# Regulation of protein biogenesis at the endoplasmic reticulum membrane

TRANSLOCATION SERIES

Ramanujan S. Hegde and Vishwanath R. Lingappa

*The biogenesis of most secretory and membrane proteins involves targeting the nascent protein to the endoplasmic reticulum (ER), translocation across or integration into the ER membrane and maturation into a functional product. The essential machinery that directs these events for model secretory and membrane proteins has been identified, shifting the focus of studies towards the molecular mechanisms by which these core components function. By contrast, regulatory mechanisms that allow certain proteins to serve multiple functions within a cell remain entirely unexplored. This article examines each stage of protein biogenesis as a potential site of regulation that could be exploited by the cell to effectively increase the diversity of functional gene expression.*

The authors are in the Dept of Physiology (R. S. H. and V. R. L.) and Medicine (V. R. L.), University of California, San Francisco, CA 94143-0444, USA.
E-mails: manu@itsa.ucsf.edu vrl@itsa.ucsf.edu

Our understanding of the biogenesis of secretory and membrane proteins at the ER has advanced dramatically during the past decade. The evidence in favour of receptor-mediated events involving an aqueous translocation channel as the mechanistic paradigm for protein transport across the ER membrane is now overwhelming[1]. The core components that mediate these reactions have been identified, at least for simple secretory and integral membrane proteins[2]. Together with associated machinery for the modification of the nascent chain, such as signal peptidase, oligosaccharyl transferase and various chaperones, these proteins comprise a 'machine' for protein biogenesis termed the 'translocon'.

Now that a basic conceptual framework and minimal machinery are in place, the stage has been set to ask a different type of question involving translocation of nascent chains across the ER membrane: is this key cellular event subject to regulation? Just as transcription, splicing, translation and protein degradation are carried out by core machinery subject to the action of regulatory factors, the translocation process is likely to be regulated similarly. This article considers the potential mechanisms available to the cell for regulating protein translocation and biogenesis. Principles likely to emerge from future studies are discussed in the light of current findings implicating regulated protein biogenesis as the basis for the observed functional diversity of certain proteins.

**Topogenesis**

The topology of a protein targeted to the secretory pathway is characterized by whether it spans the membrane, the number of times it spans the membrane and the relative orientation of the membrane-spanning segments. The mechanisms by which the topology of a protein is directed by the cellular machinery of translocation, especially for polytopic (multispanning) membrane proteins, remain largely a matter of speculation[3–5]. Most work aimed at elucidating the determinants of topology has focused on defining specific properties of topogenic sequences within the substrate. Features such as length and hydrophobicity of membrane-spanning segments, charge distribution of the flanking residues and size and folding state of surrounding domains have each been documented as affecting topology[4–8]. These studies have led to several basic guidelines correlating physical features of topogenic sequences to final topology[9–11]. However, the mechanism by which these physical features are used during topogenesis remains unknown. Moreover, many proteins appear not to obey these rules, either being made in a topology different to that predicted or displaying a heterogeneous population of topological isoforms.

For example, several proteins, such as the mp26 bovine lens fibre protein[12], the rat muscle glucose transporter[13] and the mouse glycoprotein PC1[14], do not obey prediction algorithms based on the tendency of a net positive charge of flanking residues on the cytosolic side of a transmembrane (TM) domain[9]. Similarly, algorithms based on hydrophobicity incorrectly predict TM domains, such as in the ER-lumenal chaperone GRP94[15] or the epidermal growth factor (EGF) receptor[11], and often fail to predict amphipathic TM segments found in many transporters and channels. Indeed, a statistical method designed to take into account both structural and topogenic features, while useful, still failed to predict the correct topology of 19 out of 83 proteins[11]. Even more unpredictable by any sequence-based algorithms are proteins that are made in more than one topology. This behaviour has been observed in several proteins, including the p-glycoprotein product of the multidrug resistance gene (MDR1)[16,17], the prion protein (PrP)[18,19], the hepatitis B virus envelope glycoprotein[20] and the transporter ductin[21].

How can these examples, some more complex than others, be reconciled into a unifying model with simpler, more predictable membrane proteins? One way of accounting for all these observations is to consider a qualitatively different model for the topogenesis of membrane proteins. Rather than viewing the topogenic process from the point of view of the substrate (and sequence determinants



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therein), we propose a model by which topogenesis is regulated by the action of *trans*-acting components of the translocation machinery. In this manner, not only can simple proteins that appear to disobey present rules be explained but the existence of proteins that achieve multiple topological forms can be explained both mechanistically and physiologically.

