View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

FEBS Letters 581 (2007) 2854–2861

Minireview

A proteasome for all occasions

John Hanna, Daniel Finley*

Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA

Received 8 March 2007; accepted 18 March 2007

Available online 30 March 2007

Edited by Horst Feldmann

Abstract In the ubiquitin-proteasome system, substrates fated for destruction first acquire covalent modification by ubiquitin, and are subsequently destroyed by the proteasome. Traditionally, 26S proteasomes have been seen as largely uniform in their composition and functional capacity. Accordingly, cells can control proteasome abundance via transcriptional pathways that mediate concerted regulation of all known proteasome genes. However, recent evidence suggests that the proteasome is also subject to subunit-specific modes of regulation, which serve to alter proteasome function and may generate ensembles of compositionally distinct proteasomes. These modes of proteasome regulation provide varied means to adapt protein degradation pathways to changing conditions in the cell.
© 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Ubiquitin; Proteasome; Stress; Rpn4; AIRAP; Ubp6

1. The ubiquitin-proteasome system for protein degradation

The ubiquitin-proteasome system represents the major pathway for intracellular protein degradation in eukaryotes. Substrates are designated for destruction in this pathway by the covalent attachment of a polyubiquitin chain. The generation of such polyubiquitin chains requires the activity of three classes of enzymes [1]. A ubiquitin molecule is first activated for transfer via formation of a thiolester bond with the ubiquitin activating enzyme, or E1. This activated ubiquitin is then transferred to a ubiquitin conjugating enzyme, or E2, again via formation of a thiolester bond. Typically, the activated ubiquitin molecule is then transferred to the substrate in collaboration with a ubiquitin ligase, or E3. Two general paradigms for ubiquitin ligation exist. In the first, the E2 transfers the ubiquitin molecule to the E3, which possesses its own catalytic activity and is responsible for ultimate transfer of ubiquitin to substrate. In the second, the E3 functions non-catalytically with E2 to promote ubiquitin transfer.

Ubiquitin itself is a highly conserved 76 amino acid protein which invariably ends in a diglycine motif. A covalent bond is formed between ubiquitin and substrate typically via the carboxyl group of the terminal glycine in ubiquitin and the ε-amino group of a lysine residue within the substrate. Less commonly, other nucleophiles may be modified by ubiquitination. Polyubiquitin chain formation proceeds typically via isopeptide bond formation between the G76 carboxyl group of the "$n + 1$" ubiquitin to the ε-amino group of a lysine within the preceding ubiquitin. Seven lysines are found in ubiquitin, allowing for the generation of a variety of polyubiquitin chain types [2]. Preventing ubiquitin chain formation abrogates or attenuates the degradation of many substrates (e.g., see Ref. [3]). The minimum length of a polyubiquitin chain capable of supporting degradation appears to be approximately four [4], although many substrates appear to acquire polyubiquitin chains that are significantly longer.

The fundamental role of the substrate-linked polyubiquitin chain is to serve as a recognition motif for a large multi-subunit protease known as the proteasome, which is responsible for hydrolyzing the substrate's peptide bonds, thereby reducing a folded protein into oligopeptides. Other proteases convert these peptide products into free amino acids, which can then be used anew in biosynthetic processes.

The proteasome is an approximately 2.5-MDa protein complex thought to consist of at least 33 subunits in the budding yeast *Saccharomyces cerevisiae* [5]. The proteasome can be divided by biochemical methods into two smaller complexes (Fig. 1). The first is the 670 kDa core particle, also known as CP or the 20S particle. The CP is a barrel-shaped complex consisting of four stacked rings, each ring composed of seven proteins [6]. The two outer rings are identical, as are the two inner rings, giving the CP an "αββα" configuration along a vertical axis, as well as a sevenfold pseudo-symmetry within each ring of subunits. The peptidolytic active sites of the proteasome are sequestered within a cavity formed at the center of the CP by the two inner (beta) rings [6]. There are three distinct peptidolytic activities in the CP, each represented twice: a tryptic activity (i.e. cleaving after basic residues), a chymotryptic activity (i.e. cleaving after hydrophobic residues), and a post-acidic activity [7]. On either side of the central cavity is a second cavity formed between an inner (beta) and an outer (alpha) ring, but the exact function of these outer chambers remains unknown. Substrates gain access to the CP only though narrow pores present at either axial end of the CP and structurally represented by the N-termini of alpha ring subunits [8]. Importantly, the pores leading to the interior of the CP are shut in the basal state, and require a mechanism of gate opening to facilitate substrate degradation. This function, among others, is carried out by the second subcomplex of the proteasome: the regulatory particle, also known as the RP or 19S particle or PA700.

*Corresponding author. Fax: +1 617 432 1144.
E-mail address: Daniel_Finley@hms.harvard.edu (D. Finley).

0014-5793/$32.00 © 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2007.03.053

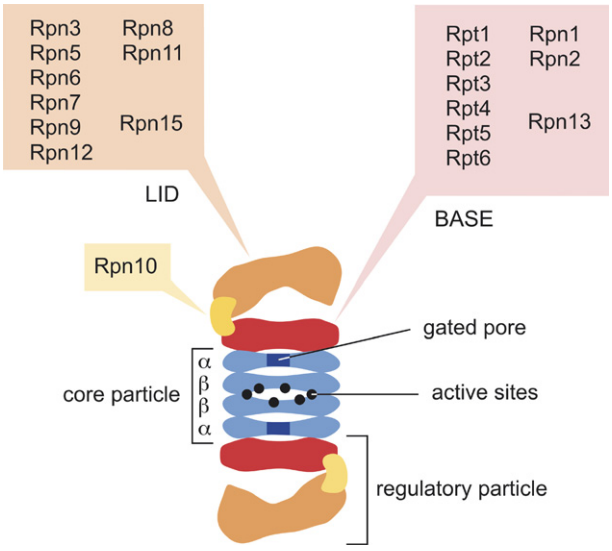

Fig. 1. Schematic representation of the 26S proteasome. The canonical 26S proteasome is composed of one core particle and one or two regulatory particles, as indicated. The regulatory particle can be further subdivided into the lid and the base. The base contains six ATPases (Rpt1-6), and three non-ATPase subunits. The lid contains six PCI domain-containing proteins (Rpn3, Rpn5–7, Rpn9, and Rpnl2), two MPN domain-containing protein (Rpn8 and Rpn11), and one additional protein, Rpnl5 (also known as Sem1). Adapted from [5], with permission.

