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Competing interests
The authors declare no competing interests.

QUALITY CONTROL

Unlocking the door for ERAD

Misfolded proteins in the endoplasmic reticulum (ER) are returned to the cytosol and destroyed by a process known as ER-associated degradation (ERAD). Hrd1 has been implicated as the channel that mediates the transport of ERAD substrates to the cytosol. A study demonstrates that Hrd1 is gated by autoubiquitination and a soluble ERAD substrate.

Miguel Betegon and Jeffrey L. Brodsky

The endoplasmic reticulum (ER) receives approximately one-third of the proteome in eukaryotes and thus plays an active role during the folding and post-translational modification of diverse integral membrane and soluble proteins in the secretory pathway. However, genetic mutations, cellular stress, and stochastic errors during synthesis and folding compromise the ability of nascent proteins to attain their native states in the ER. To prevent these misfolded proteins from exerting toxic effects, aberrant proteins are recognised by molecular chaperones, ubiquitinated, and degraded by the 26S proteasome, a process known as ER-associated degradation (ERAD). Because the proteasome resides in the cytosol, soluble ERAD substrates (i.e., those initially confined entirely to the ER lumen) must be transported, or 'retrotranslocated', to the cytosol[1]. In recent years, evidence from Rapoport and colleagues indicates that Hrd1, an ER membrane protein that exhibits E3 ubiquitin ligase activity, serves as a retrotranslocation channel for ERAD[2–5]. Nevertheless, the mechanism that triggers the opening of the channel is incompletely understood. In this issue, Meinecke and colleagues report on the route by which the Hrd1 channel opens to direct the expulsion of misfolded proteins from the ER[6].

Hampton and colleagues isolated Hrd1 as a gene required for the regulated degradation of Hrd2, an enzyme that catalyses the rate-limiting step in sterol biosynthesis[7]. In parallel, Wolf and colleagues identified Hrd1 as a gene that, when mutated, increases the steady state expression of misfolded soluble and integral membrane vacuolar proteases CPY* and PrA*, respectively[8]. In yeast, Hrd1 assembles into a multiprotein complex that ubiquitinates and targets misfolded soluble and membrane proteins for ERAD (Fig. 1a)[9,10]. Several lines of evidence suggest that Hrd1 might also serve as the channel to allow egress of these ERAD substrates. First, Hrd1 overexpression in yeast was sufficient to stimulate ERAD substrate degradation in the absence of other Hrd1 complex members, and Hrd1 interacted directly with ERAD substrates[2]. Second, Hrd1 can retrotranslocate an ERAD substrate in a reconstituted system with purified factors, an event that requires Hrd1 autoubiquitination[3,4]. Third, a cryo-electron microscopy (cryo-EM) structure of Hrd1 in complex with its partner Hrd3 revealed that the architecture of Hrd1 resembles Sec61, a channel required for protein translocation into the ER[5]. However, the function of Hrd1 as a channel had not been directly measured and, because its structure differs from that of any known ion channel, the mechanism(s) that directly regulate Hrd1 gating were mysterious. To this end, Meinecke and colleagues set out to investigate the effect of Hrd1 autoubiquitination and substrate binding on Hrd1 channel activity[6].

Using an in vitro system consisting of purified Hrd1 incorporated into a planar lipid bilayer, the authors first measured whether Hrd1 formed an ion channel and how this activity might be regulated under different conditions. Single-channel recordings revealed that Hrd1 alone lacks channel activity[6], consistent with its closed state in the cryo-EM structure[5]. However, Hrd1 autoubiquitination, induced by addition of E1 and E2 enzymes as well as a fragment of an E2-activating protein, led to small conductance events. This effect was reversible, as Hrd1 deubiquitination inactivated the channel. Notably, the addition of CPY* led to larger and more frequent channel conductance events, and the results suggested that the channel can reach diameters comparable to those found in other polypeptide translocation channels[11,12]. Notably, Hrd1 that had not been ubiquitinated remained inactive, even in the presence of CPY*. Moreover, the ability of CPY* to augment channel gating was only observed when the substrate interacted with the lumenal side of Hrd1 (ref. [6]). Because an ERAD channel must allow the retrotranslocation of aberrant but not native proteins, channel selectivity was also examined. As anticipated, the authors demonstrated that the folded, wild-type version of CPY*, CPY, was

