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Protein Movement Across Membranes

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PREFACE

For cells to function properly, correct protein localization is essential. This is true for both prokaryotes, i.e., Bacteria and Archaea, where proteins may be directed outside the confines of the cytoplasm to take up residence in the plasma membrane or beyond, as well as for eukaryotes, which also have to ensure that selected proteins are correctly distributed between the various organelles found inside the cell. Such non-cytoplasmic proteins must, therefore, be effectively recognized and targeted to their designated subcellular locations, where translocation across one or more membranes takes place. Across evolution, cells have developed complex systems dedicated to the transfer of proteins across a variety of biological membranes. In this volume, aimed at both the newcomer seeking an introduction to the subject and the expert wanting to keep abreast of recent discoveries in the field, the reader will learn about various aspects of protein translocation across a variety of membranes.

Translocation of exported proteins in each of the three domains of Life is the focus of the first four chapters. In Chapter 1, recent findings and outstanding questions regarding protein translocation across the membrane of the endoplasmic reticulum, the first step on the eukaryal secretory pathway, are presented. Chapter 2 provides insight into the latest discoveries in bacterial Sec-dependent translocation. In Chapter 3, current understanding of protein translocation in Archaea is discussed. Chapter 4 reveals how structural biology joins genetics and biochemistry as experimental approaches being employed to better understand translocation through the Sec translocon.

Indeed, as we learn more about protein translocation, previously hidden aspects of the process are being uncovered. Chapter 5 addresses strategies adopted by Bacteria for the integration of membrane proteins from a structural perspective. In Chapter 6, the twin arginine transport system, a more recently-defined translocation system largely employed for the transit of folded and complexed proteins across the membrane, is discussed. Chapter 7 describes how the endoplasmic reticulum exploits the Sec-based translocon for retrograde translocation of defective proteins back into the cytosol, where they undergo proteasome-based degradation.

Finally, several chapters examine the manner by which proteins are imported into different cellular organelles. Playing central roles in cellular metabolism, the chloroplast, mitochondria and peroxisome obtain most, if not all, of their proteins from sites of synthesis in the cytoplasm. Chapter 8 addresses how protein translocation into and across the membranes surrounding the chloroplast and the various sub-compartments contained therein takes place. Chapter 9 considers how proteins are delivered from outside the mitochondria into either the matrix or the inter-membrane space, as well as how outer and inner membrane proteins are inserted. In Chapter 10, current

understanding of one of the least-well described protein import systems, namely that of the peroxisome, is considered.

With biological investigators now able to simultaneously address numerous complex processes at the cellular, system and even entire organism levels, a more thorough understanding of protein translocation is essential. This volume represents a step in that direction.

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CHAPTER 1

Protein Translocation Across the Endoplasmic Reticulum Membrane

Ramanujan S. Hegde*

Abstract

Proteins to be secreted from eukaryotic cells are delivered to the extracellular space after trafficking through a secretory pathway composed of several complex intracellular compartments. Secretory proteins are first translocated from the cytosol into the endoplasmic reticulum (ER), after which they travel by vesicular trafficking via various intermediate destinations en route to the plasma membrane where they are released from the cell by exocytosis. By sharp contrast, secretion in prokaryotes involves the translocation of proteins directly across the plasma membrane. While these two systems are superficially dissimilar, they are evolutionarily and mechanistically related. This relationship between the prokaryotic and eukaryotic systems of secretion forms the backdrop for this chapter focused on protein translocation into the ER. In the first part of this chapter, the essential steps and core machinery of ER translocation are discussed relative to evolutionarily conserved principles of protein secretion. The last section then explores the concept of regulation, a poorly understood facet of translocation that is argued to be evolutionarily divergent, relatively specific to the ER, and likely to be most highly developed in metazoans.

