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# Degradation of ubiquitin: The fate of the cellular reaper

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# Degradation of ubiquitin
## The fate of the cellular reaper

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**Key words:** ubiquitin, ubiquitination, degradation, proteasome, UBB$^{+1}$

**Abbreviations:** Ub, ubiquitin; UPS, ubiquitin-proteasome system; DUB, deubiquitinating enzyme; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein (ubiquitin conjugating enzyme); E3, ubiquitin-protein ligase; 20S CP, 20S core particle of the 26S proteasome; 19S RP, 19S regulatory particle of the 26S proteasome; Ubl, Ubiquitin-like; HECT, homologous to E6-AP carboxyl terminus; ODC, ornithine decarboxylase

Ubiquitin (Ub), a centrally important component of the ubiquitin-proteasome system (UPS), is covalently attached to numerous cellular proteins through a highly regulated process. The attached Ub serves as a recognition element in trans, to which a variety of downstream effectors bind. These complexes play roles in a broad array of cellular functions, the best studied is targeting of the conjugated proteins to degradation by the 26S proteasome. Regulated degradation plays key roles in basic processes such as cell cycle, differentiation, transcription, and maintenance of the cellular quality control. In addition to its conjugated form, there is also a free pool of Ub that is essential to ascertain its immediate availability for the many tasks it serves. Ub is considered as a stable protein, particularly due to its unique globular structure and ability to be recycled by deubiquitinating enzymes (DUBs). However, alterations in its steady state which occur under different pathophysiological conditions have suggested more complex, yet elusive, regulatory mechanisms that govern Ub stability. Recent findings have demonstrated that Ub can be degraded by the proteasome via three routes along with its conjugated substrate, when extended with a C-terminal tail, and as a monomer.

### Introduction

Modification of many key regulatory proteins by Ub controls a variety of essential cellular processes, such as cell cycle, transcription, signal transduction, DNA repair, the immune and inflammatory responses, growth and differentiation and cellular quality control.[1] Ub is a small, (76 amino acid residues) highly conserved protein. It is encoded by a family of multiple genes, and their contribution to the basal level of the protein varies among different organisms, tissues and cell types.[2-8] In yeast, Ub is encoded by four genes: Three of them—*Ubi1*, *Ubi2* and *Ubi3*—encode Ub fused to ribosomal subunits and provide all of the Ub necessary to support cell activities under basal metabolic conditions.[4] The fourth, *Ubi4*, encodes a tandem, head-to-tail repeat of five Ub moieties and is essential during stress.[5] Mammalian Ub is similarly encoded by four genes. Two of them—*Uba52* and *Uba80*—are coding Ub fusion proteins with ribosomal subunits, whereas *Ubb* and *Ubc* are coding for tandem-repeat, head-to-tail spacerless Ub units.[2,6-11] *Ubb* and *Ubc* are highly inducible by various stresses.[2,6,9,11] Thus, none of the genes encodes for monomeric Ub, and the generation of single and functional Ub units requires post-translational cleavage of the Ub precursor at the C-terminus of each unit by cytoplasmic Ub-specific proteases (which are essentially DUBs).[2,4,6,12] Ubiquitination is mediated by the concerted action of three enzymes: the Ub-activating enzyme (E1), a Ub carrier protein [Ub-conjugating enzyme (E2), and a Ub-protein ligase (E3). During the process, a polyUb chain is formed. In most cases, the first Ub moiety is conjugated via its C-terminal glycine to an ε-amino group of a lysine residue of the substrate to generate an isopeptide bond. It can also be conjugated to the N-terminal residue of the substrate to generate a linear peptide bond[13] or to a threonine, serine or cysteine residue to generate an ester or a thiolester bond.[14-16] In successive reactions, additional Ub moieties are typically attached to internal lysine 48 of the previously conjugated Ub moiety to generate isopeptide bond-based polyUb chain.[1,17] Attachment to other internal lysine residues of Ub (that also generates an isopeptide bond-based chain) or to the N-terminal residue of Ub (to generate a linear polyUb chain), has also been described.[18-21] Generation of heterogeneous rather than homogenous polyUb chains where Ub moieties are linked to different internal lysine residues within a single chain, or where more than one Ub moiety is attached to a single Ub (multiply branched chains) have also been reported.[22-24]

