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SPOTLIGHT

The HERCulean task of recognizing, ubiquitinating, and shielding misfolded integral membrane proteins

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During ER-associated decay, unfolded membrane-resident proteins are targeted for removal and degradation by ubiquitin ligases whose identities and precise operations remain unclear. In this issue, Guerriero and Brodsky discuss new results from Kamada et al. (https://doi.org/10.1083/jcb.202308003) showing the clearance of misfolded CFTR by the E3 ligase HERC3.

Membrane protein folding and insertion into a lipid bilayer begins at the endoplasmic reticulum (ER). As a result of their transmembrane domains (TMDs), membrane proteins are co-translationally deposited within three chemically distinct environments: the cytosol, the more oxidizing ER lumen, and the hydrophobic lipid bilayer. Because their native structures also require the coordinated, high-fidelity assembly of contact sites between soluble domains and the TMDs, the folding of some membrane proteins is inefficient. This problem is compounded for membrane proteins that transport ions or hydrophilic solutes, which require hydrophilic residues within the lipid bilayer that might lack stabilizing contacts during co-translational insertion.

To rid the ER of potentially toxic TMD-containing proteins, misfolded or misassembled confomers are selected, ubiquitinated, and retrotranslocated from the ER for degradation by the cytosolic proteasome via a process termed ER-associated degradation (ERAD) (1). To date, various factors catalyzing these steps have been identified, including a subset of the ∼600 E3 ubiquitin ligases that append ubiquitin onto ERAD substrates (2). Some E3s even facilitate three critical steps in the ERAD pathway: substrate selection, retrotranslocation, and ubiquitination (3, 4). In addition, more than one E3 can act on a single substrate, perhaps due to the multiple locations in which a misfolded lesion may reside. How the actions of E3 ligases are coordinated—especially during ERAD—is poorly understood.

Based on their vital functions and intricate folding itineraries, it is unsurprising that a large and growing number of human diseases arise from genetically encoded destabilizing mutations, thus linking them to the ERAD pathway. One of the earliest and perhaps the best studied examples is the F508del mutation in the cystic fibrosis transmembrane conductance regulator (CFTR). The corresponding protein lacks a phenyalanine at position 508 within the first nucleotide binding domain (NBD1), which delays protein folding. The misfolded protein is therefore delivered to the ERAD pathway, preventing delivery to the cell surface. Approximately 90% of CF patients carry at least one F508del allele, and although a drug cocktail benefits patients who possess this allele, further modifications to protein folding agonists would improve health. Because folding/ERAD modifiers are targets for other protein conformational diseases, characterizing the E3 ligases that act on CFTR and other ERAD substrates is also critical. However, with few exceptions (5, 6), the coordinated actions of these ligases during ERAD have not been fully elucidated.

In a new study, Kamada et al. focused on a poorly characterized HECT domain-containing E3 ligase, HERC3, during the ERAD of F508del-CFTR (7). While HERC3 has been studied in cancer, its role in ERAD was undefined. This group uncovered HER3C in an siRNA screen along with six other E3 ligases that, when knocked down, increased plasma membrane levels of low-temperature rescued F508del-CFTR (rΔF508) (8). Kamada et al. (7) now provide evidence for unexpected contributions of HERC3 during CFTR quality control. Although the effects of HERC3 silencing and overexpression on overall F508del-CFTR levels were modest, the impact of HERC3 knockdown was accentuated for rΔF508. Moreover, combinatorial knockdown with other E3 ligases that act upon CFTR, i.e., RNF5 (also known as RMA1) and RNF185 (9, 10), revealed additive effects, suggesting HERC3 acts via a distinct pathway. Of greater clinical relevance, additive effects were also evident when an FDA-approved folding corrector was included in knockdown experiments.

To better define the role of HERC3 during ERAD, F508del-CFTR was next fused to the 11-amino acid HiBiT tag, which in the presence of cytosolic LgBiT reconstitutes the formation of a naturally occurring nanoluciferase and allows quantification of protein levels in real-time. The use of this assay first confirmed additive effects of HERC3 with RNF5 and RNF185. When the HiBiT tag was next fused to F508del-CFTR so it faced

