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# Membrane Protein Biogenesis: Regulated Complexity at the Endoplasmic Reticulum

Review

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A protein must be precisely localized in space and time if it is to serve its intended function in a cell. Not only is a wayward protein generally useless, it can have dire consequences. For this reason, cells have evolved numerous elaborate systems for the proper segregation of proteins to and within various compartments. One such system, the secretory pathway, is used by nearly all proteins fated for extracellular or membrane-bound destinations within the eukaryotic cell (Palade, 1975). Proteins that are transported and sorted by the secretory pathway begin their journey at the endoplasmic reticulum (ER) membrane. It is here that nascent secretory and membrane proteins are translocated across or integrated into the membrane, appropriately modified, folded, and assembled prior to subsequent transit to various parts of the cell (reviewed by Andrews and Johnson, 1996; Rapoport et al., 1996). An increasingly complex macromolecular machine, termed the translocon (Walter and Lingappa, 1986), is responsible for the proper transport and biogenesis of proteins at the ER membrane.

For secretory proteins, the major role of this translocon is to facilitate the movement of the entire polypeptide across the otherwise impermeable ER membrane. By contrast, membrane proteins demand significantly more. In addition to translocation of some but not other domains, the translocon must also recognize potential membrane-spanning domains, properly orient these domains with respect to themselves as well as the membrane, and facilitate their integration into the lipid bilayer. Because of these and other complexities, most work on the function of the translocon has thus far focused on small secretory proteins as model study systems.

These studies have led to the discovery of protein-conducting channels in the ER membrane (Simon and Blobel, 1991; Crowley et al., 1993, 1994), the isolation and functional reconstitution of their main components (Görlich et al., 1992; Görlich and Rapoport, 1993), and most recently, their direct visualization (Hanein et al., 1996). Although far from complete, a framework for the mechanism of (at least simple) secretory protein biogenesis has emerged. With the tools developed during the elucidation of this framework in hand, increasing attention is being paid to the molecular mechanisms of membrane protein biogenesis. The findings of these recent studies, although often contradictory, may be painting a rudimentary picture of the next frontier in protein translocation.

## Secretory versus Membrane Proteins: Common Aspects

In mammalian systems, secretory and membrane proteins are translocated across the ER membrane concurrent with their synthesis by membrane bound ribosomes (Morrison and Lodish, 1975; Palade, 1975). This "co-translational" translocation begins in the cytosol with the synthesis of the first hydrophobic segment of a nascent polypeptide, either a signal or transmembrane (TM) sequence. Following the emergence of this hydrophobic sequence from the ribosome, it is recognized by the signal recognition particle (SRP), which mediates the targeting of the ribosome–nascent chain–SRP complex to the ER membrane in a GTP-dependent manner (reviewed by Siegel, 1995; Millman and Andrews, 1997). Once this complex is bound to the membrane, the nascent chain is transferred into the aqueous translocation channel, which is subsequently sealed from the cytosolic environment by a tight ribosome–membrane junction (Crowley et al., 1993; Jungnickel and Rapoport, 1995). Up to this point in biogenesis, both secretory and transmembrane proteins use the same SRP-dependent pathway of targeting. Furthermore, the "generic" translocon in which this early nascent chain resides must be capable of facilitating the subsequent cotranslational biogenesis of both secretory and transmembrane proteins, directed by what is translated next (e.g., Kehry et al., 1980; McCune et al., 1980; Yost et al., 1983).