Degradation of the polyUb-conjugated target substrate is carried out by the 26S proteasome which is composed of a catalytic 20S core particle (20S CP) and two 19S regulatory particles (19S RP). The 19S RP contains binding sites for ubiquitinated proteins and six ATPases that most probably unfold the substrates and facilitate their translocation into the 20S CP for proteolysis.[25] As noted, many other processes such as signal transduction along the NFκB activation pathway or nucleosomal histone modification are regulated by non-destructive modes of ubiquitination. Here, the target proteins can be modified by a single or few Ub moieties (mono and oligoubiquitination, respectively), or by

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polyUb chains where the internal linkages are via lysine residues other than lysine 48.[1,20,21,26-29] Most polyUb chains appear to be cleaved, at least in part, by DUBs that hydrolyze the peptide bond downstream to the C-terminal glycine of Ub regardless of whether it is of the linear or the isopeptide type (and regardless of which lysine residue the isopeptide bond is based).[12]

Regulation of the UPS own components must play an important role in the ability of the system to function properly. It was demonstrated that certain E2s and E3s can be efficiently degraded by the 26S proteasome following ubiquitination.[30-35] However, the mechanisms that govern the stability of Ub have not been studied in detail, partially because Ub was conceived as a physically stable protein that remains properly folded even following treatment at extreme pHs and high temperature.[36-38] Despite its exceptional physical stability, different studies have shown that Ub is turning over.[39-45] Furthermore, its stability and consequently its steady state level are affected by changing physiological conditions. Thus, it is rapidly depleted under stress or when certain components of the UPS, specific DUBs for example, malfunction.[46-51] Recent findings have suggested that, in general, Ub is degraded by the 26 proteasome via three main routes—as monomer, as part of its conjugated substrate, and as a fusion protein with a C-terminal tail.[52-54] In this review, we shall discuss the mechanisms that control degradation of Ub. These mechanisms play important roles in determining Ub steady state level and availability, and are therefore affecting numerous basic cellular processes.



## Ub homeostasis in health and disease

Cellular Ub exists as a free monomer or as chains which are mostly conjugated to target proteins but can also be free. The balance between these two pools is generally maintained by the opposing activities of Ub ligating factors (E1, E2s and E3s) that catalyze synthesis of chains and DUBs that disassemble them.[12,55-57] The level of Ub in the two pools varies within different tissues which probably reflects unique degradation modes under varying physiological conditions such as development, differentiation and cell cycle.[7,42,45,58-64] Studies in different tissues showed that although the total level of Ub may vary up to three-to-four-fold, the ratio between free and conjugated Ub is less variable and approximately 40–60 % of the Ub is free .[45,63,65-67] The findings that tissues differ in the abundance and activity of their E2s, E3s and DUBs, as well as in the rate and regulation of their metabolism, suggest that Ub pools are tightly controlled at different levels to ensure availability of the protein during different pathophysiological conditions.

When cells are exposed to different insults such as amino acid analogues and oxidative and heat stresses, aberrant proteins are produced. These abnormal proteins are selectively polyubiquitinated and subsequently degraded by the 26S proteasome.[68-71] Under these conditions, more Ub conjugates are formed, and the ratio between the conjugated and free pool increases transiently.[43,45,71,72] The synthesis of Ub is also increased under stress, probably to overcome the greater consumption and to provide the cell with sufficient amount of the protein necessary for coping with the increased and continued demand.[5,72-74] The increase in consumption of Ub and in its synthesis is reversible, and upon removal of the insulting agent or adaptation to the stress, transcription of Ub returns to basal level,[5] and the generated conjugates are proteolyzed along with a portion of the tagging Ub.[52,53] It should be noted that an increased level of conjugates have been described in different pathologies, notably in nearly all neurodegenerative disorders where abnormal accumulation of Ub conjugates have been observed in different intracellular inclusions. These include Lewy bodies of Parkinson disease, neurofibrillary tangles and senile plaques of Alzheimer disease, Pick bodies of Pick disease, Rosenthal fibers within astrocytes, Lewy body-like hyaline inclusions of familial amyotrophic lateral sclerosis and intranuclear inclusions in polyglutamine expansion disorders such as in Huntington disease.[75-78] Increased conjugates have also been described in Mallory bodies which are the hallmark of alcoholic liver disease.[77] Immunohistochemical studies suggested that Ub conjugates as well as free Ub accumulate also in various malignancies.[79-81]

