The concept of translocational regulation

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Biological processes are regulated to provide cells with exquisite adaptability to changing environmental conditions and cellular demands. The mechanisms regulating secretory and membrane protein translocation into the endoplasmic reticulum (ER) are unknown. A conceptual framework for translocational regulation is proposed based on our current mechanistic understanding of ER protein translocation and general principles of regulatory control.

The cotranslational translocation pathway

Since the articulation of the signal hypothesis (Blobel and Dobberstein, 1975), a steady assault by genetic, biochemical, and structural analyses have provided considerable insight into the pathways by which proteins are translocated across the ER membrane (Rapoport, 2007). In cotranslational translocation, signal sequences (or transmembrane domains [TMDs]) within a nascent polypeptide are recognized by universally conserved factors in the cytosol and membrane to mediate their selective targeting, translocation, and/or membrane insertion (Fig. 1 A). This process is initiated when the hydrophobic core of a signal sequence or TMD emerges from the ribosome and is recognized by the signal recognition particle (SRP). The SRP-bound ribosome-nascent chain complex (RNC) is then targeted to the ER membrane via an interaction with the SRP receptor (SR). The RNC is then transferred to an adjacent translocon, and the SRP–SR complex dissociates. These steps, collectively referred to as targeting (Shan and Walter, 2005), result in the delivery of translocation substrates to sites of translocation at the ER.

However, delivery of RNCs to a translocon does not ensure translocation (or membrane insertion, in the case of TMD-containing proteins). Substrates must additionally interact with and gate open a normally closed channel formed by the Sec61 complex. This decisive interaction is also mediated by a signal sequence or TMD and serves at least two purposes. First, it may represent a "proofreading" step that prevents translocation of spuriously targeted proteins that lack a functional signal sequence or TMD. Second, it allows for appropriate positioning of the nascent chain in preparation for subsequent events (Fig. 1 B). For a signal sequence, the proper position for subsequent translocation is a "looped" orientation in which the N terminus of the signal is facing the cytosol and the mature portion of the nascent chain is inserted into the aqueous pore of the Sec61 channel. TMDs, depending on features of their hydrophobic and flanking regions, are oriented in one of two ways (Higy et al., 2004). If the orientation is looped like a signal sequence, the downstream domain is translocated into the lumen upon further elongation. In the nonlooped orientation, the downstream domain is released into the cytosolic environment through a gap between the ribosome and translocon. Additional events, such as signal sequence cleavage, TMD insertion into the lipid bilayer, and translocation, occur upon continued elongation of the nascent chain.

This paradigm paints the picture of stereotyped sequential interactions between specific domains in a nascent chain and the highly conserved targeting and translocation apparatus that culminates in a defined outcome. Hence, the sequence elements within a nascent chain would seem to predetermine the outcome in a deterministic manner. How then might translocation be regulated to influence the outcome of substrate location or topology? Which of the steps outlined in the previous paragraphs are susceptible to physiological perturbation? And of what importance might such regulation be for the cell or organism? These questions have been largely ignored, in no small part because even rather basic issues in translocation had remained unknown. With the core machinery now in hand and an increasingly mechanistic understanding of the basic steps of translocation, it seems appropriate to pose a working framework for translocational regulation.

Essential elements of a regulatory system

A common theme in all regulatory systems is the embellishment of a core machinery with accessory factors that can selectively stimulate or inhibit specific decisive reactions along the core pathway. Protein translocation is presumably regulated analogously, and would therefore require three key elements. First, even though all proteins that enter the ER share features that allow their recognition by the core translocation machinery, they must nonetheless contain distinguishing elements. Second, these substrate-specific elements must impart some functional differences that can be exploited by noncore (i.e., accessory) components to influence a key step in translocation. And finally, the cell must be able to modulate the function (or availability) of such accessory machinery to effect regulatory control in a substrate-selective manner.

Each of these three elements in translocational regulation is considered in detail in the subsequent sections. In this

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Abbreviations used in this paper: RNC, ribosome-nascent chain complex; SR, SRP receptor; SRP, signal recognition particle; TMD, transmembrane domain; TRAM, translocating chain-associating membrane protein; TRAP, translocon-associated protein complex.