The molecular components of this "generic" translocon are numerous and varied. The most recently identified components, the heterotrimeric Sec61 complex (with $\alpha$, $\beta$, and $\gamma$ subunits) and translocating-chain associated membrane protein (TRAM), turn out to be functionally crucial from the standpoint of translocation (Görlich et al., 1992; Görlich and Rapoport, 1993). These proteins, when reconstituted with pure lipids and SRP receptor into proteoliposomes, are able to catalyze both the vectorial translocation of secretory proteins into the lumen as well as the integration of membrane proteins in the bilayer (Görlich and Rapoport, 1993; Oliver et al., 1995). Numerous lines of evidence have established that the functional and structural core of the translocon is composed of the heterotrimeric Sec61 complex. This complex has been shown to be adjacent to translocating nascent chains (Mothes et al., 1994), absolutely necessary for translocation (Görlich and Rapoport, 1993), and in some instances sufficient for translocation (Jungnickel and Rapoport, 1995). TRAM, on the other hand, has been shown to be adjacent to secretory and membrane proteins only at certain (poorly defined) stages of their translocation and/or integration (Görlich et al., 1992; Mothes et al., 1994; Do et al., 1996). Functionally, TRAM was shown to facilitate the translocation of many, but not all, proteins by aiding in the initial formation of a tight ribosome–membrane junction at the translocon (Görlich et al., 1992; Voigt et al., 1996). However, the precise role of TRAM, if any, at later stages of translocation or during membrane integration remains obscure.

In addition to these "minimal" components, several other protein complexes interact with nascent translocating polypeptides. Signal peptidase (a complex of 5 proteins) and oligosaccharyl transferase (a 3-protein complex) have defined enzymatic activities that are important for the maturation of many nascent chains (Evans et al., 1986; Kelleher et al., 1992). Similarly, many of the ER lumenal proteins (e.g., BiP, GRP94, calnexin,

protein disulfide isomerase, and others) have been shown to interact with a variety of nascent chain substrates and are thought to act as molecular chaperones to promote proper folding and assembly (e.g., Munro and Pelham, 1986; Ou et al., 1993). Thus, although crucial for proper protein maturation and function, these accessory components of the translocon currently have poorly defined roles in the translocation process. It should be stressed, however, that simply because translocation (of model secretory and membrane proteins) is able to proceed in the absence of these components, it is hasty to discount them from the translocation process. Given the potentially multiple functions of each of these proteins and the limited number of translocation substrates thus far examined, it is quite likely that in certain situations one or more of these accessory factors serves an indispensable role in translocation. So how does this translocon, minimally composed of only the Sec61p complex and TRAM, handle the topologically diverse group of proteins encountered at the ER membrane?

## The Diversity of Membrane Proteins

Before one considers the question of how a universal translocon handles topologically diverse proteins, it is instructive to first ask what is required of such a translocon. For a secretory protein, one might imagine that the minimal requirement is simply an aqueous channel sufficiently large to accommodate a nascent chain that spans the ER membrane. In this situation, once the ribosome is docked tightly at this channel, continued translation of the message would result in vectorial discharge of the nascent chain into the ER lumen. The Sec61 complex can, at least in the case of the simplest secretory proteins, satisfy these requirements of tight ribosome binding and formation of a protein-conducting channel (Görlich and Rapoport, 1993; Jungnickel and Rapoport, 1995; Hanein et al., 1996). However, it is already apparent that even for secretory proteins, the situation is not always this simple. Many secretory proteins require TRAM for translocation (Görlich et al., 1992; Voigt et al., 1996), while others do not necessarily maintain a tight ribosome–membrane junction throughout their translocation (Hegde and Lingappa, 1996). While the significance of these additional requirements and events in translocation of secretory proteins is currently unclear, they already hint at a more complex and dynamic translocon than would be necessary for the simplest case.

Membrane proteins complicate matters severely. Even a simple single spanning membrane protein needs to translocate certain domains into the ER lumen, leave others in the cytosol, properly orient the TM segment, and move it from the aqueous translocation channel into the lipid bilayer. Multispanning membrane proteins face even further challenges: (i) TM segments must be oriented properly relative to each other; (ii) in many cases, a subset of the TM segments may need to assemble into a defined structure (such as formation of an ion channel) before the protein is integrated into the bilayer; and (iii) some TM segments may need to assemble with TM segments from other membrane proteins. Because many, if not all, of these events occur in an aqueous environment (i.e., before integration of TM segments into the lipid bilayer), the translocon should be adaptable to a variety of situations. It must not only be able to accommodate multiple TM segments at once, but also be capable of releasing some of these segments into the bilayer in a defined sequence, while keeping other regions of the chain in the channel. Furthermore, because TM segments synthesized by a membrane-bound ribosome would be expected to enter the translocon with the N-terminal domain facing the lumen, some may need to be reoriented to achieve the final topology of a particular multispanning membrane protein. Finally, the translocon may need to allow the nascent chain to have access to TM segments that have already integrated into the bilayer in order to facilitate proper folding or other interactions. Thus, unless all of these TM segment gymnastics are dealt with entirely posttranslocationally, the translocon must be either enormously large, or structurally and functionally flexible. How do the currently available data and our current views of the translocon compare with these requirements demanded for membrane protein biogenesis?