The RP is a nearly 1-MDa complex consisting of at least 19 proteins. One RP may associate with either axial end of the CP. When the CP is associated with one or two RP species, the complex is referred to as the 26S proteasome. The RP may be further divided by biochemical means into two subcomplexes of its own, the base and the lid [9]. The base subcomplex is located proximal to the CP and contains six AAA-type ATPases (Rptl-6) as well as four non-ATPase subunits (Rpn1, Rpn2, Rpn10, and Rpn13). The six ATPases of the base are thought to form a ring, as well as to possess a protein remodeling capacity presumed to function in substrate unfolding [10,11]. The pore leading into the CP is too narrow to be traversed by most folded proteins, and as such, unfolding is thought to be a prerequisite for the degradation of the majority of proteasome substrates. Despite very high sequence similarity among the six ATPases, early work demonstrated their non-equivalence of function [12], and delineating their precise roles remains an active area of investigation. One ATPase, Rpt2, is known to play a role in opening the pore into the CP to facilitate substrate entry [13], while Rpt5 is thought to play a role in recognition of the substrate-bound ubiquitin chain [14]. Another base component, Rpn10, is clearly a ubiquitin receptor [15,16]. The functions of Rpn1 and Rpn2, which represent two of the largest proteins of the proteasome, have remained more elusive. Rpn1 is known to bind a series of ubiquitin chain receptors which are not core components of the proteasome, but rather, substoichiometrically-associating proteins [17,18]. These proteins – Rad23, Dsk2, and Ddil – share a common domain at their respective N-termini known as a ubiquitin-like domain, and this domain mediates recognition by Rpn1. An unrelated protein, the deubiquitinating enzyme Ubp6, also contains an N-terminal ubiquitin-like domain, and is recognized by Rpn1 (see below). The functions of Rpn2 remain even more obscure, but Rpn2 is known to bind a HECT-domain containing ubiquitin ligase known as Hul5 [19].

The lid subcomplex of the RP is found distal to the base and was originally characterized as an eight-component complex with high sequence homology to two other cellular complexes, the COP9 signalosome and translation initiation factor eIF3 [9]. All three complexes are characterized by two protein motifs known as the MPN and PCI domains [9]. The lid possesses six PCI-domain-containing subunits (Rpn3, Rpn5-7, Rpn9, and Rpn12) and two MPN domain proteins (Rpn8 and Rpn11). The PCI domain remains poorly understood, but the MPN domain of Rpn11 is known to represent a metalloprotease-like deubiquitinating activity which removes ubiquitin from substrates [20–22]. A corresponding activity in the COP9 signalosome removes the ubiquitin-like modifier Nedd8 from the cullin subunits of various protein complexes [23]. No such activity has yet been identified in eIF3. The MPN domain of Rpn8 is very similar to that of Rpn11, but lacks crucial catalytic residues. In recent years, a ninth member of the lid, Sem1 or Rpn15, has been identified [5].

Initial characterization of the lid demonstrated its necessity for the degradation of ubiquitin conjugates, in contrast to base-CP molecules, which were competent for the degradation of at least some non-ubiquitinated substrates [9]. The precise basis for this requirement for the lid remains unclear, but may reflect, at least in part, the activity of Rpn11 in releasing ubiquitin chains from substrates prior to degradation (see below).

Thus the proteasome is an unusually complex protease, harboring, in addition to the CP-enclosed active sites, a number of catalytic and non-catalytic activities which facilitate the unique aspects of its function. Potential substrates must first be recognized and bound by ubiquitin receptors in the RP. Substrates must be unfolded and threaded through the pore into the CP, which can occur only after the gate leading to the CP has been opened. Finally, at some point prior to degradation, the ubiquitin molecules, which serve to target the substrate to the proteasome, must themselves be released for reuse.

2. Regulation of both the magnitude and nature of proteasome function

The proteasome functions in a wide array of cellular processes, many of them essential for viability. As such, the cell is presumably under pressure to maintain adequate proteasome function in the face of challenges which may compromise its function or create increased demand for proteasomes. A homeostatic mechanism controlled by the zinc finger transcription factor Rpn4 is thought to regulate cellular proteasome abundance [24]. Several recent reports, however, suggest that regulation of the proteasome may be even more complex. As opposed to the concerted induction of all proteasome genes by Rpn4, it now appears that individual components of the proteasome may be selectively induced under varying cellular conditions. This induction of specific proteasome components has the capacity to alter proteasome function. Thus the proteasome appears to be under at least two levels of regulatory control, and both the magnitude and nature of proteasome function may be altered by the cell to meet varying cellular requirements.

3. Regulation of proteasome magnitude: transcriptional activation by Rpn4

Rpn4 was originally identified in a screen for suppressors of the temperature sensitivity of a mutant of Sec63, a component of the endoplasmic reticulum translocon [25]. Because the particular allele of *SEC63* under study was known as *npl1-1*, the gene encoding *RPN4* was originally designated *SON1* (*s*uppressor of *npl1-1*). Although the mechanistic basis for suppression of the *sec63* mutant by loss-of-function in *RPN4* remains unclear, two subsequent screens firmly placed *RPN4* within the ubiquitin-proteasome pathway. In the first screen, Varshavsky and co-workers identified five yeast genes which, when mutated, confer defects in the degradation of model proteasome substrates consisting of ubiquitin fusion proteins [26]. Because this pathway is known as the ubiquitin-fusion degradation pathway, *SON1* acquired a second designation: *UFD5*. An unrelated screen sought yeast mutants that displayed synthetic lethality when combined with a mutant of the proteasome subunit Rpnl2 [27]. Again, mutants of Sonl/Ufd5 were recovered.

Early studies detected co-fractionation of Sonl/Ufd5 with other proteasome subunits, and for this reason, the gene was renamed *RPN4* (*R*egulatory *P*article *N*on-ATPase 4) to reflect its putative role as a subunit of the proteasome [27,28]. Indeed, Rpn4 appears to make a direct contact with the proteasome base subunit Rpn2, as recombinant versions of the two proteins interact specifically [24]. However, later purifications of the proteasome failed to identify Rpn4 in significant amounts [29,30]. This discrepancy is likely accounted for by the subsequent discovery that Rpn4 is turned over at a rapid rate (its half-life is estimated to be two minutes or less), and its steady state abundance is quite low [24].

Analysis of the *RPN4* coding sequence identified sequences similar to those found in Cys_2-His_2 zinc finger domains, as well as two acidic regions. As both of these features are often found in transcription factors, a role for Rpn4 in transcription was investigated. Indeed several groups were able to demonstrate that Rpn4 acts as a transcription factor, and that a major target of its transcriptional activity is the proteasome itself [24,31,32]. Specifically, an 8–9 nucleotide sequence, known as *P*roteasome-*A*ssociated *C*ontrol *E*lement (PACE) can be found in the upstream promoter regions of all known proteasome subunits and several proteasome-associating factors, and it is thought that Rpn4 mediates its effects on proteasome gene transcription by directly binding this element. This observation, when coupled with data demonstrating that the short half-life of Rpn4 is a consequence of proteasome-mediated degradation [24], has led to an elegant model in which Rpn4 controls cellular proteasome levels via a homeostatic negative feedback loop (Fig. 2).

