Cell, Vol. 102, 709–712, September 15, 2000, Copyright ©2000 by Cell Press

Minireview

The ER Translocon and Retrotranslocation: Is the Shift into Reverse Manual or Automatic?

Arthur E. Johnson*†‡ and Nora G. Haigh*

*Department of Medical Biochemistry and Genetics

†Departments of Chemistry, and of Biochemistry and Biophysics

Texas A&M University

College Station, Texas 77843

In eukaryotic cells, the sorting of most noncytoplasmic proteins begins at the membrane of the endoplasmic reticulum (ER). Proteins destined for secretion are transported across the ER membrane at sites termed translocons (reviewed in Johnson and van Waes, 1999). In mammalian cells, this translocation occurs cotranslationally, at the same time that the protein is being synthesized by a ribosome. Protein processing, folding, and subunit association then occur, assisted by various chaperones and enzymes located largely in the ER lumen. Similarly, membrane proteins are cotranslationally integrated into the ER membrane at translocons and are then folded, processed, and directed to various cellular surfaces to carry out their function.

However, secretory or membrane proteins that do not fold properly or do not assemble correctly into multicomponent complexes are degraded before embarking on their sorting pathways. This proteolysis eliminates nonfunctional polypeptides, and hence constitutes a quality control mechanism. Such intracellular degradation of secretory and membrane proteins was once thought to occur within the lumen of the ER, but it has become clear recently that most of this proteolysis is instead carried out by proteasomes in the cytoplasm (recent reviews include: Brodsky and McCracken, 1999; Plemper and Wolf, 1999; Römisch, 1999). The discovery that proteolysis occurs in a different compartment than folding and assembly has prompted a new set of questions about how misfolded or unassembled polypeptides are transported from the ER lumen or membrane back into the cytoplasm for degradation.

An attractive candidate for the vehicle that mediates such transport is the translocon. This complex is comprised of several membrane proteins that form an aqueous pore in the ER membrane. Since secretory proteins are translocated from the cytoplasm into the ER lumen through the translocon pore, it is reasonable to suspect that the same pore is used to move polypeptides in the opposite direction, from the ER lumen to the cytoplasm. Thus, several recent studies have examined this translocation in reverse, termed retrotranslocation or dislocation, to ascertain whether the translocon is involved. The combined results of these studies strongly indicate that retrotranslocation occurs through the translocon pore (Brodsky and McCracken, 1999; Plemper and Wolf, 1999; Römisch, 1999; Zhou and Schekman, 1999; Wilkinson et al., 2000). Although unambiguous proof has yet to be reported, it seems an appropriate time to examine the putative role of the translocon in retrotranslocation, and to consider the implications of bidirectional movement through the translocon in terms of what is already known about its structure, function, and regulation.

‡To whom correspondence should be addressed (e-mail: aejohnson@tamu.edu).

Multiple Structural and Functional States of the Translocon

The functional states of the translocon identified or postulated to date include cotranslational protein translocation, cotranslational membrane protein integration, posttranslational translocation, retrotranslocation, and a resting state (Johnson and van Waes, 1999). To carry out each of these functions, the translocon must adopt various structural states that are characteristic of, but perhaps not unique to, a particular function (Figure 1). The different states reveal two important properties of the translocon: its structure is dynamic, and its structure (and hence function) is regulated. It follows that if the translocon engages in retrotranslocation, then the conversion of the multifunctional translocon to the retrotranslocation-specific structural state must be controlled. In this scenario, retrotranslocation is simply one of several functional modes of the dynamic translocon machinery.

Requirements for Translocon-Mediated Retrotranslocation

Although the direction of polypeptide transport is reversed, the functional stages in translocation and retrotranslocation are equivalent. The transport substrate must be identified and targeted to the translocon, the translocon pore must be opened to allow substrate passage, and some mechanism for powering the transport of the substrate through the pore must be provided, all while maintaining the permeability barrier of the membrane (Figure 2). We now wish to consider each of these mechanistic stages in turn as they relate to the translocon.

How Are Retrotranslocation Substrates Recognized and Targeted to the Translocon?