The mechanisms that underlie the increased level of conjugates, particularly in neurodegenerative disorders, are still controversial. Certain studies have suggested that aberrations in the function of the UPS, such as inhibition of the proteasome, result in accumulation of conjugates.[82] However, other studies report that there is no global impairment in the function of the UPS in diseases such as polyglutamine expansion pathologies that are characterized by accumulation of aggregate-prone proteins. Rather, the Ub conjugates accumulate because of more specific defect(s) in the UPS, defects in other quality control maintaining systems such as macroautophagy, or because the conjugates are of different structure and are not recognized by the system.[83,84] Indeed, mass spectrometrical analysis of Ub conjugates from brains of mouse model of Huntington's disease, and more importantly, from brains of patients with the disease, have shown the presence of chains with atypical composition of internal linkages between the Ub moieties.[85] Curiously, it is not known whether the conjugates play a role—pathogenetic or protective—in these diseases, as they are aggregated and sequestered from critical cell machineries.[86] Interestingly, it is not all clear that a high level of Ub provides the cell with protection against stress, as overexpression of Ub in yeast increased the tolerance to osmostress, ethanol, and canavanine (an amino acid analogue), but at the same time decreased it to cadmium, arsenite, and paromomycin.[87] It is clear however that inadequate supply of Ub can be harmful. Yeast cells in which free Ub was reduced by the inactivation of the Doa4 and Ubp6 DUBs, displayed a high sensitivity to different insults such as exposure to canavanine and heat, and overexpression of Ub suppressed this sensitivity.[46,49] Also, yeast cells become sensitive to stress following deletion of *Ubi4* which encodes a head-to-tail Ub transcript that provides the cell with a large amount of Ub.[5] In mammals, several pathologies have been described where different DUBs malfunction. The disorders have been attributed, at least partially, to Ub deficiency. For example, a mutation in Usp14 causes a syndrome characterized by ataxia, retarded growth, behavioral disorders, resting tremor and hindlimb paralysis.[48] Loss of Uch-L1 causes gracile axonal dystrophy (GAD)

which is a "dying back" sensory axonopahty characterized by progressive sensory followed by motor ataxia.[51] Importantly, an inactivating mutation in a single allele of the enzyme has been associated with a familial form of Parkinson disease.[88] In line with these findings, it was proposed that decreased Ub availability is sufficient to cause neuronal dysfunction and death in mice lacking the Ubb or Ubc genes.[7,8]

## Modes of Ub Degradation

The existence of an efficient Ub recycling machinery that is governed by DUBs postponed somehow the search for a mechanisms of Ub degradation, as destruction of the protein did not appear to be necessary for regulating the activity of the UPS. The first evidence that Ub is degraded came from pulse-chase experiments in Chinese hamster ovary cells that reported a half life of ~9 hours for native Ub.[40] In this study the researchers followed the fate of Ub-conjugated histone H2A. Since the histone moiety of the conjugate was stable, the researchers concluded that it was the free deconjugated Ub molecule that was degraded. Similar results were obtained using erythrocyte-mediated microinjected $^{125}$I-labelled Ub in HeLa cells.[41-44] Here the researchers reported that WT as well as Ub74 that lacks the C-terminal diGly motif (75 and 76) necessary for its conjugation, are degraded in approximately the same rate (~9 hours), further corroborating the notion that Ub can be degraded as a free monomer. An interesting finding was the observation that increased proteolysis of cellular proteins that occurs, for example, under stress (such as during amino acid starvation, high temperature, incubation of the cells in the presence of an amino acid analog or puromycin), is accompanied by accelerated degradation of Ub.[44,45,49,52] These findings raised the hypothesis that Ub can be also degraded along with the substrate(s) to which it is conjugated.

