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# Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome

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## Abstract

The proteasome is an intricate molecular machine, which serves to degrade proteins following their conjugation to ubiquitin. Substrates dock onto the proteasome at its 19-subunit regulatory particle via a diverse set of ubiquitin receptors, and are then translocated into an internal chamber within the 28-subunit proteolytic core particle, where they are hydrolyzed. Substrate is threaded into the core particle through a narrow gated channel, and thus translocation requires ATP-dependent unfolding of the substrate. Six distinct ATPases in the regulatory particle appear to form a ring complex and to drive unfolding as well as translocation. ATP-dependent, degradation-coupled deubiquitination of the substrate is required both for efficient substrate degradation and for suppressing ubiquitin degradation. However, the proteasome also contains deubiquitinating enzymes that act on substrates prior to degradation, thus suppressing substrate degradation. Here we examine the key elements of this molecular machine and how they cooperate in the processing of proteolytic substrates.

## INTRODUCTION

The proteasome, a protease of over 2.5 MegaDaltons, functions primarily to degrade proteins that have been modified by the attachment of the small protein ubiquitin (1, 2) (Figure 1). A cascade of enzymes, known as E1, E2, and E3, conjugate ubiquitin via its C-terminus to lysine residues in target proteins. The ubiquitin-proteasome system (UPS) is the major cytosolic proteolytic system in eukaryotes, with critical functions in cell cycle control, apoptosis, inflammation, transcription, signal transduction, protein quality control, and many other biological processes. The number of genes involved in the UPS varies significantly among eukaryotes, occupying, at the high end, over 6% of the genome of *A. thaliana* (3). The breadth of the UPS owes mainly to the multiplicity of ubiquitin ligase (E3) enzymes, the specificity factors for ubiquitination, but the proteasome is the single most complex enzyme known in the system.

The proteasome works via a multistep mechanism, with peptide bond cleavage being only the last step in a complex program of substrate manipulation. Critical upstream processes–

**FUTURE ISSUES**

We are at an early stage in the functional analysis of the proteasome. The functional complexity of the RP and the paucity of detailed structural information on this particle should make it a major subject of interest in coming years. The greatest single challenge in the field is to determine the complete structure of the RP, though the structure will probably pose more questions that it answers. The key activities of the RP are all present in multiple distinct copies: 6 ATPases, 3 deubiquitinating enzymes, and over 5 ubiquitin receptors. Why is this? The relationships among paralogous functions have so far proven to be fascinating, an example being the dissimilar and often opposing properties of the three deubiquitinating enzymes. Such issues must be addressed for the ATPases and ubiquitin receptors. Much attention has been given to the substrate translocation channel of the CP, but that of the RP, which is uncharacterized, should prove to be more important and interesting. Does this involve the Rpt proteins, the Rpn1/2 complex, or both?

recognition, unfolding, translocation, and deubiquitination of the substrate–take place distantly from the site of proteolysis, and are mediated by a large separable subassembly, the regulatory particle, largely under the direction of six distinct ATPases. Structurally, the proteasome is a hybrid between a proteolytic machinery and an ATP-dependent regulatory machinery, functionally linked by a gated protein translocation channel. But more interestingly it is also a hybrid between an ancient core biochemistry consisting of mechanisms for protein unfolding and degradation, which was developed in the early precursors of all cells, and the more recent eukaryotic inventions associated with ubiquitination. These two periods of evolutionary innovation, separated by billions of years, produced an unusually complex molecular machine. The most basic questions about the proteasome are those that apply generally to molecular machines: how is the energy of nucleotide hydrolysis converted into useful work, how is the fidelity of the process ensured, how are the core mechanisms of the machine regulated to provide for robust function and adaptation to changing cellular conditions. The answers are only beginning to emerge, but have already proven novel because of the unique biochemical features of the proteasome.

The proteasome exists in multiple forms, but contains two major assemblies, the 28-subunit core particle (CP, also known as the 20S particle) and a regulatory particle of 19–20 subunits, depending on the species (RP, also known as the 19S particle, and PA700) (Figure 2). The CP is a barrel-like structure whose subunits are arranged in four stacked seven-membered rings (4,5) (Figure 3A). The proteasome's proteolytic active sites are sequestered within the large internal space of the CP (Figure 3B). Thus, for the free CP, substrate access into the proteolytic chamber is blocked through an essentially topological mechanism. In the RP-CP holoenzyme, substrate entry is controlled by the RP: the RP both opens a substrate translocation channel into the CP (6–12) and guides substrates into this channel. The RP binds to the cylinder end of the CP (13), and opens a channel located centrally within the cylinder end. Thus, the axial channel opens directly onto the RP. The RP is in turn assumed to possess its own substrate translocation channel, the outlet of which is apposed to that of the CP, although the RP channel is essentially uncharacterized.

The substrate translocation channel, even when open, is sufficiently narrow (6, 8, 14) that it prevents the bulk of cytoplasmic proteins from being degraded spuriously. The channel also imposes a constraint on true substrates, that they must be unfolded by the RP prior to translocation into the CP. Substrate entry in an unfolded state presumably also facilitates efficient hydrolysis of substrates within the CP. Finally, in contrast to the distributive mechanism typical of proteases, the proteasome degrades substrates processively, which reflects its compartmentalized structure and substrate translocation mechanism. Processive protein degradation avoids the generation of truncated reaction products, which could compromise cell function. However, in rare instances, regulatory proteins are subjected to partial proteolysis by the proteasome, which activates them through the removal of inhibitory domains. Partial proteolysis requires specialized features in the substrate, as discussed below.

The protein-unfoldase activity of the RP is thought to be mediated by its six ATPase subunits, which are members of the AAA protein family (15–20). These ATPases, known as the Rpt proteins in yeast, are thought to form a pseudo-symmetrical ring structure, which is embedded within the highly asymmetric structure of the RP (10, 13, 21). Docking of the RP to the CP is stabilized by the alignment of two ring assemblies–the Rpts and the outer ring of the CP (the α ring). Because the Rpt ring is thought to have six members and the α ring seven, RP-CP docking might induce a major break in the symmetry of either the Rpt or α ring.

It is generally assumed that the initial recognition of substrates by the proteasome is mediated by the substrate's ubiquitin tag (1, 2). This is a function of the RP; the CP itself cannot specifically degrade ubiquitin-protein conjugates. There is no indication that ubiquitin performs roles at the proteasome other than substrate tethering, though a signaling function for ubiquitin within the proteasome remains a distinct possibility. A surprising number of proteins can mediate ubiquitin recognition at the proteasome: two subunits of the RP, Rpn10 and Rpn13 (22–30); and apparently three proteasome-associated proteins, Rad23, Dsk2, and Ddi1 (26, 27, 31–33). The latter proteins will be referred to collectively as the UBL/UBA proteins. Each contacts the proteasome through its UBL (ubiquitin-like) domain and ubiquitin-conjugates through one or more UBA (ubiquitin-associated) domains. The UBL/UBA proteins are not integral proteasome subunits; they bind proteasomes only weakly and are usually substoichiometric components of purified proteasomes. Many other ubiquitin receptors exist; most are not associated with proteasome function, but instead mediate nonproteolytic functions of ubiquitination (34).

The proteasome does not degrade proteins to amino acids (35) but instead produces a highly heterogeneous mixture of peptides from a given protein (36). These peptides serve as raw material for adaptive cell-mediated immunity. Product peptides of 8–10 residues dock onto the major histocompatibility (MHC) class I molecule in the endoplasmic reticulum after their export from the cytoplasm via the peptide-specific TAP transporter. Once routed to the cell surface, the peptide-MHC complex may be recognized by epitope-specific T cell receptors carried on cytotoxic T lympohocytes. If the presented epitope is derived from viruses, tumors, or other "foreign" sources, the presenting cell is induced to undergo apoptosis.

Eukaryotic cells contain a second proteolytic pathway that is responsible for the breakdown of less rapidly degraded proteins. The lysosome, a membrane-enclosed organelle, contains numerous proteases of low specificity, ensuring that almost any cytoplasmic protein that reaches its interior will be degraded efficiently. The uptake of intracellular proteins into the lysosome, known as autophagy, is a predominantly nonspecific process in which portions of cytoplasm or whole organelles are subsumed en mass into an autophagic vesicle, whose entire contents are then delivered to the lysosome upon membrane fusion. This contrasts strongly with the mechanism of substrate selection by the proteasome, which recognizes proteins via a specific tag and sorts proteins individually. It should be noted, however, that some autophagy pathways are selective (37), and some evidence for ubiquitin-mediated targeting in this pathway has been presented (38). The autophagic pathway might serve as a back up mechanism for the proteasome for some substrates. The regulatory mechanisms associated with autophagy are complex, fascinating, and highly relevant to human health (see recent reviews [39,40]).

Because proteins that are rapidly turned over are also typically present at low levels in cells, mass-spectrometry-based searches for ubiquitinated proteins and proteasome substrates have so far provided only incomplete surveys (28, 41, 42). However, the number of proteins that are degraded via the proteasome is undoubtedly very high. The existence of hundreds of physiological substrates (43), and potentially more, implies that the proteasome's capacity to process protein substrates is robust and specifically that it can unfold a remarkable variety of proteins. However, in most cases the proteasome can only act on proteins if they are ubiquitinated.

Regulatory proteins such as cyclins, CDK inhibitors, IκB, and p53 are key substrates for the proteasome, and their ubiquitin-dependent degradation is under tight control. Other regulators are activated through partial proteolysis as described above. Finally, misfolded and aberrant proteins are substrates, and recent work has shown that numerous diseases are

associated with poor clearance of these often deleterious proteins. Thus, the proteasome is important to human health in a variety of ways.

The proteasome literature is vast, and cannot be fully reviewed here. The reader is referred to excellent summaries of the earlier literature (1, 2, 5, 44–48). Space limitations and the availability of current reviews preclude discussion of either proteasome inhibitors (5, 49, 50), which are anti-cancer agents in clinical use, subcellular localization of the proteasome (44), nonproteolytic roles of the proteasome (46), or the remarkable maneuvers of proteasome assembly (48). An emerging area that will also not be covered is the regulation of proteasome levels, in which the transcription factors Rpn4 (in yeast) and Nrf2 (in mammals) play important roles (51,52).

A nomenclatural note: unfortunately, most proteasome subunits have many different names, among species and within a species. The systematic nomenclature for RP and CP subunits is most easily apprehended, and will be used here (4, 53). For example, we will refer specifically to budding yeast Rpn10 as scRpn10, murine Rpn10 as mRpn10, and human as hRpn10. Table 1 provides, for the RP subunits, both systematic names and other names found in the literature.