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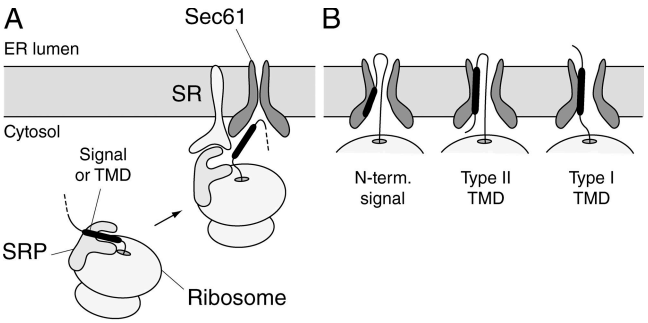
framework, signal sequences are proposed to encode substrate-specific differences that influence their interaction with the Sec61 complex, a decisive step in initiating translocation. Such differences in this critical interaction are proposed to be especially susceptible to modulation in multiple ways by several accessory factors that include membrane proteins, lumenal proteins (such as chaperones), and cytosolic proteins. And finally, the functions of these accessory factors are proposed to change by diverse means, including alternative splicing, differential expression, phosphorylation, and titration, resulting in consequences for the translocation of some but not other substrates.

Substrate diversity within a shared motif

Natural signal sequences are remarkably diverse. So much so that homology searches with one signal sequence usually fail to identify any of the thousands of other signal sequences from unrelated proteins. Signals differ markedly in length, hydrophobicity, charge, amino acid composition, and flanking mature domain (Fig. 1 C; von Heijne, 1985). The only unifying property shared by all signals is an overall hydrophobic character typified by an uninterrupted stretch of at least six nonhydrophilic residues (Fig. 1 C, underlined). This tolerability in specific sequence was strikingly illustrated by the observation that up to one-fifth of all random 20-residue sequences can serve as secretion signals in yeast (Kaiser et al., 1987). Thus, it has long been thought that signal sequence diversity represents degeneracy caused by a lack of selective pressure to maintain all but a general hydrophobic character. However, there is growing appreciation that at least some of this diversity may be biologically meaningful (discussed more extensively by Martoglio and Dobberstein [1998] and Hegde and Bernstein [2006]).

First, an evolutionary analysis of signal sequences has found that they evolve more slowly than would be expected from their apparent degeneracy (Williams et al., 2000). Second, numerous anecdotal observations in various systems suggest that signal sequences are not always interchangeable without functional consequences (e.g., Rutkowski et al. [2001]). Third, signal sequences can vary in their functional efficiency of mediating translocation in vitro (Kim et al., 2002) and in vivo (Levine et al., 2005). In many cases, these substrate-specific differences in signal sequence efficiency are evolutionarily conserved (Kim et al., 2002). Fourth, relative efficiencies among different signal sequences in vivo are influenced by the cell type and culture conditions used (Levine et al., 2005). And finally, examples have been described in which alternative splice variants or alternative translation start sites result in differentially expressed proteins differing only in their signal sequences (e.g., Nakajima et al. [1999], Damodarasamy et al. [2000], and Clark et al. [2002]). Such observations are puzzling if all signal sequences are presumed to be functionally equivalent and constitutively active in directing substrate translocation. Instead, a common motif (hydrophobicity) that imparts the minimal functionality of mediating translocation is proposed to be elaborated by substrate-specific features that are often conserved. At least some of these differences among signal sequences could be exploited for differential modulation of translocation to mediate regulation.