Several areas of Rpn4 biology remain poorly understood. Mutations in subunits of the proteasome itself are known to induce Rpn4 [33,34], consistent with a model in which the stress response mediated by Rpn4 operates via post-translational mechanisms. However, it has recently become clear that the *RPN4* gene is itself subject to a complex array of transcriptional controls, indicating that Rpn4 may respond not only to proteasome dysfunction, but to a variety of cellular stresses, some or all of which may require an augmentation of proteasome capacity. The upstream promoter region of *RPN4* is recognized by the heat shock transcription factor Hsfl, two multidrug resistance-associated transcription factors, Pdrl and Pdr3, as well as the transcription factor Yapl [34–36]. It remains unclear whether this represents an exhaustive list of inputs into *RPN4* transcription; likely it does not. Furthermore, the environmental stresses that activate each of these factors to induce *RPN4* transcription are only beginning to be worked out. Cellular stresses known to induce *RPN4* transcription include heat, the DNA damaging agent MMS, the amino acid analog AZC, and hydrogen peroxide [33,34,36]. Yapl, which is best known for its role in responding to oxidative stress, presents an unusually interesting case. Not only does Yapl recognize the *RPN4* promoter, but the promoter of Yapl itself contains the PACE box, thus potentially establishing a positive feedback loop [37]. The full elucidation of environmental inputs leading to Rpn4 induction, as well as the cellular factors carrying out these responses, should add significantly to our understanding of the cellular stress response program.

The outputs of Rpn4 function also remain incompletely understood. In addition to the proteasome, PACE boxes can be found in the promoters of more than 20 additional genes [30,31]. These genes encode a diverse group of factors regulating processes such as ubiquitination, vacuolar function, RNA metabolism, and glucose utilization. For the majority of these genes, it remains unclear to what extent regulation by Rpn4 actually occurs, but their existence suggests the possibility that proteasome induction may be only one facet of a wide-ranging cellular stress response mediated by Rpn4. The case of ubiquitination is especially interesting. PACE boxes can be found in

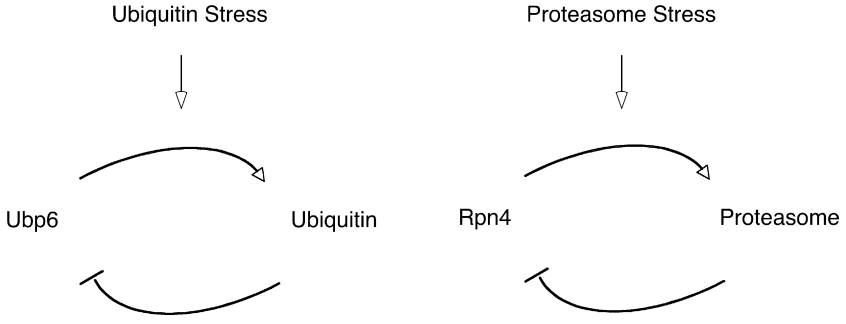

Fig. 2. Model for distinct cellular pathways mediating the proteasome stress response and the ubiquitin stress response. Proteasome levels are controlled via a negative feedback loop involving the transcription factor Rpn4. Ubiquitin levels are controlled, at least in part, via ubiquitin-dependent transcriptional regulation of Ubp6, which increases proteasomal efficiency in ubiquitin regeneration. Adapted from [54], with permission from Elsevier.

the promoters of the polyubiquitin gene, *UBI4*, as well as the El gene, *UBA1*, and Rpn4 has been shown to increase *UBI4* transcription [31,34]. As proteasome levels rise, ubiquitin induction may be necessary to support correspondingly higher rates of degradation. However, at least one report has demonstrated that supplementation of proteasome hypomorphic mutants with exogenous ubiquitin is actually detrimental [34]. A related question concerns to what extent proteasomes induced by Rpn4 resemble their basally-produced counterparts. It has been long assumed that Rpn4 changes only proteasome number, but careful studies will be required to exclude the possibility that proteasomes induced under stress by Rpn4 are actually adapted in novel ways. Finally, the maximal extent of proteasome induction by Rpn4 remains unknown. Proteasomes are highly abundant within cells under unstressed conditions, and so it seems unlikely that proteasome levels could be elevated by more than an order of magnitude. Recent advances in constructing mutants of Rpn4 resistant to degradation [38,39] may be useful in estimating the upper limit of cellular proteasome plasticity.

A third issue concerns the mechanisms whereby Rpn4 signaling is turned off, and in particular, the destruction of Rpn4 protein. Rpn4 is unusual in that despite its extremely short half-life, a major component of its degradation appears to be ubiquitin-independent [24]. Accordingly, whereas mutations in the proteasome stabilized Rpn4 against degradation, global impairment of ubiquitination by mutation of the ubiquitin activating enzyme had no detectable effect on the half-life of Rpn4 [24]. This puzzling finding may be explained by the need for the cell to regulate proteasome and ubiquitin levels independently (Fig. 2). Thus Rpn4 may have evolved to report primarily on the activity state of the proteasome, and may be inherently insensitive to secondary or indirect impairments of degradation deriving from failure of substrate ubiquitination. Consistent with this model, while mutations of proteasome subunits induce Rpn4 accumulation, mutants displaying severe deficiencies of cellular ubiquitin (e.g. *doa4*Δ) show no accumulation of Rpn4 [34]. Indeed, it may be that the capacity of Rpn4 to directly bind the proteasome is necessary for ubiquitin-independent degradation of Rpn4. The situation is made even more interesting by recent observations that Rpn4 also undergoes a secondary, but possibly weaker mode of ubiquitin-dependent degradation [38,39]. In this pathway, internal lysines of Rpn4 are recognized and ubiquitinated by the E3 ubiquitin ligase Ubr2 [40]. The significance of having both ubiquitin-dependent and ubiquitin-independent modes of Rpn4 degradation remains unexplained, and its elucidation seems fundamental to understanding Rpn4 function.