## ATP-DEPENDENT PROTEASES

The proteasome is the only ATP-dependent protease known in the eukaryotic cytoplasm and nucleus, and the only one that is ubiquitin-dependent. However, simple, compartmentalized ATP-dependent proteases have been characterized in eubacteria, archaea, and subcellular organelles such as the mitochondrion and chloroplast (54). Although always oligomeric, they can be specified by as few as 1–3 gene products, which suggests that the universal features of ATP-dependent proteolysis are less complex than might appear from consideration of the proteasome. The much greater complexity of the proteasome reflects in part adaptations within the RP for the recognition and processing of ubiquitin-conjugates. Indeed, many of the RP components that are not part of the ATPase ring function as either ubiquitin receptors, receptors for ubiquitin receptors, deubiquitinating enzymes, or ubiquitin ligases.

The ATPase domains of all compartmentalized ATP-dependent proteases belong to the same AAA+ family (55), indicating common basic features and presumably a common mechanism of protein unfolding. *E.coli* HslVU is notable for the detailed structural understanding that has been achieved (56–58), while the archaeal particle known as PAN is of special interest because of its detailed similarity to the proteasomal ATPases (10, 11, 18, 19, 59). Indeed, PAN can be viewed heuristically as the precursor to the eukaryotic RP. Innovative studies of ClpXP from *E. coli* demonstrated that ClpX is an unfoldase and that unfolding is an active process dependent on ATP hydrolysis (15). Unfoldase activity is now understood to be characteristic within the ATP-dependent protease family (18, 20, 60, 61), though some proteases are more potent unfoldases than others (61). For lack of space, the literature of other ATP-dependent proteases cannot be reviewed here, but the reader is referred to excellent treatments of this subject (47, 54, 62).

## FUNCTIONAL ELEMENTS OF THE PROTEASOME

### The Core Particle

The CP has 28 subunits, arranged into four hetero-heptameric rings (4; for a detailed review, see ref. 5) (Figure 3A). The complex is generated from 14 gene products, and exhibits two-fold symmetry. The stacked rings define an interior space whose largest dimensions are approximately 100 Å axially (in the closed state) and 60 Å along the orthogonal symmetry

axis (Figure 3B). Each inner ring is formed by β-type subunits, three of which contain proteolytic active sites (β1, β2, and β5). The proteolytic subunits belong to the N-terminal nucleophile (Ntn) hydrolase family, and are characterized by an unusual, essentially single-residue active site: the N-terminal threonine of each proteasome subunit provides both the catalytic nucleophile (on its side-chain) and the primary proton acceptor (the main chain N-terminus). Each active site can cleave a broad range of peptide sequences, with β1 preferring to cleave on the C-terminal side of acidic residues, β2 after tryptic residues, and β5 after hydrophobic residues (5). Thus, the site specificities are generally classified as caspase-like for β1, trypsin-like for β2, and chymotrypsin-like for β5. These designations are rough, however, because specificity is not dictated solely by the amino acid N-terminal to the scissile bond. The combination of multiple distinct active sites of rather low specificity, substrate whose unfolded state exposes its peptide bonds, and a high concentration (~50 mM) of active sites within a sequestered space, ensures that few proteins, if any, will survive intact after passage into the CP.

Because the active sites face the interior space of the CP, proteolytic substrates must gain entry to this space, a strictly regulated event that is central to the mechanism of the proteasome. In the closed form of the CP, the N-termini of the α subunits converge axially in an irregular but well-defined interdigitated network that closes the entrance to the channel (4, 6). The CP is activated through the disruption of this network. The process of channel opening can be evaluated by monitoring the hydrolysis of peptide substrates (6). The α subunits also contain a series of seven pockets on the RP-facing surface of the ring, which are formed at the α–α interfaces (Figure 3C). These "α pockets" provide binding sites for the RP (9, 11, 12). In the case of the RP, occupancy of the α pockets opens the channel. Thus, holoenzyme formation is generally accompanied by opening of the CP channel. Once opened, the CP channel is evidently in register with a second channel within the RP. Thus, to enter the CP, a substrate first needs to be translocated through the channel of the RP, and substrate specificity is probably imposed to a large extent at or before this step. In addition to the RP, a number of other proteins and protein complexes can open the CP channel (8, 9, 46, 63), as described below.

## The Proteasome Base

The RP is composed of 20 subunits in mammals, and 19 in yeast (Table 1). It has been subdivided into the lid and base assemblies (64), which are proximal and distal to the CP, respectively (Figure 2). The ten components of the base include six paralogous ATPases, referred to here as the Rpt proteins, following the nomenclature for yeast. The Rpts are critical for RP-CP complex formation, as the C-termini of several and perhaps all of the Rpts insert into the above-described α subunit cavities (9, 11, 12). Other base components are the scaffolding proteins Rpn1 and Rpn2, and the ubiquitin receptors Rpn10 and Rpn13.

Degradation of typical physiological proteasome substrates is strictly ATP-dependent, which indicates a central role played by the Rpts in the reaction mechanism. Nucleotide appears to have several functions in the proteasome (10, 19, 65–69). It positively regulates such distinct processes as substrate deubiquitination and proteasome stability, and supports substrate unfolding and translocation into the CP. As discussed below, this probably occurs by threading the protein through a translocation channel formed at the center of the Rpt ring. Whether nucleotide hydrolysis by the six Rpts is concerted, sequential around the ring, or simply random, is an important unresolved issue.

## The Gated Channel of the CP

Other than the RP, the most intensively studied activator of the CP is Proteasome Activator 28αβ (also known as REG and the 11S regulator; hereafter PA28), a heteromeric complex

of 28-kDa subunits. PA28 binds to the cylinder end of the CP, thus opening the CP channel (9, 46). In contrast to the RP, PA28 lacks ATPase activity and the ability to bind ubiquitin conjugates. PA28 is encoded within the major histocompatibility (MHC) locus, induced by interferon-γ, and required for efficient cell surface presentation of at least some peptide antigens on MHC class I molecules. For some antigenic peptides, PA28 enhances production substantially (35, 70–72). PA28 appears to influence the cleavage specificity of the CP active sites, despite not being in contact with the β subunits. The mechanism is unclear. In addition, it has been proposed, though not confirmed, that PA28 could facilitate the exit of peptide products from chimeric RP-CP-PA28 proteasomes, by providing an exit channel in trans from that being used by the RP for substrate entry (7, 8). Rapid peptide exit could prevent overdigestion of peptide within the interior of the CP cavity, given that MHC class I molecules have a minimum length requirement for binding. The PA28 ring has a central channel aligned with that of the CP (8, 9), and it is thought that peptides entering or exiting the CP pass through PA28's channel.

The ability of PA28 to open the CP channel was beautifully elucidated through structural studies of its homoheptameric homolog from *T. brucei*, PA26. Like PA28 (73), PA26 opens the channel via two sequence elements. The C-termini of PA26 extend from the body of the heptamer and are individually inserted into the α pockets in the cylinder end of the CP, each in a β-strand-like structure (8, 9, 63). This interaction brings an internal segment within PA26, known as the activation loop (73), into proximity with the CP. The activation loop clashes sterically with a reverse-turn loop within the α subunits, the Pro17 loop. The seven identical activation loops of PA26 nudge the seven distinct Pro17 loops of the α ring into a seven-fold symmetric array, which moves the nearby tails such that they cannot maintain the ordered state of the closed channel (8). The repositioned N-termini pack against the repositioned Pro17 loop to stabilize the open state (9, 63).

A key activation mechanism used by the RP proved to be surprisingly different from that of PA28 (11). The Rpt proteins do not use an activation loop to effect gating, nor do they act via directly forcing seven-fold symmetry on the α ring. For the RP, gate-opening results directly from engagement of the C-termini with the α pockets (11). The distinct behavior of the RP-derived C-termini owes to their sequence features. An HbYX motif, encoding a hydrophobic residue, followed by a tyrosine and an unspecified C-terminal residue, is sufficient for gate opening. For example, synthetic peptides terminating in this motif can open the gate. This motif was originally recognized within PAN, then found in four of the six eukaryotic Rpt proteins (11). Mutational studies have shown that the presence of the HbYX motifs in Rpt proteins correlates well their ability to open the gate in yeast proteasomes (11).

The distinction between the gating mechanisms of PA28 and the RP can also be seen in how the conformation of the CP α subunits is altered to achieve gate opening (12). In a cryo-electron microscopy study of the *T. acidophilum* CP, HbYX peptide binding was observed to induce a 4° rigid-body rotation of the α subunits (12). Surprisingly, as a result of the rotation, the Pro17 loop is displaced, similarly to the PA26-CP complex, and the gate is opened.

It is uncertain whether the gate is dynamic in the holoenzyme. Some evidence both for (10, 11, 66) and against (67) the idea that the gate is closed in the presence of ADP has been presented, and it is possible that this property shows some phylogenetic variation. In the case of the latent (free) form of CP, atomic force microscopy (AFM) studies have indicated that the channel flickers between open and closed states, probably accounting for the small residual peptidase activity of the latent CP (74).

Finally, future work should define more precisely the functional significance of gating in vivo. In yeast, open channel mutants lose viability prematurely in stationary phase, suggesting that a closed channel in the free CP is important for suppressing unwanted proteolysis during periods of nutritional limitation (75). Additionally, open channel mutants degrade even ubiquitinated substrates more rapidly than wild-type, raising the interesting possibility that the activity of the assembled RP-CP holoenzyme on ubiquitin-conjugates can be negatively controlled, at least in part, via the state of the channel (75).

## The Rpn1/Rpn2 Complex

Associated with the Rpt ring within the base complex are the largest proteasome subunits, Rpn1 and Rpn2. Rpn1 and Rpn2 are related (76) and probably diverged from a common ancestor. They each contain up to 11 degenerate tandem repeat elements of 35–40 residues that are predicted to form α helical hairpins (76). These repeats account for the gross structural features of each protein. The repeats were further predicted to align so as form toroids 55–60 Å in diameter, which was recently supported by AFM studies (77). The toroids formed by Rpn1 and Rpn2 are stacked in register, with a central channel estimated by AFM to be ~20 Å (77). Interestingly, this double toroid structure is capable of attaching to the CP independently of the Rpt ring, the interaction being mediated by Rpn2. In the CP-Rpn2/Rpn1 complex, the Rpn1-Rpn2 channel aligns with the CP channel, consistent with the view that the Rpn1/Rpn2 channel may be a part of the substrate translocation channel of the base, although this remains to be directly addressed. A related proposal is that the Rpn1/2 channel is inserted within the Rpt channel (77). In this scenario the substrate would not directly contact the Rpt channel, which, in other ATP-dependent proteases, houses a mobile loop that is thought to drive substrate translocation and unfolding (58, 62). A more conservative proposal would be that the Rpn1/2 and Rpt channel are in series with one another. In this case it would be appealing to place the Rpn1/2 channel between the Rpt channel and the CP channel, although data linking CP gating to the Rpt C-termini, discussed above, indicate that the Rpt ring is in direct contact with the CP. To distinguish among these and other possible scenarios, it will be interesting to localize Rpn1 and Rpn2 in the context of an intact proteasome or base-CP complex, as opposed to an Rpn1/2-CP complex.