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Protein	signal sequence	length	charge
BiP	MKLSLVAAMLLLLSAARA	18	+1
Prolactin	MNIKGSPWKGSLLLLVSNLLLCQSVAP	28	+2
TGF-beta 2	MHYCVLSAFLILHLVTVAL	19	0
TGF-beta 3	MKMHLQRALVVLALLNFATVSLS	23	+2
Growth hormone	MATGSRTSLLLAFGLLCLPWLQEGSA	26	+1
Interferon gamma	MKYTSYILAFQLCIVLG	17	+1
Osteopontin	MRIAVICFCLLGITCA	16	+1
Leptin	MHWGTLCGFLWLWPYLFYVQA	21	0
ANP	MSSFSTTTVSFLLLLAFQL	19	0
PrP	MANLGCWMLVLFVATWSDLGLC	22	0
Apo-A1	MKAAVLTLAVLFLTGSQA	18	+1
EGF-receptor	MRPSGTAGAALLALLAALCPRA	24	+1
Glucagon	MKSIYFVAGLFVMLVQG	19	+1
Inhibin beta	MPLLWLRGFLLASCWIIVRSSPTPGS	27	0
HLA-DC beta	MSWKKALRIPGGLRVATVTLMLAMLSTPVAEG	32	+2
Choriogonadotropin	MEMFQGLLLLLLSMGGTWA	20	-1
Carboxypeptidase B	MLALLVLVTVALASAH	15	0
Robo1-A	MKWKHVPFLVMISLLSLSPNHLFLA	24	+2
Robo1-B	MIAEPAHFYLFGLICLCSG	20	-1
β-Gal-related prot.	MPGFLVRILLLLLVLLLLGPTRGL	23	+1
Collagen IX	MKTCWKIPVFFFVCSFLEPWASAA	23	+2

Figure 1. **The essential steps in cotranslational translocation.** (A) SRP-dependent targeting of a signal- or TMD-containing nascent chain to the Sec61 translocon. (B) Modes of interaction between signals and TMDs with the Sec61 translocon. (C) The diversity of signal sequences: the overall length and net charge of the n domain (green) are listed for a set of typical signals. The hydrophobic core of each signal sequence is indicated in red underlined text.

Most signal sequences are intrinsically inefficient after targeting to Sec61

Because signals are recognized twice, first by SRP for targeting and subsequently by Sec61 to initiate translocation, both are potential sites for regulation. Recognition by SRP is mediated by a methionine-lined hydrophobic groove in the signal sequence binding subunit SRP54 (Keenan et al., 1998). The highly flexible side chains of methionine combined with the size and shape of this binding domain are thought to make its interaction with hydrophobic substrates very tolerant to sequence variation. Because the signal binding domain of SRP54 is precisely positioned at the polypeptide exit site of the ribosomal tunnel (Halic et al., 2004), signal recognition is typically considered to occur rapidly and efficiently. Although it is certainly conceivable that this apparently robust mechanism of signal recognition could be subject to modulation, little evidence currently exists for this view. It therefore seems that SRP has the capability of providing all potential substrates (defined broadly by hydrophobicity) a "license" to be translocated by bringing them constitutively to translocons at the ER.

Whether this license is subsequently exercised is contingent on at least one additional interaction between the signal sequence and the Sec61 complex in the translocon. Although based on limited analyses, it is clear that signal recognition by the Sec61 complex is more stringent and less efficient than that mediated by SRP. For example, mutant signals that function for SRP-dependent targeting can be essentially inactive in their

ability to initiate translocation through Sec61 (Kim et al., 2002). Furthermore, sequence differences among natural signals markedly influence their interaction with the Sec61 translocon. In studies using proteoliposomes containing purified Sec61 complex and SR, the relative translocation efficiencies among substrates varied widely (Gorlich and Rapoport, 1993; Voigt et al., 1996; Hegde et al., 1998). Remarkably, only a very few signal sequences were capable of even moderately efficient initiation of translocation. This substrate-specific variability appears to occur after successful delivery to the Sec61 complex (Voigt et al., 1996; Hegde et al., 1998), pointing to differences in the signal–Sec61 interaction.

