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Transport of Proteins Across the Endoplasmic Reticulum Membrane

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The biosynthesis of many eukaryotic proteins requires their transport across the endoplasmic reticulum (ER) membrane. The process can be divided into two phases: (i) a targeting cycle, during which, by virtue of their signal sequences, nascent polypeptides are directed to translocation sites in the ER and (ii) the actual transfer of proteins across the membrane. The first phase has been well characterized, whereas the latter until recently was completely unresolved. Key components of the translocation apparatus have now been identified and it seems likely that they form a protein-conducting channel in the ER membrane. The transport process is similar to the process of protein export in bacteria.

A great number of proteins are transported across the ER membrane as they are synthesized. These include secretory proteins and proteins of the plasma membrane, lysosomes, endosomes, and all organelles of the secretory pathway. Synthesis of these proteins begins in the cytoplasm, but they are then targeted to the ER membrane by signal sequences, which are characterized by a continuous stretch of 6 to 20 apolar amino acids and are often located at the NH_2-terminus of precursor molecules. Recognition of the signal sequence and targeting of the nascent chain generally requires the combined function of the signal recognition particle (SRP) and of its membrane receptor, but alternative targeting pathways exist. This review summarizes briefly our knowledge of the targeting process (for previous reviews, see *1*, *2*).

The main focus of this review is the translocation process that succeeds the targeting phase. Proposed mechanisms of translocation have ranged from the idea that the transport of a polypeptide chain occurs directly through the phospholipid bilayer without participation of membrane proteins to models in which polypeptides are transported through a hydrophilic or amphiphilic channel formed from transmembrane proteins (*1*). It now seems that a protein-conducting channel does exist. The evidence comes from electrophysiological data and from the identification of membrane proteins as putative channel constituents. Three powerful approaches have contributed to the recent progress—genetic screening for translocation components, identification of membrane proteins adjacent to translocating polypeptides by chemical crosslinking, and reconstitution of the translocation components into proteoliposomes after their solubilization and purification. This review summarizes our knowledge of the various components of the translocation site.

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The Targeting Cycle: Role of the SRP

In eukaryotes, most proteins are targeted to the ER membrane by the SRP. The SRP is a ribonucleoprotein particle consisting of a 7S RNA molecule and six polypeptide subunits of 9, 14, 19, 54, 68, and 72 kD (*2*). In vitro experiments with the mammalian SRP have suggested a scheme for the function of the SRP (Fig. 1). As soon as the signal sequence of a growing polypeptide chain has emerged from the ribosome, it is bound by the SRP (step 1). Next, the complex containing the nascent chain, ribosome, and SRP is specifically targeted to the ER membrane by an interaction with a membrane-bound receptor, the SRP receptor or docking protein (*3*), which consists of α and β subunits (*4*) (step 2). Guanosine triphosphate (GTP) is required for the next step, during which the SRP is released from both the ribosome and the signal sequence (*5*) (steps 3 and 4). The nascent chain is transferred into the membrane and the ribosome becomes membrane bound through its attachment to a ribosome receptor. Finally, GTP hydrolysis leads to the dissociation of the SRP from its receptor, and a new targeting cycle can begin (*6*) (step 5). The actual transfer of the polypeptide through the membrane does not require the SRP or its receptor and commences only after their disengagement (after step 4). According to this scheme, the SRP has two basic functions: First, it targets the polypeptide chain to the ER membrane by interacting both with the signal sequence and with the translocation apparatus. Second, it keeps the bound signal sequence segregated from the rest of the polypeptide chain and thereby prevents aberrant, premature folding.

The signal sequence is recognized by the 54-kD polypeptide of the SRP (SRP54) (*7*). This subunit contains a methionine-rich M domain and a GTP-binding G domain (*8*, *9*). The former domain interacts with signal sequences (*10*). The methionines in the M domain are assumed to be located on one side of three α helices and could form or contribute to the formation of a hydrophobic pocket into which the hydrophobic cores of signal sequences could be buried (*9*). The flexible side chains of methionines appear to be particularly well suited to accommodate signal sequences of different structure. The G domain, which is not needed for signal sequence binding, seems to take part in targeting (*11*). GTP hydrolysis at this site may result in the release of erroneously bound signal sequences from the M domain; it would thus be required for a proofreading mechanism during signal sequence recognition. SRP54 can bind to signal sequences in the absence of any other component of the SRP (*12*).