Another function of Rpn1 and Rpn2 is to serve as molecular scaffolds that mediate proteasome binding of a variety of factors, including both additional proteasome subunits and proteasome-associated proteins. Surprisingly, all known Rpn1 and Rpn2 binding factors act directly on ubiquitin conjugates, though in different ways. For example, the ubiquitin receptor Rpn13, an integral proteasome subunit (29), binds to the proteasome via Rpn2 (30, 78, 79). Other ubiquitin receptors, the UBL/UBA proteins (Figure 4), bind proteasomes via an interaction of Rpn1 with their UBL domains (25). However, the UBL/UBA proteins also bind proteasomes to some extent via Rpn13 (29), and, in many eukaryotes, via Rpn10/S5a (80, 81).

In addition to ubiquitin receptors, Rpn1 and Rpn2 bind enzymes that disassemble or extend ubiquitin chains. Thus, it appears that the deubiquitinating enzyme Ubp6 binds proteasomes via Rpn1 (82, 83), and the ubiquitin ligase Hul5 binds proteasomes through Rpn2 (84). In addition, the deubiquitinating enzyme, Uch37 is linked to Rpn2 via Rpn13 (30, 78, 79). Because the factors that recognize, disassemble, or extend ubiquitin chains are thus collected at Rpn1 and Rpn2, it is clear that these subunits represent a major site in the proteasome for chain dynamics and also regulation of proteasome activity, which will be discussed below. There is little known about how the Rpn1 and Rpn2 toroids and their positioning are related to their scaffolding function. Some of the binding sites for factors such as Hul5 and Ubp6 may be outside of the toroid segments. However, in the one instance where the binding site has been mapped, that for the UBL/UBA protein Rad23, it fell within the Rpn1 toroid (25).

### The Proteasome Lid

Of the 9 lid subunits, only Rpn11 (known as Poh1 in humans) has a known function. It is a deubiquitinating enzyme, and this activity is critical for proteasome function in yeast and humans (68, 69, 85, 86). Proteasome substrates, before being degraded, are first separated from attached ubiquitin groups. This early step in degradation is mediated primarily by Rpn11. The control of Rpn11 is poorly understood, but it is possible that other lid subunits serve to activate, position, or regulate the specificity of Rpn11.

A distinguishing feature of Rpn11 (68, 69) is its insensitivity to ubiquitin-aldehyde (UBal), a potent inhibitor of almost all deubiquitinating enzymes (DUBs). UBal, in which the C-terminal carboxylate of ubiquitin is derivatized to an aldehyde, reacts with the active site cysteines in those DUBs that are thiol proteases, but does not modify metalloenzyme DUBs such as Rpn11, as these DUBs do not employ a covalent enzyme-ubiquitin reaction intermediate. Instead, metalloenzyme DUBs such as Rpn11 can be inhibited by TPEN, a zinc ion chelator (69).

The structural organization of the lid has been studied by electron microscopy and by tandem mass spectrometry (87, 88). Subunits of the lid show a detailed, one-to-one correspondence to subunits of the COP9 signalosome (CSN) and the translation factor eIF3 (64, 89). Remarkably, the CSN was found to have an enzymatic activity analogous to that of the lid, though it disassembles conjugates formed from the ubiquitin-like protein Nedd8/Rub1 rather than ubiquitin. Subunit Csn5, a paralog of Rpn11, carries the metalloprotease active site (90). If the evolution of these complexes in early eukaryotic radiations is traced, the lid seems to have formed or stabilized prior to the CSN or eIF3, and thus may have been their evolutionary precursor. The primary target of the CSN is the SCF ligase and other ligases of the cullin family (90). Nedd8 modification of cullins enhances their ubiquitin-ligating activity (91). Another scenario suggested by the close relationship between the lid and the CSN is that the CSN may bind proteasomes, perhaps by replacing the lid. Several studies have proposed such interactions, though detailed characterization remains to be done (92, 93). It is not clear from our knowledge of Nedd8 why replacing the lid with the CSN should be advantageous.

The lid, CSN, and eIF3 are sometimes referred to as the PCI complexes, after a noncatalytic sequence motif found in multiple subunits (89). In the lid this motif is found in Rpn3, Rpn5, Rpn6, Rpn7, Rpn9, and Rpn12 (89). PCI motifs are thought to consist of a winged helix domain flanked at its N-terminal side by a helical segment with similarities to TPR motifs (89). PCI-PCI interactions provide extensive intersubunit contacts (94). Two remaining lid subunits, Rpn8 and Rpn11, contain MPN domains (85). The MPN domain contains the catalytic site of Rpn11, but Rpn8 is thought to lack deubiquitinating activity.

## SUBSTRATE RECOGNITION AND PROCESSING

### Degradation-Coupled Deubiquitination

The deubiquitinating activity of Rpn11 within proteasomes is tightly controlled, and exerted only on proteolytic substrates (68, 69). Deubiquitination by Rpn11 is not seen in the absence of ATP, suggesting that Rpn11 cannot act on substrates unless they are proceeding on the pathway of degradation. The mechanism of coupling between degradation and deubiquitination is a major unsolved problem in the proteasome field. Neither process is supported by ATPγS, indicating a common requirement for the hydrolysis of ATP. Based on its sequence features, Rpn11 is unlikely to be an ATPase, and this is true as well of the remaining 8 subunits of the Lid. Most likely, nucleotide hydrolysis by certain ATPases of the base allows Rpn11 to remove ubiquitin from the substrate. One view is that ATPases direct the translocation of the ubiquitin chain to the active site of Rpn11. Another notion is

that Rpn11 acts only after a “commitment step” in degradation (68). Once substrate is committed to degradation, Rpn11 would be allowed to act, perhaps via allosteric activation. However, there is no independent evidence of a discrete commitment step. The coupling of Rpn11 activity to substrate degradation is lost in the isolated (bovine) RP, indicating that strict coupling depends on specific features of properly assembled proteasomes (69).

Because substrate degradation and deubiquitination by Rpn11 are coupled, the demodified substrate can be difficult to observe. Inhibition of the CP active sites circumvents this problem (68). However, depending perhaps on the source of proteasome or substrate, some deubiquitinated product can accumulate in the absence of CP inhibition (95), indicating that a “nonproductive” pathway exists, in which degradation and deubiquitination are uncoupled.

Ubiquitin chains are polar, with the proximal ubiquitin being defined by its attachment to substrate. Rpn11 cleaves chains with a proximal specificity, thus usually removing whole chains at once (69). Proteasomes function poorly in the absence of Rpn11 activity, presumably because substrate translocation into the CP is obstructed when residual, covalently-bound ubiquitin must accompany substrate into the CP. The nature of the degradation defect resulting from Rpn11 inactivation is not known, but ubiquitin is very stable physically, so that the unfolding step might be slowed when chains are not fully removed prior to translocation. The efficiency with which the proteasome removes ubiquitin from substrate prior to degradation is also not known. It is evidently high, but probably not 100%, because proteasome-dependent ubiquitin turnover is readily observed in wild-type cells, probably via this route (96). Another potential impediment to degrading substrate-attached ubiquitin is that it would call for the proteasome to translocate two polypeptides, or perhaps more, in parallel through the narrow entry channel. However, the ability of the proteasome to act as an endoprotease on large substrates (97), and related studies (98, 99), have shown that the CP channel can accommodate two peptide segments, and this conclusion is consistent with the size of the channel as determined by crystallography (6, 8).

### Deubiquitinating Enzymes Ubp6 and Uch37

Before the substrate is committed to degradation, and becomes subject to the activity of Rpn11, two other deubiquitinating enzymes can trim its ubiquitin chains–Ubp6 and Uch37. Through early removal of the ubiquitin tag, these ATP-independent enzymes could antagonize substrate breakdown, in contrast to Rpn11. Uch37 also differs from Rpn11 in that it removes ubiquitin moeities from the distal end of the chain (100). Thus, it does not remove chains *en bloc*, but progressively shortens them. Uch37 releases only monoubiquitin from chains, indicating an obligatory “exo” mechanism (100). Presumably, as the chain shortens, the substrate’s affinity for the proteasome is attenuated (101), and substrate breakdown is slowed. Ubp6 is also able to trim conjugates progressively, but can release di- and tri-ubiquitin from conjugates (95).

How the distinct activities of Rpn11, Ubp6 and Uch37 cooperate to provide for effective processing of ubiquitin chains is unclear. The simplest view, that all chains are cut proximally by Rpn11, is not tenable, in part because mutations in *UBP6* have strong effects on ubiquitin turnover (96, 102). Some substrates may arrive at the proteasome with multiple ubiquitin chains. Perhaps, in such cases, Ubp6 is needed to remove supernumerary chains.

Uch37 has been proposed to function as an editing activity for the proteasome, that is, as a regulator of substrate degradation that selectively suppresses the breakdown of proteins that are lightly ubiquitinated (100). The editing model was based on in vitro studies showing that Uch37 can impair the degradation of mono- and di-ubiquitinated substrates but has little effect on the degradation rates of substrates carrying longer chains (100). These divergent effects seem consistent with the ATP-independence and distal-exo features of the Uch37

mechanism. However, it has so far not been conclusively tested in vivo whether Uch37 could provide a means for the proteasome to discriminate against short-chain substrates. Although deletion of the *Uch2* gene from *S. pombe* (encoding the Uch37 ortholog) does not produce an overt phenotype (83, 103), it seems likely that deeper study of this mutant will reveal interesting perturbations of proteasome function.

RNAi studies carried out in HeLa cells showed that breakdown of the model proteasome substrate Ub-R-GFP was stimulated somewhat when either Uch37 or Usp14 (the mammalian form of Ubp6) was depleted (104). This finding neither confirms nor contradicts the editing model, which predicts that Uch37 depletion should accelerate degradation of otherwise poor substrates that carry too few ubiquitin groups. Rather, the suggestion is that Uch37 and Usp14 negatively regulate the degradation of bona fide substrates, or possibly most substrates, since bulk ubiquitin conjugate levels fell when either Usp14 or Uch37 was subject to RNAi (104). These findings could reflect surprisingly rapid deubiquitination of true substrates at the proteasome, though noncatalytic effects in this setting have not been excluded. Simultaneous depletion of Uch37 and Usp14 reversed the stimulatory effect on Ub-R-GFP breakdown, for unknown reasons. One possibility is that the cellular pool of free ubiquitin becomes depleted in the double knock-down. Although Uch37 has shown higher activity than Ubp6/Usp14 (79, 83), these studies were done using a fluorigenic test substrate (ubiquitin-AMC), which may not reliably predict the relative activities of Uch37 and Ubp6/Usp14 on natural ubiquitin chains. A more significant difference between Ubp6 and Uch37 is probably in the capacity of Ubp6 to regulate proteasome activity noncatalytically, as described below.