## The Classical Model

The current view (literally) of the translocon using electron microscopy has shown the Sec61 complex to be in oligomeric rings in the ER membrane (Hanein et al., 1996). These rings, approximately 85–100 Å in diameter, appear to contain between 3 and 4 copies of Sec61 complex arranged around a central pore of approximately 20 Å in diameter. The large size of this pore clearly fits the requirement of accommodating an extended nascent chain (anhydrous diameter of 5–7 Å, ~11 Å when fully hydrated), and even chains with some secondary structure. Thus, although sufficiently large to house a TM segment in an $\alpha$-helical conformation (~10–12 Å anhydrous diameter, 15–17 Å if fully hydrated), how might a static structure such as this mediate the biogenesis of multispanning membrane proteins?

The classical model to explain this conundrum has been to propose that the ribosome cycles between membrane bound and unbound states (Katz et al., 1977; Blobel, 1980; see Figure 1). It is bound and docked at the translocon when synthesizing domains that follow a signal sequence or TM segment that is oriented with the N terminus in the cytosol. These portions of the chain are thus transferred directly into the translocon destined for the ER lumen. Furthermore, the next TM segment would enter the translocon oriented properly (opposite of the previous one) with the N terminus directed toward the lumen. Upon halting further translocation, through action of a stop transfer sequence when this TM segment reaches the translocon, the ribosome would detach and the subsequent domain (which should be cytosolic) would be synthesized directly into the cytosol. The next synthesized TM segment would provide an internal signal sequence for retargeting the ribosome (with or without SRP) to the translocon and positioning of this segment in an N-cytosolic orientation. If each TM segment exited the translocon to the lipid bilayer before

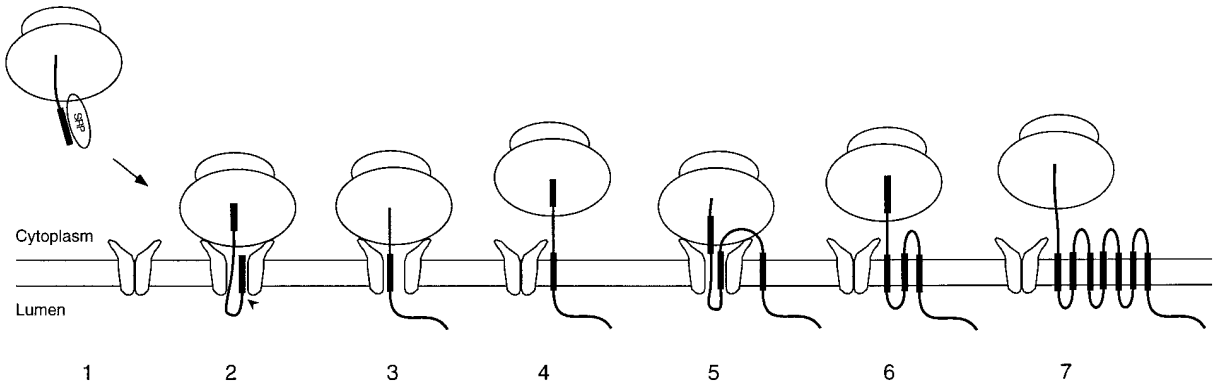


Figure 1. Conventional Model of Membrane Protein Biogenesis

In this model, hydrophobic sequences (either signal or TM segments) are recognized by SRP, targeted to the translocation channel, and inserted in the "loop" orientation with the N terminus of the segment facing the cytoplasm (steps 1 and 2 or 4 and 5). Subsequent stretches are synthesized by a membrane-bound ribosome and directly enter the translocation channel (e.g., steps 2, 3, and 5). When the next hydrophobic TM segment enters the channel (step 3), the ribosome detaches from the translocon, the TM segment(s) are released into the lipid bilayer, and the translocation channel closes (e.g., steps 4 and 6). This cycle of events repeats until all TM segments are "pooled" into the membrane (step 7).