GTP binds to both subunits of the SRP receptor. The α subunit interacts with the SRP, and the GTP binding site of the α subunit appears to be important for the targeting reaction (*13*). It seems likely that a guanine nucleotide exchange reaction is induced by the contact of the SRP with the α subunit of the SRP receptor (step 3 in Fig. 1). Occupation of the site by GTP initiates the release of the signal sequence from the SRP (step 4). The function of the GTP-binding site of the β subunit of the SRP receptor is unknown.

The SRP and its membrane receptor are found in all organisms that have been examined. Homologs to the mammalian components have been detected in plants, yeast, and even bacteria. Depletion of *Saccharomyces cerevisiae* cells of SRP components or of the SRP receptor leads to defective translocation of many exported

proteins (*14*). Similar observations have been made with a temperature-sensitive mutant of a homolog of SRP19 (Sec65p) (*15*). Thus, there is strong evidence for the function of the SRP in vivo. However, the complete deletion of the genes for the SRP constituents or for the SRP receptor is not lethal. The yeast cells adapt to the lack of the SRP pathway and grow, albeit very slowly. Thus, one or more SRP-independent targeting pathways must exist that can replace the one mediated by the SRP. How the signal sequence is recognized under these circumstances is not known.

The role of a complex like the SRP in *Escherichia coli* has been under dispute. Although a structurally related particle exists that contains a 4.5S RNA that bears similarities to the mammalian 7S RNA and a 48-kD polypeptide (P48 or Ffh) that is highly similar to SRP54 (*16*), no convincing evidence for its function in protein export had been obtained. Recent data demonstrate, however, that depletion of Ffh from *E. coli* cells leads to the accumulation of precursors of all exported proteins tested, albeit to different extents (*17*). Furthermore, in an *E. coli* lysate, Ffh is the only component that specifically interacts with signal sequences (*18*). Even though the SRP may function in protein translocation in *E. coli*, its components have not been found in genetic screens.

Alternative Targeting Pathways

The existence of at least one SRP-independent targeting pathway is not only indicated by the fact that *S. cerevisiae* cells can survive without SRP but also by the finding that various proteins can be translocated in vitro in the absence of the SRP and the SRP receptor (*19*). Posttranslational translocation of some yeast proteins has been demonstrated in vitro and in vivo (*20, 21*). Any posttranslational translocation must occur without the SRP because the latter binds only to nascent chains attached to ribosomes (*22*). It is likely that cytosolic chaperones, such as Hsp70 in eukaryotes (*23*) and SecB in *E. coli* (*24*), are required to keep precursor molecules in a translocation-competent state.

The specific functions of the SRP-dependent and SRP-independent pathways may differ. The SRP-dependent path may be the basic one (*25*). The immediate binding of SRP to a signal sequence that emerges from the ribosome may alleviate constraints that particular folding characteristics of a protein might impose on its translocation. SRP-independent translocation could serve as a salvage pathway for precursor proteins that have missed their chance for cotranslational targeting by the SRP. Short proteins or proteins with signal sequences that have low affinity for the SRP may engage the SRP relatively late during their synthesis or not at all (particularly in *E. coli* where translation is fast); they may then be rescued for transport by interaction with chaperones. Such a concept does not exclude, however, that salvage chaperones also interact with nascent chains cotranslationally.