To allow generation of multiple topological forms of a membrane protein, the function of a putative TM domain must be malleable. This versatility could in principle occur in two ways (Fig. 1). The putative TM domain is either 'recognized' or 'ignored' by the translocation machinery, resulting in integration into the membrane or translocation into the lumen, respectively. If recognized, the TM domain might be inserted into the membrane in either of its two possible orientations. The responsibility for regulating these two events is proposed to reside in specific components of the translocation machinery that we shall term as 'translocation accessory factors' (TrAFs). Combining these two modes of regulation by utilizing combinations of different TrAFs could allow for enormous variation and diversity in topogenesis.

The decision on whether a putative TM domain is 'recognized' or 'ignored' could be made in two related ways. First, a TM domain could be shielded from the machinery that is normally responsible for recognizing and subsequently integrating it into the membrane (Fig. 1a). Alternatively, a relatively weak hydrophobic stretch that normally would not be recognized by the translocon as a TM domain could be assisted to interact better with the translocation machinery (or lipid bilayer[22,23]) responsible for membrane integration (Fig. 1b). Both of these scenarios are conceptually similar: chaperoning a putative TM domain during its translocation in a manner that directs its fate. This function could be the responsibility of different components of the translocation machinery, each of which might recognize particular subsets of substrates in need of such regulation and that could be activated or inactivated selectively by cellular signal-transduction pathways[24].

In the case of some TM domains, the core translocon might suffice[2,25]. But, for other substrates, handling of a TM domain might be regulated by the action of accessory factors, or TrAFs[26]. It has been observed that, once a TM domain enters the translocation channel, it is not necessarily integrated into the lipid bilayer immediately. Instead, it appears to integrate via a multistage series of reactions in which it is in distinctly different environments[27]. This mode of integration occurs for some[27], but apparently not all[23], TM domains. Thus, during its integration, particular TM domains could be 'sampled' by various environments, potentially allowing them to be subject to regulation by different TrAFs.

In principle, TM segment orientation could also be regulated (Fig. 1b,c). This might occur in at least two distinct ways. One possibility is that the TM segment is presented to the translocon in one of its two orientations and, once in the translocon in a given orientation, is integrated into the membrane in that orientation. Alternatively, TM segments might be able to change orientation once in the translocation channel. Whether the former, latter or perhaps both mechanisms are used by the cell to regulate TM segment orientation remains to be seen.

