A platform for compartmentalized protein synthesis: protein translation and translocation in the ER
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Recent advances in the study of protein translocation across the membrane of the endoplasmic reticulum include insights into the mechanism of signal-sequence function. Biochemical and genetic studies have provided further evidence that luminal proteins perform direct roles in secretory protein translocation and in the regulation of protein-conducting-channel permeability during membrane protein integration. A hypothesis identifying the endoplasmic reticulum as a site of mRNA localization and compartmentalized protein synthesis has been suggested.

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Abbreviations
BIP binding protein
SRP signal-recognition protein

Introduction
Protein translation and translocation in the ER serves as the entry point for directing secretory and membrane protein precursors into the exocytic and protein-sorting pathways of the cell. In current models, ER-bound ribosomes reside in association with the translocon, the cadre of ER-membrane components that participate in protein translocation, to direct the transfer of nascent polypeptides across, and integration into, the ER membrane. In this view, the membrane-bound ribosomes of the ER participate solely in the translation of secretory, integral membrane, and resident ER luminal and membrane proteins. Recent investigations suggest, however, that membrane-bound ribosomes may also participate in the synthesis of cytosolic proteins.

In this review, I will cover the factual and the hypothetical. I will discuss recent advances in the study of the mechanism of protein translocation across the ER, giving particular emphasis to recent insights into the function of the signal sequence in the initiation of protein translocation across the ER. I will also discuss the role of luminal proteins in the regulation of protein-conducting-channel permeability and as essential partners in the ‘thermal ratchet’ mechanism of vectorial transport. A hypothesis will be presented to explain long-standing and enigmatic observations regarding the mechanism of ribosome exchange on the ER membrane. As a foundation for this hypothesis, I will present a model that identifies the ER as a primary site for mRNA localization in the cell and a platform for compartmentalized protein synthesis.

The shape of things to come
Insights into the mechanism of protein translocation have greatly benefited from recent structural biology studies. With the advent of three-dimensional image reconstruction models of the translocation channel, in complex with a bound ribosome and translocating nascent chain, detailed hypotheses regarding the coupling of protein translation and protein translocation can now be properly examined. My discussion will focus on the most recent structures of the ribosome–nascent-chain–Sec61p complex [1••,2••]. As noted in these landmark papers, the ribosome–membrane junction has long been thought to serve a critical role in the regulation of protein translocation. Thus, in current views, the membrane-bound ribosome participates in the formation of an ion-impermeable seal with the ER membrane, thereby providing a sterically constrained path for nascent chain transit from the ribosomal exit site to the protein-conducting-channel component of the Sec61p complex [3,4].

Interestingly, image reconstruction studies of the ribosome–nascent-chain–Sec61p complex junction display a gap of 15–20 Å that may function to provide an exit site for the nascent chain to the cytosol [1••,2••]. Although it remains to be determined how an ion-impermeable junction is maintained in the face of such a large discontinuity, the discovery of this structural feature has guided recent thinking on the mechanism of polytopic membrane protein integration. As proposed by Menetret et al. [1••] and Beckmann et al. [2••], the gap region could function to accommodate the intervening cytosolic loops of the membrane protein precursor, while providing for a stable ribosome–membrane junction. Also as noted by these authors, the gap region might also function in the process of ‘pause-transfer’, where transient exposure of the translocating nascent chain to the cytosol occurs [5,6]. Menetret et al. [1••] also propose that this structural feature might also provide an essential nascent chain exit site, in the event that a ribosome engaged in the synthesis of a cytosolic protein should bind to the channel.

In summary, the detailed images of the ribosome–nascent-chain–protein-conducting-channel complexes identify a stable macromolecular complex containing a protected region comprising both portions of the ribosomal exit tunnel and the ribosome–channel gap region. This functions to allow the nascent chain to sample the interior of the protein-conducting channel and undergo accurate selection for translocation.

