Retrotranslocation: Endoplasmic Reticulum’s Junk Disposal Mechanism

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ABSTRACT

The primary structure of polypeptides is converted to their final tertiary and quaternary structure by sequential maturation steps, and the endoplasmic reticulum (ER) provides the environment for the polypeptides to attain their proper 3-dimensional architecture. Proteins that misfold or fail to oligomerize with their partners (Chen et al., 1998; Wileman et al., 1990) are quickly degraded, as unfolded or unassembled proteins could interfere with normal cellular function. Retrotranslocation is the process by which terminally misfolded or unassembled ER proteins are translocated back into the cytosol for degradation mediated by the proteasomal machinery. Increasing amounts of evidence now support the fact that the same translocon pore that is involved in the translocation of polypeptides into the ER is also used for the retrotranslocation process. But questions, like how the misfolded proteins are recognized and targeted to the translocon pore, whether the process requires energy, and what pulls the polypeptides as they emerge out of the pore into the cytoplasm, remain to be elucidated. This review addresses our current knowledge about the retrotranslocation process.

INTRODUCTION: HOW DOES THE ENDOPLASMIC RETICULUM DISPOSE OF MISFOLDED OR UNASSEMBLED PROTEINS?

One hypothesis for the mechanism of protein disposal is selective degradation via the lysosomal pathway. However, a more plausible mechanism via a lysosome independent pathway has been recently verified by the degradation of many misfolded unassembled proteins (Bonifacino et al., 1989) in the presence of lysosomal inhibitors. The other interesting aspect was that these misfolded proteins were endoglycosidase H sensitive and acquired neither sialic acid modification nor sensitivity to the endoglycosidase D (Klausner and Sitia, 1990), which pointed out that these proteins never left the endoplasmic reticulum (ER). Now, the question is how does the cell accomplish the pre–Golgi selective degradation of ER retained misfolded proteins or “ER associated degradation (ERAD)” (McCracken and Brodsky, 1996). The idea of degradation within the ER compartment is difficult to appreciate, as it would be detrimental for nascent polypeptides undergoing the process of folding. Initial reports about cytosol independent ER protein degradation by the “protease” ER-60 (Otsu et al., 1995) could not gain popularity, as later it was shown that ER-60 is in fact a molecular chaperone (Oliver et al., 1997). Also, the presence of a sub-domain in the ER specialized for degradation of misfolded or unassembled proteins could not be verified by electron microscopy (EM) (Klausner and Sitia, 1990). The other option for the ER to dispose the junk is to redirect the misfolded or unassembled proteins to the cytosol, where the ubiquitin-proteasomal pathway might degrade them.

HOW DOES A PROTEIN THAT HAS ENTERED THE ENDOPLASMIC RETICULUM GET BACK TO THE CYTOPLASM?

Identification of Sec61p associated with the retrotranslocation process came from the study of destruction of class I MHC heavy chain molecules in human cytomeglovirus (HMCV) infected cells (Wiertz et al., 1996a). The US2 gene product of HMCV pulls the class I MHC heavy chain out into the cytoplasm, where it is deglycosylated and destroyed by the proteasomal machinery. The deglycosylated breakdown product was shown to be associated with Sec61 complex. Retrotranslocation was shown to be non-specific for virus, when non-viral cells, which do not express viral gene product US2, showed a large proportion of class I MHC heavy chain associated with the Sec61 complex only when misfolding was induced by treatment with dithiothreitol (DTT) (Wiertz et al., 1996b). Also, in yeast, a misfolded protein was shown to be associated with the Sec61p by using an ER to cytosol export defective temperature sensitive Sec61p strain (Pilon et al., 1997).

However, it should be noted that ATPase enzymes like FtsH in E. Coli (Kihara et al., 1999) and AAA proteases Yta10/Yta12 and Yme1 in mitochondrial inner membrane (Langer, 2000; Leonhard et al., 2000) have been identified that can degrade transmembrane proteins by extracting them from the lipid bilayer without the involvement of a translocon pore. Thus, a translocon independent pathway for retrotranslocation cannot be ruled out.