Assuming that signals interact with Sec61 analogously to TMDs, variability in translocation efficiencies among signals could be due in part to the efficiency with which different signals adopt the correct looped orientation in the channel (Fig. 2). In the same way that TMD orientation is influenced by its length, hydrophobic domain, and flanking regions (Higy et al., 2004), analogous differences among signals may affect their affinity, stability, and mode of interaction with Sec61. When positioned in the looped orientation, elongation of the nascent chain results in its entry into the ER lumen. Interaction in a nonlooped orientation forces the mature domain of a nascent chain to be extruded into the cytosol. Presumably, these configurations are dynamic, and the nascent chain not only samples both orientations but can switch between them at early stages of translocation (as suggested for TMDs by Goder et al. [1999]). However, increasing nascent chain length upon continued translation would decrease the capacity to change orientations, eventually resulting in "commitment" to either forward or failed translocation. The decisive point (i.e., nascent chain length) at which commitment occurs would depend on properties of both the signal sequence (its affinity for and stability within the translocon) and mature domain (its capacity to remain sufficiently unfolded to pass through the translocon). This commitment point would therefore vary from substrate to substrate, giving each a somewhat different period of time to be biased in one direction or another (as elaborated in the subsequent section).

Thus, it is proposed that the intrinsic stability of a productive interaction in a looped orientation with the isolated Sec61 complex is very low for the vast majority of signal sequences. Either the looped configuration is not achieved or, more likely, is not maintained for long enough to allow the mature domain to substantially enter the ER lumen before translocation competence is lost. This means that the "basal" translocation activity for most signal sequences in the context of the core translocation machinery is low because of dynamically unstable post-targeting interaction between the signal and the Sec61 complex relative to the constraints imposed by ongoing translation.

Substrate-selective trans-acting factors

Low basal activity of most signal sequences for the Sec61 complex in mammals necessitates a requirement for additional factors for productive translocation. In the context of translocational regulation, such trans-acting factors would need to be substrate specific and operate combinatorially to determine the net efficiency of translocation. Based on the model of a highly dynamic

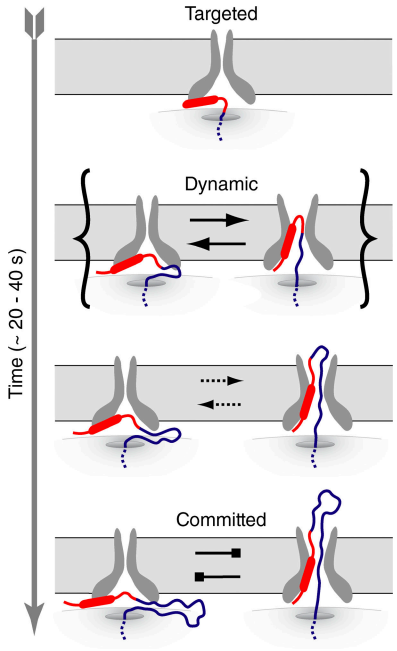

Figure 2. **A dynamic signal–Sec61 interaction.** After targeting to the Sec61 complex (top), the signal sequence is proposed to interact weakly and dynamically with the putative signal binding site on Sec61. The looped (right) and nonlooped (left) configurations are more interconvertible at shorter nascent chain lengths than at longer lengths.

temporally constrained signal–Sec61 interaction that directly determines translocation efficiency (Fig. 2), at least three qualitatively different mechanisms of action for trans-acting factors can be envisioned.

The first mechanism involves accessory factors that interact directly with the nascent chain to stabilize the looped orientation (Fig. 3 A). Such factors would therefore bias forward translocation by minimizing the ability of the signal sequence to sample the nonlooped configuration associated with translocational failure. The translocating chain-associating membrane protein (TRAM) and the translocon-associated protein complex (TRAP) may represent such accessory factors (Gorlich et al., 1992; Voigt et al., 1996; Fons et al., 2003). Both proteins stimulate translocation in a signal sequence–dependent manner, and neither protein is absolutely required because at least some substrates can be translocated in their absence. Furthermore, these proteins seem to interact directly with (or at least be very close to) the nascent chain: TRAM can be cross-linked to regions N-terminal to the hydrophobic core of the signal (High et al., 1993), whereas TRAP seems to cross-link with longer nascent chains that have access to the lumen (Gorlich et al., 1992). Cryoelectron microscopy analysis has positioned TRAP at the site of translocation with a large lumenal domain that sits very close to the lumenal aperture of the translocation pore (Menetret et al., 2005). Combined with the observation that Sec61 seems to interact most directly with the hydrophobic core of the signal sequence (High et al., 1993), a multipartite interaction can be envisaged (Fig. 3 A). Different regions of the signal sequence and nascent chain would make contacts with different subsets of factors to influence the overall positioning and stability of the looped conformation.