Whereas *ubp6* null mutants of budding yeast are ubiquitin-deficient, the expression of *UBP6* is strongly induced at the RNA level in ubiquitin-deficient cells (105), resulting in elevated Ubp6 loading onto the proteasome (105). The negative control of Ubp6 by ubiquitin, together with the positive control of ubiquitin levels by Ubp6, suggests a homeostatic negative feedback loop. The principal function of this hypothetical loop would apparently be to control fluctuations in ubiquitin levels. The signaling machinery directing this loop remains to be identified. Other, still unidentified regulatory mechanisms may also assist in stabilizing ubiquitin levels. Although little work has been done in this area, it is apparent that free ubiquitin levels can be perturbed under many circumstances (106). Consistent with these findings, modest ubiquitin overexpression provides resistance to a variety of stresses (96). Also, low ubiquitin levels might contribute to neurologic dysfunction in some murine model systems (107–109).

A particularly unexpected aspect of Ubp6 function is its capacity to reduce the efficiency of protein degradation by the proteasome by a mechanism independent of deubiquitination (95). This inhibition appears to require Ubp6 binding to the proteasome (95). The mechanism of this noncatalytic effect is unknown, but the inhibitory function has been observed in vivo and in vitro, and on multiple substrates. What fraction of proteasome substrates is susceptible to the effect is not clear. One possible function of proteasome inhibition by Ubp6 is to reduce ubiquitin consumption under conditions of ubiquitin deficiency, or “ubiquitin stress.” In this sense the noncatalytic effect would act in concert with the catalytic or chain-trimming function. A closer connection between the noncatalytic and catalytic effects is suggested by the finding that Ubp6 can trim the chains of a substrate while it imposes noncatalytic degradation delay (95). Thus, the typical mode of Ubp6 action could be to couple these two processes.

## Ubiquitin Recognition by the Proteasome

Five ubiquitin receptors associated with the proteasome are currently known: two proteasome subunits, Rpn10 and Rpn13, and three “shuttle factors,” Rad23, Dsk2, and Ddi1

(Figure 4). These proteins are conserved throughout eukaryotes. Remarkably, none of the five is essential for growth in yeast, nor are they generally essential for protein degradation, nor are they collectively essential (29, 45). Because these receptors are nonessential in yeast, additional ubiquitin receptors for the proteasome probably remain to be identified. However, Rpn10 and Rad23 are both essential in the mouse (110, 111). Strong candidates for additional intrinsic receptors for ubiquitin in the proteasome, Rpt5/S6′, Rpt1, and Rpn1, have been suggested by in vitro chemical crosslinking studies (112, 113).

The existing receptor mutants show numerous phenotypes in yeast, and some of these receptors are essential for the degradation of specific substrates (26). Thus, substrates arrive at the proteasome via multiple distinct pathways. It remains to be established how the different delivery pathways could affect the substrate's fate once at the proteasome.

Rpn10 (in humans, S5a or hRpn10) was the first ubiquitin receptor to be described (22), and remains the best characterized. hRpn10 recognizes ubiquitin via two ubiquitin-interacting motifs, or UIMs, located towards its C-terminus (114), and joined by a flexible linker region (114, 115). Variant forms of hRpn10 exist, resulting from alternative splicing, which can eliminate UIM2 (116). Each UIM consists of a single α helix (Figure 5A). UIM2 binds ubiquitin with an approximately 5-fold higher affinity than UIM1 (115, 117). If more than one ubiquitin is present in a conjugate, binding to UIM1 and UIM2 is not competitive but cooperative (K. Walters, pers. comm.) scRpn10 contains a single UIM element, which is comparable to UIM1 of hRpn10. Its deletion does not result in a null phenotype, indicating a critical role for Rpn10 other than ubiquitin recognition, localized to its N-terminus (24, 26, 64, 94). The N-terminus contains a von Willebrand A (VWA) domain, whose precise function remains to be defined. However, the VWA domain is known to be important for proteasome structure; the base and lid are readily dissociated in its absence (64).

Rpn13, the other known intrinsic ubiquitin receptor of the proteasome (29, 30), binds ubiquitin via a pleckstrin homology domain (PHD) known as the Pru domain (Pleckstrin-like receptor for ubiquitin). The Pru domain is a seven-stranded β-sandwich formed by two almost orthogonal β-sheets stabilized by an α helix (Figure 5B). The ubiquitin-binding surface is formed by three loops that bridge β strands. Thus, Rpn13's ubiquitin-interacting surface differs greatly from that of Rpn10, and indeed no other ubiquitin receptor relies exclusively on loop segments to bind ubiquitin (30). Yet Rpn13 and Rpn10 bind to essentially the same surface of ubiquitin.

Like Rpn10, Rpn13 shows interesting phylogenetic differences. hRpn13 is ~42 kDa, whereas scRpn13 is ~18 kDa, and contains only the N-terminal Pru domain. The C-terminal domain of the mammalian protein serves as the receptor site for Uch37 (78, 79, 118). The lack of this domain in scRpn13 is presumably related to the absence of a Uch37-encoding gene in budding yeast. That a deubiquitinating enzyme is recruited to the proteasome via a ubiquitin receptor is extremely interesting. Perhaps binding of a ubiquitin chain to Rpn13 would induce accelerated chain disassembly via the proximal Uch37. Some mechanism of this kind seems reasonable, and indeed Uch37 is activated when bound to Rpn13 (78, 118), but it is not known whether ubiquitin binding by Rpn13 is critical for activation. It has likewise not been demonstrated that chains bound to Rpn13 are any more prone to disassembly than chains bound to other ubiquitin receptors of the proteasome.

In addition to the proteasome, Uch37 also assembles into the Ino80 chromatin remodeling complex (119). In vitro studies have shown that, to be catalytically activated in this context, Uch37 requires the presence of proteasomes. Proteasomes from some tissues contain Rpn13 in excess over Uch37, and would thus have an unoccupied Uch37 binding site available to activate. This mechanism could potentially provide for the control of Ino80-mediated

deubiquitination at specific chromatin sites (119). The excess amounts of Rpn13 over Uch37 observed on proteasomes in this study (119) are also suggestive that Uch37 is not a proteasome subunit, as previously believed, but a proteasome-associated protein.

Rad23, Dsk2, and Ddi1 are the remaining proteasome-associated ubiquitin receptors. The UBA domain, a bundle of three α-helices, binds ubiquitin (Figure 5C), whereas the N-terminal UBL domain binds the proteasome. The UBL/UBA proteins are sometimes called "shuttle" proteins, because they may capture substrates remotely from the proteasome, then escort them to this complex. This model is still being evaluated, but several observations support it. For example, NMR studies have shown that the N- and C-termini of hHR23a (a mammalian ortholog of Rad23 [Figure 4]) interact with one another dynamically, so that in the major form of purified hHR23a the UBL domain is not free to bind proteasomes. When the UBA domain is engaged, the UBL domain is released, and proteasome binding is stimulated (120). This type of mechanism would enhance the remote capture of conjugates.

A second mechanism that appears to underlie the remote capture of conjugates by the UBL/UBA proteins is the interaction of these proteins with ubiquitin ligases. In these pathways, the UBL/UBA protein is thought to capture the ubiquitin-protein conjugate while the conjugate is still bound to the ligase that synthesized it (or as the ligase continues to build the ubiquitin chain). A remarkable example is the interaction of scRad23 and scDsk2 with the ligase Ufd2 (121, 122). Ufd2 is a ligase of the E4 class, which is defined as a ubiquitin chain extending activity (122). Ufd2 is associated with Cdc48, an ATPase ring complex that performs multiple roles in the ubiquitin-proteasome pathway, and is associated with its own cohort of ubiquitin receptors (45). Rad23 and Dsk2 bind Ufd2 via their UBL domains, and are thus positioned to bind the ubiquitin chain as it is extended by Ufd2. Dissociation of the UBL from Ufd2 would then free the UBL/UBA protein to associate with the proteasome (121, 122). Numerous proteins may follow this pathway from Cdc48 to the proteasome (121, 122).

An analogous mechanism applies to scDdi1 (123, 124). Ddi1 binds to the ligase Ufo1, and is required for the proteasomal degradation of Ufo1's substrate, the HO endonuclease. Both UBL and UBA domains of Ddi1 are required for HO degradation, as seen in the Ufd2/Rad23/Dsk2 pathway. Interestingly, Ufo1 recognition of Ddi1 involves an array of UIM elements in this ligase. This interaction promotes the degradation not only of HO but of Ufo1 itself (124). Examples of additional UBL/UBA-ligase interactions are too numerous to discuss in detail, but two interesting cases are Kpc2, a mammalian UBL/UBA protein that forms a 1:1 complex with Kpc1, a RING-ligase, which mediates degradation of the CDK inhibitor p27 (125); and the HECT ligase E6AP, which forms complexes with mammalian orthologs of both Rad23 (hHR23a) and Dsk2 (hPLIC) (126, 127).

The UBL/UBA proteins and Rpn10 show significant substrate-selectivity in vivo (26, 28). In principle, ubiquitin ligase interaction provides a simple explanation for this observation. However, considering the multiplicity of ubiquitin ligases, and the comparatively modest number of ubiquitin receptor-ligase interactions that have been identified to date, there is still a strong possibility that other major mechanisms of selectivity exist.

Another important aspect of UBL/UBA protein function is their ability to suppress both chain extension and disassembly (122, 128–130). Due to the preference of these proteins for binding chains of a certain minimum length, their effects on chain dynamics do not prevent chain formation in its early stages. Rpn10 as well can protect chains from disassembly (130). Extending the lifetime of ubiquitin chains may expand the time window for ferrying chains to the proteasome, and perhaps protect them from proteasomal DUBs. As mentioned

above, Rpn13 may be an exceptional case: it might instead destabilize the chains that it binds, as a result of its interaction with deubiquitinating enzyme Uch37.