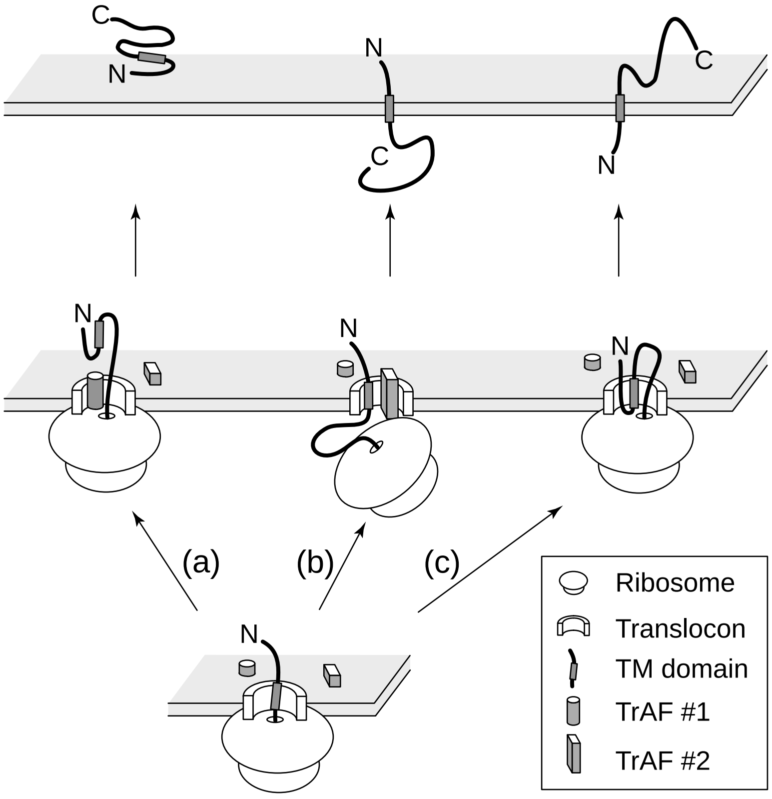


**FIGURE 1**

Potential mechanisms for generating more than one topological form of a protein. Once targeted to and docked at the translocation channel in the endoplasmic reticulum (ER) membrane, a nascent chain (heavy black line) containing a putative transmembrane (TM) segment (shaded rectangle) might have several options. The action of a particular translocation accessory factor (TrAF; cylinder) might shield the hydrophobic TM segment from being recognized by the translocon, allowing it to be translocated into the ER lumen [(a) versus (b) or (c)]. If the TM segment is allowed to be recognized by the translocation machinery, another TrAF (box) might function to position it in a specific orientation [(b) versus (c)]. If no TrAF interacts with the nascent chain, a 'default' pathway, perhaps the least energetically demanding orientation, is favoured (c). The final topology resulting from each of these possibilities is shown at the top of the diagram. A similar set of events is thought to occur during the biogenesis of the prion protein[26], although the identity of the individual TrAFs remains unknown.

The notion that TM segments can be presented to the translocon in either orientation is supported by the observation that they can be recognized almost immediately after their synthesis, perhaps by the ribosome itself[28]. Shortly after this recognition, dramatic changes in the nature of the ribosome–membrane junction can be elicited, allowing regions of the TM domain to be accessible to the cytosol[28]. Thus, it seems plausible that the TM domain could orient itself appropriately in the cytosolic environment before presenting itself to the translocation channel in either of two orientations. If this were the case, one might imagine that cytosolic factors, including perhaps molecular chaperones or even signal-recognition particle (SRP, which can recognize TM domains[29–31]), could play a role in orienting and/or presenting the TM domain to the translocon. Modulation of such factors could therefore have an impact on the topology achieved by the substrate. In support of such a model, it has been observed that the topology, and presumably therefore the function, of at least some substrates (for example, PrP and MDR1) appear to be dependent on cytosolic factors[32,33].

The alternative model, that TM segments orient themselves after entering the translocation channel, is supported by the observation that multiple TM segments can apparently assemble themselves in the translocon prior to integration into the lipid bilayer[34]. The significance of such an observation is that it allows TM segments to assemble with other TM segments, and perhaps orient or re-orient themselves with respect to each other. This assembly/orientation might be directed by various TrAFs, functioning as chaperones to facilitate the biogenesis of a complex membrane protein.

