Review

Structure, Assembly and Homeostatic Regulation of the 26S Proteasome

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The 26S proteasome is the major protease responsible for degradation of regulatory and abnormal proteins in the cell. Proteasomal degradation controls many cellular processes including, but not limited to, cell cycle control, transcription, DNA repair, apoptosis, quality control and antigen presentation. To elucidate how the proteasome is regulated is crucial to our understanding of the molecular details of proteasomal degradation and its functions in diverse biological pathways. In this article, I will highlight recent advances in understanding the proteasome structure and assembly and the regulation of proteasome gene expression. The implications of these new developments in cancer therapy will also be discussed.

Keywords: proteasome, ubiquitin, Rpn4, transcription factors, gene expression, feedback regulation, cancer therapy, chaperone

Introduction

Protein degradation by the proteasome is one of the major regulatory mechanisms in the cell (Coux et al., 1996; Hoffman and Rechsteiner, 1996; Voges et al., 1999; Hershko et al., 2000; Pickart and Cohen, 2004; Finley, 2009). The proteasome mediates the degradation of most short-lived proteins that control cell cycle, transcription, DNA repair, apoptosis and other cellular processes. The proteasome is also responsible for the degradation of abnormal or damaged proteins and therefore plays an important role in quality control. In addition, the proteasome participates in generation of major histocompatibility complex class I-presented peptides via breakdown of proteins derived from intracellular pathogens (Rock and Goldberg, 1999). The antigenic peptides can also result from proteasomal degradation of defective ribosomal products, which are newly synthesized polypeptides that never acquire native structure due to errors in translation or post-translational processes necessary for proper protein folding (Reits et al., 2000; Schubert et al., 2000). Besides its predominant roles in protein degradation, the proteasome appears to have additional non-proteolytic functions in transcription and DNA repair (Reed and Gillette, 2007; Kodadek, 2010). The complexity of the functions of the proteasome may explain why aberrant proteasome activity is often associated with the pathogenesis of cancers and other human diseases (Schwartz and Ciechanover, 1999). The proteasome has recently emerged as a drug target for cancer therapy (Orlowski and Kuhn, 2008). The introduction of proteasome inhibitors in cancer therapy originated from the observation that inhibition of proteasome activity induces apoptosis preferentially in transformed cells.

The 26S proteasome is a large complex consisting of at least 66 proteins. To elucidate the structure and assembly of the 26S proteasome and the regulation of the proteasome genes is the key to our understanding of the mechanism of proteasomal degradation and its physiological functions. Recent developments in these research areas will be reviewed in this article. I will also briefly discuss the clinical relevance of these advances.

Structure of the proteasome

The 26S proteasome is a 2.5 Mda protein complex consisting of two subcomplexes: the catalytic 20S core particle (CP or 20S proteasome) and the 19S regulatory particle (RP, also known as 19S proteasome or PA700; Groll et al., 2005; Nickell et al., 2009). The 19S RP is attached to one or both ends of the CP to form the RP1CP or RP2CP complex (Figure 1). Whereas the true sedimentation coefficient of RP2CP is actually 30S (Yoshimura et al., 1993), the 26S proteasome denotes both RP1CP and RP2CP in the literature.

The CP is a barrel-shaped structure of a stack of four seven-subunit rings in a α3β2α2β2 configuration. Both exterior rings contain one set of seven different α subunits; and both interior rings contain one set of seven different β subunits. The CP performs three types of catalytic activities inside its chamber: chymotrypsin-like, trypsin-like and caspase-like activities, which are provided by β5, β2 and β1 subunits, respectively. In immune responsive cells the constitutively expressed β1, β2 and β5 subunits are replaced by three induced β subunits (β1i, β2i and β5i) to form the immunoproteasome that has higher chymotrypsin-like and trypsin-like activities known to be
favorable for antigen processing (Boes et al., 1994; Gaczynska et al., 1994; Cardozo and Kohanski, 1998). βS1 is replaced by another proteolytically active subunit βSt in cortical thymic epithelial cells, forming the so-called thymoproteasome (Murata et al., 2007). Different from eukaryotic CP, there are only one type of α subunit and one type of β subunit in prokaryotic proteasomes, with the exception of the proteasome isolated from bacterium Rhodococcus, which contains two types of α and β subunits (Voges et al., 1997).