the next one entered, the 20 Å size of the translocon would suffice entirely, and the TM segments would simply be sequentially "spooled" into the lipid bilayer in an alternating fashion. Although this model can theoretically explain the biogenesis of a wide variety of membrane and secretory proteins while demanding very little of the translocon, evidence for it is lacking in most cases, and contradictory in others (see Rapoport et al., 1996, and references therein).

The "spooling" model predicts that the ribosome detaches from the translocon when synthesizing cytosolic domains of a membrane protein (e.g., Figure 1, steps 4 and 6). Unfortunately, not only is there little if any indication of such a detachment, but evidence to the contrary has been provided by Mothes et al. (1997). They were able to demonstrate that the cytosolic domain of the nascent chain following a TM segment could be cross-linked to components of the translocon (Sec61$\alpha$ and Sec61$\beta$). Furthermore, severing the nascent chain within this cytosolic domain between the TM segment and the ribosome did not release the ribosome from the membrane. Finally, even after severing the nascent chain, the domain of the chain still bound to the ribosome (which itself remained bound to the membrane) could still be cross-linked to the translocon. These experiments indicate that not only is the ribosome still membrane bound when synthesizing the cytosolic domains of a membrane protein, but both it and the nascent chain are still at the translocon.

A second prediction of the "spooling" model is that TM segments should integrate into the lipid bilayer shortly after they enter the translocation channel (Figure 1, step 4). This allows the ribosome to detach from the translocon with the chain firmly anchored into the membrane and permits the translocon to prepare for the next TM segment. While it seems clear that the ribosome does not detach from the translocon (see above), TM segments have been shown in some cases to integrate into the bilayer shortly after entering the translocation channel (Mothes et al., 1997). However, the immediate integration of TM segments as they are synthesized does not always occur. With some substrates, it appears that the TM segment remains in the aqueous translocon long after it enters, not integrating until translation of the entire protein has finished (Thrift et al., 1991; Do et al., 1996). In the most dramatic example, it appears that up to six TM segments of some membrane proteins could be synthesized without the protein being integrated into the lipid bilayer (Borel and Simon, 1996). That is, these nascent chains could be extracted from the membrane by treatments that do not extract completed integral membrane proteins. Thus, at a time in biogenesis when (according to the above model) several TM segments should have integrated into the lipid bilayer, the entire nascent chain was still in an aqueous environment.

Finally, the "spooling" model requires that incredibly diverse TM segments (some very hydrophobic while others are amphipathic or even quite charged) all be recognized by the translocon (and subsequently be moved into the lipid bilayer), while all domains of secretory proteins (some of which can be more hydrophobic than bona fide TM segments) need to be allowed to translocate into the lumen. While this may be possible (albeit energetically unfavorable in some cases), it makes difficult the formation of intramolecular interactions between TM segments that stabilize the final structure. Not only are such interactions functionally important, but they can allow multiple relatively hydrophilic TM segments to form a larger unit that energetically favors integration into the lipid bilayer. Indeed, during the biogenesis of some membrane proteins, individual TM segments are unable to integrate into the lipid bilayer unless they interact with other specific TM segments, which by themselves also cannot integrate into the bilayer (Skach and Lingappa, 1993; Wilkinson et al., 1996). Thus, while it remains entirely possible that some membrane proteins are integrated in the "spooling" fashion (Kuroiwa et al., 1996), other mechanisms are also likely to be involved.