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the ER lumen, retrotranslocation could be measured via cytoplasmic luminescence. Interestingly, by examining diverse ERAD substrates, including soluble (lumenal) and integral membrane protein substrates possessing destabilizing lesions in the ER lumen, in the ER membrane, or facing the cytosol, only F508del-CFTR ERAD was affected by HERC3 knockdown. The ERAD of another misfolded ABC transporter family member (ABCB1) was also unaffected. At this point, it is unclear whether HERC3 recognizes specific amino acid motifs in CFTR or non-native conformers. However, by examining the fates of CFTR fragments fused to the HiBiT tag, the E3 appeared to recognize CFTR's membrane spanning domains (MSDs) when expressed in the context of the F508del allele or in the presence of another allele that similarly affects MSD folding. The selected targeting of MSDs by HERC3 was bolstered further by domain-swap experiments. In the future, defined regions within the MSD recognized by HERC3, and perhaps RNF185 in coordination with other factors (10), could be identified with site-specific crosslinkers. This will help differentiate between the possibility that HERC3 monitors general MSD folding or identifies, for example, exposed hydrophilic residues in a TMD that are required for CFTR-dependent ion transport.

Beyond its role in MSD recognition and ubiquitination, might HERC3 also facilitate the delivery of retrotranslocated F508del-CFTR to the proteasome? Some support for this model was provided by an experiment in which HERC3 and biotinylated CFTR fragments were produced in vitro. While the proteins interacted in solution, association was absent after CFTR was encapsulated into membranes. Therefore, perhaps like the E3 ligase-associated Bag6 protein that binds solubilized MSDs in solution (11), HERC3 might also help solubilize retrotranslocated F508del-CFTR during proteasome delivery. Moreover, Kamada et al. (7) investigated the relationship between HERC3 function and the ubiquilins, which augment the shuttling of select ubiquitinated substrates to the proteasome. Overexpression and knockdown studies were consistent with HERC3 facilitating the formation of a ubiquilin-F508del-CFTR complex. Data using the HiBiT fusion to monitor retrotranslocation were also consistent with ubiquilin-associated MSD shielding. Thus, HERC3 and ubiquilin might help solubilize the retrotranslocated protein, but direct proof for this possibility—along with studies examining a role for Bag6 or other chaperone-like "holdases"—will require refined biochemical analyses.

