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Roles of p97-associated deubiquitinases in protein quality control at the endoplasmic reticulum

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Abstract

To maintain protein homeostasis in the ER, an ER protein quality control system retains unfolded polypeptides and misassembled membrane proteins, allowing only properly folded proteins to exit the ER. Misfolded proteins held in the ER are retrotranslocated into the cytosol, ubiquitinated, and degraded by the proteasome through the ER-associated degradation pathway (ERAD). By timely eliminating misfolded proteins, the ERAD system alleviates cytotoxic stress imposed by protein misfolding. It is well established that ER-associated ubiquitin ligases play pivotal roles in ERAD by assembling ubiquitin conjugates on retrotranslocation substrates, which serve as degradation signals for the proteasome. Surprisingly, recent studies have revealed an equally important function for deubiquitinases (DUBs), enzymes that disassemble ubiquitin chains, in ERAD. Intriguingly, many ERAD specific DUBs are physically associated with the retrotranslocation-driving ATPase p97. Here we discuss the potential functions of p97-associated DUBs including ataxin-3 and YOD1. Our goal is to integrate the emerging evidence into models that may explain how protein quality control could benefit from deubiquitination, a process previously deemed destructive for proteasomal degradation.

Keywords

p97/VCP/Cdc48; ERAD/retrotranslocation; deubiquitination; ataxin-3; YOD1; proteasome; ubiquitin; ER protein quality control

Introduction

The biogenesis of secretory and membrane proteins require translocation of nascent polypeptide chains into the lumen of the endoplasmic reticulum (ER) or their integration into the ER membrane. Polypeptides are then folded and assembled into proper oligomeric complexes in the ER. This process is assisted by a large number of chaperones. Interestingly, many ER chaperones can also serve a triaging role to retain polypeptides that fail to acquire proper conformation, which often causes the degradation of the retained proteins by a process termed ER-associated protein degradation (ERAD) or retrotranslocation. It appears that the partition of ER chaperones between the folding and degradation processes may determine the fate of chaperone-bound substrates. Specific communications between these triaging chaperones and certain ERAD machinery proteins in the membrane likely initiate the retrotranslocation of misfolded polypeptides into the cytosol where they are degraded by the ubiquitin proteasome system [1–3].

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The ubiquitin proteasome system is the major proteolytic machinery in eukaryotic cells that is responsible for disposal of many damaged or unwanted proteins. Proteasomal degradation usually requires the formation of ubiquitin chains on a Lys residue in the substrate, which serves as a degradation signal. Posttranslational modification of substrates with ubiquitin chains is mediated by the concerted actions of three enzymes, an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme, and an E3 ubiquitin ligase. As expected, most ubiquitin ligases involved in ERAD either contain membrane anchors or interact with ER-associated proteins, which orient the catalytic domain of these enzymes on the cytosolic side of the ER membrane [4, 5]. It is generally believed that these enzymes act on ERAD substrates while the substrates are still associated with the ER membrane, conjugating ubiquitin chains them. Ubiquitination hereby serves dual roles. In addition to its conventional role in targeting retrotranslocated ERAD substrates to the proteasome, it also allows the engagement of a cytosolic ATPase termed p97 in a step upstream of proteasomal degradation. p97 uses the energy from ATP hydrolysis to extract ubiquitinated ERAD substrates from the membrane for proteasomal targeting [6, 7]. In this context, it is intriguing that both the retrotranslocation-driving ATPase p97 and the proteasome interact with deubiquitinases (DUBs) [8, 9], enzymes dedicated to destruction of ubiquitin chains [10]. Even more surprising are the findings that many of these DUBs can positively regulate ERAD given that the disassembly of ubiquitin chains would in principle erase the degradation signal and hence inhibit the degradation process. In this review, we discuss the possible models that may explain the functions of p97-associated DUBs (PAD) in ERAD.