Additional studies have shown that Ub stability varies among different cell lines. Thus, the half life of $^{125}$I-lableed microinjected Ub in IMR-90 human fibroblasts was ~320 hours.[39] In yeast, Ub was reported to have a half-life of less than 2 hours.[50] Studies in mammalian cells have shown that Ub was stabilized by ATP depletion, its degradation was mediated by the proteasome and was inhibited only modestly by lysosomal inhibitors.[44,45,53,89]

It appears that although the degradation rates of Ub do not place it among the most unstable proteins, its turnover is nevertheless significant.

Taken together, the findings suggest that degradation of Ub can occur via at least two modes, as a free monomer and in a conjugated form as part of the targeted substrate. This latter mode does not require recognition of Ub by a specific UPS ligating factors, as it "piggybacks" the substrate-specific ligation mechanism. It is logical to assume that the most proximal part of the polyUb chain is pulled along with the substrate into the proteasome and is degraded, whereas the more distal part is rescued via the activity of DUBs. Several studies have supported this "piggyback" mode of Ub degradation. Using a reconstituted cell free system, we have recently demonstrated that addition of bona fide UPS substrates enhances proteasome-dependent degradation of Ub which was preceded by specific conjugation.[53] Other studies have demonstrated that deletion of DUBs, such as Doa4 and Ubp6 in yeast, or Usp14 and Uch-L1 in mammals, accelerate significantly the degradation/depletion of Ub.[46,48-51] Here it is assumed that failure to release Ub efficiently from ubiquitinated substrates targeted to the proteasome, causes the entire conjugate to undergo degradation. Thus it appears that DUBs, by playing a role in Ub recycling, also affect its stability, and regulation of their level and/or activity may play a role in governing the size of Ub pools in the cell, adapting it to changing requirements under varying pathophysiological conditions. Yeast Ubp6 and its human homolog Usp14 as well as yeast Doa4, are proteasome-associated DUBs.[90,91] The close association of the DUBs with the proteasome is probably aimed to secure high efficiency of removal of Ub from the targeted substrates before their entry into the 20S CP for degradation. The critical role of Ubp6 in the maintenance of the free Ub pool is underscored by the regulation of its expression by Ub: depletion of the cell from Ub results in up regulation—via control of gene expression—of Ubp6 which is recruited to the proteasome to increase the efficiency of Ub removal from conjugated target substrates.[92] Also, Ubp6 acts non-catalytically to slow down proteasomal degradation of substrates, probably in order to allow long enough time to release Ub from the substrates'-bound polyUb chains.[93] Additional levels of regulation that endow DUBs with the ability to govern the level of different Ub pools reside in their distinct expression levels and distribution in specific tissues and sub-cellular compartments, and along differentiation and development.[12,51,94-97] For example, local changes in synaptic Ub levels that are caused by deficiency in Usp14 can explain the developmental defects in the neuromuscular junction and the presence of defects in synaptic transmission in motor neuron endplates and in the hippocampus.[98,99] Despite the role of DUBs in recycling Ub at the proteasome level, it is still not known what determines the fraction of Ub in the chain that is degraded along with the substrate compared to the part that is recycled. Is there a minimal number of moieties in the proximal part of the polyUb chain that cannot be removed mechanistically? Is it related to the structure of the substrate and/or the ubiquitination sites and their nature? Also, it is not clear why degradation of part of the polyUb chain is necessary biologically, and the entire chain or all the Ub moieties are not rescued. It is possible that a certain part of the chain must be present all the time on the substrate to secure its tight binding to the proteasome, and a premature removal of the chain will result in detachment of the substrate or part of it before its complete entry and digestion by the proteasome. Thus, degradation of at least part of the polyUb chain must always accompany degradation of the substrate.