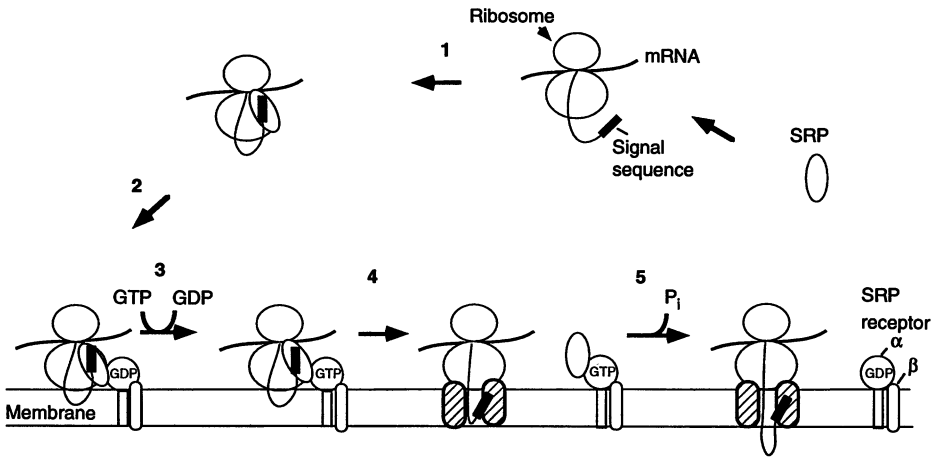

Fig. 1. The protein targeting cycle. The scheme shows the first steps in protein translocation across the ER membrane. When the signal sequence of a growing polypeptide chain has emerged from the ribosome, it is recognized and bound by the SRP (step 1). In step 2, the complex containing the ribosome, nascent chain, and SRP binds to the ER membrane through an interaction of the SRP with its membrane receptor, which consists of two subunits. In step 3, guanosine diphosphate (GDP) is exchanged for GTP at the SRP receptor. In step 4, the SRP is released both from the ribosome and from the signal sequence. The ribosome becomes membrane-bound, probably by its interaction with Sec61p or associated proteins, and translocation begins. In step 5, the SRP is released from its receptor by GTP hydrolysis and can then enter a new targeting cycle. Whether or not the two subunits of the SRP receptor dissociate during the SRP cycle is not known.

Table 1. Possible components of the translocation site of the ER membrane. For references and discussion, see text.

Protein	Occurrence	Function
Sec61p/Yp	Yeast, bacteria, mammals, fish	Constituent of a protein-conducting channel
TRAM protein	Mammals	Early function in translocation
Sec62p-Sec63p complex	Yeast	Early function in translocation
SSR complex	Mammals, fish, birds	Unknown
Signal peptidase complex	Yeast, mammals	Signal peptide cleavage
Oligosaccharyl transferase	Mammals, yeast	Asn-glycosylation
mp30	Mammals	Unknown

The Translocation Site

After targeting, polypeptides are transported at specific sites through the ER membrane. The translocation site is probably a rather complex structure, consisting of a number of proteins with different functions. Some of the components of the translocation site may be directly involved in the transport process, for example, as constituents of a protein-conducting channel. Others may take part in chemical modifications of a nascent polypeptide or in its folding and assembly. Some components may only be required for certain types of proteins, for example, for the insertion of membrane proteins into the phospholipid bilayer. The translocation site may be a dynamic structure, assembled by the membrane targeting of a nascent chain and disassembled after termination of translocation. The complexity of the translocation site is indicated by the growing number of components that have been found in it (Table 1).

Although long postulated, only recently has evidence for a protein-conducting channel been provided by an electrophysiological approach (*26*). Rough microsomal vesicles fused into planar lipids showed channels of high ion conductivity. The channels increased in number after release of the nascent chains from ribosomes by puromycin, suggesting that they had been plugged by nascent chains in transit through the membrane. They closed if the salt concentration was subsequently increased—conditions known to result in the dissociation of the ribosomes into their subunits. In similar

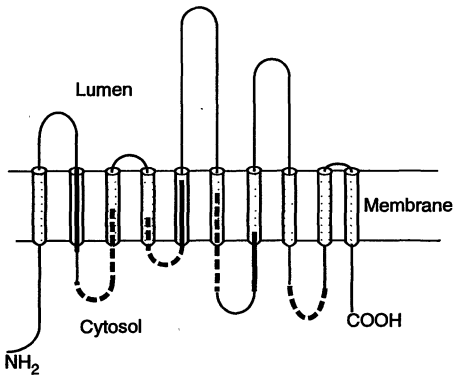

Fig. 2. Predicted membrane topology of Sec61p and SecYp and regions of similarity. Thick regions indicate sequences with conserved amino acids and high similarity between Sec61p from mammals and yeast and SecYp from various bacteria (*31*). Dashed regions indicate sequences of lower similarity. The proposed topology is based on various predictive computer algorithms and on the experimentally determined topology of SecYp of *E. coli* (*65*).