A final open area for investigation into the proteasome stress response concerns the extension of study into higher eukaryotes. Thus far, no ortholog of Rpn4 has been identified in higher organisms, nor can PACE boxes be readily recognized in proteasome genes outside of yeast. Nevertheless, the proteasome stress response does appear to be conserved in higher organisms. Studies in both *Drosophila* and in mammalian cells have demonstrated that abrogation of proteasome function, either by treatment with chemical proteasome inhibitors or by RNAi-treatment directed against proteasome subunits, increased the levels of proteasome gene transcription, and ultimately proteasome levels [41–43]. The identification of the factors required for this response, as well as the promoter sequences by which it is mediated, represent important objectives in understanding the proteasome stress response, and how the cell regulates proteasome levels to survive cellular stress.

4. Regulation of the nature of proteasome function: AIRAP and Ubp6

The proteasome has traditionally been thought of as a distinct functional entity, but it is becoming increasingly apparent that the cell's population of proteasomes may actually represent a diverse group of functionally distinct members, and moreover, that populations of proteasomes may be subject to highly dynamic regulation. Thus the cell may be able to not only increase or decrease the abundance of "canonical" proteasome forms, but may also be capable of inducing the formation of other proteasome configurations that display novel and unique properties, and which may be useful under varying cellular conditions.

Early evidence in favor of such a model can be found in the interferon inducibility of the three core particle subunits harboring active sites (known as β1i/LMP2, β2i/MECL1, and βi/LMP7). Upon transcriptional induction by interferon-γ, these subunits accumulate and are incorporated into the proteasome, replacing their constitutively expressed counterparts [44]. This modification alters proteasome function, resulting in a spectrum of peptide products that may enhance MHC Class I-dependent immunological presentation. Also supporting the idea of a cellular repertoire of diverse proteasome species are the observations that alternate structures besides the canonical regulatory particle are capable of capping the core particle. The first, known as PA28, is heteroheptameric, is also inducible by interferon-γ, and appears to function with LMP2, MECL1, and LMP7 to generate optimal substrates for MHC-I loading and subsequent recognition by the immune system [1]. A second proteasome cap, known as PA200 in mammals and Blm10 in yeast, consists of a single large (~250 kDa) polypeptide (see Ref. [5]). How PA200 alters proteasome function remains an open question.

In recent years, two new pathways of proteasome regulation have become apparent, each mediating response to a distinct stress. In the first, treatment with the toxin arsenic results in the induction and accumulation of the novel proteasome interactor, AIRAP. In the second, deficiencies of cellular ubiquitin result in the induction and accumulation of the proteasomal deubiquitinating enzyme Ubp6.

5. AIRAP

Arsenic is a naturally occurring compound associated with a wide variety of adverse effects on human health, and is found in nature in several forms. The tetraoxide derivative of arsenic (arsenate; AsO_4^{3-}) is capable of substituting for phosphate in certain phosphoryl transfer reactions. Occurrence of this substitution during glycolysis, for example, is known to decrease the yield of ATP from this process [45]. The toxicity of arsenite (AsO_3^-), on the other hand, seems to derive from its ability to form thiol adducts with free sulfhydryl groups [46]. Such modification is thought to promote protein misfolding, and accordingly, arsenite treatment results in both induction of heat shock responses as well as the accumulation of high molecular weight ubiquitinated material [47,48].

AIRAP (*A*rsenite-*i*nducible *R*NA-*a*ssociated *p*rotein) is a small (19 kDa) protein first identified in a screen for genes induced by arsenite treatment [47]. AIRAP shows remarkable specificity for the toxicity associated with arsenite, as induction could not be observed with zinc, copper, tunicamycin, thapsigargin, hydrogen peroxide, heat, or AZC [47,49]. Failure of induction by AZC, a proline analog which promotes protein misfolding, seems particularly interesting because of the suspected role of protein misfolding in arsenite toxicity. The efficacy of AIRAP induction is clear inasmuch as experimentally induced deficiencies in AIRAP give rise to markedly increased death rates of *C. elegans* exposed to arsenite [47].

An initial report suggested a role for AIRAP in binding RNA [47]. The significance of this result remains unclear, particularly in light of a second report which indicated a robust role for AIRAP in proteasome function [49]. Under arsenite-free conditions, low levels of AIRAP appear to be associated with proteasomes, but in the presence of arsenite, AIRAP can be readily co-purified with proteasomes. That these AIRAP-containing proteasomes are distinct from basally produced proteasomes was immediately suggested by the imprecise co-fractionation of AIRAP and canonical proteasome subunits. Specifically, AIRAP-containing proteasomes appear to represent a heavier-than-average subpopulation of the total proteasome pool [49]. In vivo, total cellular ubiquitin conjugate levels increased in cells treated with an siRNA directed against AIRAP, consistent with the proposed role of AIRAP in proteasome function. The exact role of AIRAP in proteasome function remains unclear, but in vitro, AIRAP has intriguing effects on proteasome stability. Conventionally purified proteasomes require ATP, at least in part, for prolonged integrity. In contrast, proteasomes containing AIRAP were not only stable in the absence of ATP, but actually appeared to disassemble in the presence of ATP [49]. Furthermore, AIRAP-containing proteasomes showed higher rates of hydrolysis of model substrates such as the peptide substrate suc-LLVY-AMC, a property possibly related to the increased in vitro proteasome stability conferred by AIRAP [49]. Notably, arsenic itself had little effect on the ability of purified proteasomes to hydrolyze suc-LLVY-AMC, suggesting that the function of AIRAP may be to adapt proteasomes to withstand an as yet poorly understood cellular toxicity arising secondary to arsenic exposure.

What might be the function of AIRAP? Sequence analysis indicates two regions containing highly conserved cysteine and histidine residues, and proteasome binding by AIRAP requires at least the C-terminal cysteine/histidine repeat [49]. The precise arrangement of the conserved residues bears similarities to the RING domain which is often found in ubiquitin ligases [49]. Ubiquitination at the proteasome is known to increase the efficiency of proteasome-mediated degradation [19]. Possibly, AIRAP works analogously to improve proteasome efficiency in the face of an increased substrate burden arising from protein modification by arsenite. However, such a model would be unlikely to explain the increased capacity of proteasomes to degrade model substrates like suc-LLVY-AMC, which are hydrolyzed in a ubiquitin-independent manner. Alternatively, the nucleotide-independent stability of proteasomes conferred by AIRAP may protect proteasomes against the ATP depleting effects of arsenic forms including arsenate. Though not excluding this model, the levels of arsenic necessary for AIRAP induction are significantly lower than those required to deplete cellular ATP [49]. A third model posits that AIRAP might in fact represent a functional homolog of Rpn4. The arrangement of conserved cysteines and histidines in AIRAP does not obviously resemble the Cys_2–His_2 sequence of Rpn4. However, arsenic is known to induce a proteasome stress response [50], AIRAP does appear to make contacts within the base of the proteasome adjacent to the site of Rpn4-proteasome interaction [49], and AIRAP, despite its specificity for arsenic, is also induced by MMS, a potent stimulus of Rpn4 induction [49]. The elucidation of the precise function of AIRAP will likely require an exploration of AIRAP's effects on the various functions carried out by the proteasome, namely substrate recognition, unfolding, deubiquitination (and possibly ubiquitination), opening of the gate into the CP, translocation of substrate into the CP, and finally, proteolysis.