Ubiquitin and ERAD

Efficient elimination of misfolded ER proteins requires cooperation between ERAD machinery proteins that reside on both sides of the ER membrane. Chaperones in the ER lumen initiate the process as they retain misfolded proteins. The retained polypeptides are then targeted to one of the few putative protein conducting channels through which retrotranslocation takes place. Cytosolic factors that associate with the putative retrotranslocons recognize the substrate emerging from the membrane, modify them with ubiquitin chains, and segregate them from the membrane for delivery to the proteasome (Figure. 1).

It is well established that ubiquitination of polypeptides undergoing retrotranslocation is crucial for ERAD [1, 2, 4, 5]. The core ERAD ubiquitination machineries comprising of ubiquitin ligases and their cognate conjugating E2 enzymes are highly conserved from *S. cerevisiae* to human. These enzymes are believed to either intimately associate with the retrotranslocation apparatus or even be an integral component of the putative retrotranslocation channels. The relevant E2s identified in budding yeast include Ubc7p, Ubc6p, and Ubc1p [11–14]. Deletion of these genes individually or in combination inhibits ubiquitination and degradation of many misfolded ER proteins. Likewise, the mammalian homologous of Ubc7p and Ubc6p, the UBE2g and UBE2j sub-families, respectively, are required for degradation of many model ERAD substrates studied to date [15–20]. In yeast, Hrd1p and Doa10p are the two major ERAD-dedicated ubiquitin ligases [14, 21, 22], whereas in mammals, Hrd1 and a Hrd1-related gene named gp78 mediate the degradation of many misfolded ER proteins [19, 23–27]. Mammalian cells also employ several additional E3 ligases such as RMA1 [27, 28], RNF121 [29], RNF170 [30], the U-box containing ubiquitin ligase CHIP [31], Parkin [32], and two F-box-containing (Fbs1 and Fbs2) multi-subunit Skp-Cullin-F-box (SCF) ubiquitin ligases [33, 34]. In addition, a ubiquitin chain-elongating factor named Ufd2p in yeast or E4a and E4b in mammals was shown to extend short ubiquitin chains on the substrate to enhance the efficiency of proteasomal degradation [35–38]. Despite that the list of ERAD specific E3s has been significantly expanded, ubiquitin ligases are clearly outnumbered by the thousands of ER proteins that could become

misfolded. This raises the question of how a small collection of E3 ubiquitin ligases can deal with a large number of substrates sharing no sequence homology. A prevailing model is that each retrotranslocation complex may contain one or a few ubiquitin ligases that are capable of modifying any substrates emerging from the same translocon. To ensure the processivity of ubiquitin chain formation on such diverse substrates bearing no common 'degron', the cell has evolved several specific mechanisms, which include preassembly of ubiquitin chains on the E2 active site cysteine (e.g. the gp78-UBE2g2 complex) or engagement of a substrate-holding chaperone (e.g. DOA10 and CHIP) [31, 37, 39, 40].

In addition to ubiquitin ligases, emerging evidence reveals that deubiquitinases, enzymes that disassemble ubiquitin chains, also play important roles in ERAD [10]. Deubiquitinases involved in ERAD can be categorized into two groups on the basis of the core ERAD machinery proteins that they bind to. Several DUBs are associated with the proteasome and their roles in proteasomal degradation are not restricted to ERAD [8, 9]. Some of the proteasome-associated DUBs cleave ubiquitin chains from the substrate to inhibit protein degradation [41, 42]. However, at least one of these DUBs Rpn11 can promote protein turnover by removing the bulky ubiquitin conjugates from the substrate and thereby facilitate substrate entry into the proteolytic compartment of the proteasome [43, 44]. Removal of ubiquitin chains from the substrates while they are being threaded into the proteasome also exempts ubiquitin from proteasomal degradation, allowing ubiquitin molecules to be recycled for use by other substrates [45]. The second class of DUBs, which associate with p97, can remove ubiquitin from the substrates that are still bound by p97 [8, 46–48].

