Regulatory subunit interactions of the 26S proteasome, a complex problem

Katherine Ferrell, Caroline R.M. Wilkinson, Wolfgang Dubiel and Colin Gordon

The 26S proteasome is the major non-lysosomal protease in eukaryotic cells. This multimeric enzyme is the integral component of the ubiquitin-mediated substrate degradation pathway. It consists of two subcomplexes, the 20S proteasome, which forms the proteolytic core, and the 19S regulator (or PA700), which confers ATP dependency and ubiquitinated substrate specificity on the enzyme. Recent biochemical and genetic studies have revealed many of the interactions between the 17 regulatory subunits, yielding an approximation of the 19S complex topology. Inspection of interactions of regulatory subunits with non-subunit proteins reveals patterns that suggest these interactions play a role in 26S proteasome regulation and localization.

THE 26S PROTEASOME is a multisubunit complex that degrades proteins that have been targeted for destruction by the ubiquitin (Ub) pathway. The targeting mechanism involves the addition of polyubiquitin chains onto lysine residues of substrate proteins. Polyubiquitin chains act as a signal enabling the substrate to be recognized by the 26S proteasome. The Ub pathway plays a central role in regulating essential cellular processes, such as the cell cycle, antigen processing, transcription and signal transduction. As depicted in Fig. 1, two subcomplexes, the 20S proteolytic core and the 19S regulatory particle, make up the 26S proteasome. The 20S subunits are classified as α and β subunits on the basis of their homology to the simpler version of the proteasome found in the archaebacterium Thermoplasma acidophilum. Electron microscopy images and X-ray crystallographic structures of 20S particles from T. acidophilum and Saccharomyces cerevisiae reveal a cylindrical structure made up of 7α7β7α7β7α rings with a narrow channel running through the centre of the structure. Proteins are hydrolysed inside the cylinder by proteolytic sites localized on β subunits. The 19S regulator is thought to carry out a number of different biochemical functions. First, it can recognize polyubiquitinated substrates. Second, it is predicted to have an isopeptidase activity to cleave the polyubiquitin chains into Ub monomers, which are then recycled; Ub is not degraded by the proteasome. Third, binding of the 19S complex to either or both ends of the 20S particle is thought to open the narrow pore at the ends of the 20S structure. Finally, proteins must be unfolded to allow entry into the 20S chamber. Therefore, the 19S regulator is thought to have reverse chaperone activity to denature substrates and translocate the unfolded polypeptides into the proteolytic compartment of the 20S particle. Peptide sequencing of the recently purified 19S regulator from S. cerevisiae identified 17 subunits, all of which are homologous to subunits identified in preparations from a number of different laboratories using materials from many different organisms. This composition can therefore be thought of as the core 19S complex. Besides these 17 subunits, additional proteins have been associated with purified preparations and might encode species- or tissue-specific subunits of the 19S regulator. Throughout the review we use the recently proposed ‘Rpn’ nomenclature originally developed for naming the 19S regulator subunits from S. cerevisiae and the ‘S’ nomenclature developed for higher eukaryotes.

Subunit–subunit interactions of the 19S regulator

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Subunit–subunit interactions of the 19S regulator

The recent discovery in S. cerevisiae by Dan Finley and co-workers that the 19S regulator can be dissociated into two subcomplexes, called the base and the lid (Fig. 1), has provided new insights into the structure and function of the 19S particle. The fact that the 19S lid can also be isolated from human red blood cells makes it likely that the complex has a universal base-lid composition.

The 19S regulator base. The base consists of six ATPases (Rpn 1–6) that all belong to the ATPase-associated with different-cellular-activities (AAA) family and the non-ATPases Rpn2(S1), Rpn3(S2) and Rpn10(S5b). It is thought to interact with the 20S particle causing a narrow pore to open, which allows access into the inner compartment of the 20S complex. The base has been shown to have chaperone-like activity in vitro, consistent with the hypothesis that it could function in vivo as a reverse chaperone, unfolding proteins before translocating them into the 20S core for proteolysis.

Genetic studies in yeast have demonstrated that the six ATPases of the 19S regulator are not redundant. Null mutants of each of the ATPases are lethal and cannot be rescued by overexpression of the other ATPases. Interestingly, mutation of each ATPase results in a distinct phenotype. Genetic and biochemical studies indicate that protein–protein interactions between the different ATPases occur via the N-terminal coiled-coil region of each protein. The interactions between the different ATPases have been characterized as pairs: Rpt2(S4) binds to Rpt1(S7), Rpt3(S6b) binds to Rpt6(S8), and Rpt5(S6a) binds to Rpt4(S10b) (see Fig. 2). Glycerol gradient sedimentation analysis demonstrated that the two pairs,
Although interactions of regulatory ATPases have been studied intensively in many laboratories, there is only one report of a possible direct interaction, based on genetic experiments, between a regulatory ATPase and an α subunit of the 20S proteasome\textsuperscript{20}. Perhaps, complete ring structures are needed before interactions between ATPase rings with complete α rings can occur. Additional evidence for contact between the ATPase subunits and 20S proteosomal α rings has been provided by mild proteolytic digestion experiments in which the ATPases were no longer accessible to digestion after assembly of the 19S complex into the 26S proteasome\textsuperscript{21}. In addition, the recent identification of the base-20S complex (Fig. 1) provides further evidence that the ATPases interact directly with the α subunits of the 20S particle.