An unresolved question is whether conjugates that arrive at the proteasome on UBL/UBA proteins are handed off to the intrinsic receptors, Rpn10 and Rpn13. A contrasting view is that long chains may engage multiple receptors at once, possibly stabilizing their interaction with the proteasome substantially. A third possible mode of cooperation among the receptors is suggested by the finding that hRpn10 and hRpn13 can both bind not only ubiquitin chains but also the UBL domains of UBL/UBA proteins (29, 80, 81). hRpn10 binds Rad23 proteins via UIM2, leaving UIM1 available for chain binding. Lacking UIM2, scRpn10 apparently does not dock Rad23 at the proteasome (26); this function is dependent on scRpn1 (26, 131, 132).

Although Rpn10 is routinely classified as a subunit of the proteasome, a significant pool of free Rpn10 exists (23, 133). In budding yeast, this fraction of Rpn10, but not the proteasomal fraction, is complexed to Dsk2 (134). Rpn10's UIM recognizes the UBL domain of Dsk2 to form the complex. Strikingly, deletion of the UIM of Rpn10 results in enhanced proteasome binding by Dsk2. Consistent with this finding, genetic data indicate that the extra-proteasomal Dsk2-Rpn10 interaction regulates Dsk2 function negatively. Another protein that sequesters UBL/UBA proteins from the proteasome by binding their UBL domains is Pth2 (135). Protein degradation is significantly stimulated when this gene is deleted. Remarkably, Pth2 also has peptidyl-tRNA hydrolase activity. How the degradative and translational functions of Pth2 are linked is an intriguing topic for future study.

Among the UBL/UBA family, Ddi1 stands out in containing an aspartyl protease domain (136–138). This domain is conserved from yeast to humans, whereas the UBA domain is absent in humans. Active site mutants exhibit some but not all phenotypes of a *ddi1* null mutant (138). Thus, Ddi1 is apparently not only a proteasomal cofactor but also a protease itself. It will be interesting to determine whether Ddi1 can cleave bound ubiquitin-protein conjugates.

Mammals show a greater diversity of UBL/UBA proteins than does yeast. For example, four Dsk2-like proteins are known (Figure 4), including A1Up/CIP75, which was identified via its interaction with the polyglutamine repeat protein ataxin-1 (139), and more recently implicated in the degradation of connexin43 (140). More divergent UBL/UBA family members include Nub1 (see below), AIRAP-L (see below), and p62. Mutations in the p62 gene lead to Paget's disease of bone, characterized by dysfunctional osteoclasts. Evidence from different laboratories has implicated p62 in delivering ubiquitin conjugates to the proteasome and also to the lysosome (38, 141). Further clarification of p62's mode of action will be of high interest.

## Ubiquitin Chain Structure and Proteasome Targeting

The role of lysine-48-linked ubiquitin chains in targeting proteins for degradation was discovered (142) through studies of the artificial substrate X-β-galactosidase (a substrate of the N-end rule ligase Ubr1). When Lys48 of ubiquitin was substituted with Arg, chain formation was blocked, indicating that, in this conjugative pathway, the other six lysines in ubiquitin cannot substitute in chain synthesis. Consistent with these findings, Lys48 of ubiquitin is an essential residue in yeast, the only lysine of ubiquitin to have this property (143). However, Lys63 is required for efficient DNA repair, in a process that does not appear to involve protein degradation (144, 145). These and many subsequent observations led to the idea that proteasome function involves K48 chains, and nonproteolytic functions of ubiquitin involve K63 chains and perhaps other linkage types (for review, see ref. 146).

Recent studies have led to a more complex picture, which contains significant ambiguities. Firstly, linkages other than K48 appear to be proficient in targeting proteins to the proteasome for degradation (147–151). Likewise, in binding assays, the known proteasomal ubiquitin receptors do not cleanly distinguish chain types from one another, though preferences exist (152). Even K63 chains, which have been found in many instances to provide a signaling rather than degradation function in vivo, can target attached protein to the proteasome to be degraded in vitro (149). The discordance between in vivo and in vitro assessments of the proteasome-targeting capacity of K63 chains is an important unsolved issue. Also, most studies have focused on chains with uniform linkages, but whether these or mixed-linkage chains predominate in vivo is unclear. The proteasome may even encounter "forked" chains in which an acceptor ubiquitin is modified by more than one donor ubiquitin. Forked chains have been proposed to be unfavorable substrates (153).

It is also of interest whether there is a strict requirement for polyubiquitin chains to be formed at all, or whether, for proteins with a sufficient number of modified lysines (150), the addition of a critical number of single ubiquitin groups directly to the substrate is sufficient for delivery to the proteasome. While most proteins studied require multiubiquitination of some kind, monoubiquitinated forms of proteins can also be targeted to the proteasome and degraded (69, 100), including one physiological substrate, Pax3 (154). To what extent one can generalize from these few examples is unclear.

**Ubiquitin-Independent Protein Degradation—**If ubiquitin's main function at the proteasome is to tether substrates in proximity to the unfolding site, it should not be surprising that some substrates can be degraded without ubiquitin modification. The first example of this class of substrates was ornithine decarboxylase (ODC; 155). ODC is targeted to the proteasome by antizyme (AZ). Although the ODC-AZ complex is not covalent, the proteasome-tethering function of AZ in this pathway is analogous to that the ubiquitin for typical substrates. Indeed, AZ even competes for proteasome recognition with ubiquitin chains, suggesting a proximal binding site (156). However, ODC may be an exceptional member of this class; other known ubiquitin-independent substrates do not seem to use separate targeting proteins such as AZ. The role of proteasome activators in ubiquitn-independent protein degradation is discussed in the following section.

Of the ubiquitin-like protein modifiers, only Fat10 is a good candidate to act as a signal for proteasomal degradation (157). Fat10 itself is turned over rapidly by the proteasome (half-life, ~1 hr), and, because proteasomes do not appear to have demodifying activity for Fat10, proteins conjugated to Fat10 may suffer the same fate. There is little known about Fat10 substrates or functions, but its induction by γ-interferon and tumor necrosis factor, as well as the location of the Fat10 gene within the MHC locus, point to a possible immunological function. Proteasomal degradation of Fat10 has not been reconstituted from purified components, so it is unclear whether it is directly recognized by the proteasome. NUB1, a UBL/UBA protein, binds both proteasome and FAT10, and thus might mediate Fat10 degradation (158).

## COMPOSITIONAL VARIANTS OF THE PROTEASOME

### Proteasome Activators

Proteasome activators bind to the cylinder end of the CP and open its channel. Three are known: REGγ, PA200/Blm10, and the above-described PA28. These activators can bind on one side of the CP while the RP occupies the other side, to generate chimeric proteasomes (72, 159–161). Thus, they help to generate a complex ensemble of proteasome species. REGγ and PA28 are of similar size and are approximately 25% identical. PA28 is essentially vertebrate-specific, while REGγ, being found in both vertebrates and

invertebrates such as worms, is apparently more ancient, and PA28 may have evolved from REGγ (162). Whereas PA28 is interferon-inducible, REGγ is not, and its localization to the cell nucleus also differs from that of PA28.

To be degraded, ubiquitin-independent substrates must be unfolded and must pass through an open CP channel. The case of SRC-3, a nuclear steroid hormone receptor coactivator, illustrates strategies for this alternative proteolytic mechanism (163). In the ubiquitin-independent pathway of SRC-3 degradation, the RP is not involved, but rather the proteasome activator REGγ (also known as PA28γ). REGγ is a ring heptamer, structurally analogous to PA28. It apparently has no ATPase activity to support unfolding, nor ubiquitin-recognition capacity, but does open the CP channel. SRC-3 has several loosely structured segments, so the need for active unfolding is presumably bypassed. REGγ further promotes SRC-3 breakdown by binding to SRC-3. Other substrates of REGγ, which are loosely folded and, where tested, targeted to REGγ-CP complexes through an interaction with REGγ, are the cyclin-dependent kinase inhibitors $p21^{Cip1}$, $p16^{Ink4}$, and $p19^{Arf}$ (164). Another such substrate might be the hepatitis C virus core protein (165). Studies of REGγ knockout mice seem to verify the importance of such ubiquitin-independent proteolytic pathways, with phenotypes such as stunted growth, cell-cycle defects and elevated spontaneous apoptosis being reported (166, 167). The relevance of REGγ to viral pathogenesis, cancer, and other diseases was recently reviewed (168).

In addition to PA28 and REGγ, the proteasome can be activated by PA200/Blm10 (161, 169, 170). PA200/Blm10 is actually the most conserved of proteasome activators, being found from yeast to mammals. (It is known as PA200 in mammals, and Blm10 in budding yeast; formerly Blm3 in yeast.) A distinctive feature of PA200/Blm10 is that it apparently binds the cylinder end of CP as a monomer, as opposed to the heptameric assemblies of PA28αβ and REGγ, whose 7-fold symmetry and pseudosymmetry match that of the CP. Accordingly, PA200/Blm10 is a ~245-kDa protein, as opposed to PA28, whose monomers are only 28 kDa. Internal HEAT repeats (171) confer a periodic and near-circular structure on free PA200/Blm10 (161), which allows it to lie like a turban atop the cylinder end of the CP (172). PA200/Blm10 binding leads to channel opening (170) and activation of the CP for peptide hydrolysis (161, 169). The functions of PA200/Blm10 remain to be described in detail; it is required for optimal proteasome function (161) and for normal spermatogenesis (172). scBlm10 copurifies with immature CP precursor complexes (173) and appears to promote correct CP maturation, a function it shares with the RP (174). It is unlikely, however, that scBlm10 functions exclusively in CP maturation, because it is found in both nascent and mature proteasomes (161).

To determine which proteasome variants exist in vivo and in what amounts has been attempted only a few times (159–161). HeLa cell CP-containing complexes were estimated to be 40% free CP, 25% RP-CP-PA28, 20% PA28-CP (including PA28-CP-PA28), and only 15% the species that is usually considered the canonical proteasome, RP-CP (including RP-CP-RP) (159). PA200/Blm10 was not examined in this study. It is unclear whether the immunoprecipitation protocol used in these experiments may have affected the results, but proteasome complexes can be labile. Running fresh extract on native gels may be preferable. Using this approach with budding yeast, which lack PA28, Blm10-CP-RP species were seen to be equivalent in amounts to CP-RP, though RP-CP-RP was more abundant than either (161). The prevalence of free CP in the HeLa cell study (159) raises the question of whether the free CP could be a significant mediator of protein degradation. In vitro studies do not exclude this possibility (97), but it will be challenging to resolve the issue in vivo.