p97/CDC48: a ubiquitin mastermind

p97 (also termed VCP for valosin-containing protein or CDC48) was originally identified as an AAA (ATPase associated with various cellular activities) ATPase that regulates cell cycle progression in both yeast and mammals [49]. p97 contains two walker type ATPase domains. The first ATPase domain assembles the enzyme into a barrel-like hexamer with a central pore [50]. This configuration is commonly found in members of this ATPase family. For instance, the base of the 19S proteasome also contains six AAA ATPases that are assembled into a similar ring-like structure. p97/CDC48 also bears an amino-terminal domain and a short unstructured carboxyl tail, which connect p97 to various cofactors [51]. In ERAD, a dimeric cofactor complex termed Ufd1-Npl4 uses a ubiquitin binding motif in Ufd1 to facilitate substrate recognition by p97 [6, 52, 53]. Existing evidence suggest that ATP hydrolysis by p97 leads to the extraction of ubiquitinated ER proteins from the membrane, but the precise mechanism by which p97 extracts substrates is unclear [6, 54–56]. Because the action of p97 generally results in the separation of polypeptides from a relatively large immobile subcellular structure, p97 has been dubbed “segregase” [57]. It is noteworthy that the “segregase” function of p97 has been extended to other substrates involved in biological processes such as the regulation of transcription, DNA replication, nuclear envelop formation, membrane fusion, endocytic trafficking, and autophagosome formation [49]. The functional diversity of p97 is at least in part owing to its ability to engage a large number of cofactors, which are thought to link the ATPase to different substrates in the cell [51].

As mentioned above, p97 can associate with both ubiquitin ligases and DUBs, enzymes with opposing biochemical activities. Among the ERAD specific E3 ligases that directly interact with p97, the best characterized example is gp78, which use a small p97-interacting motif to bind to the N-domain of p97 [58, 59]. Conceptually, the association of p97 with ubiquitin ligases provides an obvious means to synchronize the retrotranslocation and the ubiquitination processes, which would explain why only fully ubiquitinated substrates can

be extracted from the membrane [60]. p97 may even be able to influence the ubiquitination process by modulating the activity of E3 ligases or their interactions with the substrate. By contrast, the logic underlying the involvement of PAD in protein degradation at a step upstream of substrate targeting to the proteasome is elusive because ubiquitin chains with four ubiquitin moieties have been established as the minimum targeting signal [61]. Thus, disassembly of ubiquitin chains on the substrates that are still associated with p97 could possibly have a detrimental effect on their turnover because it could erase or damage the degradation signal. So what are the functions of PAD in ERAD? In the next section, we will discuss this issue using a few examples that have been studied to date. We should point out right up front that the available information on this topic is scarce and many aspects of the proposed models remain speculative in nature. Nonetheless, we hope that these models would serve as a starting point for further investigations on this intriguing problem.

ERAD relevant DUBs

Ataxin-3

Ataxin-3 was initially identified as a disease-associated gene because its coding product contains a short polyglutamine (polyQ) segment (13–36 residues) that is expanded in many patients suffering a neurodegenerative disorder termed spinocerebellar ataxia type 3 or Machado-Joseph disease (SCA3/MJD) [62–64]. In addition to the polyQ tract, Ataxin-3 also contains a Josephin domain and 2–3 ubiquitin-interacting motifs (UIMs) [65]. It is widely expressed in a variety of mouse tissues and the protein is localized in both nucleus and cytoplasm [66, 67]. Clinical studies have demonstrated a strong inverse correlation between the length of the polyQ repeat and the age of disease onset [64], suggesting a causal relationship. Recent studies demonstrated that ataxin-3 belongs to the MJD DUB family, which harbors a cysteine protease activity in the Josephin domain towards ubiquitin isopeptide bond [68]. *In vitro*, purified ataxin-3 protein can cleave ubiquitin from ubiquitinated substrates or disassemble free ubiquitin chains [68]. Structural studies confirmed that the Josephin domain forms a typical papain-like cysteine protease fold similar to many other DUBs in the same family [69, 70].