Reductionistic View of ER Translocation

The eukaryotic secretory pathway is thought to have evolved by a series of steps that were initiated by specialization of the prokaryotic plasma membrane (Fig. 1). This specialized region of membrane was then expanded, internalized, and eventually subdivided into many compartments. Hence, the lumenal space of compartments in the secretory pathway is topologically equivalent to the extracellular space, and the transport of proteins across the prokaryotic plasma membrane is directly analogous to transport into the ER. Both processes face the same basic challenges: (a) substrates to be transported need to be **recognized**, (b) selectively **targeted** to the site of transport, (c) vectorally **translocated** across the membrane, and (d) maintain a **permeability barrier** during these events. At the most fundamental level, these obstacles must have been solved in even the earliest life forms. This realization, together with the evolutionary relationship between the eukaryotic ER and bacterial plasma membrane, suggests a substantial conservation of the core principles of secretory protein translocation. Thus, assorted data using various model substrates from multiple systems (e.g., Bacteria, Archaea, yeast, and mammal)

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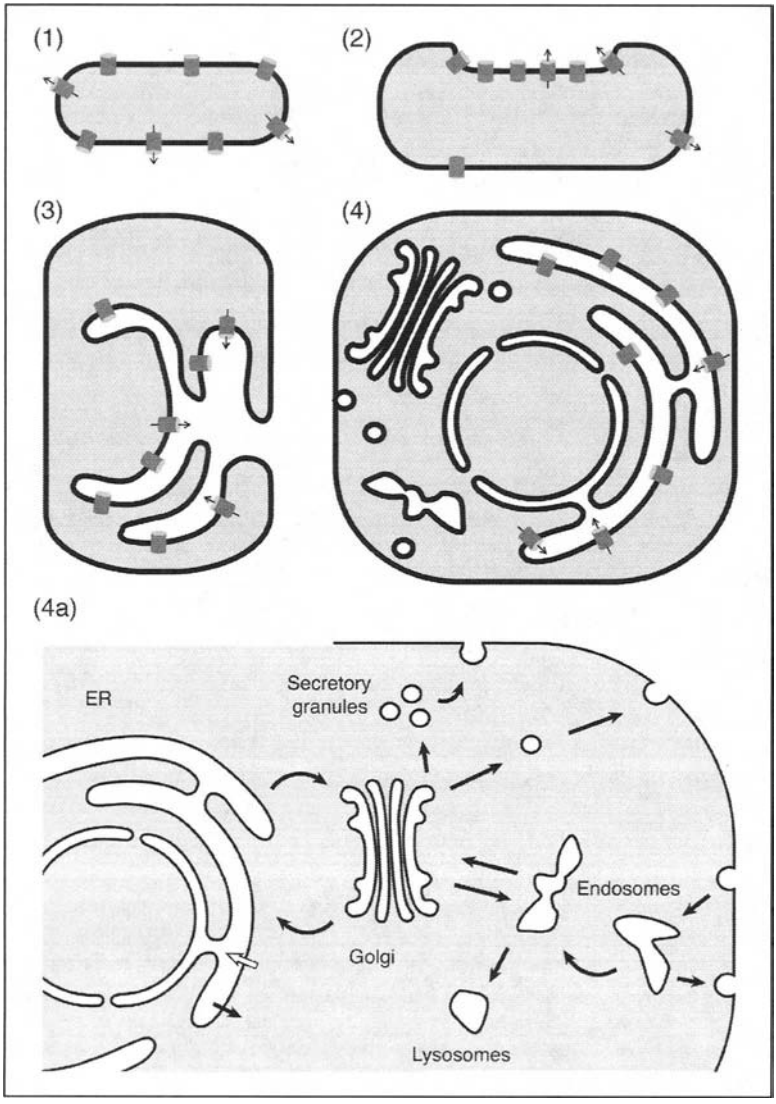