6. Ubp6

Ubp6, along with Rpn11, is one of two deubiquitinating enzymes associated with proteasomes of budding yeast. Higher eukaryotes possess yet a third proteasomal deubiquitinating enzyme, Uch37. The precise roles of these three deubiquitinating enzymes in proteasome-mediated degradation, their substrate specificity, and their relationships with one another remain incompletely understood. Nevertheless, current studies point to proteasome-mediated deubiquitination as a particularly complex aspect of the ubiquitin-proteasome pathway.

Deubiquitination by Rpn11 is thought to facilitate substrate degradation because mutations in the active site of Rpn11 stabilize various substrates in vitro and in vivo [20,21]. In contrast, Uch37 appears to negatively regulate proteasome-mediated degradation [51]. Recognition of substrates by the proteasome generally requires previous modification of the substrate by ubiquitin, and increased levels of ubiquitin modification appear to increase affinity of the substrate for the proteasome, possibly by allowing for greater interaction of the substrate with proteasomal ubiquitin receptors. Thus, premature removal of these ubiquitin molecules could thwart substrate degradation by decreasing substrate affinity for the proteasome. Uch37 is capable of slowing degradation in this manner, although just why Uch37 has evolved to accomplish this remains unclear. One plausible model is that Uch37 serves as an "editing" enzyme for ubiquitin conjugates, biasing degradation towards more highly ubiquitinated substrates [51]. Such a system could correct for "errors" in ubiquitination, in which inappropriate substrates have been aberrantly recognized by the ubiquitin system, but only modestly ubiquitinated due to a low affinity for the ubiquitin ligase. A second model posits that the nature of ubiquitin signals may inherently prioritize substrates. Substrates that acquire long ubiquitination chains in a single round of interaction with a ubiquitin ligase (termed "processive" ubiquitination) may be degraded more quickly than substrates that require multiple rounds of interaction with their ubiquitin ligase (termed "distributive" ubiquitination), the difference in rates of degradation being accounted for by increased susceptibility of distributive substrates to deubiquitination [52]. Possibly, Uch37 functions to orchestrate such ordering of degradation rates.

The third deubiquitinating enzyme, Ubp6, also appears to inhibit proteasome-mediated degradation [53]. However, in

contrast to Uch37, a catalytically dead mutant of Ubp6 retains its capacity for proteasome inhibition both in vitro and in vivo [53,54]. Thus, Ubp6 has both deubiquitinating activity and proteasome-inhibitory activity. Presumably, these two functions are coordinated in Ubp6's overall activity, but direct evidence on this point is lacking. The catalytic activity of Ubp6 is crucial in controlling cellular levels of ubiquitin. In the absence of Ubp6's catalytic activity, the rate of ubiquitin degradation increases dramatically, and this degradation occurs via the proteasome [30,53,55,56]. Thus, Ubp6 functions in regenerating ubiquitin from ubiquitin conjugates, and when this does not occur, ubiquitin is apparently translocated into the core particle along with substrate and destroyed. The physiologic significance of Ubp6 function is highlighted not only by the many stress hypersensitivities of *ubp6* mutants [55,56], but by the *ataxia* mouse, which derives from loss-of-function mutations in murine Ubp6, known as Usp14 [57]. This mouse is subject to a broad array of neurologic dysfunctions, and uniformly undergoes premature death.

The exact stoichiometry of Ubp6 with respect to the proteasome remains an unsettled question, but Ubp6 is visible by conventional Coomassie staining in standard preparations of wild-type proteasomes, indicating that it is an abundant proteasome component under steady-state conditions [30]. Indeed, Ubp6 contains a PACE sequence [30] and is co-regulated with other proteasome subunits by Rpn4 [42,54], but a recent study indicates that Ubp6 is subject to additional modes of regulation outside of the proteasome stress response [54].

An initial insight came from examination of cellular levels of Ubp6 and the catalytically dead mutant, *ubp6-C118A*, when expressed from the *UBP6* promoter. Ubp6-C118A accumulated to significantly higher levels than the wild-type protein [54]. Because Ubp6-C118A is devoid of catalytic activity, and because the catalytic activity of Ubp6 is necessary to maintain ubiquitin levels, it was hypothesized that accumulation of Ubp6-C118A might reflect a cellular stress response designed to correct ubiquitin levels. Indeed, when ubiquitin levels were corrected by exogenous expression of ubiquitin, levels of Ubp6-C118A were restored to nearly wild-type levels [54]. Furthermore, accumulation of wild-type Ubp6 protein could be observed in multiple unrelated yeast mutants deficient in cellular ubiquitin levels, suggesting that ubiquitin-dependent induction of Ubp6 represents a physiologic cellular stress response [54]. In every case, induction of Ubp6 or Ubp6-C118A protein was accompanied by increased transcription of the respective gene, analogous to the situation of AIRAP [54]. Importantly, and again likewise for AIRAP, induction of Ubp6 in response to ubiquitin deficiency resulted in increased loading of proteasome with Ubp6 or Ubp6-C118 A. Because of the specific proteasomal enrichment of Ubp6, and because ubiquitin deficiency does not seem to necessarily stimulate Rpn4 accumulation (34, 54), this pathway of ubiquitin-dependent induction of Ubp6 has been termed the "ubiquitin stress response" to distinguish it from the proteasome stress response (Fig. 2).

Several important aspects of the ubiquitin stress response remain poorly understood. The *cis*-acting element within the *UBP6* promoter, as well as the factors that mediate recognition of this element, remain unknown. Furthermore, the physiologic efficaciousness of Ubp6 induction remains unexamined. Does ubiquitin-dependent induction of Ubp6 truly buffer ubiquitin levels against stress? Identification of the ubiquitin-regulated element within the *UBP6* promoter should allow for rigorous testing of this model.

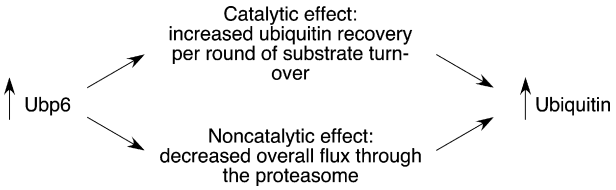

Fig. 3. Model for dual modes of ubiquitin regeneration by Ubp6. The primary effect of Ubp6 on ubiquitin levels is mediated by the catalytic deubiquitinating activity of Ubp6. However, Ubp6 also appears to directly inhibit the proteasome via non-catalytic functions, and this slowing of total flux through the proteasome might provide a second mechanism for ubiquitin stabilization.