Recent studies have further substantiated and extended the "piggyback" mode of degradation. We and others have found that Ub with a C-terminal extension of 20 amino acids is rapidly and efficiently degraded by the proteasome, and the degradation is not dependent on further ubiquitination.[52-54] The extension can represent a most proximal proteolytic intermediate where a peptide derived from the substrate is bound to Ub. Whereas most such intermediates (that have not been isolated thus far) should contain Ub bound to the peptide in an isopeptide bond, linear

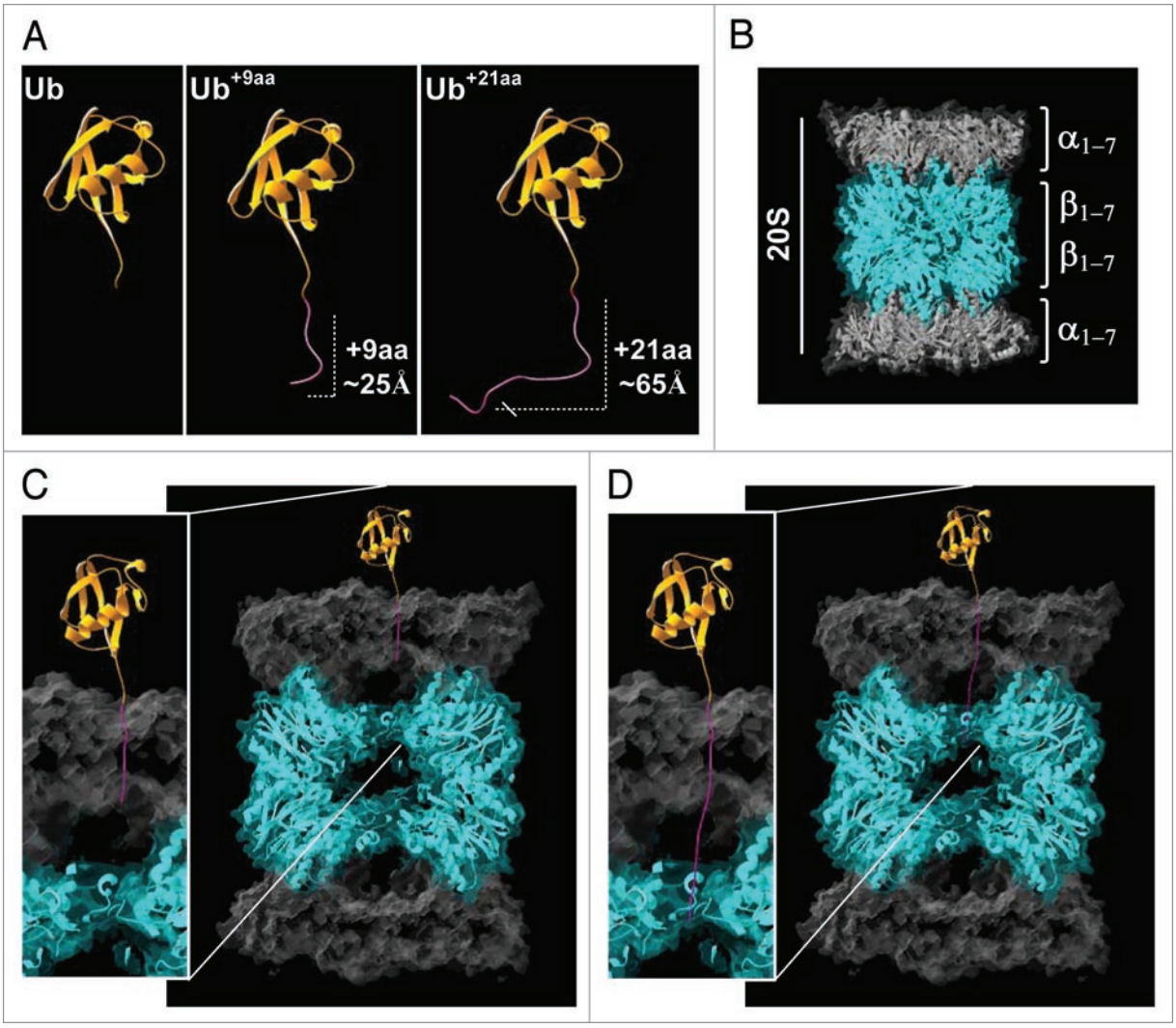


**Figure 1.** Structural modeling of the minimal length of C-terminal extension that is required for proteasomal degradation of monomeric Ub. (A) Structure of Ub (yellow) and the predicted structure the C-terminal extension (9 and 21 residues)(red). (B) Structure of the 20S CP of the 26S proteasome. Two seven α subunits rings form the gate(s) (grey), and the two seven β subunits rings contain the catalytic sites (cyan). (C) Short tailed-Ub (Ub+9aa) cannot reach the catalytic sites even when bound to its putative 19S RP binding subunit(s) (not shown). In contrast, Ub+21aa can reach the 20S catalytic core (D). Structural modeling was based on X-ray crystallography studies (PDB: 1UBQ, 1G0U).128, 129

