Mechanism of Polypeptide Translocation into the Endoplasmic Reticulum*

Ann K. Corsi and Randy Schekman‡

From the Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, California 94720

All polypeptides destined for transit through the secretory pathway begin their journey by crossing the endoplasmic reticulum (ER) membrane, whether their final destination is the ER lumen or membrane, the Golgi apparatus, the vacuole or lysosome, the plasma membrane, or the extracellular milieu. This translocation step presents eukaryotic cells with a number of problems. First, precursors destined for the ER must be distinguished from those that will remain in the cytosol or that are targeted directly to another organelle, such as the nucleus or the mitochondrion. Second, once precursors arrive at the ER membrane, they must engage the integral or peripherally associated membrane proteins of the translocation apparatus (or translocon) that directly aid in protein import. Finally, translocation must be controlled so that precursors of soluble proteins completely cross the membrane while those of membrane proteins only partially translocate so as to attain proper final orientation in the lipid bilayer. In this review, we describe a number of recent observations that help to create an emerging picture of the initial step of protein secretion (for recent reviews, see Refs. 1–3).

Targeting to the Endoplasmic Reticulum

Two pathways of targeting to the ER are known. The first is co-translational whereby the precursor remains tethered to the ribosome, and the second is post-translational whereby the precursor is fully translated and released from the ribosome before engaging the translocation apparatus. In mammalian cells, precursors are primarily targeted co-translationally whereas in yeast (Saccharomyces cerevisiae), both pathways are utilized.

Co-translational Targeting—As secretory proteins emerge from the ribosome during translation, the N-terminal amino acids that constitute the signal peptide provide the “zip code” for initial trafficking destinations. Although the sequences of signal peptides are not conserved, ER targeting is specified by key structural features, for example a central stretch of 7–20 hydrophobic amino acids (reviewed in Refs. 3 and 4). The extent of hydrophobicity of this region helps determine, at least in yeast, the pathway of targeting that a precursor will take to the ER (5). Chimeric precursor substrates were used to demonstrate that a more hydrophobic core dictates co-translational import into the yeast ER (5).

The signal recognition particle (SRP), a complex of six polypeptides and an RNA component, targets substrates for co-translational translocation to the translocon (for reviews see Refs. 6 and 7). Careful analysis of the protein subunits of SRP (named for their mass in kDa) has identified subcomplexes that are responsible for the various functions of SRP. SRP54 binds to the signal sequence as it emerges from the ribosome, probably through a direct interaction with the hydrophobic domain of the signal sequence (6, 7) (Fig. 1a). After a signal sequence has been identified, elongation of the polypeptide is arrested by a subcomplex consisting of SRP9 and SRP14 (6, 7). Although this translocation arrest is not absolutely necessary for translocation (6, 7), this pause may help ensure proper targeting to the ER membrane before significant portions of the polypeptide emerge from the ribosome and begin to fold. A recent observation by Ogg and Walter (8) emphasizes the importance of elongation arrest for co-translational translocation. They found that temperature-sensitive yeast mutants with decreased SRP levels are suppressed by sublethal doses of the translation inhibitor cycloheximide. Thus, slow translation, mimicking arrest, allows the low levels of SRP to complete targeting (8).

Once SRP has engaged the nascent chain-ribosome complex, it targets the complex to the ER translocation machinery (Fig. 1b). A heterodimeric SRP receptor (SR) composed of SRα and SRβ mediates docking (6, 7), after which SRP transfers the precursor to the translocon (Fig. 1c) and then recycles back to the cytosol (Fig. 1d). Interestingly, the SR components, as well as SRP54, have GTP binding domains suggesting that GTP hydrolysis may aid in the regulation of targeting (6, 7). Indeed, reciprocal stimulation of GTP hydrolysis exists between SRP and SR (9, 10). Functional GTP binding domains must be present in both SRP54 and SRα in order for targeting to occur although GTP hydrolysis does not seem to be required (6, 7). Surprisingly, Bacher et al. (11) found that a component of the ribosome stimulates GTP binding to SRP54 suggesting that the presence of the ribosome contributes to regulation of SRP activity. SRP54 in the GTP-bound state promotes high affinity association of SRP with SR (12). Subsequent transfer of the signal sequence to the other translocation components depends on GTP binding by SRα (13). Finally, release of SRP from the receptor induces GTP hydrolysis by SRP54 completes the cycle (10). The function of the GTPase domain in SRβ remains undefined; this subunit can bind GTP, and as an integral membrane protein it has been proposed to anchor the peripherally associated SRα to the ER membrane (14).