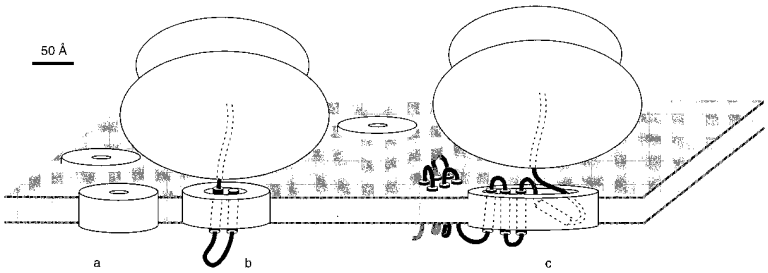


Figure 2. The Size of the Translocation Channel as Estimated by Various Methods

Direct visualization of the structures formed by purified Sec61 complex reveals a channel with a pore of ~20 Å in diameter (structure a; Hanein et al., 1996). Biophysical examination of a pore in the process of translocating a secretory protein shows the pore to be significantly larger, at 40–60 Å in diameter (structure b; Hamman et al., 1997). Based on the ability of the translocation pore to house at least six TM segments (suggested by the data in Borel and Simon, 1996), a speculative maximum pore size of ~100 Å is depicted (structure c). The ribosome (~250 Å in diameter) is shown for comparison. Bar, 50 Å.

**A Bigger, More Dynamic Translocon**

It is clear that most alternative mechanisms of membrane protein biogenesis, especially if they allow for multiple TM segments to interact within the aqueous translocation channel, are likely to require a channel that is significantly larger than the 20 Å pore visualized by Hanein et al. (1996). Evidence that these exist comes from two sources (see Figure 2).

First, Hamman et al. (1997) recently utilized a biophysical approach to estimate the pore size of a functioning translocon containing a nascent chain intermediate. Their approach was to prepare translocation intermediates of a secretory protein in which a fluorescent probe was incorporated into the nascent chain and positioned within the translocation channel. These translocation intermediates were then incubated with various quenchers of the fluorescent probe. By determining the maximum size of the quenching molecules that are able to physically enter the translocation channel containing the nascent chain and effectively quench the fluorescence, the channel was estimated to be 40–60 Å in diameter (Figure 2b).

The second line of evidence that the translocation channel may in some cases be larger than 20 Å is provided indirectly by the data of Borel and Simon (1996). Although they did not address this question directly, they were able to demonstrate that up to six TM segments were able to assemble at the membrane before any of them integrated into the lipid bilayer. Although they did not directly demonstrate that these TM segments were in the translocation channel per se (e.g., by cross-linking studies), it is the most likely aqueous arena in which they were contained. If this were the case, it is quite unlikely that the six TM segments (each in an $\alpha$-helical conformation with an anhydrous diameter of 11 Å) all fit within a pore of 20 Å diameter.

If the conclusions of both the direct and indirect measurements of translocation channel pore size are taken at face value, it might be concluded that the channel has the potential to modulate its size, maybe by recruiting more copies of Sec61. Indeed, even the direct electron microscopic observations of purified Sec61p complex–formed channels (Hanein et al., 1996) revealed significant structural heterogeneity. This was suggested to potentially be due to variable numbers of Sec61 complex per ring or perhaps differences in subunit composition. Furthermore, if all portions of a multispanning membrane protein are synthesized by a translocon-bound ribosome (as suggested by Mothes et al., 1997; see above), and several TM segments can actually assemble within this translocon (Borel and Simon, 1996), a unifying model of membrane protein biogenesis begins to emerge.

In order to accommodate several TM segments within the translocon and allow their reorientation (which would be necessary if all of them entered the translocon in the same orientation due to a membrane bound ribosome), the translocation channel may need to expand to 80 Å in diameter or more (see Figure 2c). For example, six TM segments positioned in the translocon perpendicular to the plane of the membrane may require at much as 40–50 Å, assuming that each TM segment is in an $\alpha$-helical conformation with a width of 11 Å. When the next TM segment is synthesized, there should be room to properly orient it, requiring an additional ~30–35 Å (the length of an average TM segment) or more (see Figure 2c).