Crystal structures of the CP from archaeon, yeast and mammals have been solved (Löwe et al., 1995; Groll et al., 1997; Unno et al., 2002). There is a very narrow pore or gate at the center of the α subunit ring where protein substrates enter the CP chamber. The gate is closed in a free CP by interactions among the N-termini of the α subunits, mainly α2, α3 and α4, blocking substrate entry into the proteolytic chamber. In the 26S proteasome, the RP acts as an activator to facilitate the opening of the gate by interacting with the α subunits (Smith et al., 2007; Rabl et al., 2008). Besides the RP, the PA200/Blm10 protein and the PA28/11S complex can also bind the α subunits and partially or fully open the gate of the CP, thereby facilitating substrate access to the proteolytic chamber (Whitby et al., 2000; Sadre-Bazzaz et al., 2010).

Unlike the CP, no crystal structures of the 19S RP have been solved, perhaps due to its unstable conformation and/or variable composition. Our current understanding of the 19S RP structure mainly relies on the information obtained from cryo-electron microscopy and biochemical analyses (da Fonseca and Morris, 2008; Nickell et al., 2009). The 19S RP is a multi-subunit complex, which can be divided into two subcomplexes called the base and the lid (Figure 1). The base consists of six AAA+ ATPases (Rpt1-6) and three non-ATPase subunits (Rpn1, Rpn2 and Rpn13), whereas the lid includes at least nine non-ATPase subunits (Rpn3, Rpn5-9, Rpn11, Rpn12 and Rpn15/Sem1). The connection between the lid and the base is stabilized by the Rpn10 subunit. The lack of structural data makes it difficult to fully understand how the RP subunits are arranged in the subcomplexes and how the RP stimulates the opening of the CP gate and facilitates substrate unfolding and translocation. The recent publication of several crystal structures of the essential domains of the proteasomal ATPases from archaea (named proteasome-activating nucleotidase or PAN) and actinobacteria (named ATPase forming ring-shaped complex or ARC) finally provides some important clues to understand the action of the 19S RP (Djurunic et al., 2009; Zhang et al., 2009a). PAN and ARC are orthologous to the eukaryotic Rpt subunits, sharing more than 40% sequence identity (Smith et al., 2006). The N-terminal region of PAN contains a coiled-coil (CC) segment followed by an oligonucleotide/oligosaccharide-binding (OB) domain. The OB domains from six PAN N-terminal fragments form a hexameric ring with a pseudo 6-fold symmetry. The axial pore of the OB ring is ~13 Å. The CC segments of the six PAN fragments display only 3-fold symmetry. Thus, the N-terminal fragment of PAN is assembled into a crown-shaped subcomplex: three CC segments sprouting from the hexameric OB ring (Djurunic et al., 2009; Zhang et al., 2009a). The N-terminal region of ARCCarry a CC segment and two OB domains. The OB domains of ARCC form double hexameric rings (Djurunic et al., 2009). The ATPase domains of PAN and ARC are located at their C-termini. The ATPase domain of PAN was also crystallized (Zhang et al., 2009a). It appears to form the canonical AAA+ ATPase ring with a narrow channel. Together, these crystal structures suggest a model of double-ring structure for the PAN hexamer. This model is consistent with a three-dimensional model of PAN generated from tomographic reconstruction of negatively stained particles (Medalia et al., 2009). Zhang et al. (2009b) further demonstrated that the PAN ATPase subcomplex associates with the CP and drives ATP-dependent substrate unfolding, whereas the CC–OB subcomplex serves as the entry port of the substrate translocation channel. This PAN–CP model explains why an unfolded segment is an essential determinant of a proteasomal substrate (Schrader et al., 2009). According to this model, only the unfolded segment can enter the narrow port of the CC–OB channel. The leading segment is then captured by the ATPases and drawn deeper into the channel by the force generated by ATP hydrolysis. At the same time, the pulling of the leading segment results in unfolding of the substrate.

How are the six different Rpt ATPase subunits arranged in the eukaryotic proteasome? Taking advantage of the observation that the residues in the interfaces between the CP and the 19S RP have already pointed out that the Rpn1 and Rpn2 subunits may be more peripherally connected to the
ATPase ring (da Fonseca and Morris, 2008; Nickell et al., 2009). It will be of interest to define the exact roles of the six different Rpt subunits in substrate engagement, i.e. substrate binding, unfolding and translocation. Do they have distinct functions or simply act in a probabilistic manner like the PAN ATPases? Early genetic analysis by Rubin et al. (1998) suggested that different Rpt proteins may have different functions but cooperate in substrate degradation.