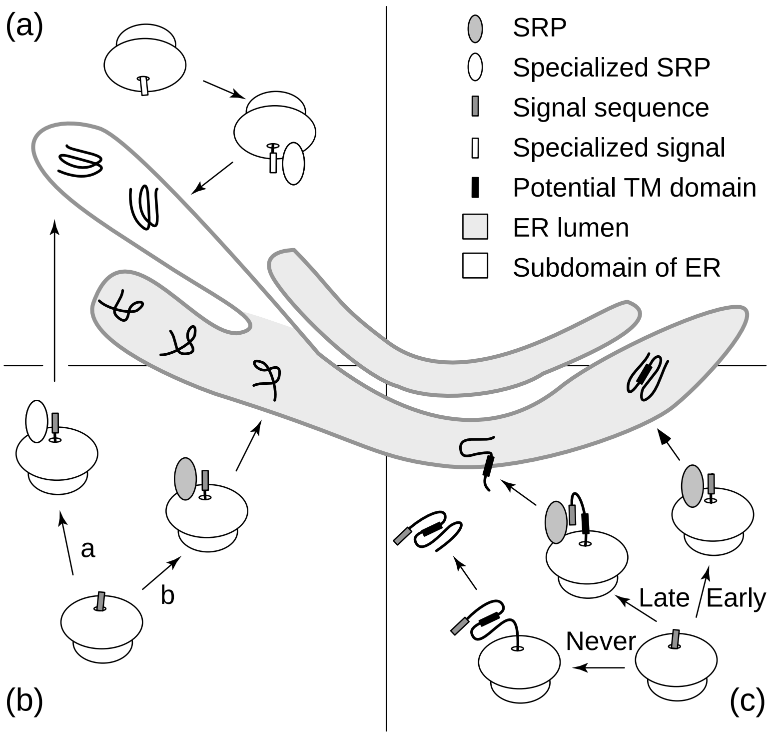


**FIGURE 2**

Modulation of protein targeting as a means of regulating protein biogenesis. The endoplasmic reticulum (ER) is shown as a heterogeneous structure containing a specialized subdomain (white area). The biogenesis of a particular substrate within this specialized subdomain is indicated to result in a folded conformation different from that at other regions of the ER [compare (a) with (b)]. Mechanisms for targeting a protein to this specialized subdomain might include the presence of a specialized signal sequence [white rectangle in (a) versus grey rectangle in (b)], specialized signal-recognition particles [SRPs; shaded versus striped ovals in (b)] or specialized receptors for the SRP (not indicated). (c) Illustration of how the timing of nascent chain targeting to the ER could have an impact on protein biogenesis. Recognition and targeting of the nascent chain early during chain growth might result in an outcome (translocation into the lumen of the ER) different from that arising if the nascent chain were allowed to elongate further before targeting (resulting in a membrane-spanning protein). If the nascent chain fails to target at all, a cytoplasmic topology results. The shaded box in the nascent chain indicates a signal sequence, and the black box a potential transmembrane (TM) domain.

While the above speculative model can explain certain observations, what evidence exists in its favour, and of what significance is a model of regulated protein biogenesis in the broader cell-biological or physiological sense? Intriguing answers to some of these questions have been provided by recent studies of PrP, which can be made in multiple topological forms at the ER[18,19]. The three topological forms, corresponding to those depicted in Fig. 1, are termed $^{sec}$PrP (fully translocated across the membrane), $^{Ntm}$PrP (transmembrane with the N-terminus in the lumen) and $^{Ctm}$PrP (transmembrane with the C-terminus in the lumen). Studies utilizing proteoliposomes containing subsets of ER proteins have defined crucial roles for TrAFs in controlling the relative amounts of each topological form synthesized[26] (Fig. 1). The potential importance of this topological regulation was highlighted by the demonstration that mutations that increase expression of the $^{Ctm}$PrP form cause neurodegenerative disease in transgenic mice and humans[19]. Thus, the currently mysterious normal function of PrP, as well as development of some of its associated diseases[35], might be regulated at the level of protein biogenesis and topology.

The prevalence of such regulatory phenomena in membrane protein biogenesis is currently unknown. It is clear that even apparently simple proteins with only one putative TM domain (such as PrP) can be regulated in their biogenesis. Thus, careful examination of the topology and biogenesis of additional proteins, especially polytopic membrane proteins, may well reveal that these events are not unusual. If so, the concept of topological regulation by a diverse family of TrAFs might become the rule rather than the exception.

### Targeting

The early phases of protein translocation involving targeting a protein to the ER and transport of non-TM domains across the membrane have been studied more extensively than the later events of topogenesis[36,37]. Because these steps are often considered mechanistically simpler and experimentally more tractable, their potential for regulation has largely been underestimated. The current understanding of protein targeting is that, as signal sequences of nascent chains emerge from the ribosome, they are recognized in the cytosol by SRP. The SRP–nascent-chain–ribosome complex is targeted to the ER via an interaction between SRP and the SRP receptor. Once targeted, the nascent chain is transferred from SRP, in a GTP-dependent step, to the protein-conducting translocation channel. However, the relatively recent appreciation that signal sequences might be more multifunctional than previously recognized[38,39] raises the possibility that their role in protein targeting is a more complex process than originally thought. But, might this step be regulated in novel ways to achieve diversity of gene expression? In principle, at least three steps could be regulated: whether to target a protein, where to target the protein and when to target the protein.

The question of whether to target a protein is perhaps the easiest to conceptualize. It is entirely plausible that a protein could have roles in more than one compartment in the cell and that, in order to 'be in two places at once', the mechanisms involved in sorting it are regulated. For example, a protein might 'inefficiently' target to the ER, yielding two populations of the protein (one in the secretory pathway and the other in the cytosol). In this manner, a single protein could serve its function in two different compartments. More dramatically, the protein might get folded or modified differently in the two compartments, allowing a single protein to serve two different functions.

Such diversity of function from a single gene has apparently been observed for the protein calreticulin[40]. This protein has been independently observed in the ER lumen (where it appears to serve as a $Ca^{2+}$-binding and storage protein[41]), the cytosol (where it appears to regulate cell adhesion by direct interaction with the cytosolic tails of integrins[42]) and in the nucleus (where it regulates steroid-receptor function[43,44]). Because some of these functions are ubiquitous (e.g. $Ca^{2+}$-binding in the ER), whereas others are cell specific, it is likely that the differential sorting is regulated in an as-yet-unidentified tissue-specific manner. Similarly, dual localization of other proteins or protein domains, such as the plasminogen activator protein[45] or hepatitis B virus envelope protein[20], has also been described previously. Whether the mechanism of multiple cellular localization in these particular examples involves regulation of the protein-targeting machinery or is on some other basis remains to be seen. However, the knowledge that the cell has evolved a means to maximize the utility of a single protein by differential compartmentalization lends substantial support to the possibility that one of the mechanisms used to achieve this is regulated protein targeting.