extensions can represent intermediates in the degradation of N-terminally ubiquitinated substrates.[100] These findings probably define the minimal requirements for recognition and degradation by the proteasome that is ubiquitination independent - a binding Ub moiety and a long enough tail (>20 residues) that allows the molecule to reach the catalytic sites or to be pulled into the 20S CP (**Fig. 1**). Similar to what have been described for substrates of the UPS, the tail probably has to be unstructured and/or flexible to allow its entry and passage through the 19S complex, reaching the 20S catalytic chamber.[101,102] Interestingly, it was shown that when Ub is extended by a peptide shorter than 20 residues, it is relatively stable.[52-54] One such naturally extended form of Ub, $UBB^{+1}$, is extended by 19 residues. It is generated as a result of molecular misreading of the Ubb gene transcript leading to translation of Ub with the C-terminal glycine residue substituted by tyrosine which is followed by an additional 19 amino acid C-terminal extension.[103] This form of Ub cannot be activated by E1 and cannot tag other proteins.[104] $UBB^{+1}$ was implicated in the pathogenesis of an early onset form of Alzheimer's disease and other neurological and conformational disorders.[103,105] It was suggested that the toxicity of the protein is due to its ability to be ubiquitinated irreversibly, where the generated Ub conjugate of this aberrant form of Ub is resistant to the activity of DUBs.[104] It was also shown that $UBB^{+1}$ inhibits the proteasome.[53,104,106] To the best of our knowledge there are no additional reports of other naturally occurring extended forms of Ub, though as noted, such Ub-peptides can be generated as intermediates in the normal proteolytic process where Ub is bound to a short peptide derived from the substrate or from the more proximal Ub moiety in the polyUb chain. They can also result from adventitious reactions in which activated Ub attacks short peptides generating amides, esters, and thiolesters. These Ub-extended species can be

recognized as "new" substrates and further degraded by the proteasome (in a "second" cycle) or subjected—as suggested—to the activity of DUBs.[107,108] While the exact nature and mechanism of formation of the extension(s) are not clear, its requirement may highlight the tight linkage between the stability of Ub and that of cellular proteins at large. It should be re-emphasized that the degradation of Ub that is extended by a short peptide is related, though conceptually different from the "piggyback" model where the conjugated Ub is co-proteolyzed along with the Ub-tagged substrate using the substrate-specific E3. The "extension" model involves two cycles of degradation, one that targets the substrate and releases the peptide-extended Ub, and the other that degrades this intermediate, a step that does not require ligation enzymes and further ubiquitination. This is of course true also for products of attachment of active, high energy E1- or E2-loaded Ub, to short cellular peptides, amino, thiol, or hydroxyl groups (see above). Still, in both cases, it is the Ub moiety(ies) that is adjacent to the substrate that is degraded. Nonetheless, it was recently demonstrated that C-terminal-extended Ub does not require any ubiquitination for its binding to the proteasome and for its subsequent degradation.[53] The notion that the proteasome recognizes and degrades monoubiquitinated targets was previously described.[29,109] It is possible that when the substrate is short enough and has a flexible tail that can reach the catalytic sites in the 20S CP (β subunits) and pull behind it the entire protein, a single Ub moiety (**Fig. 1A–D**), an oligoUb chain, or modification of several internal lysines by single Ub moieties (multiple monoubiquitinations) are sufficient to target it for proteasomal degradation. In such cases, the proteasome can bind the substrate stably, ensuring processivity of the proteolytic process. However, when the substrate is large, stabilization of its binding to the proteasome will require polyubiquitination.