The first clue for the involvement of ataxin-3 in ubiquitin dependent protein turnover comes from yeast two-hybrid studies, which revealed an interaction between ataxin-3 and two ubiquitin receptor proteins of the proteasome (HHR23a and HHR23b) [71, 72]. These proteins use a ubiquitin like domain to interact with the proteasome and a ubiquitin-associated domain (UBA) to bind ubiquitinated substrates, and therefore can facilitate substrate recognition by the proteasome [73, 74]. The association of ataxin-3 with HHR23 proteins suggests a possible role for ataxin-3 in substrate delivery to the proteasome. A subsequent genetic study in *Drosophila* demonstrated that expression of wild type ataxin-3, but not a deubiquitinating defective mutant suppresses polyQ-induced neurodegeneration of the photoreceptor cells in fly eyes. The action of ataxin-3 in this context requires its DUB activity as well as the intact proteasome function, suggesting that ataxin-3-mediated deubiquitination may promote proteasomal degradation of misfolded or dysfunctional proteins to alleviate polyQ-associated toxicity [75]. Consistent with this notion, ataxin-3 was also found to interact directly with p97 [72, 76].

Given the biochemical link between ataxin-3 and p97, we and Pittman's group independently evaluated the potential involvement of ataxin-3 in ERAD [46, 77]. Interestingly, although similar observations were made, different conclusions were reached. Both groups have tested the effect of ataxin-3 overexpression and knockdown on the degradation of model ERAD substrates. The results showed that expression of wild type ataxin-3 moderately inhibits ERAD, but its downregulation in the cell has marginal effect on ER protein homeostasis. Zhong et al. proposed that ataxin-3 may normally serve as a break

that negatively regulates the flow of substrates from ER to the proteasome [77]. Accordingly, inhibition of ERAD by ataxin-3 may allow more proteins to fold in the ER, and thus boosts the secretory capacity. By contrast, for the reasons outlined below, we favor a different interpretation. We proposed that ataxin-3 might play a positive yet non-essential role in ERAD, perhaps, as a facilitator that helps to channel the retrotranslocated substrates to the proteasome. First, the deubiquitinating activity of ataxin-3 only acts on substrates that have been extracted from the ER membrane following ATP hydrolysis by p97 because treatment of cells with either an ATPase defective p97 mutant or a p97 inhibitor drastically abrogates ataxin-3's ability to remove ubiquitin conjugates from p97-bound substrates [46, 78, 79]. In this regard, if ataxin-3 were a negative regulator of ERAD, its action in such a late step would stabilize ERAD substrates in dislocated form in the cytosol, which could not boost secretory capacity because the ER targeting signal sequence of these substrates had been removed. In addition, the model proposed by Zhong et al. is inconsistent with the genetic study in *Drosophila* mentioned above and with recent studies in *C. elegans* (see below). Moreover, since both wild type ataxin-3 and the catalytic inactive ataxin-3 mutant can inhibit ERAD, the ERAD inhibition phenotype associated with overexpression of wild type ataxin-3 likely results from a dominant negative effect rather than a gain in ataxin-3 function. Consistent with this interpretation, overexpression of many ERAD machinery proteins in wild type form can dominantly inhibit ERAD, as noted recently [80]. Studies in *C. elegans* and mice also support the notion that ataxin-3 is a promoter of protein turnover. Worms lacking ataxin-3 or mice containing an ataxin-3 mutant allele have increased levels of stress response, suggesting that they bear defects in protein homeostasis regulation [66, 81]. Finally, the degradation of a p97 substrate Ub-V-GFP is inhibited in ataxin-3 knockout worms [82]. Putting together, the evidence described above clearly suggests that ataxin-3 likely supports rather than inhibits proteasomal degradation of misfolded ER proteins, but we cannot exclude the possibility that in other cellular contexts ataxin-3 may function as an inhibitor of protein turnover.