In the example with TRAM and TRAP (Fig. 3 A, right), their requirement for translocation for any given substrate would be directly dependent on the relative stability of the basal signal–Sec61 interaction. Furthermore, depending on the relative contributions of each factor in stabilizing the looped orientation, substrate translocation could be dependent specifically on TRAP, specifically on TRAM, on either protein, on both proteins, or on neither protein. A stabilization role for TRAP would be consistent with the observation that TRAP dependence is highest for signals that are relatively inefficient (Fons et al., 2003). And finally, substrate-specific stabilization could conceivably be contributed by any of several other proteins at the site of translocation, even if this is not their primary function. The only requirement would be a capacity to interact, at least weakly, with specific regions of a nascent chain to bias its orientation transiently. Thus, numerous components near the translocon, such as mammalian Sec62 and Sec63 (Meyer et al., 2000; Tyedmers et al., 2000), signal peptidase complex (Kalies et al., 1998), oligosaccharyl transferase complex (Kelleher and Gilmore, 2006), p180 (Savitz and Meyer, 1993), Erj1p (Dudek et al., 2005), RAMP4 (Gorlich and Rapoport, 1993), and others, could contribute in a substrate-specific manner to the overall efficiency of translocation independently of (or in addition to) other putative functions.

The second mechanism of trans-acting factor function involves the biasing of translocation by trapping. Here, a transiently sampled configuration (such as the looped orientation with a portion of the nascent chain exposed to the lumen) is trapped by preventing its ability to fully interconvert with alternative configurations (Fig. 3 B). For example, binding of the lumenally exposed nascent chain to a chaperone would prevent its slippage into the nonlooped conformation even if the signal–Sec61 interaction subsequently fails. Other analogous examples of trapping could potentially include glycosylation (Goder et al., 1999) or nascent chain folding (Kowarik et al., 2002). Thus, the signal–Sec61 interaction is not stabilized per se. Rather, the commitment step is biased such that it occurs both earlier and in favor of one outcome (in this example, forward translocation). This mechanism is directly analogous to a "ratchet"-based model of translocation that is often involved in posttranslational systems of translocation (e.g., Matlack et al. [1999])

Substrate specificity in this mechanism would be imparted by features of the mature domain (e.g., the presence or absence of good chaperone binding sites, the presence of appropriately positioned glycosylation sites, etc.) as well as the signal sequence, which would determine how long a particular trapping-competent configuration is sampled. These complexities may explain why clearly delineating the functional role of lumenal chaperones in cotranslational translocation has been complicated by conflicting results (Gorlich and Rapoport, 1993; Nicchitta and Blobel, 1993; Tyedmers et al., 2003). Indeed, recent experiments have illustrated that dependence on lumenal proteins for translocation is significantly influenced by the signal sequence (Kang et al., 2006). Such substrate dependence on lumenal proteins was proposed to explain the differential effects on translocation of different proteins during acute ER stress, when chaperone availability is reduced.

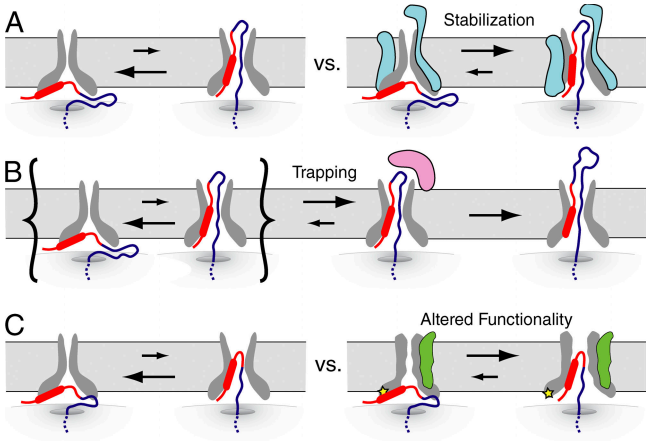

Figure 3. **Potential mechanisms of translocational regulation.** (A) Selective stabilization of the looped conformation by accessory factors (light blue; right). (B) Trapping of transiently sampled conformations by nascent chain binding proteins (such as a chaperone; pink). (C) Alteration of Sec61 functionality by an accessory factor (green) or by a modification (yellow star) that changes its signal recognition properties.