The question of where a protein directed to the secretory pathway should be targeted is raised by observations that the ER is heterogeneous and contains specialized subdomains. For example, the certain $Ca^{2+}$-binding proteins can be localized to specific places within the ER and used for generating rapid $Ca^{2+}$ waves[46]. Similarly, selected regions of the ER appear to interact with mitochondria for as-yet-unknown purposes[47]. Thus, different regions of the ER might be better equipped than others to handle the biogenesis or function of certain proteins (Fig. 2a,b). Conversely, by targeting a protein to different regions of the ER, the cell could produce multiple functional products (Fig. 2b). It could therefore be important to regulate the targeting of certain substrates to particular subdomains of the ER[48].

Interestingly, such a phenomenon has apparently evolved in at least some systems. For example, in rice endosperm, mRNAs coding for two different proteins dock at distinctly different regions of the ER[48,49]. These different ER subdomains are uniquely specialized in the biogenesis of the different substrates. It seems quite plausible that the mechanism by which this segregation occurs is by differential targeting. Perhaps there are slightly different versions of SRP and/or SRP receptor, or perhaps SRP assumes slightly different conformations when interacting with different signal sequences, resulting in the ability to modulate the site and manner of targeting (Fig. 2a,b). Reconstituting model regulatory events of targeting in a cell-free translocation system should allow these and other questions to be addressed.

Perhaps the least intuitive aspect of targeting that might be subject to regulation is the issue of when to target. It is generally assumed that the SRP binds to and targets nascent chains as their signal sequences emerge from the ribosome[36]. During the targeting process itself, SRP appears to slow down translation (in a process originally termed 'SRP arrest') apparently to limit the amount of nascent chain that is synthesized before docking at the translocation channel. This phenomenon has been studied carefully in only a handful of model substrates; hence, the potential for variations on this theme that affect targeting or topology remains unexplored.

It is possible that modulating the length of nascent chain that is synthesized before targeting occurs can modify the final outcome (Fig. 2c). Some proteins need to be targeted before a certain length of chain is synthesized, at which point they lose 'translocation competence', while other substrates maintain their translocation competence substantially longer and can clearly target at different times during their synthesis[50]. What might be the consequence of such a property? Perhaps, in some cases, chains that target early in their synthesis have a fate different from those that target later (Fig. 2c).

Thus, relatively subtle kinetic variations at one of several steps in the targeting of a protein might potentially have quite profound implications for the biogenesis of that protein. An extreme example of such kinetic variability is the difference between co- and post-translational modes of translocation used in yeast. Current evidence indicates that a combination of differences in the translocation machinery (SRP-dependent vs SRP-independent) and signal sequences differentiate the mechanism of targeting between these translocation pathways[39,51]. Whether similar variations on the mechanism of targeting exist in mammalian systems remains unknown. Given that, at present, the kinetics of targeting have been examined in very few substrates, and our tools to assess final topology and folded conformation (particularly of polytopic membrane proteins) are quite crude, it is likely that this type of regulation is used by the cell far more frequently than is currently appreciated.

**Transport**

Once targeted to the ER membrane, the complex of ribosome and nascent chain is transferred to the translocation channel. After a second signal-recognition event mediated by the translocation channel itself (presumably to further increase the fidelity of sorting)[52], the ribosome–nascent-chain complex is tightly bound to the translocon. This interaction is

Note added in proof:

One of the *headlines* on p.129 describes an ER stress response that regulates translation by phosphorylating the translation initiation factor eIF2α.

