Overview

The eukaryotic cell is characterized by the presence of membrane-bound organelles that perform a variety of highly specialized functions. The structure and function of each organelle is largely defined by its unique complement of constituent proteins. Clearly, the biogenesis and maintenance of these compartments demands that newly synthesized proteins must be accurately and efficiently targeted to their appropriate cellular location. Since most proteins begin their synthesis in the cytosol, their delivery to an organelle normally involves their translocation across at least one lipid bilayer. The selective translocation of large hydrophilic proteins across biological membranes is a complex process that is made all the more demanding by the need to maintain the integrity of the membrane so as to prevent the mixing of cytosolic and organellar contents.

The endoplasmic reticulum (ER) plays a major role in intracellular protein trafficking. Proteins translocated into the ER can either remain resident there, or may be transported to the Golgi apparatus, the lysosomal network, or the cell surface. Transport from the ER occurs by vesicular budding and fusion, and so does not require cargo proteins to undergo any further membrane-translocation events. Protein translocation across the ER membrane is therefore fundamental to organelle biogenesis and protein secretion in eukaryotes. Here I propose to review some of the most important recent findings that have revolutionized our understanding of this translocation mechanism.

Background

Ten years ago the nature of the ER translocation machinery remained unknown. Biochemical reconstruction studies had identified the signal recognition particle (SRP) and its cognate membrane receptor, known as ‘docking protein’ or SRP receptor. These factors were required to target proteins to ER-derived microsomes but played no role in the subsequent translocation of targeted polypeptides across the bilayer. Evidence suggested an aqueous transmembrane channel but the identity of the putative channel-forming components remained elusive. When I joined Randy Schekman’s laboratory in 1988, a genetic approach to the study of this problem had already begun. Deshaies & Schekman (1987) had described an elegant selection that led to the identification of several conditional-lethal mutants with defects in protein translocation. However, while these mutants were defective in the translocation of several secretory precursors, they had little or no effect upon the insertion of integral membrane proteins into the ER membrane. This raised the possibility that the mechanisms for targeting and translocation of membrane proteins might differ substantially from those effecting secretory-protein translocation. In order to address this question I began by isolating the first yeast mutants specifically defective in membrane-protein insertion into the ER. These have revealed that there are indeed two parallel targeting pathways to the yeast ER that converge at a common translocation pore complex.

Protein targeting to the ER in yeast

Proteins entering the secretory pathway contain an ER targeting signal, commonly referred to as the signal sequence. This signal sequence is usually located towards the N-terminus and consists of a continuous short stretch of hydrophobic residues flanked by one or more basic residues to the N-terminal side of the hydrophobic core (Von Heijne, 1990). In most cases the signal sequence is cleaved during translocation and the remainder of the polypeptide is translocated into the lumen of the ER. Integral membrane proteins are targeted to the ER either by a cleavable signal sequence or by a signal anchor domain that becomes stably integrated in the bilayer (for a review see High & Dobberstein, 1992).

Mammalian SRP and SRP receptor mediate a cotranslational targeting reaction which delivers nascent secretory precursors to the ER membrane, thus enabling translocation to be coupled to translational elongation (for a review see Walter & Johnson, 1994). SRP binds to the nascent polypeptide signal sequence as it emerges...
Co-translational targeting and translocation

Translating ribosome

SRP-cycle

Closed channel and/or free Sec61 trimer

Oligomeric translocation channel comprising several Sec61 trimers

TRP receptor

Trimeric Sec61 complex

(normal Sec61p, Ssa1p, Ssh1p)

Sec63p

Sec62p

Sec72p

Kar2p (BiP)

Amino-terminal signal sequence

Fig. 1. Protein translocation across the ER membrane in yeast. Nascent polypeptides are targeted to the ER co-translationaly by the concerted action of SRP and the SRP receptor. The targeted ribosome/mRNA/nascent chain complex then associates with the translocon, which comprises 3-4 copies of the Sec61 complex. Post-translationally targeted pre-proteins interact initially with the Sec62p/Sec71p/Sec72p components of the Sec63 complex before being delivered to the Sec61-containing translocon. It is not yet certain whether the translocon assembles de novo from free Sec61 trimers only in the presence of precursor, or whether it exists in a tightly closed state prior to the arrival of the targeted precursor.

from the translating ribosome. There follows a specific interaction between SRP and the ribosome which inhibits translational elongation, thus preventing the completion of the nascent polypeptide in the cytosol. The resultant complex is targeted to the ER by virtue of a specific interaction between SRP and SRP receptor. This stimulates the dissociation of SRP allowing the ribosome/nascent chain to interact directly with the Sec61 protein complex which then mediates the translocation process (see Fig. 1).