The third potential mechanism for influencing translocation is by an indirect effect on Sec61 functionality (Fig. 3 C). Because signal recognition and gating of the Sec61 translocation channel are presumably dependent on dynamic conformational changes, factors that influence these properties would impact translocation. For example, opening of the translocon may involve the movement of a "plug" domain in Sec61 that ordinarily occupies the region forming the translocation pore (van den Berg et al., 2004). Interactions between Sec61 and any factors that facilitate or hinder plug movement would affect translocation, presumably to differing extents for different substrates. The feasibility of such a mechanism is supported by the idea that the ribosome may loosen the plug domain (perhaps to prime the channel for accepting a signal sequence). Indeed, electrophysiological and biochemical assays suggest that translocons can be conductive to small molecules when bound to a nontranslating ribosome (for review see Lizak et al., 2008). Indirect effects on the stability of specific Sec61 conformations may also explain how trans-acting factors such as BiP can influence gating and conductivity of the Sec61 channel (Hamman et al., 1998). Other conformational changes, such as lateral opening of the Sec61 complex toward the lipid bilayer, are also likely to be involved in signal recognition and may therefore be subject to modulation by trans-acting factors. Alterations in such properties of the Sec61 complex would alter the basal translocation activity for many substrates, which in turn could influence their relative dependence (either increased or decreased) on trans-acting factors that operate by the first two mechanisms proposed in the previous paragraphs. In this manner, the substrate range of the Sec61 complex could be tuned. Subtle differences in gating or lateral opening might underlie the observed differences in substrates accommodated by two homologous Sec61 complexes in yeast (Wittke et al., 2002).

And finally, the converse of each of these mechanisms can also be envisaged: factors that selectively weaken, destabilize, or otherwise obstruct some signal sequences; cytosolic proteins

that trap the nontranslocated conformation upon its transient exposure; or factors that stabilize the closed conformation of the Sec61 complex. How or when such mechanisms are used remains unknown. Nonetheless, the concept that the substrate selectivity of Sec61 can be reversibly altered is dramatically illustrated by the discovery of small molecules that inhibit translocation in a signal sequence–dependent manner (Besemer et al., 2005; Garrison et al., 2005) by direct binding to Sec61α (MacKinnon et al., 2007). Thus, by a combination of both positive and negative mechanisms that act substrate selectively by multiple interdependent mechanisms, a highly selective and graded regulation of translocation becomes plausible. It is worth emphasizing that the Sec61 complex directly associates with numerous partners in mammalian systems (e.g., Sec62, Sec63, p180, Mtj1, RAMP4, TRAP, TRAM, and others), the full functions of which are very poorly defined. Given that these factors were all identified solely from one highly specialized tissue (exocrine pancreas), additional (nonessential but regulatory) factors may remain to be discovered. The recent identification of multiple forms of oligosaccharyl transferase that seem to be differentially expressed illustrates that even universal functions such as glycosylation are regulated (Kelleher et al., 2003).

Diversity in trans-acting factor functions

A key facet of regulation is that putative trans-acting modulatory factors need be responsive to cellular need. In this manner, contextual inputs from the environment or other cellular pathways can be converted into appropriate outputs, which in this case would involve a selective change in the translocation of some but not other substrates. Several possibilities can be envisioned for how this might occur. One of the simplest mechanisms is differential expression, either in a developmental or tissue-specific manner. Although this has not been studied in any systematic manner, components of the TRAP complex do appear to be under regulatory control in some organisms (Holthuis et al., 1995). The α subunit was recently also found to be made in two forms (generated by alternative splicing) that differ only in a small charged cytosolic domain (Mesbah et al., 2006). Remarkably, the two isoforms are differentially expressed both developmentally and tissue selectively. Similar alternative splice variants have been described for Sec62 in *Drosophila melanogaster* (Noel and Cartwright, 1994). Again, the difference lies exclusively in a short highly charged region that faces the cytosol. Other examples of translocon-associated proteins being regulated by expression, splicing, or other means may also exist but have not been investigated systematically.