A major mechanistic issue is how the system distinguishes between polypeptides that have stalled during processing and those that are progressing normally to their native states. It seems unlikely that the retrotranslocation signal is a stretch of protein primary sequence because this would be present in every copy of a particular protein and hence could not be used to distinguish between a misfolded protein and a not-yet-folded protein. A more general signal for retrotranslocation is the prolonged exposure of a polypeptide sequence, surface, or glycosylation state that would elicit chaperone binding. Proteins that are stalled at some point during folding and/or assembly would bind to chaperones, so retrotranslocation substrates could be identified by a mechanism that selects long-lived chaperone•polypeptide complexes. Consistent with this possibility, many different long-lived complexes between ER proteins and misfolded or misassembled proteins have been observed, most notably with BiP, calnexin, ERp57, and protein disulfide isomerase (PDI) (e.g., Liu et al., 1999; Gillece et al., 1999; Chillarón and Haas, 2000; Wilson et al., 2000). If this mechanism is correct, the next issue is how many different resident ER proteins select substrates for retrotranslocation. Do the misfolded or unassembled substrates get transferred to a single chaperone that then targets each substrate to the translocon,

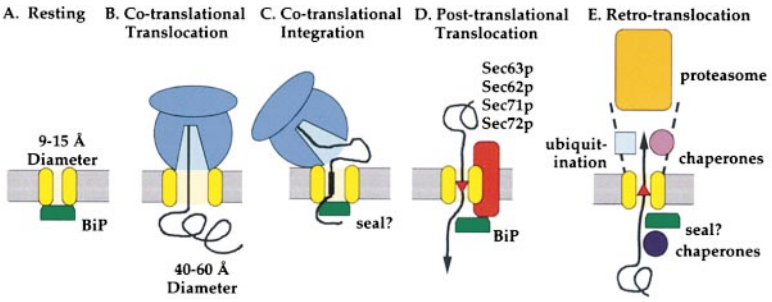

Figure 1. Operational Modes of the Translocon

The translocon complex (yellow) is shown performing various functions in the ER membrane. The cytoplasm and the ER lumen are located above and below, respectively, the membrane (gray) in each panel. BiP may close the aqueous pore itself, as shown in (A), or may effect closure indirectly through interactions with other proteins. The diameter of the translocon pore and the origin of the seal are unknown for states (C), (D), and (E). Posttransational translocation in yeast requires the additional translocon components indicated in (D). During retrotranslocation (E), chaperones may be involved on both sides of the ER membrane. The substrate is retrotranslocated to the cytoplasm where it is ubiquitinated and degraded by the proteasome. The dotted lines indicate that some or all of these functions may occur at the membrane.

or does the translocon (or a receptor; see below) accept substrates from multiple chaperones?

A number of ER proteins have been proposed to play a role in retrotranslocation, but the exact nature of their involvement has yet to be clarified (Brodsky and McCracken, 1999). It is conceivable that some or all of these proteins, or some of the many as-yet uncharacterized proteins that are expressed during the unfolded protein response (Travers et al., 2000), are directly involved in identification and targeting of retrotranslocation substrates. The multiplicity of potential unfolded states suggests that a single common mechanism is unlikely, but it cannot yet be ruled out. It is also possible that cytoplasmic or membrane proteins may be involved in the identification of misfolded or unassembled membrane proteins, as well as other proteins that are exposed to both sides of the ER membrane during processing (e.g., apolipoprotein B; Mitchell et al., 1998).

An especially intriguing question is the mechanism by which retrotranslocation substrates that leave the translocon (Plemper and Wolf, 1999) are targeted back to the translocon. Does the translocon itself serve as the initial substrate receptor, or does another protein(s) act as the receptor? No matter how substrates arrive at the translocon, it must decide whether to accept the substrate and initiate retrotranslocation. While having the translocon itself serve as the receptor would be economical, it seems more reasonable to have another protein(s) carry out the receptor function (Figure 2). This approach would have the advantage of providing a means for identifying those translocons ready to accept retrotranslocation substrates, and of establishing the operational mode of the translocon. The SRP receptor performs a similar role in cotranslational targeting (Johnson and van Waes, 1999). However, no one has yet identified a protein that functions as a retrotranslocation substrate receptor. Two groups have identified specific regions of Sec61p that are important for retrotranslocation (Zhou and Schekman, 1999; Wilkinson et al., 2000). These regions could be involved in substrate recognition, gating, or interacting with the putative retrotranslocation receptor or other proteins. They could also be involved in the initiation of retrotranslocation and the insertion of one end of the substrate into the pore, a process that is currently difficult to envision because of the absence of a signal sequence-equivalent in retrotranslocation substrates. Whatever the case, the targeting issue is arguably the most important black box in retrotranslocation.

