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PROTEIN TRANSLOCATION

Channelling the route for ER misfolded proteins

> auto-ubiquitylation of Hrd1 is crucial for its role in protein retro-translocation during ERAD

The endoplasmic reticulum (ER) possesses intricate mechanisms for detecting and removing potentially toxic misfolded proteins in a process known as ER-associated protein degradation (ERAD). ERAD involves protein translocation from the ER to the cytoplasm (retrotranslocation), their ubiquitylation and subsequent proteasomal degradation. Despite the importance of ERAD for protein homeostasis, its mechanisms are not fully understood — in particular, how misfolded proteins in the ER lumen cross the membrane lipid bilayer to reach the cytoplasm has remained elusive. Baldridge and Rapoport reveal that the transmembrane ubiquitin ligase Hrd1 is sufficient for efficient protein retrotranslocation and that its autoubiquitylation has a pivotal role during this process.

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To investigate the mechanisms of retrotranslocation during ERAD, the authors used a previously established technique that reconstitutes ERAD *in vitro* and a membrane-anchored version of yeast misfolded carboxypeptidase Y (CPY*-TM) as a model misfolded protein. They generated liposomes containing both CPY*-TM and Hrd1,which ubiquitylates misfolded proteins during ERAD and has been suggested to function as an important component of the retrotranslocation machinery in yeast. When these liposomes were incubated with the remaining components of the ubiquitylation machinery (that function with Hrd1 to complete ubiquitin conjugation), both CPY*-TM and Hrd1 were found to be polyubiquitylated. This showed that Hrd1 is able to catalyse its own ubiquitylation, in addition to ubiquitylating the misfolded protein substrate. Importantly, even CPY*-TM molecules that initially were facing the lumen of the liposome were ubiquitylated in this assay, indicating that they were translocated across the liposome membrane. This translocation was abolished when Hrd1 lacked some of its transmembrane segments or an enzymatically inactive Hrd1 mutant was used, collectively demonstrating that the membrane-embedded domain of Hrd1 and its ubiquitin ligase activity are sufficient and necessary to translocate proteins across the lipid bilayer.

To understand the role of ubiquitylation in protein retrotranslocation, Baldridge and Rapoport generated a mutant of CPY*-TM that lacked the Lys residues that are targeted for ubiquitin conjugation. As expected, the Lys-free CPY*-TM variant was not efficiently ubiquitylated. However, it was efficiently translocated across the liposome membrane in the *in vitro* assay and successfully degraded when introduced into yeast, indicating that ubiquitylation of the misfolded protein is dispensable for its retrotranslocation. Moreover, mutation of Hrd1 identified three Lys residues, removal of which interfered with CPY*-TM retrotranslocation in liposomes, without significantly affecting Hrd1 ubiquitin ligase activity. Importantly, the expression of this Hrd1 Lys mutant in yeast interfered with the ERAD pathway. Together, these results suggest that autoubiquitylation of Hrd1 is crucial for its role in protein retrotranslocation during ERAD.

As retrotranslocation most likely requires the formation of protein channels, and the membrane-embedded domain of Hrd1 is essential, the authors propose that Hrd1 functions as such a channel and that Hrd1 autoubiquitylation controls its gating. How Hrd1 autoubiquitylation is regulated and how exactly ubiquitylation contributes to channel-mediated protein retrotranslocation remain to be elucidated.

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ORIGINAL ARTICLE Baldridge, R. D. & Rapoport T. A. Autoubiquitination of the Hrd1 ligase triggers protein retrotranslocation in ERAD. *Cell* http://dx.doi.org/10.1016/j.cell.2016.05.048 (2016)

FURTHER READING Vembar S. S. & Brodsky J. L. One step at a time: endoplasmic reticulum-associated degradation. *Nat. Rev. Mol. Cell Biol.* **9**, 944–957 (2008)