In the beginning
The process by which signal sequences regulate protein-conducting-channel permeability — a process referred to as
‘gating’ — has proven to be a challenging topic of study. Following the pioneering studies of von Heinje [7,8], which identified a canonical structure for the signal sequence, and the many observations demonstrating that signal sequences were autonomous and transferable, the predominant view has been that signal sequences are degenerate domains whose function requires a central hydrophobic core region. A recent study from the Hegde laboratory indicates that this view is oversimplified, however [9•]. In detailed biochemical studies of signal-sequence function in the early stages of translocation, wherein the ‘tight’ ribosome–membrane junction is formed, Kim et al. [9•] report that signal sequences are not strictly autonomous. Rather, signal-sequence composition and activity can be functionally linked to that region of the nascent chain immediately carboxy-terminal to the signal sequence. In this context, it appears that signal sequences, although in absolute terms functionally autonomous, have undergone evolutionary selection to direct efficiently the translocation of their companion mature domains. This phenomenon is likely to be of particular relevance to those signal sequences whose mature domains, because of charge, hydrophobicity or propensity to rapidly assume stable secondary structure, are relatively poor substrates for protein translocation.

Welcome to the ER lumen

In early theoretical analyses of protein translocation in the ER, a ‘thermal ratchet’ mechanism was proposed whereby interactions of the nascent protein chain with lumenal chaperone proteins provide a biasing function, allowing net vectorial transport [10]. In this model, thermal energy supports the random, bidirectional movement of the translocating nascent chain in the protein-conducting channel. Lumenal chaperone proteins, by virtue of their ability to bind unfolded proteins, associate with the nascent chain on each random excursion into the ER lumen and thereby prevent retrograde motion. The sum of many such interactions provides for vectorial transport, in the absence of any directed, motor-like function of the translocation machinery. Experimental evidence supporting this view was first provided in studies of the translocation activity of ER proteoliposomes, reconstituted with or without the native cohort of lumenal proteins [11]. In these studies, proteoliposomes lacking lumenal proteins were observed to allow bi-directional transport of signal-cleaved nascent chains [11]. In the following years, these observations were confirmed and extended, and this system has now undergone extensive mathematical modeling [12•,13•]. Such analyses are yielding valuable predictions concerning the rate constants for chaperone–nascent-chain interactions, and the structural features of the nascent chain necessary for efficient translocation [12•,13•]. Interestingly, in the mammalian ER it is thought that binding protein (BiP) serves the predominant — if not dominant — function in translocation. Recent studies in yeast [14•], however, have identified novel lumenal chaperone proteins whose function is essential for vectorial transport. Interestingly, in yeast, BiP (Kar2p) function is also essential for co- and post-translational translocation. In the context of any thermal ratchet model, it is likely that all structural modification of the nascent chain — in particular, disulfide-bond formation and N-linked glycosylation, because they can occur co-translationally — also contribute to biasing [15,16•].

Lumenal proteins — particularly BiP — serve additional roles in the regulation of protein translocation. The Johnson laboratory has now demonstrated that BiP functions in a process of concerted lumenal/cytosolic gating of the protein-conducting channel, which accompanies the integration of a polytopic membrane protein [17••]. These studies extend previous observations from the Johnson laboratory demonstrating that BiP performs a gating function on the luminal side of the protein-conducting channel, coincident with secretory chain access to the ER lumen [3].

These studies, combined with recent structural insights discussed above, indicate that the protein-conducting channel receives regulatory input from both the cytosolic domain — provided by the bound ribosome and the relevant topogenic signals of the nascent chain — and the luminal domain — through interactions of luminal chaperones with both the nascent chain and, apparently, the luminal domains of the Sec61 complex.

What about mRNA?

Recent advances in the structural biology of the ribosome–nascent-chain–protein-conducting-channel complex have provided a compelling framework for the next level of studies into the regulatory biology of protein translocation. It now becomes necessary to integrate these findings into a broad cell biological context of protein translation and translocation in the ER. In particular, I wish to draw attention to mRNA, a long-ignored, yet obviously essential, component of this process.