In addition to differential expression, many of the translocon components can be phosphorylated, typically on the cytosolic side (Prehn et al., 1990; Ou et al., 1992; Gruss et al., 1999). The reasons are not known, but phosphorylation could influence the stability, association with the translocon or ribosome, or functional activity. The observation that the charge distribution of the cytosolic face of the translocon can be influenced by both phosphorylation and alternative splicing is intriguing from the standpoint of signal sequence recognition. The n region of signals (preceding the hydrophobic core; Fig. 1 C, green residues) is highly variable in length and charge, and the analogous region of TMDs influences its orientation relative to the membrane (Higy et al., 2004). Thus, alterations in translocon charge distribution could easily influence the orientation or stability of signals as it can for TMDs (Goder et al., 2004). Such an effect could be highly selective depending on the translocon site that is altered, the precise features of the signal sequence, and other factors influencing signal–translocon interactions. In this manner, a commonly used reversible modification, such as phosphorylation, can be envisioned to rapidly change substrate translocation in a highly selective manner.

And finally, regulatory factor availability could be modulated simply by titration. This is thought to occur with lumenal chaperones during acute ER stress, resulting in a selective reduction in translocation only for those proteins whose signal sequences dictate their dependence on the titrated factors (Kang et al., 2006). This appears to be a simple yet effective way to match maturation factor availability with substrate entry into the ER, thereby minimizing the risk of excessive protein misfolding. A similar titration effect might operate on TRAP, which was recently implicated in the ER-associated degradation pathway (Nagasawa et al., 2007). Thus, elevated flux of substrates through the degradation pathway might result in selective translocational attenuation of particular (TRAP-dependent) substrates.

Physiological implications

The broader physiological importance of translocational regulation remains to be studied. However, at least two general reasons for translocational regulation are foreseeable. The first is quantity control: regulating the entry of a protein into the ER controls the precise amount that engages the biosynthetic versus degradative machinery. Indeed, this appears to be the purpose of stress-dependent translocational attenuation (Kang et al., 2006). By controlling the amount of certain substrates that are allowed to engage the biosynthetic machinery in the ER during stress, the limited maturation capacity of a stressed ER can be prioritized to the most essential secretory and membrane proteins. Conversely, by directly routing these translocationally attenuated proteins for degradation by the proteasome (a process termed preemptive quality control; Kang et al., 2006), the retrotranslocation machinery can be spared unnecessary burden at a time of high flux. More selective regulation under some conditions (e.g., in response to specific signaling pathways) or more generalized regulation at other times (such as during mitosis) are plausible but have not been investigated. Quantity control might also be affected at the targeting step, perhaps by modulation of the function or abundance of normally limiting amounts of SR (Lakkaraju et al., 2008).

A qualitatively different purpose for translocational regulation is to control a protein's localization and therefore function. For this purpose, the nontranslocated population would need to both avoid degradation and be functionally useful for some cellular process. Several signal-containing proteins have been proposed to have functional properties in the cytosol (or other compartment outside the secretory pathway). Thus, intrinsic inefficiency in signal sequence function that originally necessitated degradation of the nontranslocated protein is proposed to have been exploited during evolution for functional benefit.