experiments with *E. coli* membranes, synthetic signal peptides were found to open large channels (*27*). Other experiments with signal peptide mutants and mutants of translocation components are required to confirm these results. Further support for a hydrophilic channel comes from experiments in which the environment of membrane-inserted nascent chains was investigated by measuring the fluorescence life time of incorporated probes (*28*).

Translocation Site Components

Sec61p was discovered in genetic screens for translocation defects in *S. cerevisiae* (*29*). Certain temperature sensitive mutations in Sec61p lead to the accumulation of precursor molecules of both secretory and membrane proteins at nonpermissive temperatures. Some alleles affected translocation of all proteins tested, other alleles affected only a subset of the tested proteins (*30*).

A mammalian homolog of Sec61p was recently identified (*31*). Its amino acid sequence is 56% identical with that of the yeast protein. Sec61p can be crosslinked to various translocating secretory proteins in ER membranes from *S. cerevisiae* (*32, 33*) or canine pancreas (*31*). At late stages of the translocation process, when the nascent chains have a sizable lumenal domain, Sec61p is the major protein that becomes crosslinked to the translocating polypeptide. P37, a major crosslinking partner of nascent membrane proteins, is identical with Sec61p (*34*), indicating that secretory and membrane proteins use the same translocation sites. The crosslinking of nascent chains to Sec61p is abolished by agents that disrupt either translocation intermediates or ribosomes (*35*). Thus, it seems that Sec61p is a component of a delicate structure closely apposed to polypeptides that are moving through the membrane.

A second, closely related gene for Sec61p was found in cDNA libraries of various mammalian cells and of fish (*31*). It is not yet clear if the corresponding proteins are present in equivalent concentrations.

Sec61p has sequence similarity to SecYp of bacteria (*31*) (Fig. 2). The proteins have identical topologies with ten predicted membrane spanning segments. Several hydrophilic amino acids within membrane-spanning regions are conserved, suggesting that they are essential for a hydrophilic environment within the membrane. Interestingly, all of the similar regions are located either within the membrane or on the cytoplasmic side in the topological model of Sec61p or SecYp (Fig. 2).

SecYp is likely to be a major component of the translocation apparatus in bacteria (*24*). The gene has been implicated in the export of proteins from *E. coli* by experiments with two different genetic selection schemes. SecYp and SecEp may be the only membrane components required for the in vitro translocation of proteins into proteoliposomes (*36*).

Sec61p has many of the properties expected for a constituent of a protein-conducting channel: (i) It is adjacent to translocating nascent chains in different organisms; (ii) it may form a hydrophilic environment in the membrane; (iii) its structure is highly conserved in evolution; and (iv) Sec61p from yeast and its prokaryotic counterpart SecYp are essential for translocation in vivo.

Association of Sec61p with Ribosomes

Mammalian Sec61p is tightly bound to ribosomes after solubilization of rough microsomes in detergent at high salt concentrations (*31*). The binding cannot be exclusively via the nascent chain because the latter can be removed from the ribosome by puromycin treatment without causing detachment of Sec61p. However, if the salt concentration is increased, so that the ribosome dissociates into its two subunits, Sec61p is released. The conditions required to strip rough microsomes from ribosomes are the same as those needed for the dissociation of the isolated Sec61p-ribosome complex. These results are consistent with those from the electrophysiological experiments that provide evidence for a protein-conducting channel. It seems that the closure of the channel coincides with the dissociation of the ribosomal subunits from Sec61p during termination of translation. The physiological relevance of the interaction between Sec61p and ribosomes is also supported by the fact that the interaction is induced by the targeting of a nascent polypeptide chain to the ER membrane (*31*). The ribosome seems to sit on Sec61p, because it shields the crosslinks of nascent chains and Sec61p from proteolytic attack (*35*). Ribosomes lacking nascent chains interact more weakly with Sec61p (*31*). This reaction probably corresponds to the binding of such ribosomes to microsomes, an assay used in previous studies on ribosome receptors (*37*). Because Sec61p is probably a core component of the translocation site and is tightly associated with ribosomes, it is likely that Sec61p alone or in association with one or more adaptor proteins constitutes the ribosome receptor.