According to current opinion, protein translocation in the ER serves, by default, to segregate mRNAs. This process of mRNA segregation is a simple extension of the accepted model of signal-recognition protein (SRP) function, wherein SRP selects those ribosomes engaged in the synthesis of secretory or integral membrane proteins and directs their targeting to the ER. Intrinsic to this model is an important assumption: all protein synthesis is initiated in the cytosol. And it is in the cytosol that nascent chain selection and segregation is initiated. This view is a logical extension of the finding that SRP can mediate the targeting of newly initiated ribosome–nascent-chain complexes from the cytosol to the ER [18]. The complementary reaction to ribosome targeting then becomes the dissociation of ribosomal subunits and mRNA from the ER membrane upon the termination of protein synthesis. This then yields a cycle of ribosome binding and release wherein ribosome residence on the ER is transient, and limited to the time necessary to complete protein translation and translocation.
A model for ER compartmentalization of protein synthesis. In this hypothesis, mRNAs are targeted to the ER by either of two pathways. (a) In one pathway (SRP-dependent), cytosolic ribosomes initiate the translation of a secretory, membrane or resident ER luminal protein, and the ribosome-mRNA-nascent chain complex is targeted to the ER membrane via the signal recognition particle (SRP) pathway. The targeted ribosome-mRNA-nascent chain complex then binds to the Sec61 component of the translocon and the nascent chain engages the translocation machinery. A sequence of de novo initiation events leads to the formation of a polysome on the ER membrane, where all ribosomes are bound to translocons. A circular form of the polysome is depicted; this circular conformation arises through direct, physical association of the 3′ and 5′ termini of the mRNA, as mediated by polyA-binding protein (3′ terminus) and the eIF4G component of the eIF4F complex (5′ terminus) [29]. (b) In a second pathway (SRP-independent), a subset of mRNAs encoding cytosolic proteins is targeted to the ER. In this pathway, such mRNAs are proposed to bear cis ER-targeting signals in their 3′ and/or 5′ untranslated regions. The cis ER-targeting signals undergo recognition by one or more trans-acting RNA-binding proteins. The trans RNA-binding proteins could be either resident ER or soluble proteins. In the case of the latter, a resident ER protein would serve as the interacting partner to provide ER localization. Once targeted to the ER, this class of mRNAs would serve as a template for protein synthesis by translocon-bound ribosomes. As in the first pathway, the mRNA is depicted to reside in a circular conformation. The two pathways may not be entirely exclusive; it is possible that all mRNAs whose translation need occur on ER-bound ribosomes are targeted to the ER before translation. It is predicted that ER-associated mRNAs will reside in association with ER-bound ribosomes for the lifetime of the mRNA, and thus the synthesis of the respective protein substrates will be compartmentalized to the ER for this time period.

Recent in vivo and in vitro studies from my laboratory indicate, however, that the termination of protein synthesis is accompanied by the continued association of ribosomal subunits with the ER membrane [19,20]. Furthermore, using an in vitro system whereby protein translation activity was limited to membrane-bound ribosomes, it was demonstrated that membrane-bound ribosomes are fully capable of initiating de novo protein synthesis, while remaining membrane-bound [19].

Importantly, very recent studies have demonstrated that in the cell, ribosomes reside in association with the Sec61p protein-conducting channel complex throughout the cycle of protein translation [21]. Intriguingly, membrane-bound ribosomes do not distinguish between mRNA substrates and therefore can initiate the translation of any protein, regardless of whether it is cytosolic or destined for translocation [19]. However, when membrane-bound ribosomes were provided with mRNAs encoding model cytosolic proteins, subsequent translation yielded the release of the ribosome–nascent chain complex from the ER to the cytosol [19,22•]. Thus, in contrast to the models where membrane-bound ribosomes engaged in the synthesis of cytosolic proteins would be expected to extrude the nascent chain through the 15–20 Å junctional gap [1••], the absence of a topogenic signal leads to ribosome dissociation from the protein-conducting channel [19].

To explain these results, Potter et al. [19,22•] proposed that the affinity of the ribosome for the protein-conducting channel is decreased during the protein elongation cycle. In the absence of a topogenic signal, this postulated decrease in affinity would ultimately lead to ribosome dissociation from the membrane. Importantly, this model provides a mechanism for the observation, established decades ago, that cytosolic and membrane ribosomes...
comprise a common pool [23,24] and therefore must undergo exchange (although following termination of secretory or membrane protein synthesis, ribosomes, as noted, do not leave the membrane).