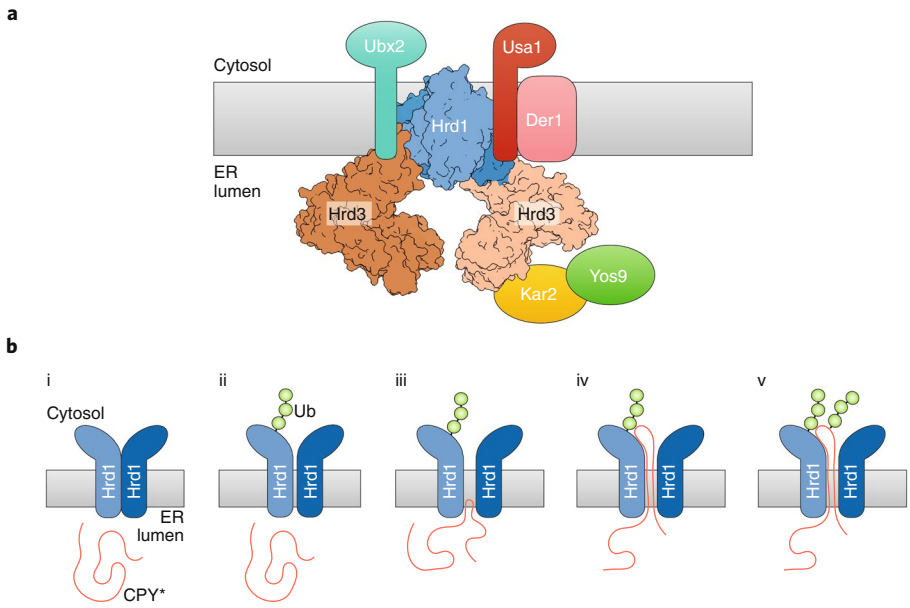

Fig. 1 | The Hrd1 complex and the schematic for Hrd1-dependent retrotranslocation of ERAD substrates. a, Schematic of the Hrd1 multiprotein complex. A Hrd1 dimer associates with two molecules of Hrd3 (adapted from the cryo-EM structure[5]). On the lumenal side, Hrd3 associates with Kar2 and in turn Yos9, which survey the structures of nascent glycoproteins and then recruit misfolded substrates to the ERAD machinery. Along with Hrd1, Der1 also intimately contacts retrotranslocating polypeptides. Usa1 associates with Hrd1, promoting its dimerisation, and the cytosolic domain of Ubx2 recruits Cdc48, an AAA$^+$-ATPase (not shown), to the Hrd1 complex for retrotranslocation. **b**, A model of Hrd1 substrate retrotranslocation and ubiquitination. i. Hrd1 is in the closed inactive state. ii. Hrd1 autoubiquitination (Ub) leads to initial channel formation. iii. Binding of a misfolded substrate to the lumenal side of Hrd1 further opens the channel, allowing for substrate (i.e., CPY*) insertion. iv. The presence of a high-affinity binding site on the cytosolic side of ubiquitinated Hrd1 drives substrate retrotranslocation. v. Retrotranslocated CPY* is ubiquitinated by Hrd1.

unable to open ubiquitinated Hrd1. They also observed that mutations that prevent the autoubiquitination of the Hrd1 RING domain reduced channel activity[6]. These data are congruent with the previously observed contribution of RING-domain-dependent ubiquitination to ERAD activity in yeast[4].

To better define the Hrd1 gating mechanism, the authors then asked whether CPY* and CPY also bind to the cytosolic face of liposome-incorporated Hrd1 (ref. [6]). As might be expected, CPY* was unable to associate with non-ubiquitinated Hrd1. By contrast, although CPY* did not facilitate Hrd1 channel formation when added to the cytosolic surface of Hrd1 (see above), it unexpectedly bound to the cytosolic domain of ubiquitinated Hrd1 with high affinity (~20 nM). The authors then incorporated Hrd1 into nanodiscs to measure substrate interaction with both the lumenal and the cytosolic sides of Hrd1 and discovered that the association between CPY* and ubiquitinated Hrd1 was comparable to that when CPY* bound to the cytosolic side of ubiquitinated Hrd1. Thus, the high-affinity substrate-binding site resides at the cytosolic domain of Hrd1. Nevertheless, CPY* was unable to bind to the cytosolic side of non-ubiquitinated Hrd1. Surprisingly, the authors also determined that CPY* interacted with non-ubiquitinated Hrd1 in the lumen with an affinity of ~200 nM. Because this interaction was unable to gate the channel, binding via this lower affinity interaction must be unproductive for gating. Potentially, the lumenal association between CPY* and non-ubiquitinated Hrd1 could hold the substrate in a retrotranslocation-competent state until Hrd1 is ubiquitinated, or it might facilitate Hrd1 ubiquitination. Together, these data indicate that the interaction between an ERAD substrate and ubiquitinated Hrd1 is stronger on the cytosolic side than on the lumenal side of Hrd1, which could facilitate substrate retrotranslocation. Yet, only lumenal interactions with an unfolded substrate lead to channel formation, consistent with Hrd1 functioning as a unidirectional channel.

Finally, the authors sought to understand whether CPY* directly binds to the Hrd1 polypeptide and/or the (poly)ubiquitin moiety. By comparing the association between CPY* and polyubiquitinated Hrd1 relative to another polyubiquitinated protein, Ubc6, they showed that there was a subtle preference for Hrd1 when Hrd1 and Ubc6 possess long polyubiquitin chains. However, as the ubiquitin chains became shorter, Hrd1 bound to the substrate with a substantially higher affinity than Ubc6, likely as a result of direct interactions between Hrd1 and the substrate. Consequently, Hrd1 containing short ubiquitin chains more selectively interacts with misfolded substrates.

Overall, these data provide more definitive evidence that Hrd1 functions as a channel and offer new insights into the regulation of Hrd1 during retrotranslocation. One model envisions that autoubiquitination primes Hrd1 for lumenal substrate binding and channel formation (Fig. 1b), but substrate binding increases channel opening, allowing for substrate insertion. Next, the formation of a high-affinity binding site on the cytosolic side of Hrd1 following autoubiquitination could potentially drive substrate retrotranslocation, substrate ubiquitination, and subsequent membrane extraction. In the future, it will be exciting to investigate how other components of the Hrd1 complex, such as Hrd3 (ref. [13]), contribute to the regulation of Hrd1 channel formation, dimer stabilisation, and substrate interaction. It is also unclear whether integral membrane ERAD substrates that associate with and require Hrd1 function[14] gate the channel in a similar fashion. In addition, it will be vital to determine whether different integral membrane ubiquitin ligases, which might function as retrotranslocation channels for integral membrane substrates with misfolded cytosolic domains[15], exhibit similar gating properties. Future experiments with a more complete system and structural work with a focus on ubiquitinated Hrd1 in the presence of Hrd1-associated partners and substrates will provide a more comprehensive view of the specific events that occur during ERAD substrate retrotranslocation. ❐

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Competing interests

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