HOW TO CONTROL THE TWO-WAY TRAFFIC?

If Sec61p, a translocon channel, is involved in both the translocation and retrotranslocation process, then how does the channel regulate this two-way traffic? One possibility might be that there are at least two different classes of specialized translocon channels, where one
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The translocon pore with a regulated pore diameter of 15-60 Å (Beckmann et al., 1997; Hanein, 1996) is found in the ER membrane of yeast and mammalian cells. It is formed by the heterotrimeric Sec61 complex (Rapoport et al., 1996), which consists of a polytopic membrane protein Sec61p and two small C-terminal anchor proteins, Sss1p (Sec61g in mammals) and Sbh1p (Sec61b in mammals) (Essault et al., 1993; Hartmann et al., 1994). Sec61p (54 KDa) spans the ER membrane ten times (Wilkinson et al., 1996), with five loops on the cytoplasmic side and four loops on the ER luminal side (Stirling et al., 1992). Although different functions had been assigned to Sec61p, the role of any specific domain or amino acid in Sec61p is uncertain. Wilkinson et al. have shown that yeast strains with a deletion of transmembrane domain 2 (TM2) or transmembrane domain 3 (TM3) of Sec61p are viable but display defects in post-translational translocation. The TM3 deletion is defective in ER retrotranslocation (Wilkinson et al., 2000) and thus induced UPR. A genetic screen for a retrotranslocation specific mutation in Sec61p by Zhou and Schekman (1999) also pointed out that the fourth luminal loop and the third transmembrane domain of Sec61p greatly influence retrotranslocation but do not have much effect on the translocation step. The most intriguing aspect is that in all these mutants the UPR was upregulated, in fact the UPR was used as a read-out for these screens. So, it seems that under normal circumstances the cell disposes misfolded proteins through the translocon channel into the cytosol by using retrotranslocation process, but any inhibition in this junk disposal elicits the UPR. The cross talk between UPR and retrotranslocation seems to be an interesting field for further research.

IS THERE AN ADAPTER?

The biggest enigma of the retrotranslocational model is what directs the misfolded or unassembled proteins to the translocon pore and how does the ER distinguish polypeptides which have been terminally misfolded from those that are yet to achieve their proper tertiary and quaternary architecture and mark the former as retrotranslational substrate? The presence of a stretch of polypeptide signal sequence for retrotranslocation substrate is highly unlikely, as it would be present in all proteins and cannot be used to distinguish between unfolded and yet to be folded proteins. So, what might be the adapter?

It is known that a variety of ER resident proteins act as chaperone/chaperonins, like Bip, calnexin, ERp57, and protein disulfide isomerase (PDI) (Liu et al., 1999; Gillece et al., 1999; Chillaron and Haas, 2000; Wilson et al., 2000), and assist in folding of polypeptides. It might be that the translocon complex recognizes a long-lived chaperone, a polypeptide, as a retrotranslocation substrate. So, is it the chaperone that acts as the adapter? In order to do so they must interact with the translocon pore, but to date very few of them have been found to fulfill this criterion.

PDI, an ER oxidoreductase, is a likely candidate for this function (Tsai et al., 2002; Fagioli et al., 2001; Orlandi, 1997; Tortorella et al., 1998). It seems that PDI acts as a “redox driven chaperone” which binds the retrotranslocation substrate in reduced state and transfers it directly to the translocon channel in an oxidized state. Another candidate is Bip, although its exact function as an adapter is debatable. It has been shown that a mutation in Bip homologue Kar2 in yeast blocks degradation of unfolded soluble protein carboxypeptidase –YCS Y (CPY*), a vacuole hydrolase (Plemper et al., 1997; Brodsky et al., 1999), and stabilizes secretion defective A1PiZ, an allelic variant of alpha1-protease inhibitor (A1Pi, alpha 1 antitrypsin) (Brodsky et al., 1999). It could be presumed that the function of Bip (Kar 2 in yeast) is to maintain the substrate in a retrotranslocation competent state by preventing aggregation (Schnell and Hebert, 2003). In the case of glycoproteins, the ER employs a carbohydrate tag mechanism to monitor the process of proper protein folding and identification of hopelessly unfolded ones as a retrotranslocation substrate for degradation (Helenius and Aebi, 2001).

