocation. Cell 1993, in press.

A fluorescent probe was incorporated into nascent secretory chains. While the chain was in the ribosome, or translocating the membrane, the probe indicated it was in an aqueous environment. However, iodide ions did not quench the fluorescence. If the protein was translated in the absence of membranes, the fluorescence was quenched with iodide, even while the protein was in the ribosomal tunnel. This suggests that the seal between the ribosome and the aqueous protein-conducting channel is not freely permeable to iodide.

75. Simon SM, Blobel G: Signal Peptides Open Protein-Conducting Channels in e. coli. Cell 1992, 69:677-684.

The signal peptide of LamB was shown to cause an increase of membrane conductance when applied to the cytoplasmic side of the E. coli plasma membrane. At low concentrations (approximately one signal peptide per E. coli volume) individual channels were observed whose size was similar to those channels seen in mammalian ER (see [72]). This indicates that the signal sequence is the ligand that opens the protein-conducting channels.

76. Schieble E, Wickner W: Preprotein Translocation Creates a Halide Anion Permeability in the Escherichia coli Plasma Membrane. J Biol Chem 1992, 267:7505-7510.

Addition of pre-proteins dissipated the membrane potential across inverted plasma membrane vesicles of E. coli. The rate was affected by the halide concentration.

77. Thieffry M, Neumark J, Pellechich M, Frem H, Henry J-P: Properties of the Mitochondrial Peptide-Sensitive Cationic Channel Studied in Planar Bilayers and Patches of Giant Liposomes. Biochips J 1992, 63:333-339.

A voltage-dependent cationic channel in mitochondrial membranes was blocked when a peptide with some of the properties of a mitochondrial signal sequence was added. The results indicate that the peptide enters and crosses the channel, which is observed as a blockage of ionic flow.

78. Joly JC, Wickner W: The SecA and SecY Subunits of Translocate are the Nearest Neighbors of a Translocating Preprotein, Shielding it from Phospholipids. EMBO J 1993, 12:255-263.

A photoactivatable cross-linker was attached to the cysteine residues of proOMP-A fused to dihydrofolate reductase. Partially translocated polypeptides were crosslinked to SecA and SecY. They were not observed to be linked to SecE, Bank I or the membrane phospholipids. This suggests that a translocating chain is completely shielded from the lipid bilayer by proteins.

79. Simon SM, Peiskin CS, Oster GF: What Drives the Translocation of Proteins? Proc Natl Acad Sci USA 1992, 89:5770-5774.

Proteins were proposed to translocate by biased random thermal motion. Translocating chains fluctuate back and forth in protein-conducting channels. Chemical asymmetries (binding of sugar groups or chaperones, differences in folding due to ions or pH, disulphide bond formation, signal peptide cleavage) bias the diffusion from the cytosol to the lumen. The model can quantitatively account for observed rates of translocation.

80. Brodsky JL, Hamamoto S, Feldheim D, Scherman R: Reconstitution of Protein Translocation from Solubilized Yeast Membranes Reveals Topologically Distinct Roles for Bip and Cytosolic Hsp70. J Biol Chem 1993, 12095-12097.

Protein translocation was reconstituted in vesicles from wild-type yeast ER or from sec5 or kar2 (Bip mutants). Translocation was deficient with vesicles from the mutants. However, the vesicles from kar2 transfected wild-type Bip was included during the reconstitution.

81. Nicchita CV, Blobel G: Lumenal Proteins of the Mammalian Endoplasmic Reticulum are Required to Complete Protein Translocation. Cell 1993, 73:989-998.

Depletion of luminal proteins from reconstituted mammalian microsomes had no effect on the targeting of proteins but blocked subsequent translocation of proteins. The inclusion of luminal ER proteins restored translocation as assayed by increased protease protection of the nascent polypeptide and co-fractionation of the peptide with the microsomes.

82. Ooi CE, Weiss J: Bidirectional Movement of a Nascent Polypeptide Across Microsomal Membranes Reveals Requirements for Vectorial Translocation of Proteins. Cell 1992, 71:87-96.

A truncated secretory polypeptide moved bidirectionally across the ER. After signal peptide cleavage it did not fractionate with microsomes. However, addition of a glycosylation site to the translocating chain ratcheted the peptide in the microsome and bidirectional transport was no longer observed.

83. Arconitz RA, Joly JC, Wickner W: Translocation can Drive the Unfolding of a Preprotein Domain. EMBO J 1993, 12:243-253.

A construct of proOMP-A dihydrofolate reductase stabilized with its ligands NADPH and dihydrofolate was trapped as a translocation intermediate. Upon removal of the ligands, the dihydrofolate reductase unfolded and translocated. This occurred with neither ATP nor ΔηH.

84. Kang PJ, Ostermann J, Shirling J, Neuert W, Craig EA, Opper N: Requirement for hsp70 in the Mitochondrial Matrix for Translocation and Folding of Precursor Proteins. Nature 1990, 348:137-143.

85. Sakaguchi M, Iwamoto K, Kurwia I, Miura K, Omura T: Functions of Signal and Signal-Anchor Sequences are Determined by the Balance Between the Hydrophobic Segment and the N-Terminal Charge. Proc Natl Acad Sci USA 1993, 89:16-19.

Mutations were made in a signal sequence that affected either the charge at the amino terminus or the length of the hydrophobic segment. Short hydrophobic segments acted as signal sequences. Longer hydrophobic segments formed transmembrane domains unless preceded by positive charges at the amino terminus.

86. Green N, Walter P: C-Terminal Sequences can Inhibit the Insertion of Membrane Proteins into the Endoplasmic
Reticulum of *Saccharomyces cerevisiae*. Mol Cell Biol 1992, 12:276–282.

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

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

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

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

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

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

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

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

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

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

92. **SKACH WK, CALVAG MC, UNGAPPA VR**: Evidence for an Alternate Model of Human P-Glycoprotein Structure and Biogenesis. J Biol Chem 1993, 268:6903–6908.

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

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

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

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