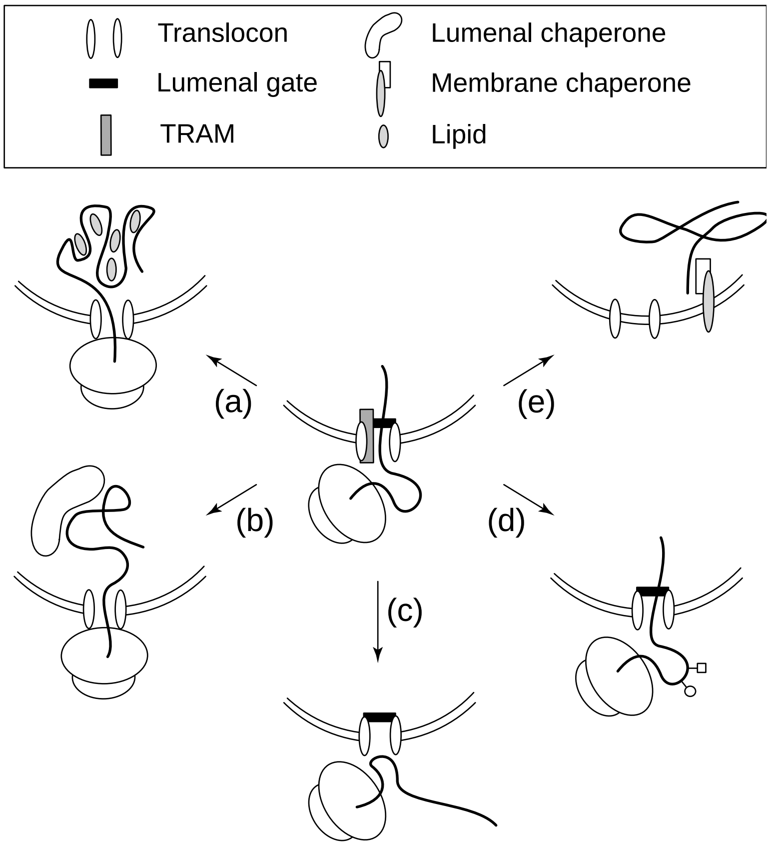


**FIGURE 3**

Potential consequences of a translocational pause. A paused nascent chain that is exposed transiently to the cytosol during translocation is depicted in the centre. By diminishing or exaggerating such an event, the nascent chain could subsequently interact differentially with other cellular components. These might include phospholipids, cholesterol or triglycerides (a), all of which assemble noncovalently with apolipoprotein B (Ref. 59). Similarly, interactions with lumenal (b) or membrane-bound (e) chaperones might be favoured by kinetic changes in the rate of translocation, as occurs during translocational pausing. Alternatively, the paused nascent chain could be modified by cytosolic enzymes (d) or perhaps targeted for degradation by removal to the cytosol (c). Cytosolic degradation of endoplasmic reticulum (ER) proteins[60], including apolipoprotein B (Ref. 61), has been demonstrated previously, although the relationship to translocational pausing remains unknown. The presence of a lumenal gate (black rectangle), preventing direct communication between the cytosol and ER lumen, is inferred from studies of translocon gating during membrane protein biogenesis[54].

such that the tunnel within the ribosome that houses the nascent chain is continuous with the tunnel formed by the translocon[53]. The junction between the ribosome and translocon is sealed tightly to shield the nascent chain from the cytosol and ensure that the ER lumenal and cytosolic contents are not in communication. Only then is a 'gate' on the lumenal side[54] (possibly formed by BiP[55]) opened to allow co-translational translocation of the growing nascent chain into the ER.

Although these mechanisms provide an explanation for how a hydrophilic protein can cross a hydrophobic barrier without compromising the integrity of the barrier, usually substantially more is involved. For many proteins, translocation is coupled to various modifications (signal-sequence cleavage, glycosylation, etc.), association with other components of the cell (chaperones, other subunits of a multiprotein complex, cofactors, ions, lipids) and intramolecular folding. Many of these events can occur only when the nascent chain is relatively unfolded and key sequence elements within the substrate are readily accessible. Thus, even ignoring the complexities of membrane protein biogenesis, vectorial transport of secretory proteins is only one part of a carefully orchestrated set of events occurring at or near the translocon.

Given that the 'space' around the nascent chain is limited, it is likely that the translocon is dynamic to give the nascent chain access to the appropriate enzymes. With the exception of Sec61$\alpha$, which is thought to form the core of the translocation channel, other components, such as translocating chain-associated membrane protein (TRAM), signal peptidase complex, oligosaccharyl transferase complex, various chaperones (both lumenal and membrane spanning) and other ER proteins, are likely to be at the translocation site only transiently when they are needed[1]. How might such events be regulated? In order to specialize the translocon for particular substrates, it seems plausible that topogenic sequences within the nascent chain are recognized by the translocation machinery to initiate the dynamic changes in the translocon.