A second question concerns the mechanisms whereby Ubp6 contributes to ubiquitin homeostasis. Without doubt, the majority of ubiquitin sparing mediated by Ubp6 is catalytic in nature, as *ubp6-C118A* mutants display serious ubiquitin deficiencies, and recapitulate the null phenotype when challenged with various ubiquitin-dependent stresses [53]. However, Ubp6 also mediates a non-catalytic inhibition of proteasome-mediated degradation, and such inhibition does not appear to be highly restricted in the scope of its substrates [54]. Thus, Ubp6 may contribute a second, indirect effect on ubiquitin levels via a decreased overall flux of ubiquitin conjugates through the proteasome. Such a model predicts that ubiquitin levels in the *ubp6-C118A* mutant should be at least modestly elevated compared to the null mutant (Fig. 3). Visual inspection of the data [54] suggests that this may indeed be the case, but careful quantitations will be required.

A third question concerns the provenance of increased Ubp6 levels on proteasomes. Transcriptional induction of the *UBP6* gene may account for the entire difference in Ubp6 levels on proteasomes. However, it should be noted that several groups have reported minor, yet significant, non-proteasomal pools of Ubp6 in cells [56,58]. Whether proteasomes can recruit additional Ubp6 from these pools remains an intriguing open question. Indeed, such a scenario would allow for more rapid induction of the stress response.

It is also worth exploring why the ubiquitin stress response appears to be distinct from the proteasome stress response, especially as the latter has components of ubiquitin regulation in addition to proteasome regulation [31,34]. It is thought that the majority of degradation mediated by the proteasome proceeds via ubiquitination of substrates. Thus, under conditions of proteasome induction, it may be necessary to provide corresponding increases of ubiquitin levels, which might be achieved primarily through induction of the polyubiquitin gene, *UBI4* [34]. It remains unclear whether an increased *UBI4* mRNA level actually elevates cellular ubiquitin pools, as opposed to ameliorating attrition of these pools, and indeed, artificially raising ubiquitin levels during proteasome stress actually exacerbates the phenotypes of some proteasome mutants [34]. On the other hand, it must be appreciated that ubiquitin performs an astounding array of cellular tasks independent of the proteasome, including regulation of cell surface receptors, histones, membrane trafficking, transcription, and DNA repair, to name a few [59]. All of these ubiquitin-dependent processes may be subject to some degree of dysfunction when ubiquitin levels fall. Thus, it may be advantageous for cells to be able to

control ubiquitin levels separately from proteasome levels. Indeed, even in wild-type cells, some fraction of ubiquitin is degraded by proteasomes [54,56,60]. Raising proteasome levels under conditions of ubiquitin deficiency might actually exacerbate the deficiency by increasing basal rates of ubiquitin degradation.

A final point of intrigue derives from the interesting convergence of AIRAP and Ubp6 onto a single proteasome subunit, Rpnl. Ubp6 and Rpnl directly interact in their bacterially produced, purified forms [30]. In the case of AIRAP, specific crosslinking to Rpnl/PSMD2 has been observed, and bacterially produced, purified AIRAP is bound by extract-derived Rpnl [49]. Possibly, these findings represent coincidence, but it is tempting to speculate on the function of Rpnl. Rpnl and Rpn2, two of the largest subunits of the proteasome, are related in sequence, and are thought to occupy a central position in the RP, between the ring of six ATPases in the base and the subunits of the lid. Furthermore, Rpnl and Rpn2 are known to bind a number of substoichiometric or more loosely-binding components of the proteasome including not only AIRAP and Ubp6, but also the ubiquitin receptors Rad23 andDsk2 [17,18], and the ubiquitin conjugating enzyme, Hul5 (KIAA10 in mammals) whose function appears to be highly related to that of Ubp6 [19]. Thus Rpnl and Rpn2 may represent a scaffold for the assembly of a variety of factors which can modulate the nature of ubiquitin chain processing and substrate degradation. Possibly, the array of factors bound to Rpnl and Rpn2 may be variable and highly dynamic, such that Rpnl and Rpn2 may serve as an "expansion port" for the proteasome – a specific site at which subunit-specific proteasome remodeling occurs.

7. Concluding remarks

The term "proteasome" is often utilized in such a way as to imply a uniformity of form and function. However, it has become increasingly clear from recent studies that the cell's population of proteasomes may actually represent a diverse group of functionally distinct entities, and furthermore, the capacity to convert between forms as well to generate novel forms may be highly developed and subject to sophisticated modes of regulation. The versatility of the proteasome and its capacity for regulation in both magnitude and nature likely play key roles in the ability of cells to survive a wide variety of environmental stresses.