The translocon, in addition to allowing multiple TM segments to accumulate and assemble, should also permit the exit of some or all of these TM segments at any time during translation. This may be necessary in some cases to prevent certain inappropriate interactions between TM segments, while allowing fully assembled sections of a protein to integrate into the bilayer as appropriate. Consistent with this proposed model of a laterally gated translocon, it has been demonstrated that some TM sequences are adjacent to lipid early in the biogenesis of a protein while sometimes remaining adjacent to translocon components (Martoglio et al., 1995; Mothes et al., 1997). Furthermore, some TM segments can in fact exit to the bilayer before synthesis of subsequent domains (Mothes et al., 1997), while other TM domains remain in an aqueous environment until the completion of translation (Thrift et al., 1991; Do et al., 1996). Together, these data suggest that the translocon is capable of, but not obligated to release TM segment(s) before synthesis of translation, with the decision perhaps determined by sequences within the nascent chain.

Several questions arise with the idea of a dynamic, expandable translocon of such enormous sizes. Is the ribosome–translocon–nascent chain complex capable of such dynamic changes during cotranslational translocation? When and how might the translocon expand and contract in size? How is the permeability barrier of the ER membrane maintained during these changes that

create such an enormous pore? Although definitive answers to these questions require further studies, recent observations suggest some possibilities.

Evidence that the translocon is a dynamic structure has been provided in multiple ways. First, it was demonstrated that early in translocation the translocon is gated on the lumenal side, regulated by the nascent chain (Crowley et al., 1994). The translocation channel is initially closed to the lumenal side, even after nascent proteins are targeted and docked at the cytosolic side. However, upon further elongation of the nascent chain to a length of ~70–80 amino acids, a lumenal "gate" opens and the ER lumen is continuous with the ribosome via the pore of the translocation channel.

At later times in the translocation of a secretory protein, the ribosome membrane junction at the cytosolic side of the translocon was also shown to be gated, again regulated by the nascent chain. In these instances, pauses in translocation are accompanied by an opening of the ribosome-membrane junction that exposes large domains of the nascent chain to the cytosolic environment (Hegde and Lingappa, 1996). Although the significance of these findings remains to be determined, the rearrangements of the ribosome–membrane junction were sufficiently protracted to allow interactions of the nascent chain with macromolecules in the cytosol.

In addition to dynamic events in gating, rearrangements of membrane proteins of the translocation channel have been suggested to occur. Perhaps the most consistently observed variable is the presence of the TRAM protein. By contrast to Sec61α, TRAM is not always found adjacent to a nascent chain positioned in the translocation channel (Mothes et al., 1994). Rather, it appears as if it may be nearby only during specific events in translocation such as tight insertion of some signal sequences into the translocon (Voigt et al., 1996) or integration of some TM segments into the bilayer (Do et al., 1996). Additionally, changes in the cross-linking pattern during certain points in translocation provide further evidence that the proteins neighboring the nascent chain are malleable (Mothes et al. 1994; Hegde and Lingappa, 1996).

Recently, many of these changes were observed to occur in regulated fashion during the biogenesis of simple model membrane proteins: the lumenal gate was observed to close promptly after synthesis of a TM segment, the ribosome membrane–junction was observed to open shortly thereafter, and cross-linking patterns varied during these events (Liao et al., 1997). Furthermore, since these events were shown to occur while the TM segment was still inside the ribosome, some nascent chain sequences may be first recognized by the ribosome itself. Thus, it appears that components in multiple compartments (the cytosol, membrane, and/or lumen) may recognize regulatory sequences in the nascent chain to elicit reorganization of the translation–translocation machinery.

The observation that the translocon can be quite dynamic is certainly consistent with an ability to expand its size by the recruitment of more Sec61 complex, TRAM, or other components. In fact, the recognition of TM segments by the ribosome, before it reaches the translocon, may facilitate translocon expansion. This early detection system could allow the lumenal gate to close and the translocon to expand before the TM segment arrives, allowing it ample room to be rotated and/or positioned within the translocon. Some of this space may be created by the opening of the ribosome membrane junction. Alternatively, the opening of the junction (as judged by accessibility of the nascent chain to cytosolic probes) may be an epiphenomenon reflecting a growing translocon. The phenomenon of translocational pausing, where the junction is also observed to open (Hegde and Lingappa, 1996), may reflect a similar enlarging of the translocon for the purpose of allowing specialized folding or modifications within the translocon.