An additional mode of degradation is that of free monomeric Ub. Studies in a cell free system[53] as well as in cells[44,110] have demonstrated that inactive Ub (UbΔGG or UbGly75,76→Val75,76, UbVV; see above) is degraded. The monomeric Ub is more stable than the C-terminally-extended species, and its degradation appears to be proteasome- and ATP-dependent.[44,53] Since monomeric Ub does not have a tail long enough to pull it into the 20S CP, it probably has to be ubiquitinated to allow it to tether on the proteasome-bound polyUb chain. Indeed, several studies have shown that monomeric Ub can be ubiquitinated where it serves as substrate to which single moieties are conjugated sequentially. E2-25K can catalyze the formation of K48-based chains.[111] The HECT domain E3 TRIP12 (thyroid hormone-interacting protein 12) was reported to ubiquitinate monomeric Ub, targeting it to degradation.[110] We have also shown that UbVVHis can be ubiquitinated on both its internal lysines and the N-terminal residue.[52]

An intriguing implication of the "monomeric/free Ub degradation mode" is that a certain portion of the cellular Ub can exist as unanchored free Ub chains.[55,56,112-114] Furthermore, it was reported that pre-assembled polyUb chains can be generated on E2 prior to their transfer to the target substrate,[32,115] suggesting that they can also be released as free chains. Given that polyUb chains are typically bound to a target substrate, free intact chains can also be derived from a cleavage at the "trunk" of the chain which is catalyzed by DUBs. The role of the free chains is still elusive. Interestingly, Lys63-based polyUb chains were reported to activate directly the TAK1 kinase complex in the NFκB signaling pathway.[116,117] The free chains can also serve as a Ub reservoir that rapidly provides monomeric Ub when it is urgently required.[118] Nevertheless, the physiological roles of free Ub chains remain largely unknown, especially when numerous and intricate internal Ub-Ub linkages within the chains can occur.

The degradation of the Ubl protein FAT10 is an interesting case that may represent the three modes of Ub degradation - as part and along with the conjugated substrate, as a C-terminally tailed fusion, and as a free monomer. FAT10 is composed of two Ubl domains with a free C-terminal diglycine, that similar to Ub, is required for its conjugation to its targets. Free FAT10 was found to be degraded by the proteasome without further modification.[119] This process is reminiscent of the degradation of tailed Ub: it is possible that here the proximal Ub domain anchors the molecule to the proteasome, whereas the distal moiety serves as the leading tail that penetrates into the 20S CP catalytic chamber. Degradation of FAT10 can be also compared to the degradation of monomeric Ub that requires further ubiquitination: here the structure of FAT10 is homologous to di-Ub where the distal domain which is homologous to Ub has upstream to it a proximal domain, also homologous to Ub. Last, it was reported that FAT10 can be degraded along with a substrate (GFP) to which it was fused.[119] In this case it appears that FAT10 serves as a degradation signal both to itself and to the conjugated substrate without undergoing further ubiquitination, demonstrating that not only Ub can be recognized by the proteasome, targeting its conjugated substrates for degradation, but also other Ubl proteins.

The three modes of Ub degradation of Ub are schematically depicted in **Figure 2**.

## Involvement of Other Proteins in Ub Proteolysis

Besides the roles of DUBs in the degradation of Ub, several other proteins have been recently found to participate in the process. One of these proteins is the fission yeast Lub1 (low Ub content) and its budding yeast homolog Ufd3.[47,120] Disruption of either *lub1* or *ufd3* resulted in an accelerated degradation of Ub and impaired UPS activity, which rendered cells susceptible to different stresses. Although no enzymatic activity has been attributed to Lub1 or Ufd3, it was suggested that they may cooperate with DUBs.[120] Another protein is Rfu1 (regulator of free Ub chains 1), which is a natural inhibitor of Doa4. It was recently found to regulate the cellular levels of monomeric and free Ub chains.[118,121] Thus, a delicate control of DUBs via a concerted activity of positive and negative regulators may play an important role in the maintenance Ub homeostasis.