A 180-kD protein (*38*) and a 34-kD protein (*39*) have been proposed as ribosome receptors but counterarguments have been raised against both candidates (*31, 40*). Of course, more than one ribosome receptor may exist and different membrane proteins may interact with the ribosome during the course of protein translocation.

The interaction of membrane-bound ribosomes with Sec61p supports the idea (*41*) that nascent chains are transferred directly from the channel or groove in the ribosome into a protein-conducting channel in the membrane. This would prevent the premature folding of a polypeptide chain in the cytoplasm into a translocation-incompetent conformation. However, at least in yeast, Sec61p also functions in the posttranslational translocation of some proteins, such as the secretory protein prepro-α-factor (*32, 33*). Presumably, these proteins maintain a translocation-competent state even after their release from the ribosome. It is possible that this mode of translocation is more common in *S. cerevisiae* than in mammals. The bacterial SecYp may have no affinity for ribosomes at all because in bacteria ribosomes do not seem to be tightly bound to the cytoplasmic membrane. Many bacterial proteins must therefore have special folding properties that allow them to be exported at late stages of their synthesis (*42*).

The TRAM Protein

Another component of the translocation site is the TRAM protein (*43*). Its identification was based on the work of Nicchitta and Blobel (*44*), who reconstituted translocation activity in proteoliposomes, and other studies that showed that short nascent chains of a secretory protein, after their transfer from the SRP into the ER membrane, can be crosslinked to a glycosylated membrane protein (*45, 46*). To purify the crosslinking partner, proteoliposomes with a defined composition of glycoproteins were

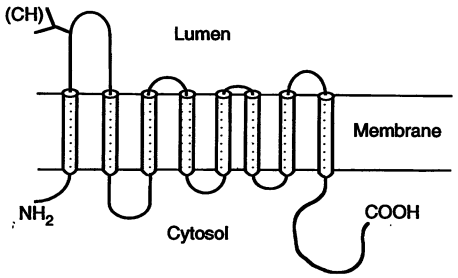

Fig. 3. Predicted membrane topology of the TRAM protein. The proposed membrane topology is based on various prediction algorithms (*43*). (CH), Asn-linked carbohydrate chain.

reconstituted from a cholate extract of canine pancreas microsomes, and tested for the appearance of a crosslinked product. A single glycoprotein, the translocating chain associating membrane (TRAM) protein, was sufficient to allow crosslinking (*43*). The sequence of the TRAM protein, as deduced from cloning of the corresponding cDNAs from different mammalian cells, suggests that it spans the membrane eight times and that it has a cytoplasmic tail of about 60 amino acids (Fig. 3). Several amino acids in the membrane-spanning regions are hydrophilic or charged. The TRAM protein is about as abundant as ER membrane–bound ribosomes, suggesting that it is present in each translocation site.

The effect of the TRAM protein on the translocation of secretory proteins was tested in an improved reconstitution system with an overall transport efficiency approaching that of the original membranes (*43*). Proteoliposomes depleted of glycoproteins had reduced transport activity for some secretory proteins (prepro-α-factor and pre-β-lactamase) but had only slightly reduced activity for preprolactin. Addition of the TRAM protein alone was sufficient to restore translocation to the original level.

One explanation for the differential effect of glycoprotein depletion on various translocation substrates may be that the TRAM protein is required for transport of some but not all proteins. The effects of several Sec mutants on different translocation substrates may also indicate different requirements for translocation components (*21, 30, 47*). However, the depletion of the TRAM protein might not have been complete (*43*). The number of translocation sites containing the TRAM protein may have only been reduced and proteins with a high affinity for them, like (presumably) preprolactin, still could have been translocated. In a reconstitution system in which a different detergent was used, preprolactin translocation was indeed reported to be dependent on glycoproteins (*44, 48*).