What further predictions are evident from such a model? Perhaps the most significant is the prediction that ribosome exchange on the ER membrane requires that membrane-bound ribosomes engage in the synthesis of cytosolic proteins. If this is indeed the case, at steady state mRNAs encoding cytosolic proteins should be readily evident on membrane-bound polysomes. This prediction is a difficult one to accept, as current models stipulate that it is the charge of SRP to direct the segregation of mRNAs, by virtue of their functional association with ribosomes, into membrane-bound (secretory/membrane protein-encoding) and free (cytosolic protein-encoding) pools. Nonetheless, early studies comparing the population similarities of mRNAs derived from membrane-bound and free polysomes demonstrated significant overlap between the two pools [25,26].

This observation has, to a degree, been lost in the literature. The advent of cDNA microarray analyses has again brought it to the forefront: in a microarray-based screen for novel secretory and membrane proteins, the Brown and Botstein laboratories recently reported that mRNAs encoding cytosolic proteins are well represented in the ER-membrane-bound polysome fraction [27••]. Significantly, the overlap was not a simple stochastic event, indicative of a contamination artefact, but rather was mRNA-specific. Therefore, a given mRNA was found to reside either in association with cytosolic polysomes, with both cytosolic and membrane-bound polysomes [27••]. Importantly, these authors speculated that ‘the apparent misclassification of some mRNAs [as determined by their recovery in membrane-bound polysomes] may reflect features of sub-compartmentalization’. With regard to the current discussion, these findings provide important evidence for the proposal that protein translation on the ER is not limited to secretory and membrane protein synthesis. They also provide corroborative evidence for the hypothesis that ribosome exchange on the ER membrane is dependent on, and driven by, the translation of cytosolic proteins by membrane-bound ribosomes.

In keeping with the intended purpose of this review, I propose that the phenomenon of mRNA localization to the ER extends beyond the regulation of ribosome exchange on the ER membrane. Thus, I formally hypothesize that mRNAs encoding a subset of cytosolic proteins are localized to the ER, such that the synthesis of their encoded products is compartmentalized to the ER membrane. Drawing from established examples of mRNA localization during early development [28], I further propose that such mRNAs bear distinct cis-localization signals, presumably present in the 3′- or 5′-untranslated regions of the mRNA, or both, and that trans-acting factors exist that function in the localization of such signal-bearing mRNAs to the ER. This is illustrated in Figure 1. The biological requirement for such localization is not yet clear, but it can be readily speculated that the protein translation products of this subclass of mRNAs might contribute to functions such as membrane-protein folding and/or the identification and processing of membrane-protein misfolding, signal transduction from the ER, and the regulation of protein traffic into the nucleus. Regarding the latter, it is interesting to note that in yeast, HAC1 mRNA, which encodes a soluble transcription factor, is highly enriched on ER-membrane-bound ribosomes [27••]. Perhaps by localizing the synthesis of Hac1p (and possibly other transcription factors) to the surface of the ER, a scenario is created wherein highly efficient, regulated nuclear transfer could occur, in much the same manner as higher-order organization of metabolic enzymes provides the kinetic advantages of substrate shuttling.

Conclusions

With the recent acquisition of high-resolution structural data on the translocation machinery, future studies on the mechanism of protein translocation in the ER can now be driven by structural considerations and, equally as important, structural constraints. The future should therefore promise insights into the mechanism of topogenic signal gating of the protein-conducting channel, the physical properties and regulation of the channel, and ultimately, a complete kinetic and thermodynamic understanding of protein transport across the ER membrane. It is my hope that such studies will extend to the broader cell biological context of the compartmentalization of protein synthesis, to yield much-needed insights into the mechanism and biological basis for mRNA localization to the ER. It is, after all, an RNA world.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


Using image reconstruction of purified ribosome-translocon complexes, the authors provide significant details of the structural organization of the protein-conducting channel and its interactions with the bound ribosome.


In providing high-resolution image reconstructions of the ribosome–protein-conducting channel complexes, significant new details of the ribosomal-pore interface are provided. Of particular interest is the finding that ribosomal RNA expansion segment 27 displays a dynamic relationship with the protein-conducting channel.