Another set of proteins, the nascent polypeptide-associated complex (NAC), is involved in maintaining the fidelity of co-translational precursor targeting to the translocon (15). NAC protects nascent chains as they emerge from the ribosome regardless of the presence of a signal sequence (16) and inhibits interactions between the ribosome and SRP (17) although it does not inhibit SRP from targeting nascent chains with signal sequences (18). By sequestering nascent proteins lacking signal peptides, NAC not only prevents inappropriate targeting to the ER membrane but also precludes ribosomes that are not translating secretory precursors from associating with membranes.

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‡ To whom correspondence should be addressed: Dept. of Molecular and Cell Biology, HHMI, Barker Hall, University of California, Berkeley, CA 94720. Tel.: 510-642-5686; Fax: 510-642-7846.

The abbreviations used are: ER, endoplasmic reticulum; SRP, signal recognition particle; SR, SRP receptor; NAC, nascent polypeptide-associated complex; TRAM, translocating chain-associated membrane protein.
In the absence of NAC, polypeptides lacking signal sequences target to the ER in an SRP-independent manner (15, 19). Still unknown, however, is the precise mechanism of NAC action and how NAC and SRP together mediate correct targeting in vivo.

In co-translational translocation, the majority of available data indicates that the main ribosome receptor in the ER is Sec61α whose function we discuss later (20). Once the ribosome is positioned at the membrane, the signal sequence associates with the bilayer such that the N terminus extends into the cytosol as the rest of the precursor threads into the lumen as an expanding loop (21, 22). Görlich and colleagues (23) postulate that the nascent chain inserts into the ER in a channel continuous with the ribosome channel. At the termination of translation the ribosome dissociates from the ER, possibly as individual subunits that may have lower affinity for proteins in the membrane.

Another early participant in mammalian cells is translocating chain-associated membrane protein (TRAM). Secretory nascent chains can be cross-linked first to SRP and then to TRAM upon the addition of mammalian membranes (24). High et al. (25) introduced a photoactivatable cross-linker at defined positions in a signal sequence to reveal that TRAM contacts the most N-terminal portion of the signal sequence (25). Not all precursors require TRAM for their transit (24, 26); its participation may be dictated by features of specific signal sequences, such as length of the hydrophobic central region and the hydrophilic N terminus (27).

Post-translational Targeting—Unlike co-translational translocation, post-translational translocation occurs independently of SRP in both yeast and mammalian cells (5, 28, 29) (Fig. 2). Because the secretory precursor is completely translated and released from the ribosome, post-translational targeting requires cytosolic components to maintain the polypeptide in an incompletely folded state; in yeast this function is performed by cytosolic Hsp70 proteins (for review see Ref. 30). If urea treatment is used in vitro to unfold precursors, import can occur in the absence of Hsp70s (30). In vivo this process may require regulation of Hsp70 ATPase activity by partner proteins homologous to the Escherichia coli DnaJ protein, known to stimulate ATP hydrolysis by DnaK, a bacterial Hsp70 (reviewed in Refs. 31 and 32). In yeast, this regulatory partner is a DnaJ homolog, Ydj1p (32). Cells harboring mutations in YDJ1 are defective for ER import of multiple precursors (33). Genetic interaction between mutant alleles of YDJ1 and the Hsp70 genes (SSA) confirms the notion that the ATPase cycle of Hsp70, promoted by physical interaction with Ydj1p, serves actively to promote the import of certain secretory precursors (34) (Fig. 2). The details of how Ssa proteins and Ydj1p regulate translocation events at the ER membrane have not been described.

The Translocation Complex

Genetic studies in yeast have identified several proteins that are required for translocation in vivo and in vitro (reviewed in Ref. 1) (Fig. 2). The most prominent of these is Sec61p, which is predicted to span the ER membrane multiple times and is a candidate subunit of the translocation channel (35). Sec61 mutations block the import of all tested precursors (35), and in the presence of ATP, stalled precursor proteins can be cross-linked to Sec61p (36, 37). The gene SSS1 encodes a high copy suppressor that relieves translocation defects in a sec61 mutant strain (38). Ssa1p, together with a third protein Sbh1p, has been purified with Sec61p as a heterotrimERIC complex required for post-translational import in reconstituted proteoliposomes (39). In mammalian cells, Sec61α (homologous to Sec61p) comprises the major multispanning membrane protein (23), and biochemical studies have also identified Sec66α and Sec61γ, which are homologs of Sbh1p and Ssa1p, respectively (26).