YOD1

YOD1 is a DUB whose role in ERAD has just become unmasked recently. YOD1 possesses an Otubain (OTU) domain and thus belongs to the OTU DUB family. Additionally, it has an N-terminal ubiquitin-like UBX domain and a C-terminal C2H2 Zinc finger motif. In a screen for potential functional partners of YOD1, Ernst et al. discovered an interaction between YOD1 and the p97-Ufd1-Npl4 complex, linking this DUB to the ERQC pathway [47].

To characterize the role of YOD1 in ERAD, Ernst et al. first confirmed that YOD1 has DUB activity by demonstrating its ability to cleave both Lys48- and Lys63-linked ubiquitin chains *in vitro*. This activity is independent of the UBX and the Zinc finger domains [47]. Immunoprecipitation experiments showed that YOD1 also interacts with the ERAD machinery components such as Derlin-1 and UBXD8. Importantly, expression of a catalytically inactive YOD1 mutant (YOD1 C160S) dominantly inhibits the degradation of misfolded RI332, a model ERAD substrate, whereas wild type YOD1 has no effect on RI332 turnover. Although the UBX and ZnF domains are dispensable for the DUB activity of YOD1, they are required for the dominant negative effect imposed by the YOD1 C160S mutant. Since UBX domain is a known p97-interacting motif, it is tempting to speculate that the dominant negative effect of YOD1 C160S results from an interaction between YOD1 C160S and p97 that blocks the access of p97 to DUBs and/or other cofactors. Substrates stabilized by YOD1 C160S appear to accumulate in part in a polyubiquitinated form [47]. These data suggest a positive regulatory role for YOD1 in ERAD. However, no study has been thoroughly carried out to examine whether lack of YOD1 would result in any defects in ERAD. This is crucial given that expression of the catalytically inactive ataxin-3 mutant has

a similar dominant negative effect on ERAD, yet loss-of-function in ataxin-3 does not lead to any obvious ERAD defects. For this reason, whether or not the function of YOD1 in ERAD is essential remains to be established. In addition, although polyubiquitin conjugates were found to accumulate in YOD1 C160S-expressing cells, because the immunoprecipitation experiments were performed using a buffer that preserves protein-protein interactions [47], it is unclear whether ubiquitin conjugates precipitated under this condition are attached to RI332 or to an ERAD machinery protein co-purified with RI332. It is therefore unclear through which substrate YOD1 acts to regulate ERAD.

On the basis of their findings, Ernst and colleagues proposed that YOD1 may assist p97 in extraction of ERAD substrates from the membrane because ubiquitinated RI332 and another ERAD substrate UBC6e accumulated in YOD1 C160S-expressing cells are mostly associated with the membrane, a phenotype reminiscent of p97-inhibited cells. Furthermore, overexpression of YOD1 C160S together with a p97 ATPase defective mutant (p97QQ) causes ubiquitinated proteins to accumulate to a higher level than expression of either protein alone. By contrast, expression of wild type YOD1 reduces ubiquitin conjugates in association with both wild type p97 and the p97 QQ mutant. Together, these results showed that the DUB activity of YOD1 is not dependent on the ATP hydrolysis by p97, and therefore suggest that YOD1 may act upstream of or in parallel to p97-mediated retrotranslocation. This stands in sharp contrast to ataxin-3, which acts downstream of p97 [46]. Together, these findings seem to suggest that deubiquitination may be required at two distinct steps in ERAD, one upstream (e.g. YOD1) and one downstream of p97-mediated retrotranslocation (e.g. ataxin-3).

Other DUBs

A recent proteomic study identified at least three additional DUBs that can bind p97, which are USP13, USP50 and VCIPI1 [8]. Preliminary studies demonstrate that at least one of these enzymes USP13 is functionally implicated in ERAD, but the precise mechanism by which USP13 acts in ERAD is unclear [8]. In addition to p97, several other ERAD machinery proteins such as Ufd1, Npl4 and UBXD8 were also identified as USP13-interacting partners. Knockdown of USP13 stabilizes the model ERAD substrate TCR α and sensitizes 293T cells to ER stressor-induced cell death, suggesting that like ataxin-3, USP13 functions as an ERAD promoter instead of an antagonist.

