

EDEM an ER quality control receptor

Tao Wang and Daniel N. Hebert

Defective proteins in the endoplasmic reticulum (ER) are tagged as terminally aberrant by the composition of their N-linked glycans. The ER carbohydrate-binding protein EDEM selectively recognizes such tags and sorts the defective proteins for degradation *via* the ERAD pathway.

The fidelity of the protein production process is monitored by quality control machinery, which ensures that aberrant proteins are not deployed throughout the cell. For proteins that travel through the secretory pathway, surveillance begins in the endoplasmic reticulum (ER) where the majority of their maturation occurs¹. Defective proteins are retained in the ER through binding to resident chaperones. If the abnormality is not rectified, the non-native substrate is selectively degraded while the flow of properly folded proteins through the secretory pathway is maintained. Two recent reports in *Science*^{2,3} have helped to solidify the role of the mammalian protein EDEM (ER degradation-enhancing α -mannosidase-like protein) as a quality control receptor responsible for sorting terminally misfolded glycoproteins for destruction and provide valuable insight into its binding mechanism.

Aberrant proteins are cleared from the ER by a process termed ER-associated protein degradation or ERAD⁴. In this process, both soluble and membrane

proteins are degraded by the 26S cytosolic ubiquitin-dependent proteasome, necessitating the requirement for a retranslocation step wherein the malformed substrate is translocated back to the cytosol. As with most protein translocation processes, ERAD requires a signal (indicative of protein defectiveness), a cognate receptor for the signal, a mechanism for targeting to a membrane channel or translocon and an extraction method (Fig. 1)⁵. In the case of ERAD, this is also coupled to ubiquitination and subsequent proteasomal degradation.

N-linked glycans have recently emerged as luminal tags or signals employed by the cell, to aid in the navigation of proteins through the secretory pathway^{6,7}. The secretory pathway contains multiple carbohydrate binding proteins, or lectins, that interact with glycans in a manner that is dependent on the composition of the glycan. Therefore, lectin binding is controlled by the transfer and trimming of glycans on the substrate. The bulky, extended and hydrophilic nature of *N*-linked glycans makes them ideal

modifications for recruiting protein machinery within the crowded confines of the ER.

The first carbohydrate binding proteins a glycoprotein encounters during maturation in the ER are the lectin chaperone calnexin (CNX), an integral membrane protein, and its soluble paralog, calreticulin (CRT). These chaperones facilitate proper folding by binding proteins possessing monoglucosylated glycans (Fig. 2, blue boxes)⁸. Release from CNX/CRT is initiated by the removal of the final glucose (of the three originally transferred glucoses) by glucosidase II. Rebinding of CNX/CRT to malformed conformers is triggered after the reglucosylation of these aberrant proteins by the UDP-glucose: glycoprotein glucosyltransferase (GT)⁹. The CNX/CRT binding cycle serves to assist in the maturation process by protecting immature proteins from aggregation and retaining misfolded or unassembled subunits within the ER.

Native proteins that emerge from the CNX/CRT binding cycle are generally trimmed to high mannose forms contain-