In yeast, homologs of Sec61p and Sbh1p (Ssh1p and Sbh2p, respectively) exist that along with the shared protein, Ssa1p, form a second trimer in the ER called the Ssh1p complex (40). This homolog is proposed to function solely in the co-translational pathway because the subunits do not associate with the Sec62p-Sec63p complex discussed below, do associate with ribosomes, and neither Ssh1p nor Sbh2p is an essential gene (co-translational import in yeast is not an essential process because null alleles in SRP are viable) (6, 40). Reconstitution of the Ssh1p complex in a co-translational import assay will help address this hypothesis.

In addition to the Sec61p complex, a second set of proteins called the Sec62p-Sec63p complex is required for post-translational translocation in yeast. This complex includes three integral membrane proteins, Sec62p, Sec63p, and Sec71p, as well as Sec72p, which is peripherally associated with the cytosolic face of the ER, probably through association with Sec71p (1, 39). Point mutations or null alleles in these proteins cause a subset of precursors to accumulate in the cytosol (41–44). Visible null mutations in either SEC71 or SEC72 cause a loss of the other protein from the complex (43, 44).

Sec63p has a luminal region homologous to the J domain, the most highly conserved region of the DnaA family (for review see Refs. 31 and 32). This protein has been shown to form a subcomplex with Sec71p, Sec72p, and BiP (45). BiP is a luminal Hsp70, and genetic data indicate this protein is also re-
required for post-translational translocation (46, 47). BiP probably acts in translocation in a cycle of ATPase activity regulated by interaction with the J domain of Sec63p (45, 48)2 (Fig. 2). Several subunits of the yeast translocon have been implicated as receptors for post-translational precursors. A stalled precursor intermediate can be cross-linked to Sec62p in the absence of ATP, presumably prior to interaction with the ER channel (36). Mutations in SEC72 affect precursor proteins differentially depending on the nature of the signal peptide (44). Recent results suggest that together Sec62p, Sec71p, and Sec72p create a surface for secretory precursors to bind before crossing the ER membrane3 (Fig. 2) and thus may be responsible for signal sequence recognition by the translocon. Sec63p and BiP also function early in transport, because specific mutations in each of these proteins cause precursors to stall in the membrane prior to signal sequence cleavage (37, 48).

### Crossing the Membrane

The minimum translocation apparatus from mammals is comprised of surprisingly few proteins. Görlich and Rapoport (26) found that purified SRP, its receptor, and the Sec61p complex are the only components necessary to achieve co-translational translocation of some precursors into reconstituted proteoliposomes. They also observed that TRAM is required for a subset of precursors and stimulates the import of others (26, 27). In yeast, the minimum translocation apparatus for post-translational translocation into a reconstituted proteoliposome consists of the Sec61p and the Sec62p-Sec63p complexes (39). The additional proteins necessary for yeast translocation could perform gating or target recognition functions that SRP and the ribosome perform in mammals.

Because translocated proteins contain hydrophilic and charged amino acids, the ER membrane must contain a proteinaceous pore that acts as a channel for passage through the hydrophobic ER lipid bilayer. Evidence from electrophysiological studies (49) and investigations of the environment sensed by fluorescent probes incorporated into nascent chains (50) demonstrate the existence of such a pore. Two approaches have been used to identify the Sec61p complex as a channel: cross-linking of precursors in transit and reconstitution of the translocation machinery into artificial proteoliposomes (reviewed in Ref. 51). Nascent chains undergoing translocation may be cross-linked to Sec61p (in yeast) or the homologous Sec61α (in mammals) (see Ref. 1 and references therein). Sec61α is also tightly associated with ribosomes from detergent-solubilized membranes whereas other proteins adjacent to the translocon apparatus are not ribosome-associated (23).

How does a channel present in the ER membrane allow specific passage of proteins while maintaining the environment inside the ER lumen? Simon and Blobel (52) showed that the addition of signal peptides to reconstituted E. coli plasma membranes increases ion conductance across the membranes. In contrast, Crowley and colleagues (53) observed that in mammalian membranes the quenching of fluorescent probes inside the channel by iodide ions introduced into the ER lumen is only possible after the nascent chain reaches a critical length of about 70 residues. Such differences may be explained by the preponderance of post-translational secretion in E. coli and co-translational import into the mammalian ER. In the latter process, the ribosome creates a tight seal around the open translocation pore, rendering the translocating nascent chain inaccessible from the cytosolic side (50). There may be exceptions to this; Hegde and Lingappa (54) recently found that specific sequences in a precursor protein that induce a pause in translocation also cause the nascent chain to be transiently exposed to the cytosol.