## Proteasome-Associated Proteins

Proteins residing in the proteasome can be classified as subunits or proteasome-associated proteins. A subunit is ideally present in stoichiometric amounts, and its association with the proteasome should not readily be disrupted by high-salt buffers. Although these distinctions are not always clear from the literature, there is agreement among researchers in the field on reasonable assignments. The original proteasome purification schemes involved high-salt washes, and thus the existence of proteasome-associated proteins was not recognized for roughly 15 years. With epitope-tagging of the proteasome, omitting the salt wash became a more practical option. Subjecting such preparations to mass spectrometry has led to the discovery of large numbers of proteasome-associated proteins, many with major regulatory roles (82, 84, 96, 105, 175–179). The recognition of these proteins significantly revised our understanding of proteasome function. However, in such biochemical surveys, true proteasome components are generally mixed in with nonspecific contaminants as well as substrates. Thus to have a reliable list of proteasome-associated proteins, as we have for subunits, will require much additional effort. As described elsewhere (180), the study of numerous proteasome-associated proteins, and especially UBL-containing proteins, is beginning to link proteasome function to various aspects of cell biology, such as cell death regulation (181) and chaperone function. Proteasome-associated proteins discussed above include the UBL/UBA proteins, Uch37, and Ubp6/Usp14. We discuss below several additional examples of proteasome-associated proteins that have recently been characterized in some detail.

In yeast, the most abundant ubiquitin ligase on the proteasome is apparently Hul5 (82, 84). Its ortholog KIAA10/E3a is an abundant component of mammalian proteasomes as well (177). When gently purified proteasomes are incubated with ubiquitin and ubiquitin activating enzyme (E1), they synthesize conjugates readily in a Hul5-dependent reaction. This finding apparently reflects a chain-elongating (E4) activity of Hul5, which may be acting on ubiquitin-conjugates that associate with proteasomes (84). In budding yeast, *hul5* null mutants, and active-site *C878A* mutants, show attenuated degradation of multiple proteins, consistent with an E4 model (84). Because longer ubiquitin chains seem to associate more tightly with the proteasome (101), chain extension should promote the degradation of attached substrates.

The chain-extending activity of Hul5 opposes the chain-trimming activity of Ubp6 (84). Whether Hul5 or Ubp6 has the upper hand may depend on a variety of factors, including the nature of the substrate and the relative levels of the two proteins on the proteasome. To cite one example, ubiquitin deficiency induces Ubp6, but not Hul5 (105; unpubl. data). Ligase-deubiquitinating enzyme pairs of this kind, which seem predisposed to futile cycling, are not uncommon in the ubiquitin pathway (182). The connection between Hul5 and Ubp6 may be fairly intimate, because Hul5 is easily dissociated from the proteasome in the absence of Ubp6 (84). The receptors for Ubp6 and Hul5, which are Rpn1 and Rpn2, respectively (82, 84), form a dimeric complex (77; see above), suggesting that Ubp6 and Hul5 may likewise be close neighbors within the proteasome.

The substrate showing the strongest dependence on Hul5 is an engineered test protein for endoplasmic reticulum-associated degradation, or ERAD (183). This chimera contains a canonical ERAD substrate, the ER-luminal protein CPY*, fused to cytoplasmically localized Leu2, a selective marker, via a transmembrane domain. While wild-type cells efficiently degrade the chimera, *hul5* mutants degrade the CPY* domain but not the remainder of the polypeptide (183). The result suggests that proteasomes lacking *hul5* have a reduced capacity to degrade what appears to be a difficult substrate for the proteasome to process. The difficulty may be due in part to the substrate's being embedded in the ER membrane. Thus, proteasomes lacking Hul5 appear to be compromised in their robustness or fidelity.

Certain E2 and E3 enzymes also associate with the proteasome, apparently enhancing their potency. Thus, mutations that abrogate the interaction of the E3 enzyme Ufd4 with the proteasome in yeast impair the degradation of its substrate without affecting substrate ubiquitination (184). In other cases, associations have been documented biochemically, but without mutational evidence for the functional significance of the interaction. Examples include Ubc4 (185), SCF complexes (175, 186), E6AP/E3C (127, 177), and the APC (132). Parkin, the E3 ligase whose loss results in familial Parkison's disease, interacts with the proteasome via an N-terminal UBL domain, similarly to UBL/UBA proteins (187). Interestingly, targeting of F-box proteins (the substrate-recognizing components of SCF complexes) to the proteasome may in some cases serve to enhance the degradation rates of the F-box proteins themselves. The Cic1 adaptor protein, which binds both proteasomes and certain F-box proteins, mediates this pathway (188).

The proteasome is a target of the cellular response to arsenicals, which compromises protein folding and thus stresses the ubiquitin-proteasome system. This response is mediated in the mouse by the factors AIRAP (arsenic-inducible proteasomal RP-associated protein) and AIRAP-L (AIRAP-like) (189, 190). The AIRAP gene, which appears restricted to mammals, is induced by arsenite and other arsenicals, while AIRAP-L, which is conserved in metazoans, is expressed constitutively but recruited to the proteasome upon treatment. Loss of function results in arsenite hypersensitivity, as shown in studies of the *C. elegans* homolog, *aip-1. aip-1* function is more generally protective against proteotoxicity, because *aip-1(RNAi)* conferred sensitivity to aggregation-prone polyglutamine tracts and reduced life span in worms.

AIRAP-L's organization is reminiscent of the UBL/UBA proteins, with an N-terminal proteasome-interacting domain and a C-terminal ubiquitin-binding element of the UIM type (190). Thus, it might function primarily as a ubiquitin receptor for the proteasome. AIRAP-L also has a conserved C-terminal CAAX box, which provides for prenylation and endoplasmic reticulum localization. AIRAP, which resembles AIRAP-L in its proteasome-binding domain, is neither ER-localized nor obviously capable of ubiquitin chain binding, as it lacks the UIM. How AIRAP alters proteasome function is under study, but in its presence peptide hydrolysis and proteasome assembly are altered (189).

**Inducible and Tissue-Specific Subunit Replacement—**The proteasome exhibits remarkable variation through the assembly of the CP with different activators, and also through the assembly of the RP with a myriad of proteasome-associated proteins. In addition, structural variation is achieved through the replacement of one subunit for another during *de novo* assembly. In response to interferon γ, the three proteolytically active subunits of the CP, β1, β2, and β5, are each replaced (5, 35). The induced subunits–β1i, β2i, and β5i–are also proteolytically active, but have altered cleavage specificities. Species containing β1i, β2i, and β5i are known as immunoproteasomes. As described above, the oligopeptide reaction products of the proteasome serve as raw material for antigen presentation by MHC class I molecules. The product spectrum is regulated in parallel by the immunoproteasome subunits and by PA28 (see above). For immunoproteasomes, the regulation of cleavage specificity is achieved by amino acid substitutions in the substrate-binding S1 pocket (5), which helps to select the residue N-terminal to the scissile bond. Crystallographic studies of bovine proteasomes indicated that β1i should enhance the production of peptides with hydrophobic C-termini in place of acidic ones, while substitutions in β2i should enhance the production of peptides with basic C-terminal residues (191). The product peptides are overall a better match for the binding specificity of MHC class I molecules at the peptide C-terminus, thus accounting for the enhanced antigen presentation conferred by immunoproteasomes (35). The peptide C-termini are, perhaps

surprisingly, preserved on their journey from the proteasome to the MHC class I molecule, because the cytosol has very low carboxypeptidase activity (35).

MHC class I molecules function not only in the immune response, but in the ontogeny of the immune system, when the spectrum of peptides presented in the thymus trains the developing immune system to distinguish self from non-self through positive and negative selection from a large pool of immature T cells (192). One aspect of this process is the elimination of T cell clones whose T cell receptors, which recognize peptides in the context of the MHC class I molecule, are activated by presented self-antigens; a failure in this will lead to autoimmunity. Unexpectedly, a recent study found that a tissue in which thymic education of T cells takes place, the cortical thymic epithelium, expresses a novel variant of β5 (193). This subunit, β5t, is assembled together with β1i and β2i to form the thymoproteasome, the predominant proteasome species in cortical thymic epithelium. β5t knockouts show a 5-fold deficiency in the generation of mature (CD8+) T cells generated in the thymus, suggesting the critical importance of β5t in thymic education (193).

The functional difference between β5t and β5 appears to be much like that described above for the immunoproteasome, involving at least principally a change in the S1 pocket. β5-mediated cleavages preferentially produce hydrophobic C-termini in the product peptides (as does β5i). However, S1 substitutions in β5t reduce the hydrophobicity of this pocket, and consequently a more hydrophilic ensemble of peptides is produced (193). The hydrophilic C-termini weaken but do not necessarily eliminate peptide-MHC class I molecule interactions. As with the immunoproteasome, it seems highly unlikely that β-subunit substitution functions by altering what proteins are degraded, but rather in what peptides result from degradation.

Whereas thymoproteasomes are expressed in thymic cortical epithelial cells (cTECs), the thymic medullary cells (mTECs) constitutively express a mixture of conventional proteasomes and immunoproteasomes. The distinct proteasome populations of mTECs and cTECs are apparently dedicated to negative and positive T cell selection, respectively. mTECs produce the self-peptides that drive negative selection of T cells, thus preventing autoimmunity, while cTECs produce “non-self” peptides that impersonate foreign antigens, or rather, allow for the maturation of a large set of T-cells whose reactivity covers the space of all possible non-self class I epitopes as well as can be mustered. The linkage of β5 cleavage specificity to the immunological definition of self and non-self is a remarkable insight into the proteasome.

Subunit replacement mechanisms as described above have not been observed in yeasts, and in vertebrates they appear to be specific to the enzymatically active β-subunits. In *A. thaliana*, and other plants, such as rice, many proteasome subunits are encoded by two isoforms, with sequence identities often in the range of 80–90% (194). This duplication of subunits could result in the formation of hundreds of distinct proteasome assemblies. However, many genes are duplicated in *A. thaliana*, and it is unclear whether this heterogeneity has any functional significance.

## MODELS OF PROTEIN FOLDING AND TRANSLOCATION BY THE PROTEASOME

### Is Translocation Coupled to Unfolding?

A detailed understanding of protein unfolding and translocation by the proteasome has not been reached. The most general distinction among existing models is whether they postulate the substrate unfolding and translocation steps to be dissociable. Model I holds that unfolding occurs on the surface of the proteasome, and translocation is a distinct process that

can begin only after unfolding has generated the loose structure needed to thread the narrow translocation channel (18). Studies carried out with the PAN protease are in agreement with this model (18). Model I envisages the establishment of multipoint contacts between the substrate and the surface of the ATPase ring, followed by ATP-driven domain motion within the ring, which mechanically destabilizes the folded state of the substrate. An alternative model (model II) holds that unfolding of the substrate is driven by substrate interactions within the translocation channel (2, 20, 195, 196). In this model, the motor for translocation is the same as the motor for unfolding, and unfolding occurs as a result of collisions between the substrate and the entry port of the translocation channel. These collisions would be induced by the pulling action of the translocation motor on substrate.