Thus, a translocon that is dynamic in both its size and gating in all three dimensions (cytosolic, lumenal, and in the plane of the bilayer) could explain much of the disparate initial findings on the biogenesis of membrane proteins (see Figure 3). Such a translocon would be flexible enough to handle each substrate slightly differently to accommodate subtle variations and requirements crucial to achieving a functional end product. Indeed, the contradicting conclusions resulting from many of the studies which each used different test substrates may reflect this flexibility.

Finally, this model does not necessarily postulate the existence of components in addition to the major ones identified for secretory proteins. Although the lumenal gate has not yet been identified, it could involve conformational changes in the Sec61 complex or one of the several known lumenal proteins. Similarly, a gate in the plane of the bilayer may be composed of either components of the Sec61 complex or TRAM, both of which have been shown to be adjacent to TM segments during particular steps of the integration process (Do et al., 1996). Furthermore, expansion of this structure could be accomplished by simply recruiting more copies of Sec61 complex into the ring. These features, along with the recent possibility that the ribosome may play an active part in the dynamics of the translocon (Liao et al., 1997) provide more than enough players and wobble room for complex events in membrane protein biogenesis.

## With Complexity Comes Regulation?

The enormous diversity of proteins that transit the secretory pathway demands a mechanistic complexity in biogenesis that has yet to be fathomed. The current understanding of the molecular components that direct the translocation of a limited subset of simple secretory proteins has revealed a remarkably (and probably deceptively) simple picture. As the lessons learned from these studies are being extended to slightly more complex substrates, it is becoming more and more obvious that our understanding is neither complete nor clear. Many substrates, especially membrane proteins, appear to require the translocation machinery to make "decisions" specific to a particular situation or substrate: Which domains of a protein are TM segments? Which TM segments should be held in the translocation channel for purposes of folding or association with distal domains, and which should be integrated immediately

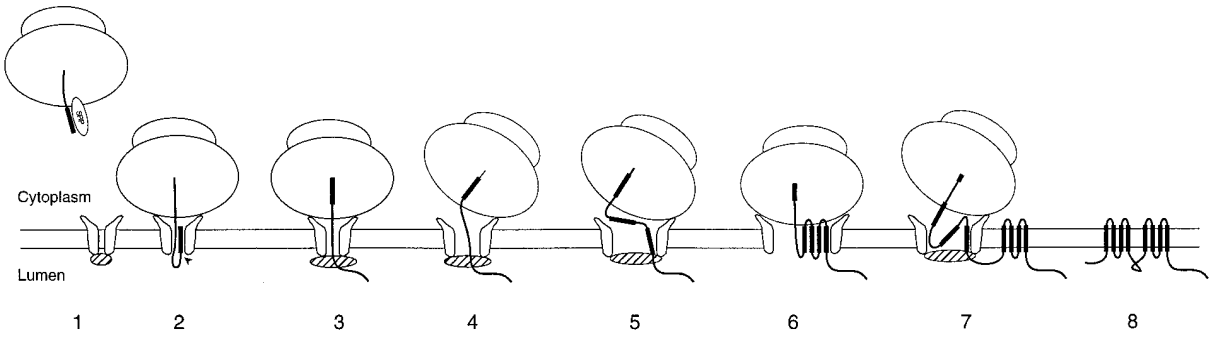


Figure 3. Speculative Model of Mechanism of Membrane Protein Biogenesis

A lumenal gate (striped oval) is opened upon tight ribosome binding to the translocon, and translocation of the nascent chain ensues (steps 1 and 2). Upon synthesis of a TM segment, the lumenal gate is closed (step 3) and ribosome–membrane junction opened (step 4). At this point, before the TM segment emerges from the ribosome, the translocon is expanded (step 4) to accommodate orientation of multiple TM segments relative to each other (step 5). When a functional unit of TM segments is assembled within the translocation channel (step 6), they are released into the lipid bilayer (step 7) while subsequent TM segments are retained prior to assembly of the next functional unit. In this model, the permeability barrier of the membrane is maintained by a combination of the lumenal gate and ribosome–membrane junction, each of which is modulated by sequences in the nascent chain. TM segments are allowed to reorient themselves and assemble with other TM segments in the space created by an enlarged translocation channel and/or open ribosome–membrane junction (e.g., see steps 5 and 7). TM segments are allowed to leave the translocation channel prior to completion of protein synthesis, but do not necessarily leave as they enter the translocon.