References

[1] Pickart, C.M. and Cohen, R.E. (2004) Proteasomes and their kin: proteases in the machine age. Nat. Rev. Mol. Cell Biol. 5, 177–187.
[2] Peng, J., Schwartz, D., Elias, J.E., Thoreen, C.C., Cheng, D., Marsischky, G., Roelofs, J., Finley, D. and Gygi, S.P. (2003) A proteomics approach to understanding protein ubiquitination. Nat. Biotechnol. 21, 921–926.
[3] Chau, V., Tobias, J.W., Bachmair, A., Marriott, D., Ecker, D.J., Gonda, D.K. and Varshavsky, A. (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science 243, 1576–1583.
[4] Thrower, J.S., Hoffman, L., Rechsteiner, M. and Pickart, C.M. (2000) Recognition of the polyubiquitin proteolytic signal. EMBO J. 19, 94–102.
[5] Schmidt, M., Hanna, J., Elsasser, S. and Finley, D. (2005) Proteasome-associated proteins: regulation of a proteolytic machine. Biol. Chem. 386, 725–737.
[6] Groll, M., Ditzel, L., Lowe, J., Stockm, D., Bochtler, M., Bartunik, H.D. and Huber, R. (1997) Structure of 20S proteasome from yeast at 2.4 A resolution. Nature 386, 463–471.
[7] Kisselev, A.F., Callard, A. and Goldberg, A.L. (2006) Importance of the different proteolytic sites of the proteasome and the efficacy of inhibitors varies with the protein substrate. J. Biol. Chem. 281, 8582–8590.
[8] Groll, M., Bajorek, M., Kohler, A., Moroder, L., Rubin, D.M., Huber, R., Glickman, M.H. and Finley, D. (2000) A gated channel into the proteasome core particle. Nat. Struct. Biol. 7, 1062–1067.
[9] Glickman, M.H., Rubin, D.M., Coux, O., Wefes, I., Pfeifer, G., Cjeka, Z., Baumeister, W., Fried, V.A. and Finley, D. (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. Cell 94, 615–623.
[10] Braun, B.C., Glickman, M., Kraft, R., Dahlmann, B., Kloetzel, P.M., Finley, D. and Schmidt, M. (1999) The base of the proteasome regulatory particle exhibits chaperone-like activity. Nat. Cell Biol. 1, 221–226.
[11] Liu, C.W., Millen, L., Roman, T.B., Xiong, H., Gilbert, H.F., Noiva, R., DeMartino, G.N. and Thomas, P.J. (2002) Conformational remodeling of proteasomal substrates by PA700, the 19 S regulatory complex of the 26 S proteasome. J. Biol. Chem. 277, 26815–26820.
[12] Rubin, D.M., Glickman, M.H., Larsen, C.N., Dhruvakumar, S. and Finley, D. (1998) Active site mutants in the six regulatory particle ATPases reveal multiple roles for ATP in the proteasome. EMBO J. 17, 4909–4919.
[13] Kohler, A., Cascio, P., Leggett, D.S., Woo, K.M., Goldberg, A.L. and Finley, D. (2001) The axial channel of the proteasome core particle is gated by the Rpt2 ATPase and controls both substrate entry and product release. Mol. Cell 7, 1143–1152.
[14] Lam, Y.A., Lawson, T.G., Velayutham, M., Zweier, J.L. and Pickart, C.M. (2002) A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal. Nature 416, 763–767.
[15] Verma, R., Oania, R., Graumann, J. and Deshaies, R.J. (2004) Multiubiquitin chain receptors define a layer of substrate selectivity in the ubiquitin-proteasome system. Cell 118, 99–110.
[16] Elsasser, S., Chandler-Militello, D., Muller, B., Hanna, J. and Finley, D. (2004) Rad23 and Rpnl0 serve as alternative ubiquitin receptors for the proteasome. J. Biol. Chem. 279, 26817–26822.
[17] Elsasser, S., Gali, R.R., Schwickart, M., Larsen, C.N., Leggett, D.S., Muller, B., Feng, M.T., Tubing, F., Dittmar, G.A. and Finley, D. (2002) Proteasome subunit Rpnl binds ubiquitin-like protein domains. Nat. Cell Biol. 4, 725–730.
[18] Saeki, Y., Sone, T., Toh-e, A. and Yokosawa, H. (2002) Identification of ubiquitin-like protein-binding subunits of the 26S proteasome. Biochem. Biophys. Res. Commun. 296, 813–819.
[19] Crosas, B., Hanna, J., Kirkpatrick, D.S., Zhang, D.P., Tone, Y., Hathaway, N.A., Buecker, C., Leggett, D.S., Schmidt, M., King, R.W., Gygi, S.P. and Finley, D. (2006) Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities. Cell 127, 1401–1413.
[20] Verma, R., Aravind, L., Oania, R., McDonald, W.H., Yates 3rd, J.R., Koonin, E.V. and Deshaies, R.J. (2002) Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. Science 298, 611–615.
[21] Yao, T. and Cohen, R.E. (2002) A cryptic protease couples deubiquitination and degradation by the proteasome. Nature 419, 403–407.
[22] Maytal-Kivity, V., Reis, N., Hofmann, K. and Glickman, M.H. (2002) MPN+, a putative catalytic motif found in a subset of MPN domain proteins from eukaryotes and prokaryotes, is critical for Rpnll function. BMC Biochem. 3, 28.
[23] Cope, G.A., Suh, G.S., Aravind, L., Schwarz, S.E., Zipursky, S.L., Koonin, E.V. and Deshaies, R.J. (2002) Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cull. Science 298, 608–611.
[24] Xie, Y. and Varshavsky, A. (2001) RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. Proc. Natl. Acad. Sci. USA 98, 3056–3061.