Another important unresolved issue is whether membrane protein substrates are moved laterally into the translocon for retrotranslocation. It is not clear how completed polypeptides that are released into the bilayer are then targeted back to the translocon if they are unable to fold or assemble properly. This issue is particularly germane because another approach for degrading membrane proteins has recently been discovered. Enzymes have been identified that use ATP to extract membrane proteins out of the bilayer and degrade them processively. These enzymes, FtsH in *E. coli* (Kihara et al., 1999) and the AAA proteases in mitochondria (Langer, 2000), appear to function independently of translocons in bacteria and mitochondria, and hence this constitutes a separate potential pathway for membrane protein degradation in the ER membrane.

Retrotranslocation substrates may also be identified before they leave the translocon. In such cases, re-targeting to the translocon is a moot point. One example of such a substrate is apolipoprotein B, which remains associated with the translocon for an extended period of time before its final fate, translocation or degradation, is decided (Mitchell et al., 1998).

What Is the Driving Force for Retrotranslocation?

Proteins can be translocated across a membrane by pulling, by pushing, or by sterically directing their movement (see Johnson and van Waes, 1999). During posttranslational translocation at the ER membrane, BiP and ATP effect forward transport by pulling and/or concentrating the substrate protein on the lumenal side of the membrane (Figure 1D). Since this process is unidirectional, it seems unlikely that BiP would also work in reverse and power retrotranslocation in the opposite direction through the translocon. Instead, a retrotranslocation substrate is probably pulled into the cytoplasm by a protein(s) located on the cytoplasmic side of the ER membrane. Indeed, cytoplasmic proteins and ATP hydrolysis are required for retrotranslocation in in vitro and semipermeabilized cell systems (Brodsky and McCracken, 1999; Wilson et al., 2000). Candidates for this pulling function (Figure 2) include cytoplasmic chaperones (Brodsky et al., 1999), proteins involved in the ubiquitination of retrotranslocated proteins (Biederer et al., 1997), and/or the proteasome itself (Mayer et al., 1998). The expected positioning of such a protein(s) at the cytoplasmic end of the ER translocon pore might also serve a regulatory function by preventing the targeting of ribosomes to the translocon to initiate translocation or integration.

How Is the Permeability Barrier Maintained during Retrotranslocation?

The ribosome is responsible for sealing the cytoplasmic end of the translocon during cotranslational translocation and some stages of cotranslational integration

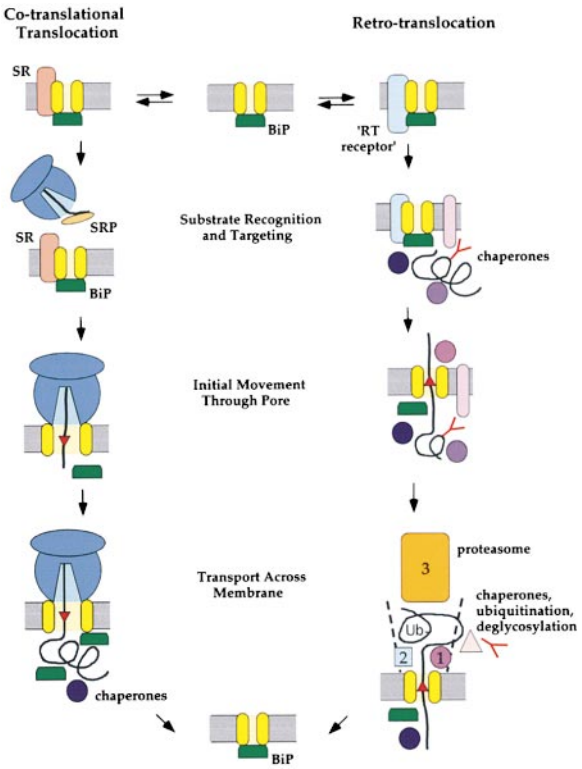

Figure 2. Stages of Cotranslational Translocation and Retrotranslocation

The operational mode of the translocon is likely to be defined by the association of accessory proteins such as the SRP receptor (SR) and the putative "retrotranslocation receptor" (RT receptor). For each process, the black arrows indicate the order of steps, while the red arrowheads indicate the direction of protein motion through the translocon. Different chaperones are depicted in various shades of purple. In the final stage of retrotranslocation, substrate movement may be powered by cytoplasmic chaperone activity (1), ubiquitination (2), and/or the proteasome (3). The process depicted here will differ for membrane protein substrates that require lateral movement back into the translocon. SRP, signal recognition particle.