Several observations support such a concept. Gating of the translocon, both at the lumenal and cytosolic sides, appears to be regulated by the recognition of specific sequences within the nascent chain[28,54]. In the case of lumenal gating, it is thought that functional signal sequences, once appropriately positioned within the translocon, trigger the opening of the lumenal gate[54]. Similarly, other topogenic sequences, such as TM sequences, appear to be recognized, perhaps by the ribosome itself, to mediate the closing and opening of the lumenal and cytosolic gate, respectively[28]. The correct orchestration of such gating events is thought to be important for maintaining the permeability barrier of the ER membrane during protein biogenesis.

A dynamic and regulated translocon appears to be important for other aspects of protein biogenesis as well. During the translocation of certain secretory proteins (such as apolipoprotein B and BiP), opening of the ribosome–membrane junction, during a phenomenon termed translocational pausing[56], transiently exposes large domains of the nascent chain to the cytosol[57,58]. The timing and extent of the cytosolic exposure appears to be regulated by both *cis*-acting sequences within the nascent chain (termed pause transfer sequences[56,57]) as well as by *trans*-acting components of the translocation machinery (such as TRAM[58]). Thus, the environment seen by a nascent chain during its translocation can be regulated. The major implication of these observations is that, by exaggerating or eliminating particular aspects of translocational pausing, a substrate could be redirected towards different structural, functional or topological fates (Fig. 3).

Although the physiological consequences of regulating translocational pausing to effect protein biogenesis await further studies, several possibilities can be envisioned (Fig. 3). It might be that this event represents a means of halting translocation temporarily, allowing the cell to decide between one of several potential fates for the substrate. Modulating the rate of translocation could affect the kinetics of cotranslational folding, give the translocon time to recruit the machinery needed for the biogenesis of each particular substrate and allow time for various enzymatic reactions to occur. The possible outcomes resulting from such a branch-point might be dependent on both the substrate and needs of the cell at any given time. Thus, even at a considerably late point in the biogenesis of a protein, its fate might be subject to regulation.

**Concluding remarks**

It is remarkable, but perhaps not entirely surprising, that several of the mechanisms needed to regulate various aspects of protein biogenesis have already been identified during the course of the study of simpler constitutive phenomena. Very small perturbations or variations of such events are all that is required to direct the fate of a protein towards dramatically different roles in the cell. Translocational regulation is a relatively easy means, of low energetic cost, to increase dramatically the diversity and adaptability of the cellular repertoire of functions. Thus, it seems quite likely that such phenomena have occurred widely during the course of evolution. In much the same way that simple constitutive processes appear to have evolved into complex ones in other realms of biology, translocation is also likely to have become a complex regulatory event. To date, the paucity of evidence of such events probably reflects the limitations of the tools available and the restricted number of complex translocation substrates that have been studied intensively. With the recent progress in understanding some of the constitutive aspects of protein biogenesis and translocation, the time has come to develop new assays and investigate more diverse and challenging substrates. As more examples of regulatory events are understood in detail, the scope and adaptive significance of translocational regulation will become clear.