[25] Nelson, M.K., Kurihara, T. and Silver, P.A. (1993) Extragenic suppressors of mutations in the cytoplasmic C terminus of SEC63 define five genes in *Saccharomyces cerevisiae*. Genetics 134, 159–173.
[26] Johnson, E.S., Ma, P.C., Ota, I.M. and Varshavsky, A. (1995) A proteolytic pathway that recognizes ubiquitin as a degradation signal. J. Biol. Chem. 270, 17442–17456.
[27] Fujimuro, M., Tanaka, K., Yokosawa, H. and Toh-e, A. (1998) Sonlp is a component of the 26S proteasome of the yeast *Saccharomyces cerevisiae*. FEBS Lett. 423, 149–154.
[28] Finley, D., Tanaka, K., Mann, C., Feldmann, H., Hochstrasser, M., Vierstra, R., Johnston, S., Hampton, R., Haber, J., Mccusker, J., Silver, P., Frontali, L., Thorsness, P., Varshavsky, A., Byers, B., Madura, K., Reed, S.I., Wolf, D., Jentsch, S., Sommer, T., Baumeister, W., Goldberg, A., Fried, V., Rubin, D.M., Toh-e, A., et al. (1998) Unified nomenclature for subunits of the *Saccharomyces cerevisiae* proteasome regulatory particle. Trends Biochem Sci. 23, 244–245.
[29] Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Dohmen, J. and Deshaies, RJ. (2000) Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. Mol. Biol. Cell 11, 3425–3439.
[30] Leggett, D.S., Hanna, J., Borodovsky, A., Crosas, B., Schmidt, M., Baker, R.T., Walz, T., Ploegh, H. and Finley, D. (2002) Multiple associated proteins regulate proteasome structure and function. Mol. Cell 10, 495–507.
[31] Mannhaupt, G., Schnall, R., Karpov, V., Vetter, I. and Feldmann, H. (1999) Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. FEBS Lett. 450, 27–34.
[32] Jelinsky, S.A., Estep, P., Church, G.M. and Samson, L.D. (2000) Regulatory networks revealed by transcriptional profiling of damaged *Saccharomyces cerevisiae* cells: Rpn4 links base excision repair with proteasomes. Mol. Cell. Biol. 20, 8157–8167.
[33] Ju, D., Wang, L., Mao, X. and Xie, Y. (2004) Homeostatic regulation of the proteasome via an Rpn4-dependent feedback circuit. Biochem. Biophys. Res. Commun. 321, 51–57.
[34] London, M.K., Keck, B.I., Ramos, P.C. and Dohmen, R.J. (2004) Regulatory mechanisms controlling biogenesis of ubiquitin and the proteasome. FEBS Lett. 567, 259–264.
[35] Owsianik, G., Balzi, L. and Ghislain, M. (2002) Control of 26S proteasome expression by transcription factors regulating multidrug resistance in *Saccharomyces cerevisiae*. Mol. Microbiol. 43, 1295–1308.
[36] Hahn, J.S., Neef, D.W. and Thiele, DJ. (2006) A stress regulatory network for coordinated activation of proteasome expression mediated by yeast heat shock transcription factor. Mol. Microbiol. 60, 240–251.
[37] Yokoyama, H., Mizunuma, M., Okamoto, M., Yamamoto, J., Hirata, D. and Miyakawa, T. (2006) Involvement of calcineurin-dependent degradation of Yaplp in Ca^{2+}-induced G2 cell-cycle regulation in *Saccharomyces cerevisiae*. EMBO Rep. 7, 519–524.
[38] Ju, D. and Xie, Y. (2004) Proteasomal degradation of RPN4 via two distinct mechanisms, ubiquitin-dependent and -independent. J. Biol. Chem. 279, 23851–23854.
[39] Ju, D. and Xie, Y. (2006) Identification of the preferential ubiquitination site and ubiquitin-dependent degradation signal of Rpn4. J. Biol. Chem. 281, 10657–10662.
[40] Wang, L., Mao, X., Ju, D. and Xie, Y. (2004) Rpn4 is a physiological substrate of the Ubr2 ubiquitin ligase. J. Biol. Chem. 279, 55218–55223.
[41] Meiners, S., Heyken, D., Weller, A., Ludwig, A., Stangl, K., Kloetzel, P.M. and Kruger, E. (2003) Inhibition of proteasome activity induces concerted expression of proteasome genes and de novo formation of mammalian proteasomes. J. Biol. Chem. 278, 21517–21525.
[42] Lundgren, J., Masson, P., Realini, C.A. and Young, P. (2003) Use of RNA interference and complementation to study the function of the Drosophila and human 26S proteasome subunit SI3. Mol Cell Biol. 23, 5320–5330.
[43] Wojcik, C. and DeMartino, G.N. (2002) Analysis of Drosophila 26S proteasome using RNA interference. J. Biol. Chem. 277, 6188–6197.
[44] Heink, S., Ludwig, D., Kloetzel, P.M. and Kruger, E. (2005) IFN-gamma-induced immune adaptation of the proteasome system is an accelerated and transient response. Proc. Natl. Acad. Sci. USA 102, 9241–9246.
[45] Stryer, L. (1995) Biochemistry, 4th Ed, Freeman.
[46] Thompson, D.J. (1993) A chemical hypothesis for arsenic methylation in mammals. Chem. Biol. Interact. 88, 14–89.
[47] Sok, J., Calfon, M., Lu, J., Lichtlen, P., Clark, S.G. and Ron, D. (2001) Arsenite-inducible RNA-associated protein (AIRAP) protects cells from arsenite toxicity. Cell Stress Chaperon. 6, 6–15.
[48] Kirkpatrick, D.S., Dale, K.V., Catania, J.M. and Gandolfi, A.J. (2003) Low-level arsenite causes accumulation of ubiquitinated proteins in rabbit renal cortical slices and HEK293 cells. Toxicol. Appl. Pharmacol. 186, 101–109.
[49] Stanhill, A., Haynes, CM., Zhang, Y., Min, G., Steele, M.C., Kalinina, J., Martinez, E., Pickart, CM., Kong, X.P. and Ron, D. (2006) An arsenite-inducible 19S regulatory particle-associated protein adapts proteasomes to proteotoxicity. Mol. Cell 23, 875–885.
[50] Zheng, P.Z., Wang, K.K., Zhang, Q.Y., Huang, Q.H., Du, Y.Z., Zhang, Q.H., Xiao, D.K., Shen, S.H., Imbeaud, S., Eveno, E., Zhao, C.J., Chen, Y.L., Fan, H.Y., Waxman, S., Auffray, C., Jin, G., Chen, S.J., Chen, Z. and Zhang, J. (2005) Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia. Proc. Natl. Acad. Sci. USA 102, 7653–7658.
[51] Lam, Y.A., Xu, W., DeMartino, G.N. and Cohen, R.E. (1997) Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome. Nature 385, 737–740.
[52] Rape, M., Reddy, S.K. and Kirschner, M.W. (2006) The processivity of multiubiquitination by the APC determines the order of substrate degradation. Cell 124, 89–103.
[53] Hanna, J., Hathaway, N.A., Tone, Y., Crosas, B., Elsasser, S., Kirkpatrick, D.S., Leggett, D.S., Gygi, S.P., King, R.W. and Finley, D. (2006) Deubiquitinating enzyme Ubp6 functions noncatalytically to delay proteasomal degradation. Cell 127, 99–111.
[54] Hanna, J., Meides, A., Zhang, D.P., and Finley, D. (2007). A ubiquitin stress response induces altered proteasome composition. Cell (in press).
[55] Chernova, T.A., Allen, K.D., Wesoloski, L.M., Shanks, J.R., Chernoff, Y.O. and Wilkinson, K.D. (2003) Pleiotropic effects of Ubp6 loss on drug sensitivities and yeast prion are due to depletion of the free ubiquitin pool. J. Biol. Chem. 278, 52102–52115.
[56] Hanna, J., Leggett, D.S. and Finley, D. (2003) Ubiquitin depletion as a key mediator of toxicity by translational inhibitors. Mol. Cell. Biol. 23, 9251–9261.
[57] Wilson, S.M., Bhattacharyya, B., Rachel, R.A., Coppola, V., Tessarollo, L., Householder, D.B., Fletcher, C.F., Miller, R.J., Copeland, N.G. and Jenkins, N.A. (2002) Synaptic defects in ataxia mice result from a mutation in Usp14, encoding a ubiquitin-specific protease. Nat. Genet. 32, 420–425.
[58] Stone, M., Hartmann-Petersen, R., Seeger, M., Bech-Otschir, D., Wallace, M. and Gordon, C. (2004) Uch2/Uch37 is the major deubiquitinating enzyme associated with the 26S proteasome in fission yeast. J. Mol. Biol. 344, 697–706.
[59] Hicke, L. (2001) Protein regulation by monoubiquitin. Nat. Rev. Mol. Cell Biol. 2, 195–201.
[60] Ryu, K.Y., Baker, R.T. and Kopito, R.R. (2006) Ubiquitin-specific protease 2 as a tool for quantification of total ubiquitin levels in biological specimens. Anal. Biochem. 353, 153–155.