(Johnson and van Waes, 1999), while BiP seals, directly or indirectly, the lumenal end of a translocon at rest (Hamman et al., 1998). It is not yet clear how the ER membrane maintains its permeability barrier during posttranslational translocation or retrotranslocation. A critical unknown in each of these cases is the nature of the translocon pore: is it an aqueous pore, and if so, what is its diameter?

Assuming that the polypeptide moves through an aqueous pore in the translocon, though in opposite directions in these two processes, simultaneous ion flow through the pore could be eliminated or minimized by gating the pore and/or allowing the pore to expand or contract as necessary to accommodate the polypeptide chain. Such gating or packing would not have to form van der Waals-level contacts everywhere, but would only need to exclude particles with a diameter larger than 6 Å (the hydrated diameter of Mg^{2+}, the smallest common metal ion, is near 6 Å). The size of the translocon pore can vary since it expands/contracts in response to ribosome binding/dissociation (Hamman et al., 1998), but it seems likely that retrotranslocation and posttranslational translocation occur through the smaller pore. If the polypeptide were transported in an unfolded state through a 9–15 Å pore, then the polypeptide would occlude the pore and thereby prevent significant ion movement across the ER membrane (Figure 2). Although it remains to be seen whether a small aqueous pore is used for these processes, such a scenario would only require the gating of the pore before and after polypeptide transport. This gating would presumably be effected by BiP (Hamman et al., 1998; Brodsky and McCracken, 1999; Römisch, 1999), and may be regulated by interactions with PDI and/or other proteins involved in targeting retrotranslocation substrates to the translocon (e.g., Gillece et al., 1999).

Yet in some cases, glycosylated proteins appear to be retrotranslocated across the ER membrane and deglycosylated in the cytoplasm (Wiertz et al., 1996; Plemper and Wolf, 1999; Römisch, 1999). The carbohydrate moieties would require considerably larger holes for transport than the 9–15 Å pore, so the translocon must either expand to accommodate glycosylated proteins or retrotranslocation must occur through a larger translocon pore. One interesting possibility is that the proteasome could bind to the cytoplasmic end of the translocon and effect pore expansion, as does the ribosome during cotranslational translocation, and thereby allow glycoproteins to move through the pore (Chillarón and Haas, 2000).

Are there other possibilities for maintaining the permeability barrier? Since BiP can mediate pore closure from the lumenal side, it is conceivable that a similar protein, such as Hsp70, gates the pore on the cytoplasmic side of a translocon engaged in or primed for retrotranslocation. It is also possible that lumenal chaperones gate the lumenal end of the translocon during retrotranslocation and help to seal off the pore much like the ribosome does during translocation. The maintenance of the permeability barrier during retrotranslocation of membrane proteins may be more complicated than for soluble proteins, as is true for movement in the forward direction (Johnson and van Waes, 1999).

How Does the Translocon Switch Gears?

The retrotranslocation and translocation operational modes of the translocon are distinct and separable because certain mutations in Sec61p and BiP can affect one process without altering the other (Brodsky et al., 1999; Römisch, 1999; Zhou and Schekman, 1999; Wilkinson et al., 2000). How then is a particular operational mode selected and maintained? The distribution of translocons into different functional modes will almost surely change to reflect a cell's metabolic state. Therefore the mechanism by which this distribution is altered constitutes an important regulatory process of the cell.

It is possible that the functional state of the translocon is dictated simply by the substrate or targeting complex that it encounters. That is, a resting translocon is directed into either translocation or retrotranslocation depending upon what collides with the translocon first, either a signal sequence-containing polypeptide on the cytoplasmic side or a misfolded protein on the lumenal side. The initiation of transport on one side of the translocon would then have to be communicated to the other side of the translocon to avoid collisions. In such a "first come, first served" mechanism, the relative substrate concentrations would dictate the extent of protein flow in each direction through the translocon. Since a shift in translocon function would be effected by the direct

interaction of an individual substrate or targeting complex, this mechanism is analogous to manually shifting the operational mode of the translocon machinery.