Short nascent chains of secretory proteins representing early stages of translocation can be crosslinked to both the TRAM protein and Sec61p (*31*). The TRAM protein seems to interact with amino acid residues preceding the hydrophobic core of the signal sequence whereas Sec61p seems to interact primarily with the core and with residues succeeding it (*49*). Therefore, the two proteins together may orient the signal sequence in a loop structure in the membrane.

With longer chains of secretory proteins crosslinks to the TRAM protein have not been observed. It is possible that these longer chains lack suitably located amino acids for crosslinking, but it seems more likely that the TRAM protein is only adjacent to nascent chains at the beginning of their membrane passage. It seems possible that signal peptide cleavage causes displacement of the TRAM protein. If the TRAM protein indeed senses the presence of a signal sequence, it may function as a signal sequence receptor. Alternatively, the TRAM protein may only be needed for proteins with weak signal sequences; those with strong ones might be transferred into the translocation site containing Sec61p directly.

The Sec62p-Sec63p Complex

Sec62p and Sec63p were detected in *S. cerevisiae* in genetic screens for translocation components (*21, 29*). They span the ER membrane two and three times, respectively (*50, 51*), and form a complex that also includes a glycoprotein of 31.5 kD and a non-glycoprotein of 23 kD (*52*). There is more Sec63p than Sec62p in yeast cells and a dynamic interaction between them has been suggested (*52*). Sec63p has a lumenal segment similar to a portion of DnaJ (*51, 53*), a partner of the heat-shock protein and chaperone DnaK of *E. coli*. It was therefore predicted that Sec63p would interact with BiP (Kar2), a eukaryotic homolog of DnaK, located in the lumen of the ER. Evidence for such an association comes from genetic data, demonstrating synthetic lethality in a *sec63, kar2* haploid mutant (*32*), and from the isolation of a complex containing both proteins (*54*). Because temperature-sensitive mutations of BiP result in rapid appearance of translocation defects at nonpermissive temperatures (*55*), it seems possible that the chaperone directly participates in the transport process, perhaps by pulling the polypeptide chain across the membrane. Alternatively BiP may act indirectly on the folding of a translocation component such as the Sec62p-Sec63p complex. In favor of the second possibility, proteoliposomes reconstituted from mammalian microsomes contain little BiP but translocate proteins with high efficiency. The Sec62p-Sec63p complex may function at early stages of the translocation process during which nascent polypeptides crosslink weakly to Sec62p (*33*). Also, mutations in Sec62p or Sec63p prevent the interaction of translocating chains with Sec61p (*32*).

Enzymes in the Translocation Site

The two known enzymes in the translocation site, the signal peptidase and the oligosaccharyltransferase, catalyze cotranslational modifications of the polypeptide chain. They are unusual enzymes in that they are as abundant as their substrates. A special structural arrangement is probably needed for the enzymes to act on uncompleted nascent chains, which may be surrounded by other membrane proteins. It seems likely that the two enzymes are a permanent part of each translocation site as long as it remains assembled.

The signal peptidase has been purified as a complex of five different proteins from mammals and yeast (*56*). The amino acid sequences of some subunits of the pancreatic enzyme from dog are similar to each other and to Sec11p, a subunit of the yeast enzyme identified in genetic screens (*57*). The proteins probably span the ER membrane only once and may have a second hydrophobic segment for interaction with signal sequences. There is no obvious sequence similarity to the bacterial signal (leader) peptidase.

The oligosaccharyltransferase is responsible for the transfer of the oligosaccharyl moiety from a dolichol intermediate to Asn residues located in the context Asn-X-(Ser or Thr) (where X is any amino acid) in a nascent polypeptide. The enzyme has been purified from dog pancreatic microsomes and consists of three subunits, the two ribophorins (I and II) and a 48-kD polypeptide (*58*). Ribophorin I has a putative binding site for the dolichol moiety in its membrane spanning region. The two ribophorins span the membrane only once. The oligosaccharyltransferase complex is among the membrane components most tightly bound to ribosomes after solubilization of rough microsomes (*31*). Its association with ribosomes may serve to localize it in the vicinity of translocating nascent chains. Both the oligosaccharyltransferase and the signal peptidase seem to be dispensable for the actual translocation process because protein translocation occurs in proteoliposomes depleted of all glycoproteins (including both enzymes) except the TRAM protein (*43*).