A number of early reports proposed a role for the 19S regulatory ATPases in other cellular functions, in particular transcription\textsuperscript{14}. However, most of these results could also be explained by altered 26S proteasome activity, and the role of the 19S regulatory ATPases in other cellular functions has yet to be unambiguously demonstrated.

How do the other components of the base complex interact with the hexameric ring of ATPases? As shown in Fig. 2, Rpn1(S2) physically interacts with Rpt2(S4)\textsuperscript{22} and Rpt1(S7)\textsuperscript{13}. Rpn2(S1) interacts with Rpt4(S10b) and can also interact with a modified form of Rpt6(S8)\textsuperscript{23}. There are also a number of interactions with lid components. It appears that, in addition to Rpn10(S5a), Rpn1(S2) and Rpn2(S1) link the base and lid complexes. These large proteins contain KEKE (rich in lysine and glutamate residues) domains\textsuperscript{24} and have LRR (consisting of one leucine and two arginine residues) β/α repeats\textsuperscript{25}, both of which are thought to be involved in protein-protein interactions and could therefore explain numerous subunit-subunit interactions.

Rpn10(S5a). One of the best studied subunits of the 19S complex, Rpn10(S5a), is the only subunit known to bind to polyubiquitin conjugates\textsuperscript{26}. Deletion of the gene encoding the Rpn10 subunit in yeast is not lethal\textsuperscript{27}, presumably because this activity is redundant. Unlike the other subunits of the 19S complex, it appears to be the only subunit found in significant quantities outside of the 26S proteasome.

It has been proposed that Rpn10 might be involved in binding and shuttling polyubiquitin conjugates to the 20S proteasome\textsuperscript{28}. How do the other components of the base complex interact with the hexameric ring of ATPases? As shown in Fig. 2, Rpn1(S2) physically interacts with Rpt2(S4)\textsuperscript{22} and Rpt1(S7)\textsuperscript{13}. Rpn2(S1) interacts with Rpt4(S10b) and can also interact with a modified form of Rpt6(S8)\textsuperscript{23}. There are also a number of interactions with lid components. It appears that, in addition to Rpn10(S5a), Rpn1(S2) and Rpn2(S1) link the base and lid complexes. These large proteins contain KEKE (rich in lysine and glutamate residues) domains\textsuperscript{24} and have LRR (consisting of one leucine and two arginine residues) β/α repeats\textsuperscript{25}, both of which are thought to be involved in protein-protein interactions and could therefore explain numerous subunit-subunit interactions.

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Phosphorylation-dependent proteases are represented by the prokaryotic HslV/HslU-like enzymes. These complexes possess homohexameric ATPases that bind to and regulate a proteolytic core and interact directly with substrates. The leap in complexity came with the interaction of the Rpn1 and Rpn2 subunits with the hexameric ring, which allowed the proteasome to adapt to a substrate signalling system, the Ub pathway. By recruiting Rpn10(S5a) and the lid complex, the proteasome evolved into a complex capable of degrading polyubiquitinated substrates, adding additional layers of regulation required for eukaryotes.

The 19S regulator lid. The 19S regulator lid complex can be isolated from yeast and human red blood cells. As little is known about its structure, we have represented it as an octameric ring-like shape in Figs 1 and 2. The lid consists of eight non-ATPase subunits. It is connected to the base complex by interactions between Rpn12(S14) and Rpn3(S1), and between Rpn11(S5a) and Rpn3(S2). Rpn10(S3a) might be involved in stabilizing these interactions. As the purified base complex has not yet been shown to degrade polyubiquitin conjugates, it is assumed that the lid is essential for polyubiquitin substrate processing prior to degradation.

The lid components possess significant sequence homologies with the eight subunits of the recently characterized COP9 signalosome complex and with subunits of the 26S proteasome. As studies show that the highly conserved N-terminal region of the protein is of more importance for certain aspects of Ub conjugate degradation than the dispensable Ub-binding region found in the C terminus, its role in the 19S regulator cannot be limited to conjugate shuttling.