Another yeast protein, Rsp5, was found to have a role in Ub homeostasis. Rsp5 is a member of the HECT (homology to E6-AP carboxyl terminus) family of Ub ligases, and was found to be involved in numerous cellular processes such as transcriptional regulation and endocytosis.[122] Interestingly, inactivation of Rsp5 resulted in 50% reduction in the cellular Ub level, similar to the

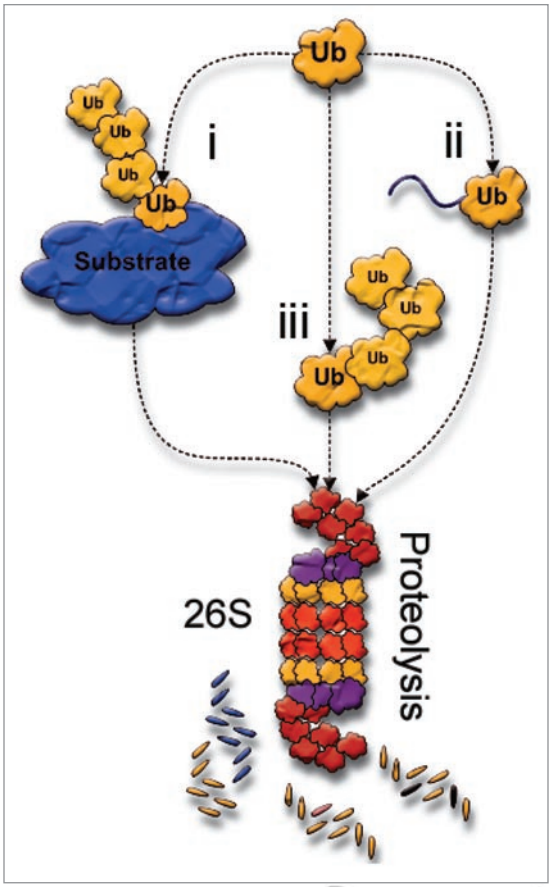


**Figure 2:** The three modes of Ub degradation. (i) Ub is pulled into the 26S proteasome along with its conjugated substrate and degraded. (ii) Ub with a flexible/unstructured tail longer than 20 residues is rapidly degraded without further modification. (iii) Monomeric Ub is targeted for proteasomal degradation, probably after ubiquitination catalyzed by E1, E2, and E3 and/or other adaptors.

reduction observed in Δdoa4 mutants.[123] It was proposed that the low level of Ub seen in the *rsp5* mutant is due to reduction in Ub synthesis under stress, and the effect of Rsp5 is not related to its E3 activity. It is not clear whether Rsp5 affects Ub synthesis directly, or it has a more general effect on the recovery of protein synthesis following stress.

## Implications of Aberrations in Ub Metabolism

Since both deficiency, but also excess of Ub and Ubl proteins have been described to induce stress in cells and in animal models, it conceivable to assume that aberrations in the mechanisms that govern Ub pools, are also involved in human pathologies. The toxicity of the non-degradable $UBB^{+1}$ is due to two mechanisms. For one, its tail of 19 amino acids is too short to penetrate into the 26S proteasome catalytic chamber and to carry behind it the Ub moiety. Importantly, we found along with its relative resistance to degradation, its ability to be ubiquitinated converts it into a proteasome inhibitor.[53] This finding further substantiates the hypothesis that the role of the polyUb chains is to increase the affinity of substrates to the proteasome, thus ensuring processivity of the proteolytic process. The inhibitory effect was found to be restricted to ubiquitination-dependent substrates of the 26S proteasome,[53] as the degradation of ornithine decarboxylase, ODC, a ubiquitination-independent substrate,[124] was not affected. This finding also demonstrates that the proteasome can bind substrates via at least two sites, only one of them is via the polyUb chain.

Evidently, 26S proteasome has recently become a successful drug target, initially for the treatment of the B cell leukemia multiple myeloma.[125-127] However, the central place it occupies in proteolytic cascades where it degrades thousands of ubiquitinated substrates along with some non-ubiquitinated substrate makes it a non-specific target. Not surprisingly, the treatment with proteasome inhibitors is accompanied with some serious side effects that at times necessitate its cessation. Identification of additional substrate/ancillary proteins/Ub binding sites, and better understanding of the role of polyUb chains and their modes of association with the proteasome, that will be achieved via additional studies on Ub metabolism, can pave the road to the development of better and more specific inhibitors.



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