In yeast, the temperature-sensitive sec65-1 mutation was first isolated as being conditionally defective in membrane-protein insertion (Stirling et al., 1992). The SEC65 gene encodes a 32 kDa subunit of SRP which is homologous to mammalian SRP19 (Stirling & Hewitt, 1992). Like its mammalian counterpart, the Sec65 protein is required for the assembly of SRP and, in particular, is required to recruit the SRP54 subunit (Hann et al., 1992; Regnacq et al., 1998). The temperature sensitivity of sec65-1 mutant cells is not due to any toxic effect of the mutant protein, but rather due to its thermolability which leads to its rapid depletion in vivo following a shift to 37 °C. This rapid loss of Sec65p disrupts SRP structure and function, leading to a severe defect in protein targeting and cell death (Stirling & Hewitt, 1992; Stirling et al., 1992; Regnacq et al., 1998). Yet despite this, gene disruption experiments have shown that SEC65 is not an essential gene. The sec65 null mutant grows very slowly, but the fact that it grows at all indicates that it must be possible to target proteins to the ER in the absence of functional SRP (Stirling & Hewitt, 1992). Similar results have been obtained with disruptions of genes encoding the other subunits of SRP and the SRP receptor (see Brown et al., 1994 and references therein). The anomaly between the conditional-lethal sec65-1 allele and the viable null mutant has yet to be resolved, but evidence suggests that the null mutant undergoes some adaptation during sporulation that reduces its dependence upon SRP. This contrasts with the rapid loss of SRP function during vegetative growth (either in sec65-1 cells or when SEC65 expression is shut-off under the control of the GALI promoter) where the consequences are lethal. The lethality associated with the rapid loss of SRP function in vegetative cells might merely reflect their failure to adapt in sufficient time, or may indicate that sporulation is specifically required to predispose cells to adaptation.

The extreme conditional nature of the sec65-1 allele has proven very useful in discriminating between targeting pathways in yeast. At their non-permissive temperature, sec65-1 mutant cells exhibit severe defects in the insertion of various integral membrane proteins and also in translocation of several secretory polypeptides. These observations are consistent with the expected role for SRP in protein targeting to the ER. However, the translocation of a subset of precursors, including carboxypeptidase Y (CPY), remains quite unaffected in sec65-1 cells. Previous evidence had demonstrated that CPY could be translocated into yeast ER membranes in a post-translational reaction in vitro. The failure of sec65-1 to block CPY translocation indicates that this post-translational targeting pathway occurs by a novel
mechanism which is independent of SRP. Recent studies indicate that the hydrophobicity of the signal sequence dictates which of the two targeting pathways a specific precursor will follow, with more hydrophobic sequences being substrates for the SRP pathway (Feldheim & Schekman, 1994; Ng et al., 1996).

**SRP-independent protein targeting**

The post-translational route is independent of SRP/SRP receptor but requires cytosolic Hsp70s and Ydj1, which maintain precursors in a translocation-competent conformation (Deshaies et al., 1988; Chirico et al., 1988), plus a further membrane protein complex comprising Sec62p, Sec63p, Sec7lp and Sec72p (Deshaies et al., 1991; Panzner et al., 1995; Wilkinson et al., 1997a).

Precursors are first targeted to this so-called Sec63 complex and then delivered to the Sec61 complex which again mediates the translocation reaction (Fig. 1). The role of the Sec63 complex in precursor targeting is thus analogous to that of the SRP receptor, but the Sec63 protein plays a further role in that it recruits a lumenal Hsp70, Kar2p, to the inner surface of the translocation site. This Hsp70 interacts directly with the translocating polypeptide and is believed to drive the post-translational translocation reaction by sequential rounds of substrate binding and release that effectively ‘pull’ the pre-protein into the ER lumen (Sanders et al., 1992; Lyman & Schekman, 1995, 1997). More recently we have identified a second lumenal Hsp70, Lhs1p, which is also required for efficient post-translational translocation in vivo (Craven et al., 1996). Sequence comparisons indicate that Lhs1p is conserved throughout eukaryotes and that it represents a novel class of the Hsp70 superfamily of molecular chaperones (Craven et al., 1997). Any direct role for Lhs1p in the translocation reaction remains to be established. An lhs1 mutant is defective in protein folding within the ER lumen (Craven et al., 1996; Saris et al., 1997) and it may be that this folding defect contributes only indirectly to the observed translocation phenotype.