Model II has the advantage of simplicity, because it reduces unfolding and translocation to a single process. Also, according to model II the proteasome needs only one strong interaction site to drive unfolding, because the opposing force is provided not by a second interaction site but by the constriction of the substrate entry port. In contrast, model I requires two interaction sites, one to pull and one to resist. Another aspect of model II is that molecular machinery that could plausibly mediate the required operations has been characterized. Groundbreaking work on HslU identified a loop within the axial channel of this ATPase ring, the pore-1 loop, which moves up and down along the channel axis during cycles of ATP hydrolysis (58, 197). Mutagenesis studies on HslU and numerous other ATP-dependent proteases showed that pore-1 is critical for protein degradation, perhaps as a result of a role in substrate translocation (54, 62, 197–199). HslU also contains coiled-coil elements that lie on the distal surface of the ATPase ring, away from the CP-interacting surface (the CP being HslV in this case). These helices also undergo major rigid motions in an ATP-dependent manner (56–58), consistent with model I. An analogous set of coiled-coils is also present in the Rpt proteins of the eukaryotic proteasome. However, in contrast to the pore-1 loop, there is so far little evidence that coiled coils of this type mediate unfolding. The precise role of the coiled-coils in proteasome function remains unclear.

## Initiation Signals

A key problem with Model II is that, while engagement of the unfolding mechanism requires contact between the pore-1 loop and the substrate, the pore-1 loop is sufficiently buried within the axial channel that a fully folded substrate could not gain access to the pore-1 loop. Studies with proteasomes and other ATP-dependent proteases point to a common requirement for an “initiation site” on a substrate, which, lacking structure, is free to access the buried site (2, 20, 97, 195; see especially 196). In support of this model, the capacity of the proteasome to degrade a protein appears to depend less on the overall thermodynamic stability of the structural domains of the substrate (20, 195, 196) than on the existence of at least one discrete, intrinsically unfolded segment (196, 200). As described below, some initiation sites are apparently endoproteolytic sites; they may be sites where substrate translocation is initiated and, subsequently, hydrolysis as well (97, 99). Initiation sites do not have strict sequence requirements (196, 200). Although the mapping of degradation signals in numerous physiological substrates of the proteasome had not pointed to such elements, it is recognized that canonical test substrates such as cyclin B, Sic1, and β-galactosidase do have such unstructured elements. To test Model II further, the key sequence features of the initiation sites should be defined, and, ultimately, related to the structural features of the proteasome, ideally determinants within the RP’s translocation channel.

## Partial Degradation and Stop-Degradation Signals

Sequences that interfere with a protein’s degradation are powerful probes of proteasome mechanism. One such signal is found in the EBNA-1 protein, the Epstein-Barr virus nuclear antigen (201). The signal (GAr) is a glycine and alanine rich segment from 60–300 residues

in length, depending on the virus isolate. Through suppressing EBNA-1 proteolysis, the GAr allows the virus to escape host immunity via the MHC class I pathway (201). One possible model is that the GAr has a high affinity for some key binding site on the proteasome, and prevents it from functioning properly. However, it does not function by interfering with ubiquitin recognition, because the GAr can suppress degradation of ODC in chimeric proteins (202). GAr does not fully suppress ODC degradation, but instead truncation products accumulate, which contain both the ODC domain and the GAr (202, 203). Although in vivo studies on EBNA itself had not revealed such partial proteolysis, the observation may be revealing of the GAr mechanism. The results may be plausibly interpreted in the context of Model II above, in which an ATP-dependent lever (such as the pore-1 loop) in the RP's substrate translocation channel drives both translocation and unfolding. After engagement of the lever by the substrate's initiation site, other segments of the substrate should progressively engage the lever as translocation proceeds. If a segment should interact poorly with the lever, translocation could stall. The GAr would represent such a poorly-interacting segment (202, 203).

A signal for proteasome-dependent processing that has been analyzed in far more detail is that found in a set of transcription factors from yeast to humans, which are activated by partial proteolysis. This mechanism, known as regulated ubiquitin-proteasome-dependent processing, or RUP (204), was originally described for the p105 precursor to the p50 subunit of NF-κB (204), but applies as well to Cubitus interruptus (Ci) of *D. melanogaster*, and the yeast proteins Spt23 and Mga2 (205, 206). In most cases, partial proteolysis activates the transcription factor by removing a segment that sequesters it in the cytoplasm. In the case of Ci, a transcriptionally active form of the protein is converted into a repressor when truncated.

Partially proteolyzed substrates appear to have a common design in which a tightly-folded domain is adjacent to a low-complexity sequence often of 20–30 residues (element I; ref. 206). This element was first defined for p105, where it is known as the glycine-rich region (204), but its exact composition differs in other substrates. It is generally located near the middle of the protein. Element I serves to initiate Spt23 degradation by being drawn into the CP as a loop (99). Initial cleavage within this loop seems to be followed by bidirectional degradation towards the protein termini. Degradation then stalls when a tightly folded domain (element II) is reached. Stalling is very sensitive to the stability of the folded domain; if it is destabilized, degradation will proceed to completion. Stalling is also sensitive to the spacing between elements I and II (206).

The features of elements I and II are reminiscent of behavior of ODC-GAr chimeras, and the same explanation may apply: mechanical force applied to element I in the translocation channel acts on the nearby folded domain, which is first reeled to the entry to the channel, and then, when the folded domain can go no further, the resulting tension drives unfolding. However, the sequence features of element I result in poor interaction with the lever, so that unfolding fails and degradation halts midway. If elements I and II are improperly spaced, the tugging force can be applied outside of element I and productive unfolding is possible (206). A curious feature of element I is that it shows properties of an initiation site, since it inserts as a loop to initiate degradation (99), as well as a stop-degradation signal, because these sequences are responsible, together with element II, for the cessation of degradation (206). It might be therefore be interesting to dissect element I with greater resolution. In any case, the interpretations above require further testing and a closer comparison of the behavior of the different substrates under study.

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## SUMMARY POINTS

1. The proteasome CP has three important types of elements: proteolytic active sites, a gated channel for substrate import, and binding pockets (the seven α pockets) in its cylinder end.
2. A diverse set of CP activators exists, with distinct mechanisms for opening the CP channel, and a common mechanism of CP binding via the α pockets.
3. The RP, the most important of the activators, and the only one to mediate ubiquitin-conjugate degradation, is composed 19–20 subunits, divided between base and lid subassemblies.
4. Substrate recognition by the RP involves five known ubiquitin receptors and still others that remain to be conclusively identified. Ubiquitin receptors function with some degree of substrate specificity, partly due to interactions with ubiquitin ligases.
5. The activity of deubiquitinating enzyme Rpn11 is ATP-dependent, coupled to substrate degradation, and controls substrate degradation positively. Two other deubiquitinating enzymes, Uch37 and Usp14/Ubp6, can disassemble ubiquitin chains prior to substrate degradation, and these proteins control substrate degradation negatively.
6. The RP unfolds ubiquitinated proteins most likely by binding an unstructured segment of the substrate, known as an initiation site, and, using energy from ATP hydrolysis, pulling the substrate into a channel that leads to the CP and is too narrow for folded globular domains to enter.

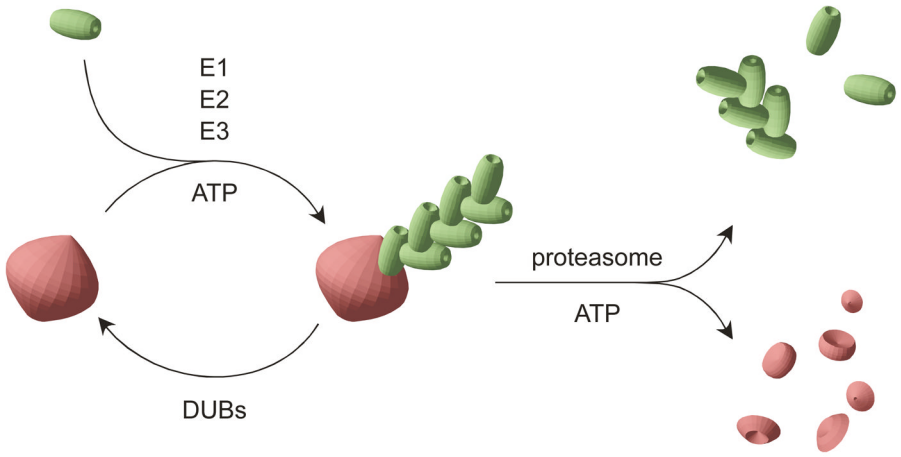


**Figure 1.**
The ubiquitin-proteasome pathway for protein breakdown. Ubiquitin in green, substrate in yellow. A ubiquitin chain, synthesized via a cascade of E1, E2, and E3 enzymes, is thought to be the predominant signal for substrate recognition by the proteasome. For some substrates, however, modification by a ubiquitin chain is not obligatory (see text for details).

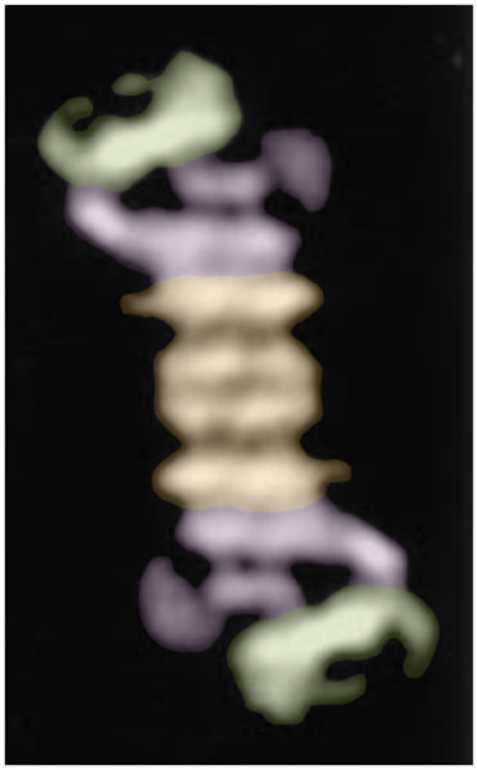

**Figure 2.**
Structure of the proteasome holoenzyme. The lid is highlighted in green, the base in purple, and the CP in orange. The position of the joint between the lid and base is only an estimate. The image was generated by averaging of electron micrographs of negatively stained *X. leavis* proteasomes. Modified from ref. 13, with permission.