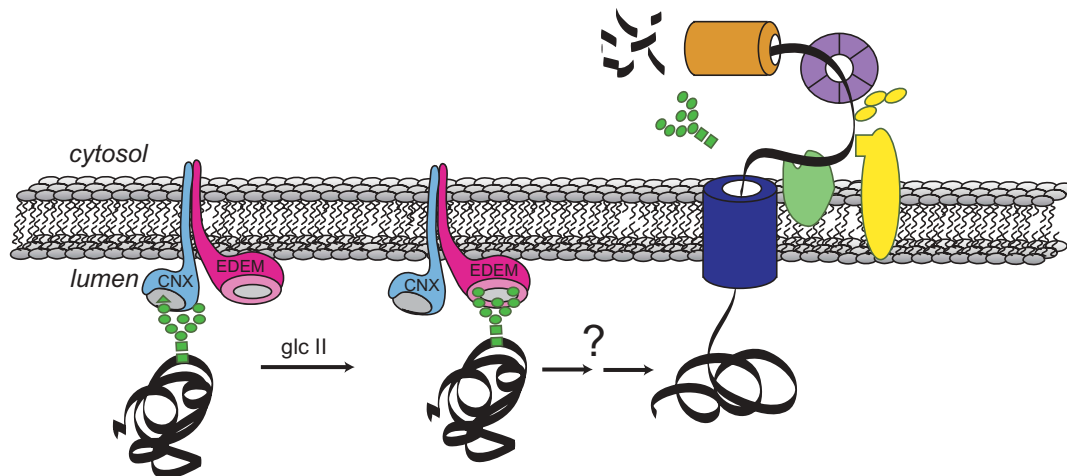


Fig. 1 ERAD recognition by the sequential binding of CNX and EDEM. Defective proteins remain monoglucosylated due to their reglucosylation by the glucosyltransferase, which supports CNX binding. The glycans are eventually trimmed to Man8 by the slow-acting mannosidase I. Release of Man8 glycans from CNX by glucosidase II allows EDEM to extract the glycoprotein from the CNX binding cycle. The EDEM bound substrate is targeted for retranslocation to the cytosol through a translocon (blue membrane channel) by an unknown mechanism, where it is deglycosylated by an *N*-glycanase (green membrane-associated protein), and ubiquitinated (yellow ovals attached by membrane associated E2/E3) prior to degradation by the 26S proteasome (orange tube). The AAA ATPase p97 (purple wheel) helps with the extraction of the glycoprotein to the cytosol. The *N*-linked glycan-containing glucoses, mannoses and GlcNAcs are denoted by triangles, circles and squares, respectively.

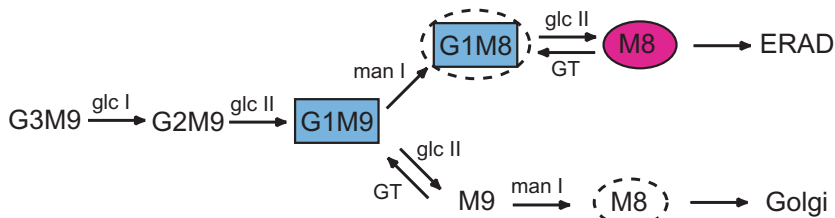


Fig. 2 Proposed mechanism for EDEM binding. *N*-linked glycans comprised of Gluc₃Man₉GlcNAc₂ (G3M9) structures are transferred co-translationally to consensus glycosylation sites. These are trimmed by glucosidases I and II (glc I and II) to the G1M9 form, which CNX binds (blue boxes). Rebinding to CNX after deglucosylation is re-initiated by the reglucosylation of non-native conformers by glucosyltransferase (GT). Mannose trimmed forms bound by CNX can be extracted by EDEM (pink oval) for ERAD. Unglucosylated glycans that are trimmed to Man8 (M8) by mannosidase I (man I), can be transported to the Golgi or retained in the ER as resident proteins. Dashed ovals denote other possible EDEM binding structures, which cannot be excluded at this time.

ing eight (Man8) or seven (Man7) mannose residues prior to their transport to the Golgi. The mannose-binding protein ERGIC53 is proposed to aid in the selection of native substrates for transport by binding these high mannose-containing proteins¹⁰.

Mannose trimming has also been implicated in protein destruction. Pharmacological^{11,12} or genetic^{13,14} inhibition of the ER mannosidase I, which is responsible for cleaving the terminal mannose residue to generate Man8B structures, results in the stabilization of ERAD substrates. Because mannose trimming in the ER is a slow process, it has been proposed to act as a ‘timer’ of ER residency. Substrates that have been retained in the ER through repeated deglucosylation-reglucosylation cycles and CNX/CRT binding would eventually be cleaved by mannosidase I and marked for degradation by the resulting Man8B structures. These findings led to a search for an ER residing mannose-binding protein involved in the sorting of aberrant proteins for ERAD.

A search for mannosidase homologs (proteins possessing obvious mannose-binding properties) uncovered ER proteins in both *Saccharomyces cerevisiae*^{15,16} and mouse cells¹⁷, referred to as Mnl1p/Htm1p and EDEM, respectively. The deletion of Mnl1p/Htm1p in yeast increased the half-life of glycosylated ERAD substrates but not non-glycosylated substrates^{15,16}. The mouse protein was found to lack mannosidase enzymatic activity and its overexpression in HEK293 cells accelerated the degradation of a misfolded variant of α -1 antitrypsin (A1AT) through the ERAD pathway¹⁷. Together these results suggested that Mnl1p/Htm1p and EDEM might be the ER receptors responsible for directing aberrant proteins for ERAD through the recognition of a mannose-trimmed signal.

Recent reports using defective variants of A1AT and β -secretase by Oda *et al.*³ and Molinari *et al.*², respectively, now demonstrate that overexpression of EDEM accelerates the release of the aberrant proteins from CNX and their subsequent degradation by the proteasome. They demonstrated that abolishing the initial interaction of the ERAD substrates with CNX by accumulating proteins in a triglucosylated state (using glucosidase inhibitors or cell lines lacking glucosidase I) stabilized the substrates in the presence of EDEM overexpression. This indicated that EDEM acts to extract defective proteins from the CNX binding cycle (Fig. 1). This role was supported by the identification of pre-established EDEM–CNX complexes that exist in the absence of bound substrate³. The C-terminal transmembrane region or cytosolic tail of CNX mediates this interaction, placing EDEM directly next to CNX — optimally positioned for the extraction of substrate from the CNX binding cycle³.