Figure 1. Evolution of the eukaryotic secretory pathway. Steps (1) through (4) depict successive stages in the generally accepted view of eukaryotic secretory pathway evolution from a prokaryotic ancestor. The cytoplasm is shown in gray, and translocons for protein secretion are depicted by cylinders with the direction of polypeptide transport indicated by an arrow. Note the relationship between secretion across the bacterial plasma membrane (in stage 1) and translocation into the ER (in stage 4). Diagram 4a shows a more detailed view of the mammalian secretory and endocytic pathways, with the primary pathways of protein traffic indicated by arrows. Essentially all of these pathways have been discovered to be regulated in a manner that allows some, but not other substrates to be trafficked in appropriate amounts to meet the changing demands of the cell. Notable examples include quality control at the ER, exit from the ER, sorting at the Golgi, regulated exocytosis, and endocytic sorting and degradation. By contrast, translocation into the ER (open arrow) is often regarded as a constitutive process where the presence of a signal sequence in a protein predetermines its entry into the ER.

and multiple approaches (biochemical, genetic, and structural) have often been consolidated into unifying models of protein translocation that are extrapolated to all systems.[1-4] While this provides a convenient framework for understanding protein translocation in general, it is apparent that further experiments will be required to either validate or revise the models for each individual system.

Basic Principles

Secretory and membrane proteins destined for the secretory pathway are recognized by the presence of hydrophobic domains in either signal sequences or transmembrane segments. N-terminal signal sequences (typically ~15-35 amino acids long) contain a hydrophobic core of at least 6 residues, while transmembrane segments have a hydrophobic stretch of between 16-25 residues. Aside from hydrophobicity, sequences used for the segregation of secretory and membrane proteins have no other features in common.[5,6] Indeed, the requirements are so degenerate that signals and transmembrane domains from prokaryotic and eukaryotic proteins are often functionally interchangeable,[7-9] and a surprising 20% of random sequences can at least partially mediate secretion from yeast.[10] Despite this tremendous diversity, signal sequences direct substrates into one of only two main translocation pathways in eukaryotes. In the cotranslational pathway (studied most extensively in the mammalian system), substrates are translocated across the membrane concurrent with their synthesis by membrane-bound ribosomes. In the post-translational pathway (studied primarily in the yeast system), the substrate is fully synthesized in the cytosol first, and translocated in a ribosome-independent fashion.

In cotranslational translocation, emergence from the ribosome of the first hydrophobic domain (either the signal sequence or transmembrane segment) allows its recognition in the cytosol by the signal recognition particle (SRP).[2,3] The complex of SRP and the ribosome-nascent chain (RNC) is then targeted to the membrane by an interaction with the SRP receptor (SR). At the membrane, the signal sequence is released by SRP, the RNC is transferred to the translocon, and the SRP-SR complex is dissociated. Thus, the targeting cycle culminates with delivery of the RNC to the translocon and recycling of components of the targeting machinery (SRP and SR) for the next substrate.

Nascent chains that are cotranslationally targeted to the translocon must then engage the translocation channel, mediate its opening, and be transported through it across the membrane. The central component of the translocation channel is the evolutionarily conserved heterotrimeric Sec61 complex.[11,12] The Sec61 complex, which has a high affinity for ribosomes,[13] provides a docking site for RNCs without the need for other components. However, docking of an RNC at the translocon is not sufficient to initiate translocation. Rather, engagement of the channel requires a functional signal sequence (or transmembrane domain), whose association with the Sec61 complex represents a second substrate recognition event during cotranslational translocation.[14]

This second recognition step may serve a 'proofreading' purpose to ensure that no non-signal-containing substrates that inadvertently target to the channel can engage it. More importantly, binding of the signal to the Sec61 complex triggers at least three essentially simultaneous changes in the RNC-translocon complex: (a) an increase in stability of the interaction between the RNC and translocon, (b) insertion of the nascent chain into the translocation channel, and (c) opening of the translocation channel towards the lumen.[14-18] Upon successful completion of these steps, the substrate resides in a continuous path running from the peptidyl transferase center in the ribosome, through the translocation channel, and into the ER lumen.[14,15,19] From this point, continued protein synthesis is thought to result in 'pushing' of the nascent chain through the channel and across the membrane.