**Components of the ER translocon**

Genetic studies in the budding yeast Saccharomyces cerevisiae led to the identification of the Sec61 protein (Sec61p), a multi-spanning integral membrane protein of the ER required for the translocation of both secretory and membrane proteins (Deshaies & Schekman, 1987; Stirling et al., 1992; Wilkinson et al., 1996). Cross-linking experiments have shown that prepro-α-factor is in intimate contact with Sec61p during its membrane translocation, suggesting that Sec61p corresponds to a component of the translocon per se (Sanders et al., 1992; Musch et al., 1992). A mammalian homologue of Sec61p (Sec61a) sharing 56% identity with the yeast protein has been identified which can also be cross-linked to translocating chains (High et al., 1991; Gorlich et al., 1992a; High & Stirling, 1993) and is in tight association with membrane-bound ribosomes during translocation (Gorlich et al., 1992a; Kalies et al., 1994). Sec61a has been isolated from dog pancreas microsomes as a complex with two other integral membrane proteins, Sec61β and Sec61γ, both belonging to the C-terminal-anchor class (Gorlich & Rapoport, 1993; Hartmann et al., 1994). A similar Sec61 complex is found in yeast where Sec61p interacts directly with Ssl1p (homologous to Sec61γ) (Esnault et al., 1993, 1994; Wilkunson et al., 1997b) and a Sec61β homologue, Shb1p (Panzner et al., 1995). Significantly, mammalian Ssl1p/Sec61γ has been shown to function in budding yeast, as has the Sec61p/Sec61α homologue from Yarrowia lipolytica, emphasizing the extent of functional conservation of the Sec61 complex amongst eukaryotes (Hartmann et al., 1994; Broughton et al., 1997). The tripartite nature of the Sec61 complex also draws comparison with the molecular nature of the SecY/E/G translocase of Escherichia coli (Brundage et al., 1990; Douville et al., 1994). Bacterial SecY appears to be related to Sec61p/Sec61α (Stirling, 1993; Stirling et al., 1992), whilst SecE shares a lesser degree of sequence identity with Ssl1p/Sec61γ (Hartmann et al., 1994). Interestingly, bacterial SecG and yeast Shb1p show no convincing sequence similarity but, perhaps significantly, neither of these components is essential for viability in their respective organisms (Finke et al., 1996; Nishiyama et al., 1994).

**Multiple roles for the Sec61 complex**

The genetic data and cross-linking evidence discussed above strongly suggest a direct role for the Sec61 complex in protein translocation. This has been confirmed by the reconstitution of protein translocation using purified components. Co-translational translocation of proteins into mammalian-derived proteoliposomes requires only SRP, SRP receptor and the Sec61 complex, although some precursors display an additional requirement for the presence of the TRAM protein (Gorlich et al., 1992b; Gorlich & Rapoport, 1993; Oliver et al., 1995; Voigt et al., 1996). The role of TRAM is uncertain but it has been suggested that it may be involved in regulating the activity of the translocon (Hegde et al., 1998a). Interestingly, no TRAM homologue is encoded by the yeast genome indicating that specialized components may have evolved in some systems.

The Sec61 complex co-purifies with ribosomes from detergent-solubilized membranes and these ribosomes can be removed by treatment with puromycin and high salt (Gorlich et al., 1992a; Gorlich & Rapoport, 1993; Kalies et al., 1994). The same treatment had been previously demonstrated to remove ribosomes from rough microsomal membranes (Adelman et al., 1973). Puromycin stimulates the premature release of nascent polypeptides from the translating ribosome and thus implicates the nascent chain itself in the ribosome/Sec61p interaction. This is consistent with a direct interaction between the Sec61 complex and the ribosome/nascent chain complex thereby providing a molecular basis for coupling between translation and translocation.

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In addition to its interaction with ribosomes, the Sec61 protein also interacts specifically with signal sequence prior to the initiation of translocation. This allows the translocon to proof-read the signal sequence on a targeted precursor, thus enhancing the accuracy of the overall targeting process (Jungnickel & Rapoport, 1995; Plath et al., 1998). It seems highly likely that lipids also play a role in discriminating signal sequences, especially given that there is a strong correlation between the hydrophobicity of a peptide sequence and its ability to interact with lipid and to function as a signal sequence (Hoyt & Gierasch, 1991). Moreover, photocrosslinking studies have shown that lipids are in close contact with the signal sequence during translocation, leading to the proposal that the protein-conducting channel is open laterally toward the lipid bilayer during an early stage of protein insertion (Martoglio et al., 1995).