EDEM alone was insufficient to accelerate degradation. However, it should be noted that EDEM itself is a glycoprotein containing five consensus *N*-linked glycosylation sites. Therefore, EDEM likely engages the CNX/CRT binding cycle for its own maturation. The necessity of this cycle for proper maturation of a specific protein is highly variable. Whereas some glycoproteins mature efficiently in the absence of CNX/CRT binding, others are synthesized completely inactive⁶. Thus, ablating the CNX/CRT binding cycle may directly affect the efficient maturation of EDEM, which could provide an alternative explanation for why EDEM is unable to accelerate substrate degradation in the absence of CNX binding.

The transfer of glycoproteins from CNX to EDEM could potentially be mediated by a change in carbohydrate structure, provided CNX and EDEM have

differential carbohydrate-binding specificities (Figs. 1, 2). This possibility was supported by the increase in mobility observed on SDS-PAGE with the total β -secretase population when EDEM was overexpressed, suggestive of the rapid accumulation of nonglycosylated protein, an isoform that does not bind CNX². While these results suggest that EDEM binds unglucosylated glycoproteins with Man8 structures, a direct demonstration of the sugar binding specificity for EDEM is lacking.

If EDEM does bind Man8 glycoforms, the question remains: how do resident ER glycoproteins and native secretory proteins on their way to the Golgi evade EDEM detection, because both of these protein types can contain Man8 glycans? One possibility is that EDEM substrates are generated solely from glucosidase II digestion of monoglucosylated Man8 glycoforms bound to CNX (Fig. 2, G1M8; top pathway). The orientation of CNX–EDEM and the ability of EDEM to extract substrates from the CNX binding cycle support this scenario^{2,3}. Alternatively, the three forms of Man8 (A, B and C), which are generated by the removal of the terminal mannose from the individual carbohydrate triantennary, could be recognized differentially by the quality control machinery. Furthermore, EDEM may also recognize protein determinants such as exposed hydrophobic regions, which are hallmarks for misfolded proteins, as suggested by the observation that the EDEM– β -secretase complex remained intact after digestion of the majority of the glycans with endoglycosidase H².

It appears that at least two types of CNX complexes are found in the ER: CNX associated with the thioredoxin-like protein ERp57 through its extended flexible arm, which assists with the folding and oxidation of nascent chains; and CNX in complex with EDEM through their transmembrane regions, to aid in the sorting of defective proteins for degradation. Overexpression of EDEM increases the fraction of CNX bound to EDEM, permitting the rapid degradation of ERAD substrates. A similar fate is observed upon exposure of cells to prolonged ER stress, which activates a pathway that leads to the transcription induction of EDEM¹⁸. Since CNX levels remain constant with stress, the increase in EDEM concentration should increase the fraction of CNX–EDEM complexes.

Valuable advances have been made recently in our understanding of how



ERAD substrates are tagged and recognized as defective and how they are extracted into the cytosol for proteasomal degradation¹⁹. However, a large gap remains in our understanding of how the ERAD substrates are targeted to a membrane channel for retranslocation. Further studies will be required to determine if EDEM itself helps with the initial retranslocation process by targeting ERAD substrates to the translocon.

Tao Wang and Daniel N. Hebert are in the Department of Biochemistry and Molecular Biology, Program in Molecular and Cellular

Biology, University of Massachusetts, Amherst, Massachusetts 01003, USA. Correspondence should be addressed to D.N.H. e-mail: dhebert@biochem.umass.edu