Hence, the architecture of the RNC-translocon complex[20-22] biases the direction of nascent chain movement, thereby harnessing the energy of protein synthesis to simultaneously drive translocation.

Post-translational translocation operates in several qualitatively different ways. In eukaryotes, this pathway has been studied most extensively in yeast, where a seven protein Sec complex at the ER membrane and the lumenal chaperone BiP (known as Kar2p in yeast) have been identified as the essential translocation apparatus.[23-27] This Sec complex can be conceptually (and experimentally) divided into two sub-complexes: the trimeric Sec61 complex (homologous to the mammalian Sec61 complex), and the tetrameric Sec62/63 complex.[23] The Sec61 complex presumably forms a similar channel in the post-translational Sec complex as it does in the cotranslational translocon.[28] This means that the remaining components (the Sec62/63 subcomplex and BiP) must fulfill the functions otherwise provided in cotranslational translocation by the targeting machinery (SRP and SR) and ribosome, neither of which are involved in post-translational translocation.

Consistent with this idea, the Sec62/63 complex (but not BiP) is essential for signal sequence recognition by the Sec61 complex.[23,27,29] Thus, the Sec complex, by selectively binding signal-containing substrates, mediates targeting to the translocon in a single mechanistic step that replaces the series of targeting reactions involving the ribosome, SRP, SR, and translocon. Once substrate is bound to the Sec complex, the Sec61 translocation channel is thought to be engaged and opened in a similar fashion to the signal-mediated gating step in cotranslational translocation.[30] The substrate would then need to be moved unidirectionally through the Sec61 channel across the membrane.

Since vectorial movement of the substrate through the channel cannot exploit the energy of protein synthesis (as during cotranslational translocation), the actual transport step needs to occur differently. This function of biasing the direction of polypeptide movement is provided by BiP, a chaperone that binds the substrate on the lumenal side of the translocation channel to prevent its back-sliding into the cytosol.[23,25-27,31] Subsequent rounds of binding and release, stimulated by ATP hydrolysis, allows BiP to act as a molecular ratchet to drive substrate transport into the lumen.[32] The ATPase activity of BiP is regulated by Sec63p, a J-domain containing component of the Sec complex, which presumably also serves the function of recruiting BiP to the translocation channel.[27,33] Thus, the substrate is largely 'pulled' across the membrane from the lumenal side in the post-translational pathway, in contrast to being 'pushed' from the cytosolic side in cotranslational translocation.

A comparative analysis of these basic features of eukaryotic cotranslational and post-translational translocation reveals an important central theme (Fig. 2). It has become clear that the actual channel through which the polypeptide is translocated acts as a relatively passive conduit. It only acquires its functionality for substrate recognition and vectorial transport upon interaction with various binding partners. In cotranslational translocation, a key binding partner is the ribosome which acts to mediate translocon assembly, 'primes' the Sec61 complex for signal recognition, and couples the energy of protein synthesis to translocation. In post-translational translocation, the key binding partner is the Sec62/63 complex which, like the ribosome, facilitates translocon assembly, allows signal sequence recognition, and provides the driving force for translocation by recruiting and regulating the function of BiP at the translocation site. Indeed, even in the bacterial system, the homolog of the Sec61 complex (termed the SecY complex) interacts with the cytosolic SecA ATPase that both receives the substrate at the channel and drives its subsequent translocation across the membrane.[34] Thus, the highly conserved Sec61 channel can be exploited in several markedly different ways by various coassociating partners that mediate protein translocation across the eukaryotic ER or prokaryotic plasma membrane.[1,4]