The polypeptide conduit must possess a degree of flexibility that allows the passage of precursor molecules both through and into the plane of the membrane. For example, hydrophobic transmembrane domains must be transferred out of the aqueous translocation pore during the biosynthesis of a membrane protein. Using a photoactivatable cross-linker, Do et al. (55) observed that a single membrane-spanning domain first contacts Sec61α and TRAM and then only TRAM before the completed polypeptide is transferred into the lipid bilayer. Borel and Simon (56) showed that during the integration of a truncated mult spanning transmembrane protein into mammalian microsomes, translocation intermediates still attached to the ribosome could be extracted with urea (indicating incomplete integration into the hydrophobic bilayer) but were converted to urea-resistant extraction once the polypeptide was released from the ribosome. Additionally, Martoglio and colleagues (57) demonstrated that the hydrophobic regions in both the signal sequence and transmembrane domains of two different nascent secretory proteins could be cross-linked not only to protein components of the translocon but also to phospholipids during transit. It is possible that the channel converts between configurations that preclude or permit contact with the neighboring phospholipid bilayer, but this topic warrants further study.

### Completing Precursor Transit across the Membrane

For soluble secretory proteins, the final step in the completion of translocation is full transfer from the pore into the ER lumen. This step can be performed in reconstituted proteoliposomes containing the SRP receptor and the mammalian Sec61p complex but devoid of lumenal proteins (26). However, alkaline extraction of luminal proteins (reticuloplasm) from native mammalian microsomes produces a translocation defect that is only repaired by restoring reticuloplasm within resealed vesicles (58). Reticuloplasmatic proteins such as BiP may be necessary in the context of the native ER membrane to protect the nascent chain as it folds in an intricate protein environment. Alternatively, luminal proteins may be required for the translocation apparatus to fully recover from the alkaline treatment used to extract the reticuloplasm (26, 58). Finally, luminal proteins may be necessary to catalyze the cycling of the translocation complex between active and inactive states in a native membrane whereas reconstituted proteoliposomes may already contain an unplugged pore or a translocon in an active state.

In yeast, completion of precursor transit depends on functional Sec63p and BiP; mutations in either protein cause a precursor to stall in the Sec61p pore (37, 48). The presence of BiP and ATP in proteoliposomes reconstituted with the yeast translocation complex promotes efficient translocation (39). We recently have found that association of BiP with the translocon (through binding to the J domain of Sec63p) is required for its function in translocation.2 Interaction with Sec63p may allow BiP to act as a translocation motor (59, 60) reeling in the precursor in a cycle of ATP hydrolysis.

### Energy Requirements

Movement of precursor proteins across membranes requires energy. We discussed earlier how GTP hydrolysis is used in the targeting of SRP to the ER membrane (Fig. 1). In co-translational translocation, as amino acids are added to the growing chain, the elongating polypeptide emerges from the ribosome and continues through the Sec61p channel (26). However, during post-translational translocation, in which polypeptide chain elongation is uncoupled from protein translocation, additional energy (in the form of ATP) is needed (30). Energy is

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2 A. K. Corsi and R. Schekman, submitted for publication.
3 S. Lyman and R. Schekman, submitted for publication.
probably consumed by the luminal ATPase protein BiP, which is required for translocation (37, 46, 47, 61). How does ATP get into the ER lumen? Mayinger and colleagues (62, 63) found that an ATP transporter is required both in vitro and in vivo for efficient translocation. A candidate transporter, Sac1p, is required for efficient protein translocation as well as for ATP-transporting activity in vitro (62, 63) but has not been proven to be the actual ATP transporter.

Perspectives

It now appears that the full complement of translocation components has been identified for both mammalian co-translational and yeast post-translational import. In addition, our understanding of the mechanistic events during translocation has increased. However, a number of intriguing questions remain. What is the stoichiometry of the subunits of the Sec61p complex in the membrane, and how do the three subunits of the complex cooperate in translocation? How is the translocation pore gated both with respect to the cytoplasm (especially in post-translational translocation) and to the surrounding bialayer? How are transmembrane domains transferred from the pore to the membrane? In the coming years, the availability of the pure translocon and continuing biochemical and biophysical investigations should enable us and other interesting questions to be answered.

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