The yeast Sec61 complex (Sec61p, Sss1p and Sbh1p) has also been purified in association with the Sec63 complex (Sec62p, Sec63p, Sec71p, Sec72p; see Fig. 1). This heptameric complex, together with Kar2p, is sufficient to reconstitute the post-translational translocation reaction into proteoliposomes (Panzner et al., 1995). Intriguingly, components of the heptameric complex have also been implicated in a process that has become known as ‘dislocation’. This involves the transport of misfolded polypeptides from the ER lumen to the cytosol where they are then degraded by the cytosolic ubiquitin-independent proteasome. This pathway appears to be an essential aspect of the quality-control of protein biogenesis within the ER. Whilst the details of the reaction have yet to be determined, the involvement of the heptameric complex has led to the seductive hypothesis that this pathway may correspond to a direct reversal of the post-translational translocation reaction (for reviews and further reading see Sommer & Wolf, 1997; Brodsky & McCracken, 1997).

**The nature of the translocation channel**

Until recently, it was assumed that the translocation channel would be so narrow as to accommodate only unfolded polypeptide chains. This assumption seemed consistent with the co-translational translocation of nascent polypeptides, and with the perceived role of cytosolic Hsp70s in preventing precursor folding prior to translocation by the post-translational route. However, a variety of recent data indicate that the channel is very much larger than expected. Firstly, electrophysiological techniques identified a high-conductance aqueous channel that was opened when partially synthesized proteins were released with puromycin under conditions where the ribosome remained membrane-bound. Removal of the ribosome by increasing salt concentration led to closure of this channel, observed as a loss of conductance (Simon & Blobel, 1991). Fluorophore quenching experiments also revealed the presence of an aqueous channel with a diameter of between 40 and 60 Å (1 Å is 0.1 nm) formed in the presence of a ribosome/nascent chain complex (Hamman et al., 1997). This would be the largest hole identified to date in any semi-permeable biological membrane. The translocation of a polypeptide through such a large channel raises a number of interesting questions. The most obvious being how such a large pore could be regulated so as to remain impermeable even to ions. The regulation of the channel will be discussed in the following section, but first another unexpected possibility is that this very large channel might provide a protected environment within which the translocating polypeptide might begin to fold. To put this in perspective, biophysical measurements indicate that a variety of polypeptides behave as spheres (in aqueous solution), with diameters smaller than that of the ER translocation channel. For example, ribonuclease (14 kDa) has a hydrodynamic volume of less than 40 Å, whilst ovalbumin (45 kDa) has an effective diameter of 36 Å (Tanford, 1961). It would therefore appear that the channel is large enough to accommodate very substantial degrees of protein folding. Some degree of folding during translocation might be desirable as opposed to the simple extrusion of an unfolded polypeptide into the milieu of the ER lumen where non-productive protein–protein interactions might be more likely. Moreover, the formation of secondary structure may be essential prior to the translocating chain becoming exposed to the modifying enzymes located within the luminal compartment. This might favour the formation of disulphide bonds only between appropriate cysteine residues, or the addition of asparagine-linked core oligosaccharides only to those glycosylation consensus sequences (Asn-Xaa-Ser/Thr) located on the exposed surfaces of the protein.