A recent study also reported on another DUB named USP19 that may have a function in ERAD [83]. Unlike other DUBs that are all soluble proteins, USP19 contains a carboxyl tail anchor that can target it to the ER membrane. It was shown that USP19 can interact with Derlin-1 and overexpression of wild type USP19 can inhibit the degradation of a few model ERAD substrates. However, it is unclear whether this is due to a dominant negative effect or a biologically relevant DUB activity of USP19. In addition, it is also unclear whether or not USP19 can interact with p97.

The functions of p97-associated DUBs in ERAD

Thus, at least three DUBs have been confirmed to associate with p97 and function in ERAD. This raises many questions. How can p97 cooperate with so many DUBs? How many steps in ERAD may require a deubiquitinating event? What are the roles of these DUBs in ERAD? Do they have overlapping or redundant functions? What are the substrates of these DUBs? To our knowledge, very few studies have thoroughly examined the functions of PADs in ERAD. On the basis of the available evidence, we propose a working hypothesis that covers the potential functions of PAD, which include ubiquitin chain trimming for retrotranslocation and proteasome targeting.

Deubiquitination during dislocation

Since overexpression of YOD1 C160S blocks the dislocation of an ERAD substrate from the membrane by p97, Ernst et al. proposed that a deubiquitination event may be required for p97-mediated retrotranslocation. They further speculate that removal of ubiquitin chains from the substrate undergoing retrotranslocation may be required for p97 to thread the substrate through its central pore, a process that would lead to the extraction of the substrate from the ER membrane (Figure 2). This model also hints that a second round of ubiquitination is required downstream of p97 for the substrate to regain the proteasome targeting signal [84]. Although retrotranslocation by p97 may be facilitated by a DUB like YOD1, experimental data in support of the proposed “DUB-assisted threading” model is currently lacking. In fact, several lines of evidence question this model. First of all, it is yet to be proven that p97 actually extract ERAD substrates by threading them through its central pore. Although some AAA ATPases do seem to unfold the substrates using the threading mechanism [85], many AAA ATPases employ other strategies to re-model their substrates [86–88]. Interestingly, the same research group previously demonstrated that a p97 substrate can be released into the cytosol in a folded state by p97 [89], an observation that is inconsistent with the threading model because the size of the p97 central pore is too small to accommodate a folded protein. Secondly, as mentioned above, it is unclear whether the substrates of YOD1 are misfolded ER proteins or an ERAD machinery factor. Importantly, YOD1 seems unlikely to cause a complete removal of ubiquitin chains from the substrate in a biologically relevant time scale given its weak deubiquitinating activity (Deubiquitination by purified YOD1 takes a few hours to complete, whereas the half life of ERAD substrates can be as short as 5 minutes in the cell). Although the activity of some DUBs can be enhanced in the cell by unknown cofactors, such regulation has not been demonstrated for YOD1. We therefore hypothesize that YOD1 may act as a ubiquitin chain trimming or editing enzyme that modulates the length or topology of ubiquitin chains rather than a chain eliminator. It is conceivable that modulation of ubiquitin chains on a p97-bound ERAD substrate or a p97-associated adaptor may regulate the retrotranslocation process by several means even if the substrates do not pass through the central pore of p97 (Figure 3). For example, it has been demonstrated that the proper recognition of ubiquitin chains by the p97-Ufd1-Npl4 complex is required for its dislocating function [6, 7]. It is possible that ubiquitin chain trimming or editing may fine-tune substrate-p97 interaction, allowing p97 to hydrolyze ATP and execute the extraction function at the correct timing (e.g. when substrates are ready to be pulled out of the membrane). Alternatively, it may regulate the interactions between substrates and an upstream ERAD machinery factor, allowing the substrate to be handed off to p97 for dislocation.