An alternative possibility is that the translocon is constrained to either translocation or to retrotranslocation as a result of a direct or indirect structural modification of the translocon. For example, an accessory protein(s) such as the SRP receptor (or putative retrotranslocation receptor) may associate with the translocon and the resulting assembly may operate in only a single functional mode. In this model, the functional state of the translocon would be dictated by its collisions with accessory proteins rather than substrate. Such a mechanism would be easier to regulate in a more global sense. For example, by modifying the expression level (Travers et al., 2000; Casagrande et al., 2000) or the structure (e.g., by phosphorylation) of a particular accessory protein and/or translocon component, the cell could alter the balance of translocons engaged in a particular function(s) and thereby exert more direct control over its metabolism. It is easy to imagine that such higher-order or automatic control over the direction of protein flow through translocons would be advantageous under certain circumstances (e.g., an unfolded protein response).

What Is Next?

As noted above, the molecular mechanisms involved in many important aspects of retrotranslocation and the nature of the involvement of the translocon are largely unknown. The concerted efforts of many, using a variety of experimental approaches, will be required to elucidate these unknowns. Perhaps the highest priority is a well-defined and purified in vitro retrotranslocation system that would allow systematic identification of the roles of individual proteins in the process. The use of proteoliposomes reconstituted with various combinations of translocon components and/or substrates will further clarify the involvement of the translocon in retrotranslocation. Introduction of retrotranslocation substrates into microsomes by reconstitution would yield samples with known and homogeneous substrate species, and would eliminate the current complexity of proteins moving in both directions as microsomes are loaded with substrate by translocation from the cytoplasm. Another goal is to devise a mechanism for generating intermediates at different stages of retrotranslocation. Such intermediates could be analyzed using biophysical approaches to characterize the environment and interactions of the polypeptide undergoing retrotranslocation, as well as to monitor changes in translocon structure. Although future experiments to identify the mechanisms and regulation of retrotranslocation and the role of the translocon will be challenging, the high probability of exciting and unpredictable discoveries will drive continued interest in this important research area.

Selected Reading

Biederer, T., Volkwein, C., and Sommer, T. (1997). Science *278,* 1806–1809.

Brodsky, J.L., and McCracken, A.A. (1999). Semin. Cell Dev. Biol. *10,* 507–513.

Brodsky, J.L., Werner, E.D., Dubas, M.E., Goeckeler, J.L., Kruse, K.B., and McCracken, A.A. (1999). J. Biol. Chem. *274,* 3453–3460.

Casagrande, R., Stern, P., Diehn, M., Shamu, C., Osario, M., Zúñiga, M., Brown, P.O., and Ploegh, H. (2000). Mol. Cell *5,* 729–735.

Chillarón, J., and Haas, I.G. (2000). Mol. Biol. Cell *11,* 217–226.

Gillece, P., Luz, J.M., Lennarz, W.J., de la Cruz, F.J., and Römisch, K. (1999). J. Cell Biol. *147,* 1443–1456.

Hamman, B.D., Hendershot, L.M., and Johnson, A.E. (1998). Cell *92,* 747–758.

Johnson, A.E., and van Waes, M.A. (1999). Annu. Rev. Cell Dev. Biol. *15,* 799–842.

Kihara, A., Akiyama, Y., and Ito, K. (1999). EMBO J. *18,* 2970–2981.

Langer, T. (2000). Trends Biochem. Sci. *25,* 247–251.

Liu, Y., Choudhury, P., Cabral, C.M., and Sifers, R.N. (1999). J. Biol. Chem. *274,* 5861–5867.

Mayer, T.U., Braun, T., and Jentsch, S. (1998). EMBO J. *17,* 3251–3257.

Mitchell, D.M., Zhou, M., Pariyarath, R., Wang, H., Aitchison, J.D., Ginsberg, H.N., and Fisher, E.A. (1998). Proc. Natl. Acad. Sci. USA *95,* 14733–14738.

Plemper, R.K., and Wolf, D.H. (1999). Trends Biochem. Sci. *24,* 266–270.

Römisch, K. (1999). J. Cell Sci. *112,* 4185–4191.

Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S., and Walter, P. (2000). Cell *101,* 249–258.

Wiertz, E.J.H.J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T.R., Rapoport, T.A., and Ploegh, H.L. (1996). Nature *384,* 432–438.

Wilkinson, B.M., Tyson, J.R., Reid, P.J., and Stirling, C.J. (2000). J. Biol. Chem. *275,* 521–529.

Wilson, C.M., Farmery, M.R., and Bulleid, N.J. (2000). J. Biol. Chem. *275,* 21224–21232.

Zhou, M., and Schekman, R. (1999). Mol. Cell *4,* 925–934.