into the bilayer? What should the orientation of various TM segments be? With each of these decisions comes the opportunity for regulation.

Although fundamental advances toward answering such questions will undoubtedly require the development of new ideas as well as techniques, a handful of initial studies on complex substrates may suggest the functional regulation of protein biogenesis. As with transcriptional and translational control, the cell may use translocational control as an additional means of generating diversity of gene expression. Indeed, several membrane proteins have been observed to be expressed in multiple topological forms, with the diversity apparently being generated at the time of translocation at the ER membrane (see Levy, 1996, for a review). For example, the protein ductin not only has two orientations (Finbow et al., 1993), but each orientation appears to serve different functions. One of the topological forms is found as a subunit of the vacuolar $H^+$-ATPase, while the other form is a component of a connexon channel found in gap junctions. That this diversity originates at the translocation site in the ER was demonstrated by showing that ductin translated and translocated in a cell-free system results in the synthesis in both orientations (Dunlop et al., 1995).

Similarly, the P-glycoprotein product of the multidrug resistance gene (MDR1) found in various cancer cells is a membrane protein with at least two topological forms (Skach et al., 1993; Zhang et al., 1993). Although predicted to span the membrane 12 times, several of its TM segments apparently can exist in multiple orientations or locations, perhaps regulated by factors in the cytosol (Zhang and Ling, 1995). This type of structural variability appears to be qualitatively different than that observed in ductin, where the entire protein is reversed in orientation with respect to the membrane. However, similar to ductin, MDR1 has been proposed to serve multiple functions in the cell (Pastan and Gottesman, 1991). Whether the different topological forms are recruited to different regions of the cell for specialized functions, as appears to be the case for ductin, remains to be determined.

Finally, some proteins may contain potential TM segments that are not used under all circumstances. For example, the prion protein (PrP), a brain glycoprotein involved in various neurodegenerative diseases (Prusiner, 1996), contains a hydrophobic domain initially predicted to serve as a TM segment (Bazan et al., 1987). Despite this hydrophobic segment, PrP does not appear to normally span the membrane in vivo, but rather is translocated across the ER membrane, C-terminally glycolipididated, and trafficked to the cell surface (Stahl et al., 1987). By contrast, studies in cell-free systems have shown that not only can PrP span the membrane at its putative TM segment, but under some conditions, nearly all of it is found as a transmembrane protein (Hay et al., 1987). The generation of this topological form is dependent on both hydrophobic and hydrophilic sequences in the PrP molecule (Yost et al., 1990) and appears to be regulated by cytosolic factors (Lopez et al., 1990). However, just as the normal role of the PrP molecule remains enigmatic, so does the topological regulation of this unusual protein. It will be interesting to see whether the topology of PrP is regulated in vivo by *trans*-acting cellular factors, and whether dysregulation of these events at the ER plays a role in any of the wide variety of diseases attributed to PrP. If so, it seems likely that a transmembrane form, not being observed in normal brain, is involved in events related to prion disease that are carried out, in part, by as yet unidentified components of the translocon.

The identification and functional reconstitution of the core components of the translocon, using simple substrates, have now set the stage for exploring the functional complexity and structural diversity of accessory translocon components in biogenesis of more complex

secretory and membrane proteins. The initial studies on membrane proteins have already revealed unappreciated subtleties of the translocation process, pointing to a new site of regulation in the cell. Future work using the tools obtained from past work on model proteins, used to study the biogenesis of currently enigmatic substrates, will surely elucidate new functions for old machinery and new machinery involved in currently mysterious functions.

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