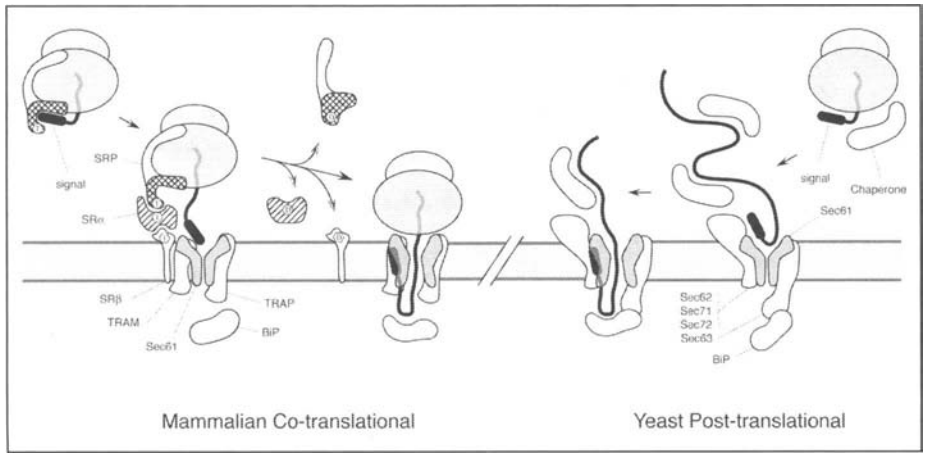

Figure 2. Pathways of ER protein translocation. The principal machinery and steps of the eukaryotic cotranslational and post-translational pathways are shown on the left and right, respectively. The components of each pathway that are conserved in all organisms (in both prokaryotes and eukaryotes) are shaded, and include the signal sequence, ribosome, SRP54 (along with a portion of its associated SRP RNA), SRα, and the Sec61 complex. Various other components that function in each pathway are also shown. The GTP- and GDP-bound states of the cotranslational targeting machinery are displayed with 'T' and 'D' respectively. The center two diagrams depict the comparable 'committed' stages of the two pathways to illustrate that in both, the Sec61 complex serves the same passive role as the channel while the associated components function to keep the polypeptide unfolded and move it vectorally into the lumen.

Molecular Details

Signal sequence recognition and targeting is understood in the greatest molecular detail for the cotranslational (i.e., SRP-dependent) pathway in eukaryotes. This is largely because the remarkable evolutionary conservation of this pathway from Bacteria to mammals has allowed the experimental results from multiple systems and approaches to be combined.[3] In higher eukaryotes, SRP is a ribonucleoprotein composed of six proteins (named by their apparent molecular weights: SRP72, SRP68, SRP54, SRP19, SRP14, and SRP9) and a ~300 nucleotide RNA (termed 7SL RNA or SRP RNA).[35,36] Of these components, SRP54 and a portion of the RNA are directly involved in both signal sequence recognition and the interaction with SR. Indeed, these two components define the minimal SRP that can be found in all organisms of every kingdom of life.[3] In almost all Bacteria, **only** these two components are found, indicating that they can perform all of the recognition and targeting functions necessary for translocation.[37-41]

Structural analysis of SRP54 homologues from several organisms[42-48] has revealed that it is universally organized into three functional segments: the M, N, and G domains. Of these, the M domain recognizes signal sequences via a deep, hydrophobic groove lined by the flexible side chains of several methionines. Phosphates of the RNA backbone are near one end of this groove, and may interact with basic residues that are often (but not always) adjacent to the hydrophobic core of signal sequences and transmembrane domains. These and other conserved features of SRP54 help to explain how it can accommodate a wide range of signal sequences whose only common feature is a hydrophobic segment, and why signals from diversely different organisms are often interchangeable.