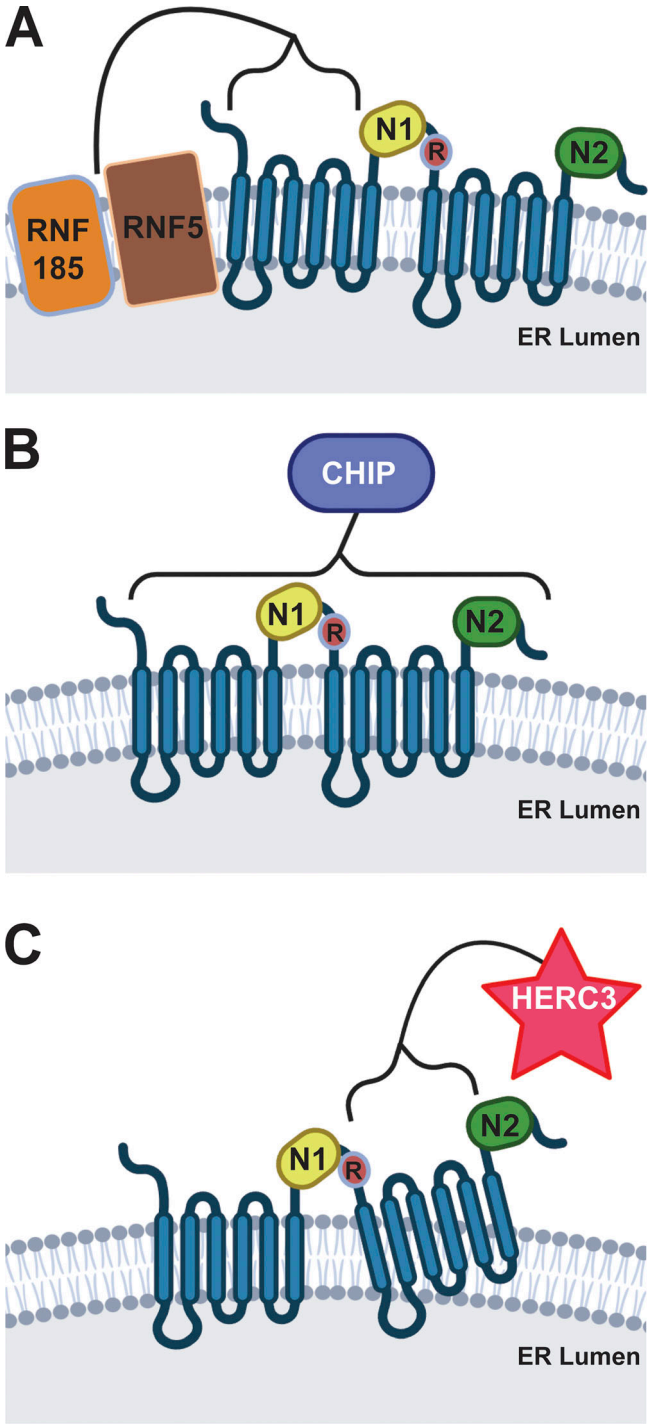

Figure 1. **HERC3 joins the cast of E3s that coordinate CFTR degradation. (A)** CFTR is depicted with transmembrane domains in blue, NBD1 (N1) in yellow, the regulatory domain (R) in red, and NBD2 (N2) in green. Also shown are two ER-resident E3 ubiquitin ligases, RNF185 (orange) and RNF5 (brown), which might recognize MSDs. **(B)** CHIP (blue oval) ubiquitinates NBD1 and potentially shorter cytosol resident loops after recognition by molecular chaperones (not shown). **(C)** HERC3 (red star) plays several roles in F508del-CFTR turnover, including ubiquitination and potentially binding to exposed MSDs to promote retrotranslocation and/or help maintain solubility. Created with https://BioRender.com.

The discovery of HERC3's role in CFTR degradation enriches our understanding of the cellular machinery involved in the quality control of complex integral membrane proteins in the ER. The study also highlights requirements for diverse ubiquitin ligases during ERAD, especially when one considers that some E3s directly facilitate retrotranslocation. One model envisions the coordinated handoff of F508del-CFTR from ER-localized E3 ubiquitin ligases, RNF5 and RNF185, to cytosolic E3 ligases, such as HERC3, and to another cytosolic E3 ligase, CHIP (12) (Fig. 1). Alternatively, or in parallel, RNF5 and RNF185 might simply recognize different non-native F508del-CFTR (sub)domains. In this context, it should be recognized that the F508del mutation exhibits long-range effects on CFTR architecture, perhaps explaining why so many ligases are implicated in its degradation. However, caution is needed in overinterpreting some data: the authors noted cell type–specific effects when HERC3 levels were modulated, an unfortunately common phenomenon in the field. Regardless, based on the results presented in this paper and other reports, HERC3 may perform dual roles by recognizing non-native structures within MSDs, potentially as F508del-CFTR begins to retrotranslocate, and possibly shielding partially retrotranslocated species (Fig. 1). It is also likely that other folding-compromised ERAD substrates similarly require a diverse cadre of E3 ligases with distinct activities and recognition sites. Finally, as hinted, the results presented here may open new opportunities for therapeutic intervention, particularly given the growing focus on "drugging" various nodes in the ubiquitin pathway.

References

1. Brodsky, J.L. 2012. *Cell*. https://doi.org/10.1016/j.cell.2012.11.012
2. Christianson, J.C., et al. 2023. *Nat. Rev. Mol. Cell Biol*. https://doi.org/10.1038/s41580-023-00633-8
3. Sato, B.K., et al. 2009. *Mol. Cell*. https://doi.org/10.1016/j.molcel.2009.03.010
4. Wu, X., et al. 2020. *Science*. https://doi.org/10.1126/science.aaz2449
5. van de Weijer, M.L., et al. 2020. *Mol. Cell*. https://doi.org/10.1016/j.molcel.2020.09.023
6. Morito, D., et al. 2008. *Mol. Biol. Cell*. https://doi.org/10.1091/mbc.e07-06-0601
7. Kamada, Y., et al. 2024. *J. Cell Biol*. https://doi.org/10.1083/jcb.202308003
8. Okiyoneda, T., et al. 2018. *Dev. Cell*. https://doi.org/10.1016/j.devcel.2018.02.001
9. Younger, J.M., et al. 2006. *Cell*. https://doi.org/10.1016/j.cell.2006.06.041
10. El Khouri, E., et al. 2013. *J. Biol. Chem*. https://doi.org/10.1074/jbc.M113.470500
11. Wang, Q., et al. 2011. *Mol. Cell*. https://doi.org/10.1016/j.molcel.2011.05.010
12. Estabrooks, S., and J.L. Brodsky. 2020. *Int. J. Mol. Sci*. https://doi.org/10.3390/ijms21020452