It seems likely that the earliest version of the proteasome-like enzymes were represented by the prokaryotic HslV/HslU-like and the Up-like ATP-dependent proteases. These complexes possess homo-hexameric ATPase rings that bind to and regulate a proteolytic core and interact directly with substrates. The leap in complexity came with the interaction of the Rpn1 and Rpn2 subunits with the hexameric ring, which allowed the proteasome to adapt to a substrate signalling system, the Ub pathway. By recruiting Rpn10(S5a) and the lid complex, the proteasome evolved into a complex capable of degrading polyubiquitinated substrates, adding additional layers of regulation required for eukaryotes.

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The 26S proteasome is a multi-subunit proteolytic enzyme complex that plays a central role in degradation of intracellular proteins. It is composed of an 11S ‘core’ particle and an 19S ‘regulator’ particle, which together form a 26S complex. The 19S regulator particle is responsible for substrate recognition and recruitment to the 26S proteasome, while the 11S core particle catalyzes the proteolytic degradation. The 19S complex is proteinaceous and contains over 20 different subunits, each with specific functions.

Subunit interactions are crucial for the activity of the 26S proteasome. Various subunits interact with each other and with regulatory proteins, which can modulate the activity of the proteasome. These interactions are dynamic and can be regulated by various factors, including phosphorylation, ubiquitination, and binding to specific proteins.

The 26S proteasome is involved in several critical cellular processes, including protein degradation, signal transduction, and antigen processing. It is also implicated in various diseases, such as cancer and neurodegenerative disorders. Further studies are needed to fully understand the role of subunit interactions in the regulation of the 26S proteasome and its contributions to these processes.

Figure 3: Possible consequences of subunit interactions with cellular and viral proteins. Subunit–non-subunit interactions have two major consequences: (a) altering the activity of 20S and 26S proteasomes and (b) localization of the 20S proteasome to discrete cellular sub-compartments. Some viral proteins seem to activate the 26S proteasome with the possible consequence of an S5b homologue has not been documented and could represent mechanisms to regulate 26S proteasome activity. ATP hydrolysis connected with substrate protein unfolding could well be the rate-limiting step in degradation by the 26S proteasome.

In general, the interactions seem to be of the two types illustrated in Fig. 3. First, the interactions with viral proteins and possibly nuclear hormone receptors and transcription factors appear to be involved in the regulation of the 26S or 20S proteasome activities. Alternatively, other interactions seem to be involved in increasing the concentration of 26S particles in discrete cellular sub-compartments.

Three viral proteins have been reported to interact with proteasomal ATPases. HIV Tat protein, human papillomavirus 16 E7 oncoprotein and adenovirus E1A protein. Binding of Tat protein leads to stimulation of Ub conjugate degradation by the 26S proteasome.

The binding of the E7 protein activates Rpt2(S4)-ATPase activity, which could result in accelerated proteolysis. One important consequence of such activated
proteolysis could be the increased turnover of the retinoblastoma protein, shown to be depleted in the presence of E7 protein with the result of oncogenic transformation.

Binding of the E1A protein has been reported to cause an increase in the degradation of the topoisomerase II alpha protein.

Tat, E1A, hepatitis B virus X (Ref. 36) and human T-cell leukaemia virus Tax (Ref. 37) have been demonstrated that in vitro interactions in the cytosolic domains of membrane receptors recruit the 26S proteasome, possibly for signal transduction purposes.

In response to signals, phosphorylated or dephosphorylated regulators of the 26S proteasome might be involved in the 20S–11S complex peptidase activities. The 11S complex, also called PA28, is only found in higher eukaryotes and is another regulator of the 26S proteasome.

The 26S proteasome has also been shown to be involved in the degradations by the 20S particle. Only preliminary data suggest that there are two general strategies employed by viruses for manipulating the proteasome. First, interfering with the 20S–11S complex activities could suppress antigen processing and shield the virus from the immune system. Second, stimulating degradation by the 26S proteasome might increase the turnover of GLS phase regulators of the cell cycle resulting in more efficient viral replication.

Initial studies on interactions of hormone receptors with substrates of the 19S regulator were confused by reports on individual 19S regulatory ATPases acting as transcriptional regulators. There is no doubt that members of the nuclear hormone receptor superfamily such as thyroid hormone (TR), retinoid X (RXR) and an orphan member of the family, MB67, interact with proteasome ATPases (see Fig. 2); however, the physiological significance of these interactions remains obscure. It is possible that the receptor proteins recruit the entire 26S complex to carry out proteolysis of transcriptional regulators that are no longer useful. For example, there is a reciprocal interference of TR and other nuclear receptors with c-Jun/Fos-dependent AP1 activity.

One underlying mechanism of this crosstalk, which leads to cell differentiation via TR or RXR transcription activity or to cell proliferation via c-Jun/Fos, might simply be proteolysis. One transcription factor might accelerate degradation of c-Jun, Fos in a similar light. The Ub-mediated degradation of c-Jun, Fos might simply be proteolysis.


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