In addition to signal sequence recognition, the other essential function of SRP is its interaction with SR to ensure the targeting of nascent secretory and membrane proteins to the

translocon. The tight coordination of the series of interactions that imparts unidirectionality to the targeting phase of translocation is through the regulated GTPase activities of SRP and SR. The GTPase component of SRP resides in the G domain of SRP54 (see ref. 35). In eukaryotes, SR is a heterodimer of α and β subunits,[49] both of which are GTPases.[37,50] Of these, SRα is highly conserved from prokaryotes to mammals and, together with SRP54 and SRP RNA, represents the minimal targeting machinery found in all organisms.[37,41] Detailed mechanistic and structural analysis of this minimal SRP pathway, mostly using the model bacterial system, has revealed the essential aspects of their regulation during cotranslational targeting.

In the current working model, free SRP in the cytosol is in the GDP-bound state. Its association with the ribosome stimulates GTP binding,[51] and subsequent association with the signal sequence inhibits GTP hydrolysis.[52,53] Thus, the signal-SRP-ribosome ternary complex is likely to be in the GTP-bound state. Although less direct evidence exists for SRα, it is thought that its association with a vacant translocon at the membrane (directly in the case of prokaryotes, and indirectly via SRβ in eukaryotes) may similarly allow GTP binding and prevent GTP hydrolysis. Thus, the SR-translocon complex would also be in the GTP-bound state. The GTP-bound forms of SRP54 and SRα have a high affinity for each other,[53] allowing the delivery of signal-containing RNCs to the close proximity of an appropriately vacant translocon.[54,55]

The interaction between the GTPase domains of SRα and SRP54 stimulate the hydrolysis of GTP by each other (thereby acting as GTPase activating proteins, or GAPs, for one another).[56] The change in conformation that accompanies this GTP hydrolysis results in a weakening of the interaction between SRα and SRP54, allowing this complex to be dissociated for another round of targeting.[54,57,58] Many of the molecular details of this generally appealing scheme remain to be elucidated. For example, SRP RNA,[59,60] as well as the translocon[61,62] and the ribosome,[51] clearly facilitate aspects of SRP-SR interactions and their GTPase activities. However, the precise mechanisms remain elusive at the present time. The recently emerging wealth of structural information on SRP and SR should help to illuminate the molecular details of this framework.

Beyond these essential functions performed by the minimal components, the significantly more complex eukaryotic SRP and SR are likely to confer additional functionality and advantages to the cell. One such eukaryotic-specific feature is the slowing of translation upon signal sequence binding by SRP, a phenomenon termed 'elongation-arrest'.[63,64] The mechanism appears to involve occlusion of the elongation factor binding site on the ribosome by the SRP9 and SRP14 subunits of SRP.[65] The resulting decrease in translational rate serves to increase the time available for targeting to the translocation channel before excessive polypeptide synthesis precludes cotranslational transport. While translational attenuation by SRP is not essential for translocation,[66] it appears to be physiologically important under at least some growth conditions in vivo.[67] Whether the other subunits of SRP (SRP68, SRP72, and SRP19), each of which is important for assembly (particularly SRP19) and stability of the complete particle,[68] confer yet additional functionality to eukaryotic SRP remains largely unknown. Similarly, SRβ, a homolog for which does not exist in prokaryotes, is likely to provide the bridge that further regulates the coordinated transfer of RNCs from SRP to the translocon. This appears to be accomplished by the regulation of SRβ GTPase activity by both the ribosome[69] and the translocon,[62] with accompanying conformational changes that are suggested to affect the RNC-SRP54-SRα-SRβ interactions.[70]

Signal sequences and transmembrane domains are also recognized by the translocon at the membrane in all modes of translocation.[14,30,71-73] The purpose of this recognition is two-fold. First, it provides a mechanism for discriminating translocation substrates from other proteins. This is the sole discriminatory step in post-translational translocation, and a secondary (or 'proofreading') step in cotranslational translocation. Second, signal recognition by the translocon is essential for its opening (or gating) in preparation for substrate transport.[14,73] Since the core