As soon as a polypeptide chain with a tripeptide sequence Asn-X-Ser/Thr emerges out into the ER lumen, a polysaccharide chain (Gluc)3(Man)9(GlucNAc)2− is added to the Asn residue. During the subsequent maturation step the (Gluc)3 is removed by the sequential actions of Glucosidase I and II respectively. In higher eukaryotes a soluble ER resident protein Uridine Diphosphate-glucose glycoprotein glycosyltransferase acts as the “watch dog,” adding on a single molecule of glucose to the unfolded protein with an exposed hydrophobic
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The process of pulling the polypeptide from the ER. It is possible that other cytosolic proteins are involved in degradation of paF or A1Piz (Brodsky et al., 1999). Thus does not play a role in retrotranslocation mediated tides. In contrast, it might be that the bulky polyubiquitination reports show that Ssa1p, a cytosolic hsc70 in yeast, during translocation (Brodsky et al., 1999), but conflict-ratchet mechanism as employed by Bip in the ER lumen out the substrate during retrotranslocation by a similar erone hsp70 has been implicated in the role of pulling the polypeptide from these chaperones. This cycle of binding and release continues until the polypeptide is properly folded; but this cannot go on forever! So, the ER has employed a “timing” mechanism. Mannosidase I cleaves one mannose residue from (Man)9(GlucNAc)2 to make (Man)8(GlucNAc)2. The mannosidase reaction rate is slow, so proteins that are retained long in the ER are trimmed to (Man)8(GlucNAc)2 (Tsai, et al., 2002). This (Man)8 is recognized by lectins that are related to Mannosidase I but are enzymatically inactive. ER degradation enhancing a mannosidase like protein (EDEM) in mammals, and HTM1 (homologous to mannosidase I) in yeast (Hosokawa et al., 2001; Jakob et al., 2001) are targeted as a retrotranslocation substrate. But the drawback to this theory is that (Man)8(GlucNAc)2 is also formed during the normal maturation steps of N-glycans as they leave the ER for Golgi. So how these lectins differentiate between normal and unfolded N-glycan is not clear.

In the case of unfolded transmembrane proteins, the scenario is hazier; do they use the same translocon pore for retrotranslocation? If so, they must move laterally on the plasma membrane to reach the translocon pore (Johnson and Haigh, 2000). Moreover, questions like what is the targeting signal or the driving force remain to be answered. It is not unlikely that yet to be characterized proteins which are upregulated during UPR act as adapters in bridging the translocon with chaperone, the unfolded protein (Johnson and Haigh, 2000).

WHAT IS THE DRIVING FORCE?

The next big question is what pulls the retrotranslocation substrate out into the cytosol; is this an energy requiring process? It has been shown that ATP hydrolysis and the cytosol are required for the process, both in vitro and in semi-permeabilized cell systems (Wilson et al., 2000). Moreover, the degradation requires a functional proteasome (Werner et al., 1996). Cytosolic chaperone hsp70 has been implicated in the role of pulling out the substrate during retrotranslocation by a similar ratchet mechanism as employed by Bip in the ER lumen during translocation (Brodschy et al., 1999), but conflicting reports show that Ssa1p, a cytosolic hsc70 in yeast, does not play a role in retrotranslocation mediated degradation of paF or A1Piz (Brodschy et al., 1999). Thus it is possible that other cytosolic proteins are involved in the process of pulling the polypeptide from the ER.

Polyubiquitination, which has been shown to be required for degradation in yeast (Hiller et al., 1996; Biederer et al., 1996) and in higher eukaryotes (Ward et al., 1995), might also be involved in the ratcheting of the polypeptides. In contrast, it might be that the bulky polyubiqui-
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