Deubiquitination downstream of retrotranslocation

Like YOD1, the *in vitro* deubiquitinating activity of ataxin-3 is also weak even when one considers the activity of a mono-ubiquitinated ataxin-3 variant, which is increased compared to the non-modified enzyme [90, 91]. Intriguingly, ataxin-3 preferentially acts on Lys63-linked ubiquitin chains, a non-degradable ubiquitin linkage [92]. Because of this specific linkage preference and the sluggish enzyme activity, we and others proposed that ataxin-3 may edit or trim ubiquitin conjugates on the substrate, which would either remove a non-degradable signal from the substrate or alter its interaction with different ubiquitin effectors/receptors along the degradation pathway (Figure 4). Given that the deubiquitinating activity of ataxin-3 in the context of ERAD is strictly dependent on the p97 ATPase activity, it is likely that ataxin-3 preferentially acts on the dislocated substrates to facilitate their targeting to the proteasome, a model consistent with the observed association of ataxin-3 with the proteasome adaptor HHR23. One possibility is that ubiquitin chain trimming on the substrate can change its affinity to ubiquitin binding effectors, which causes substrate handoff from p97 to a downstream effector such as the recently identified Bag6 holdase

complex (Figure 4) [93]. Ataxin-3 and other p97-associated DUBs may cooperate with the Bag6 complex to channel retrotranslocated substrates to the proteasome. Another possibility is that chain trimming may alter the interactions between the substrate and ubiquitin receptors. This may provide assurance that only those substrates carrying proper degradation signals are targeted to the proteasome, as proposed recently [94]. Alternatively, the substrate of ataxin-3 may be an ERAD machinery protein that has been incidentally labeled with ubiquitin conjugates by an E3 ligase in the retrotranslocation complex. In this regard, PAD may remove these ubiquitin conjugates to provide a safeguard measure that ensures the stability and functionality of the ERAD machinery. In the situation with polyQ-expanded ataxin-3, the deregulated enzyme may cause defects in the ERAD system and therefore triggers ER stress. Indeed, expanded polyQ in another disease protein huntingtin (htt) can trap essential ERAD components such as p97, Npl4, and Ufd1 and therefore disturbs protein homeostasis and induces polyQ toxicity [95]. Intriguingly, expression of wild type ataxin-3 can suppress polyQ-induced cell degeneration [75], implying that increased normal ataxin-3 activity can overcome the detrimental effect imposed by polyQ expansion, possibly through boosting ERAD capacity.

Modulating the ERAD machinery

It is noteworthy that most existing models are based on the assumption that DUBs acts exclusively on misfolded substrates undergoing retrotranslocation. However, it is equally possible that these enzymes may cleave ubiquitin chains assembled on an ERAD machinery protein to regulate its activity, and thereby affect either the retrotranslocation or the degradation processes. Interestingly, a recent study demonstrates that ataxin-3 can regulate the activity of a ubiquitin ligase termed CHIP to function in another protein quality control pathway [96]. In this case, CHIP is mono-ubiquitinated by its cognate E2 enzyme, which enhances its association with ataxin-3. The pairing of CHIP with ataxin-3 allows the latter to modulate ubiquitin chains assembled on CHIP substrates. Ataxin-3 can also cleave the ubiquitin moiety on CHIP and thus disconnects itself from the E3 and its associated substrates. Ataxin-3 can also pair with Parkin, a RING finger-containing ubiquitin ligase, to regulate its stability [97]. In the light of these findings, it is possible that ataxin-3 or another ERAD relevant DUB may modulate the ubiquitination status of an ERAD machinery protein and therefore affect the assembly of the retrotranslocation complex. Dynamic interactions between ERAD machinery proteins may be a crucial mechanism that allows the large number of ERAD factors to act in a highly organized and ordered manner to execute their functions. Alternatively, deubiquitination of an ERAD machinery protein by a PAD may increase its stability to enhance ERAD efficiency.