One example seems to be the ER lumenal protein Crt (Calreticulin), whose minor cytosolic population has been implicated in regulation of steroid hormone receptor function, nuclear export, and integrin function (for review see Michalak et al., 1999). Mechanistic studies showed cytosolic Crt to arise, at least partially, as a consequence of its slightly inefficient signal sequence (Shaffer et al., 2005). Hence, improving signal efficiency reduced the cytosolic population with a corresponding effect on glucocorticoid receptor function. Another example might be $p58^{IPK}$ which is capable of interacting with and inhibiting stress-regulated kinases such as PKR and PERK in the cytosol (Lee et al., 1994; Yan et al., 2002). Surprisingly, $p58^{IPK}$ was recently found to contain a signal sequence that directs most of it into the ER lumen, where it interacts with and modulates the activity of BiP (Rutkowski et al., 2007). Whether the putative role of $p58^{IPK}$ in regulating protein degradation (Oyadomari et al., 2006) requires its presence in the cytosol or ER lumen (or both) remains to be investigated. And finally, in *Chlamydomonas reinhardtii*, a PDI homologue appears to be partitioned between both the ER and chloroplasts where it serves independent functions (Levitan et al., 2005). Dual localization seems to be mediated by inefficient cotranslational translocation into the ER followed by posttranslational targeting to chloroplasts. Thus, translocational inefficiency can be exploited for functional benefit. Furthermore, regulation of this step could be used to modulate specific cellular processes. This concept of multifunctional proteins generated as a consequence of translocational regulation is likely to be applicable to other organelles as well (for review see Karniely and Pines, 2005).

Because translocational regulation, whether for quantity control or multifunctionality, generates multiple products in different cellular compartments, there is an increased risk of inappropriate interactions upon misregulation. Indeed, there are numerous suggestions of secretory pathway proteins making adverse interactions with cytosolic components during the course of certain diseases. For example, apolipoprotein E interacting with cytoskeletal components to incite neurofibrillary tangles (Huang et al., 2001), Alzheimer's precursor protein targeting to mitochondria and initiating apoptosis (Devi et al., 2006), prion protein interacting with Bcl2 family members (Roucou and LeBlanc, 2005) or ubiquitin–proteasome pathway (Kristiansen et al., 2007), and numerous other observations. Such findings have often been discounted in the absence of a mechanism explaining how signal-containing proteins could reside in the cytosol. With a growing appreciation that signal sequence efficiency is rather variable in vivo (Levine et al., 2005) and potentially regulated (Kang et al., 2006), such interactions now become plausible, especially under conditions of diminished cytosolic degradation capacity as might occur during aging or other disease states (Grune et al., 2004). Indeed, cytosolic prion protein accumulation upon proteasome inhibition (Yedidia et al., 2001; Ma and Lindquist, 2001) is a direct consequence of signal sequence inefficiency and can be entirely avoided with a constitutively efficient signal (Rane et al., 2004). Thus, the greater flexibility and physiological responsiveness afforded by translocational regulation may come at the cost of increased susceptibility to perturbation that can contribute to various diseases.

It is clear that in addition to developing a working framework for the plausible ways that translocation might be regulated, it will be important to identify additional tractable model systems. Although the study of essential and constitutive facets of translocation has required simple and highly robust model systems, the study of regulation will probably necessitate more complex substrates and potentially new experimental methods. From a physiological standpoint, small changes (e.g., twofold or less) of key secretory and membrane proteins, such as hormones and surface receptors, can be highly significant but difficult to study. Clearly, a move toward nonmodel substrates analyzed in more diverse experimental systems using well-defined and novel assays will be required to develop the physiological facets of translocational regulation. Furthermore, as in other fields, the consequences of misregulation may be more nuanced than defects in basic translocation (Zimmermann et al., 2006). For example, mice disrupted for the translocon accessory component RAMP4 display an ER stress–related phenotype (Hori et al., 2006). In addition, humans containing a mutant Sec63 develop polycystic liver disease (Davila et al., 2004; Waanders et al., 2006). Even disruption of TRAPα, an integral component of native translocons (Menetret et al., 2005), allows embryonic development to proceed surprisingly far (indicating that many cell types are grossly unaffected) until defects in heart development causes lethality (Mesbah et al., 2006). Hence, the study of translocational regulation may require analyses in more complex organisms and systems (such as *Caenorhabditis elegans*, *D. melanogaster*, or mouse models) than have yet to be used in this field. However, it is anticipated that as greater mechanistic insights are obtained from biochemical analyses, more precise tools to manipulate translocation in vivo will become available. Indeed, such initial insights into signal sequences and their varied dependence on trans-acting factors from in vitro studies subsequently allowed the manipulation of translocational regulation during ER stress to provide the first glimpses of its physiological importance (Kang et al., 2006). Much remains to be explored in this emerging direction of a classical area of cell biology.

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