**References**


1 Rapoport, T. A., Jungnickel, B. and Kutay, U. (1996) *Annu. Rev. Biochem.* 65, 271–303
2 Görlich, D. and Rapoport, T. A. (1993) *Cell* 75, 615–630
3 Hegde, R. S. and Lingappa, V. R. (1997) *Cell* 91, 575–582
4 Spiess, M. (1995) *FEBS Lett.* 369, 76–79
5 Gafvelin, G. *et al.* (1997) *J. Biol. Chem.* 272, 6119–6127
6 Beltzer, J. P. *et al.* (1991) *J. Biol. Chem.* 266, 973–978
7 Sakaguchi, M. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 16–19
8 Parks, G. D. and Lamb, R. A. (1991) *Cell* 64, 777–787
9 Hartmann, E., Rapoport, T. A. and Lodish, H. F. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 5786–5790
10 Sipos, L. and von Heijne, G. (1993) *Eur. J. Biochem.* 213, 1333–1340
11 Jones, D. T., Taylor, W. R. and Thornton, J. M. (1994) *Biochemistry* 33, 3038–3049
12 Gorin, M. B. *et al.* (1984) *Cell* 39, 49–59
13 Charron, M. J. *et al.* (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 2535–2539
14 van Driel, I. R. and Goding, J. W. (1987) *J. Biol. Chem.* 262, 4882–4887
15 Mazzarella, R. A. and Green, M. (1987) *J. Biol. Chem.* 262, 8875–8883
16 Skach, W. R., Calayag, M. C. and Lingappa, V. R. (1993) *J. Biol. Chem.* 268, 6903–6908
17 Zhang, J. T., Duthie, M. and Ling, V. (1993) *J. Biol. Chem.* 268, 15101–15110
18 Yost, C. S. *et al.* (1990) *Nature* 343, 669–672
19 Hegde, R. S. *et al.* (1998) *Science* 279, 827–834
20 Swameye, I. and Schaller, H. (1997) *J. Virol.* 71, 9434–9441
21 Dunlop, J., Jones, P. C. and Finbow, M. E. (1995) *EMBO J.* 14, 3609–3616
22 Martoglio, B. *et al.* (1995) *Cell* 81, 207–214
23 Mothes, W. *et al.* (1997) *Cell* 89, 523–533
24 Bonifacino, J. S. *et al.* (1990) *Nature* 344, 247–251
25 Oliver, J. *et al.* (1995) *FEBS Lett.* 362, 126–130
26 Hegde, R. S., Voigt, S. and Lingappa, V. R. (1998) *Mol. Cell* 2, 85–91
27 Do, H. *et al.* (1996) *Cell* 85, 369–378
28 Liao, S. *et al.* (1997) *Cell* 90, 31–41
29 Mize, N. K., Andrews, D. W. and Lingappa, V. R. (1986) *Cell* 47, 711–719
30 High, S., Flint, N. and Dobberstein, B. (1991) *J. Cell Biol.* 113, 25–34
31 Kuroiwa, T. *et al.* (1996) *J. Biol. Chem.* 271, 6423–6428
32 Lopez, C. D. *et al.* (1990) *Science* 248, 226–229
33 Zhang, J. T. and Ling, V. (1995) *Biochemistry* 34, 9159–9165
34 Borel, A. C. and Simon, S. M. (1996) *Cell* 85, 379–389
35 Prusiner, S. B. (1997) *Science* 278, 245–251
36 Walter, P. and Johnson, A. E. (1994) *Annu. Rev. Cell Biol.* 10, 87–119
37 Siegel, V. (1995) *Cell* 82, 167–170
38 Martoglio, B. and Dobberstein, B. (1998) *Trends Cell Biol.* 8, 410–415
39 Zheng, N. and Gierasch, L. M. (1996) *Cell* 86, 849–852
40 Dedhar, S. (1994) *Trends Biochem. Sci.* 19, 269–271
41 Smith, M. J. and Koch, G. L. (1989) *EMBO J.* 8, 3581–3586
42 Coppolino, M. G. *et al.* (1997) *Nature* 386, 843–847
43 Burns, K. *et al.* (1994) *Nature* 367, 476–480
44 Dedhar, S. *et al.* (1994) *Nature* 367, 480–483
45 Belin, D. *et al.* (1996) *EMBO J.* 15, 468–478
46 Simpson, P. B. *et al.* (1997) *J. Biol. Chem.* 272, 22654–22661
47 Cascarano, J. *et al.* (1995) *Hepatology* 22, 837–846
48 Okita, T. W., Li, X. and Roberts, M. W. (1994) *Trends Cell Biol.* 4, 91–95
49 Li, X., Franceschi, V. R. and Okita, T. W. (1993) *Cell* 72, 869–879
50 Perara, E., Rothman, R. E. and Lingappa, V. R. (1986) *Science* 232, 348–352
51 Panzner, S. *et al.* (1995) *Cell* 81, 561–570
52 Jungnickel, B. and Rapoport, T. A. (1995) *Cell* 82, 261–270
53 Crowley, K. S., Reinhart, G. D. and Johnson, A. E. (1993) *Cell* 73, 1101–1115
54 Crowley, K. S. *et al.* (1994) *Cell* 78, 461–471
55 Hamman, B. D., Hendershot, L. M. and Johnson, A. E. (1998) *Cell* 92, 747–758
56 Chuck, S. L. and Lingappa, V. R. (1992) *Cell* 68, 9–21
57 Hegde, R. S. and Lingappa, V. R. (1996) *Cell* 85, 217–228
58 Hegde, R. S. *et al.* (1998) *Cell* 92, 621–631
59 Yao, Z. and McLeod, R. S. (1994) *Biochim. Biophys. Acta* 1212, 152–166
60 Wiertz, E. J. *et al.* (1996) *Nature* 384, 432–438
61 Fisher, E. A. *et al.* (1997) *J. Biol. Chem.* 272, 20427–20434


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