Perspectives

In summary, although DUBs have been traditionally viewed as negative regulators of protein turnover because their actions are often linked to the removal of degradation signals from proteasomal substrates, recent studies have highlighted a distinct set of functions for DUBs that can promote protein turnover at least in the context of ERAD. It is apparent that our studies on the functions of PADs or deubiquitinases in general are at the infancy stage. Many questions are to be addressed by future studies. We feel that the most important task is to identify the substrate(s) for each of the p97-associated DUBs. Once we know the substrate, we can test what kind of ubiquitin chains are attached to the substrate and how complete or partial removal of these chains can benefit the degradation process. It is also currently unclear whether PAD is an evolutionarily conserved component of ERAD. Some earlier studies identified Otu1 as a YOD1 homologue in yeast and showed that it also interacts with the p97 homologue CDC48 [98, 99]. However, Otu1 seems to prefer mono-ubiquitin and Lys48-linked ubiquitin chains to Lys29- and Lys63-linked chains, which differentiates it from YOD1. Whether Otu1 is required for ERAD in yeast needs to be

investigated. Lastly, how are the activities of the ERAD relevant DUBs regulated? Ataxin-3 appears to be modulated by its own ubiquitination status, but it is unclear whether this is a general mechanism and how ubiquitination can activate ataxin-3. Along the same line, it is unclear how certain DUB can act prior to p97-mediated extraction whereas others function downstream of p97. With so many intriguing questions outstanding, the roles of deubiquitination in protein quality control and beyond will for sure become the forefront in the future studies on ubiquitin proteasome system.

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Figure 1. The ER-associated protein degradation pathway

ER-associated protein degradation proceeds through the following steps. In step 1, misfolded proteins are identified by ER chaperones (C), which target unfolded proteins to a retrotranslocation complex (R) in the membrane (step 2). In step 3, retrotranslocation is initiated as the substrate is moved across the ER membrane. The putative retrotranslocon comprises of at least one E3 ubiquitin ligase, which adds ubiquitin conjugates to the substrate (step 4). Upon modification, the ubiquitinated substrate is pulled out of the membrane by the p97-Ufd1-Npl4 complex (step 4). The retrotranslocated substrate is then targeted to the proteasome (step 5) for degradation (step 6). Due to space limits, only molecules involved in a particular step are shown. It is currently unclear whether these ERAD factors act in a single retrotranslocation complex or form a few subcomplexes that are sequentially recruited to execute their functions.

Figure 2. The proposed DUB-assisted dislocation model

In this model, retrotranslocation products are assumed to be retrotranslocated through the central pore of p97. Removal of the bulky ubiquitin chains from the substrate may facilitate substrate entry into the narrow pore of p97. However, this model indicates that the dislocated polypeptides need to be re-ubiquitinated before being targeting to the proteasome.

Figure 3. Potential roles of ubiquitin chain trimming in retrotranslocation

p97-associated DUBs may not completely eliminate ubiquitin chains from its substrates. Instead, they probably trim ubiquitin chains, which could influence the dislocating activity of p97 by several ways. (A) Proper chain length may be required to activate the p97 ATPase activity, which leads to the extraction of misfolded proteins from the ER membrane. (B) Similar to A except that the p97 dislocation activity could be fine-tuned by cleaving the ubiquitin chains on a p97-associated cofactor. (C) The modulation of ubiquitin chain length may facilitate the handoff of ubiquitinated substrates from an upstream ubiquitin binding receptor (green) to the p97 complex for subsequent dislocation.

Figure 4. A working model for ataxin-3 in ERAD

A possible role for ataxin-3 (atx3) in ERAD is to facilitate substrate targeting to the proteasome. Ataxin-3 may shorten the ubiquitin chain length, allowing ubiquitinated proteins to be released from the p97 complex to a downstream ubiquitin receptor that is associated with the proteasome such as the Bag6 holdase complex (dark blue). Ataxin-3 may also edit ubiquitin chains to maintain a desirable degradation signal.