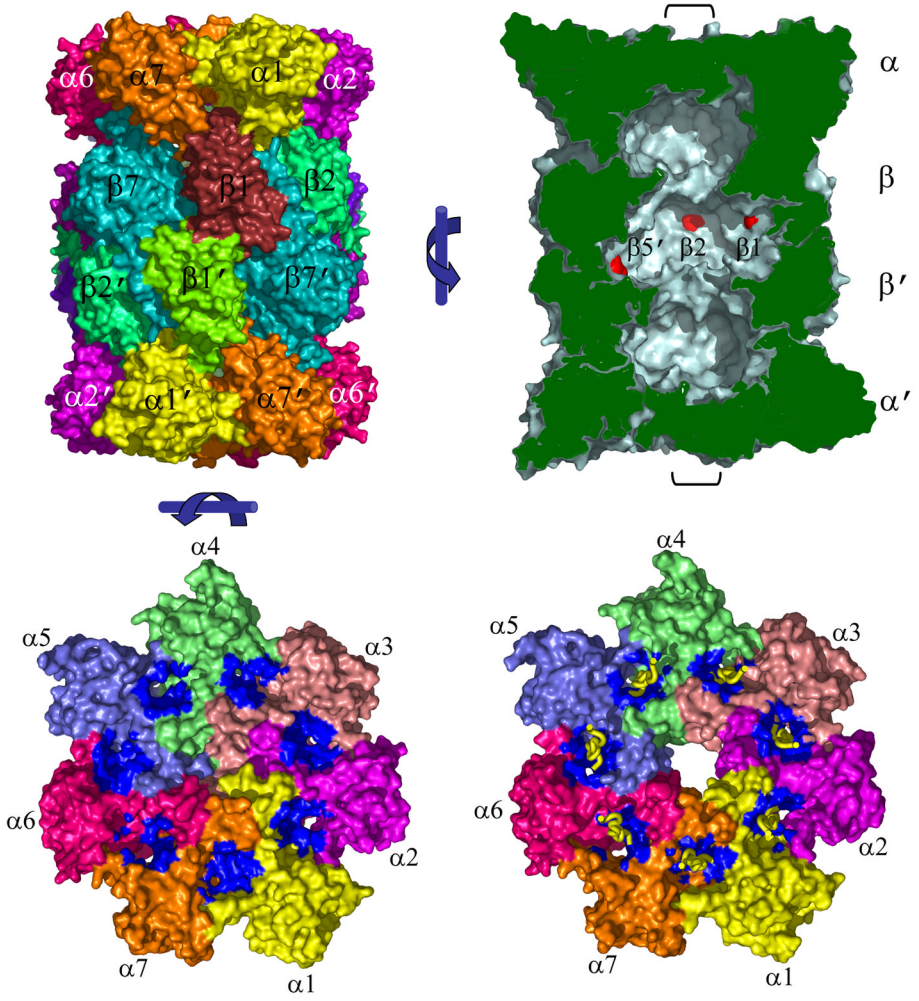


**Figure 3.**

Gallery of proteasome core particle images. *Upper left*, Surface representation of the CP, showing its organization into 4 heptameric rings of subunits. The CP is shown along its 2-fold symmetry axis. Each subunit and its symmetry mate were painted in the same color except for b1 and b1′. *Upper right*, free, closed-channel state of the CP showing sequestration of the proteolytic sites (painted in red) and the closed channel. A medial section is shown, with the slice surface in green. A bracket indicates the approximate position of the channel entry port as seen in the open state. Adapted from ref. 210 with permission. (C) α ring of the free CP as seen from the side where RP and PA26 bind, with α pockets of the CP in blue. Channel is closed. (D) α ring with bound PA26 C-termini (yellow) and the channel in the open state. Structural coordinates taken from 1RYP (closed) and 1FNT (open), from refs. 4 and 8.

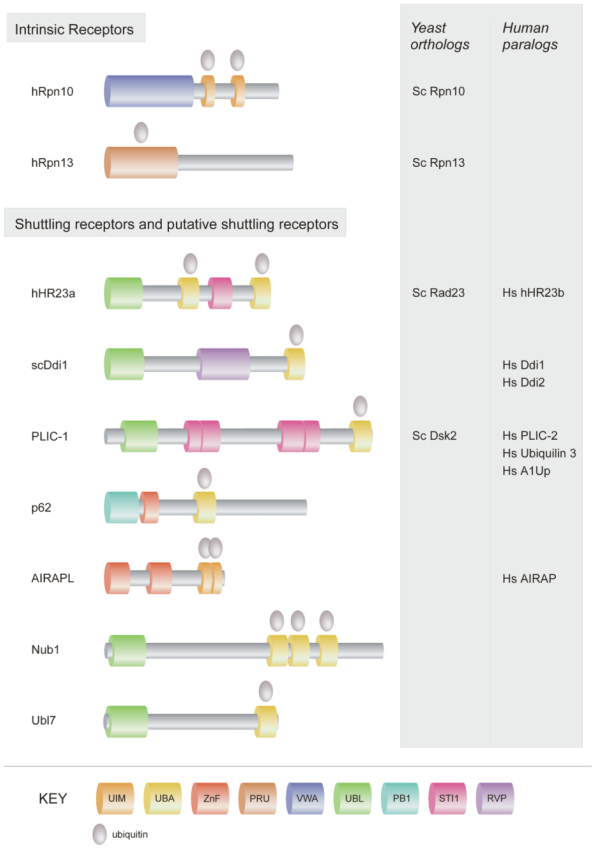


**Figure 4.**

Ubiquitin receptors of *S. cerevisiae* and humans. Authentic proteasome subunits are classified as intrinsic receptors, whereas proteins reversibly associating with the proteasome are termed shuttling receptors. The form of each receptor protein seen in humans is depicted, except for Ddi1, whose human homolog as no UBA domain. Schematics for canonical members of each class are shown, and, apart from ubiquitin, all proteins and domains are drawn to scale. UIM, UBA, STI1, VWA and PB1 are SMART domains (http://smart.embl-heidelberg.de/). RVP is a Pfam domain, and is encompassed by the slightly larger aspartyl protease Pfam domain. In all cases but two, UBL corresponds to the SMART UBQ domain. UBL domains indicated for scDdi1 and hNub1 are not recognized by SMART, but a requirement for the UBL in proteasome binding has been demonstrated (123, 131, 158). The ZnF domain refers to two different types of zinc finger domains, and specifically ZnF(AN1) in AIRAPL and ZnF(ZZ) in p62. The PRU domain is from refs. 29 and 30. Aliases and accession numbers are given in a Supplementary Table.

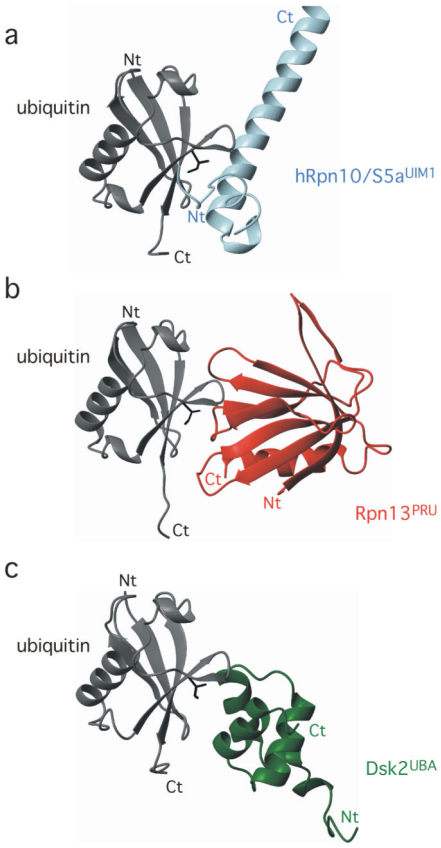


**Figure 5.**
Gallery of ubiquitin-ubiquitin receptor complexes mediating substrate recognition by the proteasome. (A) Rpn10 (PDB code 1WR1), (B) Rpn13 (PDB codes 2Z59 and 2R2Y), (C) Dsk2 (PDB code 1YX5). Figure adapted from ref. 30 with permission.

**TABLE 1**

Subunits of the Proteasome Regulatory Particle

| Base[1] | Activity or domain type | Budding yeast | kDa (humans) | Human | Human (Rechsteiner) | Bovine (DeMartino) | Comments (other names) |
|---|---|---|---|---|---|---|---|
| | AAA ATPase | Rpt1 | 48.6 | PSMC2 | S7 | p48 | (Cim5) |
| | AAA ATPase | Rpt2 | 49.2 | PSMC1 | S4 | p56 | |
| | AAA ATPase | Rpt3 | 47.3 | PSMC4 | S6 (or S6b) | p47 | |
| | AAA ATPase | Rpt4 | 44.1 | PSMC6 | S10b | p42 | (Sug2) |
| | AAA ATPase | Rpt5 | 49.1 | PSMC3 | S6′ (or S6a) | p50 | |
| | AAA ATPase | Rpt6 | 45.7 | PSMC5 | S8 | p45 | (Sug1, Cim3) |
| | scaffold | Rpn1 | 100.2 | PSMD2 | S2 | p112 | α-helical toroid |
| | scaffold | Rpn2 | 105.9 | PSMD1 | S1 | p97 | α-helical toroid |
| | Ub-receptor | Rpn10 | 40.7 | PSMD4 | S5a | p54 | UIM domain |
| | Ub-receptor Uch37 receptor | Rpn13 | 42.1 | ADRM1 | --- | --- | Pru domain |
| | --- | --- | 24.6 | PSMD5 | S5b | --- | absent in budding yeast |
| **Lid** | | | | | | | |
| | PCI | Rpn3 | 61.0 | PSMD3 | S3 | p58 | |
| | PCI | Rpn5 | 52.9 | PSND12 | --- | p55 | |
| | PCI | Rpn6 | 47.4 | PSMD11 | S9 | p44.5 | |
| | PCI | Rpn7 | 45.5 | PSMD6 | S10a | p44 | |
| | MPN | Rpn8 | 37.1 | PSMD7 | S12 | p40 | (Mov34) |
| | PCI | Rpn9 | 42.9 | PSMD4 | S11 | p40.5 | |
| | MPN; Deubiquitination | Rpn11 | 34.6 | PSMD14 | S13 | --- | (Pad1, Poh1) |
| | PCI | Rpn12 | 30.0 | PSMD8 | S14 | p31 | |
| | --- | Rpn15 | 24.6 | PSMD9 | S15 | p27L | (Sem1, Dss1) |

[1]Gankyrin, an oncoprotein (207), also known as PSMD10, and p28 in the nomenclature of DeMartino, is a possible component of the base. It binds to the C-terminal domain of Rpt3 in both mammals and budding yeast (208, 209). Its yeast ortholog is Nas6, encoded by a nonessential gene.