The Signal Sequence Receptor Complex

Short translocating polypeptide chains can be crosslinked through their signal se-

quence to an integral membrane protein that is about 35 kD, has a cytoplasmic tail of about 5 kD, and is glycosylated (*45*). On the basis of these properties the signal sequence receptor α subunit (SSRα) was purified (*59*) and received its name on the assumption that it was identical with the major crosslinking partner of short nascent chains. However, both the TRAM protein and SSRα have the above-mentioned properties, and the TRAM protein is actually the major crosslinking partner (*43*). However, SSRα can be crosslinked to a minor extent to various translocating chains, and the proportion of SSRα among the glycoproteins crosslinked to the nascent chain appears to increase as chain length increases (*43*). The protein is not a signal sequence receptor and probably not even directly involved in translocation: proteoliposomes reconstituted from a detergent extract from which SSR was removed by immuno-affinity chromatography (*60*) and proteoliposomes containing the TRAM protein as the only glycoprotein (*43*) both have undiminished translocation activity.

Nevertheless, the SSR is likely to be located in the translocation site. It is a major protein of the ER membrane in mammals, birds, and fish. It is segregated to the rough portion of the ER and can be crosslinked to membrane-bound ribosomes [for review see (*1*)]. Also, it is associated in part with ribosomes after solubilization of rough microsomes. Finally, antibodies to SSRα and Fab fragments prepared from the antibodies block the in vitro translocation of several secretory proteins (*59*).

SSRα is a constituent of a stoichiometric complex containing four membrane proteins (the SSR complex) that are adjacent to each other in intact membranes (*61*). The amino acid sequences deduced from cloning of the corresponding cDNAs indicate that the α, β, and δ subunits span the membrane only once. The γ subunit is predicted to span the membrane four times. The existence of a different complex containing SSRα, SSRβ, and two other proteins has been reported (*62*).

The function of the SSR complex remains obscure. It may have an enzymatic activity or it could be required for the translocation of only a subclass of proteins. Alternatively, it could function as a chaperone, facilitating the folding of membrane proteins that span the membrane multiple times or the assembly of membrane protein complexes. The SSR has also been proposed to function in the retention of proteins within the ER (*62*).

Other Components

Other candidates for translocation components include an abundant ER membrane protein of 30 kD (mp30) with affinity for the SRP (*4*), an unidentified adenosine triphosphate (ATP)–binding membrane protein (*63*), and a number of proteins that have been identified as crosslinking partners of translocating nascent chains (*64*). The latter are only known by their approximate molecular sizes.

Conclusions and Perspectives

It seems that a long lasting dispute concerning the participation of membrane proteins in the translocation process has come to an end. The translocation site contains not only proteins that are essential for the transport process, but also enzymes that catalyze the modification of nascent polypeptides and probably proteins needed for other functions (Table 1). A protein-conducting channel is likely to exist but further evidence is required and the direct participation of lipids cannot be excluded.

Sec61p and SecYp proteins seem to be major components of the putative protein-conducting channel. Other membrane proteins, like the TRAM protein or the Sec62p-Sec63p complex may be only transiently required. During the course of protein translocation different membrane proteins may be apposed to a nascent chain. Whether the translocation site is transiently assembled from complexes of membrane proteins or whether it only undergoes conformational changes remains to be determined. In general, at least in mammals, the nascent polypeptide seems to be transferred directly from the ribosome into the translocation site through a tight junction between the membrane-bound ribosome and Sec61p.