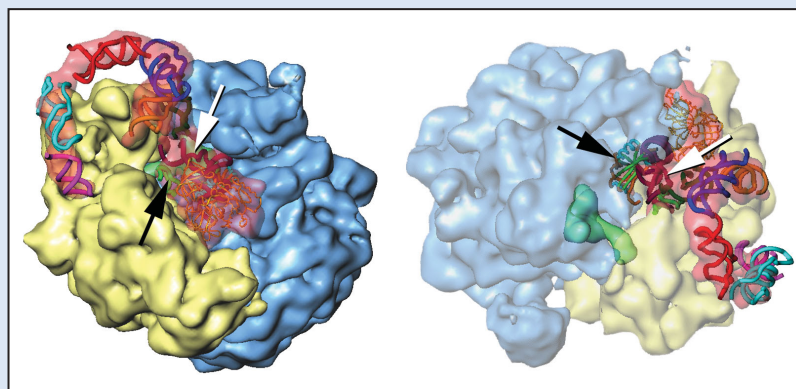
1. Ellgaard, L. & Helenius, A. *Nat. Rev. Mol. Cell Biol.* **4**, 181–191 (2003).
2. Molinari, M., Calanca, V., Galli, C., Lucca, P. & Pagnetti, P. *Science* **299**, 1397–1400 (2003).
3. Oda, Y., Hosokawa, N., Wada, I. & Nagata, K. *Science* **299**, 1394–1397 (2003).
4. McCracken, A.A. & Brodsky, J.L. *J. Cell Biol.* **132**, 291–298 (1996).
5. Schnell, D.J. & Hebert, D.N. *Cell* **112**, 491–505 (2003).
6. Helenius, A. & Aebi, M. *Science* **291**, 2364–2369 (2001).
7. Schrag, J.D., Propopio, D.O., Cygler, M., Thomas, D.Y. & Bergeron, J.J. *Trends Biochem. Sci.* **28**, 49–57 (2003).
8. Hebert, D.N., Foellmer, B. & Helenius, A. *EMBO J.* **15**, 2961–2968 (1996).
9. Sousa, M. & Parodi, A.J. *EMBO J.* **14**, 4196–4203 (1995).
10. Appenzeller, C., Andersson, H., Kappeler, F. & Hauri, H.-P. *Nat. Cell Biol.* **1**, 330–334 (1999).
11. Su, K., Stoller, T., Rocco, J., Zemsky, J. & Green, R. *J. Biol. Chem.* **268**, 14301–14309 (1993).
12. Liu, Y., Choudhury, P., Cabral, C.M. & Sifers, R.N. *J. Biol. Chem.* **274**, 5861–5867 (1999).
13. Knop, M., Hauser, N. & Wolf, D.H. *Yeast* **12**, 1229–1238 (1996).
14. Jakob, C.A., Burda, P., Roth, J. & Aebi, M. *J. Cell Biol.* **142**, 1223–1233 (1998).
15. Jakob, C.A. *et al. EMBO Rep.* **2**, 423–430 (2001).
16. Nakatsukasa, K., Nishikawa, S.-i., Hosokawa, N., Nagata, K. & Endo, T. *J. Biol. Chem.* **276**, 8635–8638 (2001).
17. Hosokawa, N. *et al. EMBO Rep.* **2**, 415–422 (2001).
18. Yoshida, H. *et al. Dev. Cell* **4**, 265–271 (2003).
19. Tsai, B., Ye, Y. & Rapoport, T.A. *Nat. Rev. Mol. Cell Biol.* **3**, 246–255 (2002).

tmRNA to the rescue

When a bacterial ribosome encounters a defective mRNA during the course of protein synthesis, the ribosome may 'stall', thereby blocking further protein synthesis at that ribosome. Such a stalled ribosome is rescued by tmRNA, a molecule that possesses the functions of both tRNA and mRNA.

tmRNA contains an alanyl-tRNA-like domain (TLD) and an open reading frame (ORF) encoding a peptide degradation tag. During the rescue process, alanyl-tmRNA binds in the ribosome A site and allows its alanine to be added to the nascent polypeptide chain. Then, in the not-so-well understood process referred to as trans-translation, the ribosome shifts from the defective mRNA to the ORF of tmRNA and resumes normal translation. The stalled ribosome is thereby rescued and the defective protein is tagged for degradation.

In a report in the April 4 issue of *Science* (**300**, 127–130; 2003), Valle *et al.* used cryo-EM to examine the ribosome rescue complex. They prepared a 70S ribosome complex stalled at the end of a short mRNA and reacted it with alanyl-tmRNA, SmpB (a small protein required for tmRNA-mediated rescue), elongation factor Tu (EF-Tu) and GTP in the presence of the antibiotic kirromycin. Kirromycin



allows GTP hydrolysis by EF-Tu but prevents the dissociation of EF-Tu-GDP from the ribosome, halting the rescue process at an early stage.

The cryo-EM map of the resulting complex (pictured in two different orientations) revealed the tmRNA TLD (burgundy ribbon within red surface, indicated by white arrow) engaged with EF-Tu (thin orange ribbon in red surface) at the ribosome A site in a similar manner to that observed for normal tRNA. SmpB (black arrow) bridges between the tmRNA TLD and the ribosome, approximating the D loop of tRNA.

A large part of the tmRNA molecule (red surface with multicolored ribbon) is loosely wrapped around a portion of the 30S subunit (yellow), positioning the ORF near the mRNA

entrance to the A site. This arrangement is suggestive of events that may occur following EF-Tu release and TLD translocation to the ribosome P site (tRNA occupying the P site is shown as a green surface). Translocation would guide the ORF into the A site where it serves as the template for the degradation tag that is attached to the defective protein.

Of course, the details surrounding other events in bacterial ribosome rescue — how tmRNA is recruited to the stalled ribosome and how trans-translation is achieved — remain unclear. Nonetheless, the study by Valle *et al.* offers a first look into the mechanism of action of tmRNA and sets the stage for future studies of ribosome rescue.

Elizabeth Cox Grzymski