**Structure of the translocon and gating of the channel**

Current data suggest that the translocon is a dynamic structure that assembles and opens only in the presence of a suitable substrate. Recent images of the purified Sec61 complex suggests that it forms an oligomeric ring structure comprising 2–4 Sec61 trimers (comprising Sec61p, Sss1p and Sbh1p). The overall diameter of the ring is some 85–95 Å, with the internal pore having a mean diameter of 20–35 Å (Hance et al., 1996; Beckmann et al., 1997). When complexed with the ribosome the central pore of the Sec61 oligomer is seen to align with the site on the large ribosomal subunit that is believed to correspond to the exit channel for the nascent polypeptide (Beckmann et al. 1997). The translocon channel can therefore be viewed as an extension of the ribosome with the translating polypeptide having nowhere to go but through the transmembrane channel. The apparent ‘hole’ in the Sec61 doughnut structure observed by cryoelectron microscopy is smaller (20–35 Å) than the ion-conductance channel discussed above (40–60 Å). This might be due to the particular cryofixation or staining procedures used or, alternatively, the complexes observed may represent the closed state of a dynamic channel. Interestingly, Hance et al. (1996) observed a similar Sec61 doughnut structure in both yeast and mammals, and most strikingly they find that...
this ring is induced by the presence of either ribosome or the Sec63 complex. This observation suggests that individual Sec61 trimers (comprising Sec61p, Ssslp and Sbh1p) are recruited to form a functional oligomeric translocon only in the presence of a suitable ligand. It is clear that a ribosome alone can hold a channel in its open state after the release of the nascent chain by puromycin (as discussed above), but current evidence indicates that the precursor is required to promote channel opening. Johnson and co-workers have examined the accessibility of fluorescently tagged nascent chains to fluorophore-quenching ions from either the cytosolic or luminal face of microsomal membranes. Their data confirm that the translocation channel can be sealed at the cytosolic face (presumably at the ribosome/Sec61 junction) (Crowley et al., 1993, 1994), but furthermore that the aqueous channel remains sealed to the ER lumen until the nascent polypeptide reaches a length of ~70 amino acids (Crowley et al., 1994), which is highly suggestive of a role for the signal sequence in initiating channel opening. The authors have proposed that the luminal seal might provide a safety mechanism that would maintain the permeability barrier of the ER membrane by preventing channel opening until the cytosolic face of the channel is sealed by a properly engaged ribosome. The luminal seal depends upon the presence of active BiP (Crowley et al., 1994; Hamman et al., 1998). In yeast, BiP/Kar2p has been shown to be recruited to the yeast post-translational translocon by Sec63p, but it is not known how it might be recruited in the co-translational reaction. Moreover, it remains to be established what factors might replace the ribosome in sealing the cytosolic face of the channel in the post-translational reaction. Obvious candidates might include the cytosolic domains of the Sec63 complex, or even the cytosolic Hsp70s associated with the targeted precursor, but any such role remains speculative.

**Membrane-protein insertion**

The ER represents the major site of membrane biogenesis in eukaryotes. Many membrane proteins are first inserted into the ER bilayer and then transported to their final destination by membrane-bound transport vesicles. Most membrane-protein precursors appear to be targeted to the ER by SRP and are thus inserted into the membrane co-translationally. Thus the initial targeting of proteins to the ER is indistinguishable from that of SRP-dependent secretory proteins. Moreover, the insertion process clearly involves the same trimeric Sec61 complex as that involved in secretory-protein translocation (Stirling et al., 1992; Gorlich & Rapoport, 1993; Oliver et al., 1995). However, the two processes can be distinguished by the fact that during membrane-protein insertion, the polypeptide chain must, at some stage, pass laterally from the translocation channel into the lipid phase to allow the integration of transmembrane domains. In principle, one might imagine that this process is rather straightforward, requiring only the partitioning of a hydrophobic domain into the lipid bilayer driven by obvious thermodynamic forces. However, cross-linking studies suggest that the mechanism is more complex, requiring the nascent protein to interact with several proteinaceous components prior to its eventual release into the lipid (Do et al., 1996; Laird & High, 1997). More recently, Hegde et al. (1998b) have reported that the human prion protein can exist in several forms, including a secreted species and at least two topologically distinct integral membrane forms. The membrane insertion of PrP into reconstituted membranes requires the Sec61 complex and TRAM, but also requires unidentified ER components termed Translocation Accessory Factor(s) (TrAFs) (Hegde et al., 1998c). The extent to which these TrAFs might be generally involved in membrane protein assembly is currently unknown.