Perhaps the most gratifying conclusions is that the mechanisms of protein transport across the ER membrane and across the cytoplasmic membrane in bacteria are basically the same. Both the discovery of a complex similar to the SRP that may function in protein export from bacteria and the obvious similarity of SecYp and Sec61p provide a mechanistic correlate to the fact that signal sequences are similarly structured and exchangeable between different classes of organisms. Further similarities may exist between the components of the translocation systems.

The mechanism of translocation remains unclear but we are now closer to a major goal of the field—to reconstitute into proteoliposomes the translocation process from purified components. With the establishment of an efficient reconstitution system and the availability of purified key components, there is a chance to reconstitute at least partial reactions. It is likely, however, that other translocation components will have to be identified to reconstitute the overall process. In fact, the driving force for the translocation process is still completely mysterious. Is there a pumping machinery that requires energy or does the polypeptide simply diffuse through the membrane with directionality determined by its folding at the lumenal side? ATP is known to be required for the membrane transfer of a sizable polypeptide domain (*33*) but the energy-requiring component is unknown.

The biosynthesis of membrane proteins presents a particular enigma. If there is a protein-conducting channel, does it open laterally to release transmembrane segments into the phospholipid bilayer? Does a channel open and close during the synthesis of a membrane protein that spans the membrane many times? If so, does it depend on the function of protein segments as translocation start and stop signals? Do membrane chaperones exist that transiently associate with transmembrane segments containing hydrophilic amino acids? Chemical crosslinking and reconstitution methods may soon yield the answers to these questions.

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Transport Proteins in Bacteria: Common Themes in Their Design

Hiroshi Nikaido and Milton H. Saier, Jr.

Bacterial transport proteins mediate passive and active transport of small solutes across membranes. Comparison of amino acid sequences shows strong conservation not only among bacterial transporters, but also between them and many transporters of animal cells; thus the study of bacterial transporters is expected to contribute to our understanding of transporters in more complex cells. During the last few years, structures of three bacterial outer membrane transporters were solved by x-ray crystallography. Much progress has also occurred in the biochemical and molecular genetic studies of transporters in the cytoplasmic membranes of bacteria, and a unifying design among membrane transporters is gradually emerging. Common structural motives and evolutionary origins among transporters with diverse energy-coupling mechanisms suggest that many transporters contain a central module forming a transmembrane channel through which the solute may pass. Energy-coupling mechanisms can be viewed as secondary features added on to these fundamental translocation units.

True bacteria are divided into Gram-positive and Gram-negative organisms, depending on their reaction to a staining protocol. In the former group, the plasma membrane is surrounded only by a mechanically rigid and rather porous cell wall (peptidoglycan). In contrast, the latter group (which includes *Escherichia coli*) produces a second membrane, the outer membrane, that is located outside the plasma (cytoplasmic) membrane and the thin peptidoglycan layer. A fundamental function of biological membranes is to serve as a selective permeability barrier. Consonant with its location, the outer membrane serves as an efficient permeability barrier that protects Gram-negative bacteria from a number of harmful compounds, such as some antibiotics, disinfectants, and detergents (*1*). Both the outer and inner membranes contain transport proteins that mediate the passage of a limited range of solutes. These prokaryotic cell membranes have proven to be excellent experimental systems for studying transporters because of the ease of biochemical and genetic manipulation in bacteria. The study of bacterial transporters is significant also because their amino acid sequences are now known to be strikingly similar to those many transporters of cells of higher animals (see below).

This review will summarize recent data obtained from the study of a variety of bacterial transporters. These data suggest a common theme in the design of many transporters. These transport proteins contain similar transmembrane domains encompassing a membrane-spanning channel (*2*). These domains are made up of transmembrane β strands in outer membrane proteins, whereas transmembrane α helices, frequently a pair of domains each containing six helices, are found in plasma membrane transporters. The diversity of the transporters often seems to originate from the specificity of these channels and peripheral domains employed to couple energy to active transport processes. Because of limited space, many transport systems are not discussed; these include primary ion pumps such as bacteriorhodopsin and P-type adenosine triphosphatases (ATPases).

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Channels in the Outer Membrane

The outer membrane contains three types of channels (Fig. 1) (*1*). (i) Proteins known as porins (Fig. 1A) contain large, open,