**Orientation of membrane-domain insertion**

Some membrane proteins possess a cleavable signal sequence followed by a hydrophobic stop-transfer domain that integrates into the bilayer, thus terminating the translocation of C-terminal sequences (see High & Laird, 1997). Alternatively, targeting may be via a non-cleavable signal-anchor domain that constitutes the first transmembrane sequence in the mature protein. A signal sequence is presumed to initiate translocation by inserting into the membrane in a loop conformation, thus exposing the C-terminal signal cleavage site to signal peptidase activity within the lumen (Wilkinson et al., 1997a). However, signal-anchor domains come in two guises, those that insert with their N-terminus in the cytosol and those with their N-terminus in the lumen (High & Laird, 1997). Exhaustive studies on the cis acting factors which dictate the orientation of a signal-anchor domain have demonstrated the significance of positively charged residues, which tend to be clustered on the cytosolic side of the membrane (Sipos & Von Heijne, 1993). This so-called positive-inside rule appears valid in both prokaryotes and eukaryotes. In bacteria it has been suggested that it may simply mirror the charge differential across the plasma membrane (i.e. negative inside). However, there is no known charge differential across the eukaryotic ER membrane, therefore any trans acting components required to decode the polarity of membrane-protein insertion remain to be defined. Intriguingly, the translocon in the bacterial plasma membrane is clearly related to the Sec61 complex, suggesting that the regulation of these events may be similar throughout biology (Stirling, 1993).

**Multispanning membrane-protein integration**

The insertion of many multispanning integral membrane proteins can be conveniently explained in terms of the signal-anchor/stop-transfer hypothesis (Wessels & Spiess, 1988). In this model, the orientation of the first transmembrane sequence determines the relative orientation of all subsequent transmembrane sequences which insert sequentially, and alternately, as either signal-anchor or stop-transfer domains. During this process each stop-transfer domain terminates trans-
location, thus requiring each subsequent signal-anchor domain to reinitiate insertion. Although this model predicts the independent insertion of each transmembrane domain, there are several examples where specific transmembrane domains are required to interact closely with downstream transmembrane sequences in order to integrate stably in the bilayer (Sengstag et al., 1990; Wilkinson et al., 1996). Indeed, in some cases it is suggested that closely spaced transmembrane domains insert simultaneously into the bilayer as a helical hairpin (Gafvelin & Von Heijne, 1994; Gafvelin et al., 1997). It is not currently known whether multispansing integral membrane proteins are fully assembled within the translocation site prior to release into the bilayer, or whether individual transmembrane domains are released to interact with putative assembly chaperones outwith the translocation site itself.

An unexpected role for the ribosome?

Data discussed above indicate that the translocation pore can be gated at either its cytosolic or luminal face. Evidence suggests that the lumenal gate remains closed until a nascent polypeptide is ~70 residues long (Crowley et al., 1994). At this point the lumenal gate opens, but the integrity of the channel is maintained by the cytosolic seal. However, the question of gating becomes more complex when one considers the cotranslational integration of multispansing membrane proteins. The initial insertion of a type II membrane domain (i.e. C-terminus in the lumen) and the subsequent translocation of its luminal domain might be terminated by stop-transfer sequences, enabling cytosolic domains to be translated directly into the cytosol. The next signal-anchor domain then reinitiates the translocation process. Thus any gating mechanism must be versatile enough to maintain the integrity of the channel as it switches from signal-anchor to stop-transfer integration modes. Perhaps the most striking observation of recent years has been the finding that both the lumenal and cytosolic gates appear to be regulated from within the ribosome (Liao et al., 1997). This has led to the suggestion that integral membrane domains are first recognized, not by the Sec61 complex, but within the translating ribosome. It has been further proposed that this recognition event may trigger a long-range conformational change that signals a switch in the translocon between translocation mode and membrane-insertion mode (Liao et al., 1997). The close association between the ribosome and Sec61p during the translocation process might mediate such a signal, although the nature of the recognition event, and associated switch, are currently unknown.

In conclusion

Considerable advances have been made both in identifying components of the ER translocon and in understanding the role that each plays in the translocation reaction. This has been achieved using a combination of approaches ranging from yeast genetics to biochemical reconstitution. Nonetheless, several key questions remain. For example, it is not yet known whether the post-translational targeting route requires any cytosolic targeting factor(s) analogous to SRP. Nor is it clear how the translocation channel can be sealed at the cytosolic face during the post-translational translocation reaction. Moreover, the very existence of a Sec62/Sec63-dependent post-translational pathway in mammals has yet to be established. In the co-translational reaction the role of the ribosome becomes ever more complex. A molecular description of its interaction with the translocon and its apparent role in modulating the integration of multispansing membrane proteins will be vital to our understanding of this most crucial aspect of membrane biogenesis. Finally, the dimensions of the translocation channel raise new and unexpected possibilities, most notably the concept that translocating polypeptides might fold extensively within a protected environment prior to release into the ER lumen. Another surprise has been the finding that the translocation channel also functions in reverse to facilitate the degradation of misfolded proteins. If the present rate of progress in this area continues then we might expect a few more surprises in the near future.

References