Assembly of the proteasome

Assembly of the 26S proteasome is not a simple task. At least 66 proteins have to be assembled into a functional RP2CP complex. Some of these proteins, including the proteolytic β subunits and non-catalytic β6 and β7, need to be processed or to mature during the assembly. Recent studies have provided a body of evidence showing that the biogenesis of the 26S proteasome involves an order of events assisted by a number of chaperone proteins.

CP assembly

Two excellent recent reviews have extensively discussed the assembly of the CP (Marques et al., 2009; Murata et al., 2009). Here I simply summarize some important findings. The assembly of the CP starts with the formation of the α ring. Because the α ring is normally composed of seven different α subunits that are placed in defined positions, there must be a mechanism in the cell that prevents 'random' oligomerization of the α subunits. In fact, deletion of the α3 subunit in yeast leads to incorporation of two α4 subunits in the α ring (Velichutina et al., 2004). It is now known that four chaperone proteins guide the assembly of the α subunits (Hirano et al., 2005, 2006; Le Tallec et al., 2007; Kusmierczyk et al., 2008; Yashiroda et al., 2008). These chaperones, named PAC (proteasome assembly chaperone) 1–4 in humans and Pba (proteasome biogenesis-associated protein) 1–4 in yeast, form a pair of heterodimers, PAC1–PAC2 (Pba1–Pba2) and PAC3–PAC4 (Pba3–Pba4). The PAC1–PAC2 and PAC3–PAC4 complexes have roles at different steps but cooperate in the assembly of the α ring. The PAC1–PAC2 complex not only assists α ring formation, but also prevents premature dimerization of α rings by stirring to the proteasome precursor until the 20S proteasome is completely assembled. It is then degraded by the newly formed 20S proteasome.

The α ring serves as a platform for the assembly of the β subunits. The detection of the 135 intermediate including an α ring and β2, β3 and β4 and the so-called half-mer (-β7) precursor complex bearing all β subunits except for β7 on the α ring indicates that the assembly of the β ring is a process of sequential steps (Li et al., 2007; Marques et al., 2007). In mammalian cells, the assembly of the β ring begins with the incorporation of the β2 subunit, followed by β3, β4, β5, β6, β1 and finally β7 (Hirano et al., 2008). In yeast, the exact order of the entrance of the β subunits remains unclear. The complex containing one α ring and one β ring is named half CP because an intact CP has two α rings and two β rings.

The assembly of the β ring involves another CP-chaperone, called Ump1 in yeast and UMP1, proteassembler or proteasome maturation protein in mammals (Ramos et al., 1998; Burri et al., 2000; Griffin et al., 2000; Witt et al., 2000). Ump1 appears to enter the β ring assembly line after β2, β3 and β4 are recruited to the α ring in yeast (Li et al., 2007). In contrast, UMP1 is incorporated into the α ring together with β2, suggesting that UMP1 is required for the initiation of β ring assembly in mammalian cells (Hirano et al., 2008). Ump1 not only facilitates the recruitment of additional β subunits, it may also act as an assembly check point protein to inhibit dimerization of half CPs until all seven β subunits are assembled on the α ring. The dimerization is triggered by incorporation of β7 into the half-mer (-β7) and intercalation of its C-terminal tail between β1 and β2 subunits of the opposing β ring (Li et al., 2007; Marques et al., 2007; Hirano et al., 2008). The formation of 20S proteasome is accompanied by the degradation of Ump1 and the maturation of the β subunits whose propeptides are crucial for their incorporation and protection of the catalytic sites (Ramos et al., 1998; Le Tallec et al., 2007, Li et al., 2007). Ump1 seems to play a role in the processing of the β subunits because loss of Ump1 results in accumulation of assembly intermediates and 20S proteasomes carrying unprocessed β subunits (Ramos et al., 1998).

The assembly of prokaryotic CP, which consists of only one type of α- and β-subunits, is relatively simple and appears not to require chaperone proteins. Properly assembled and functional 20S proteasomes can be produced by coexpression of the α- and β-subunits of Thermoplasma acidophilum in Escherichia coli (Zwickl et al., 1992; Löwe et al., 1995).

RP assembly

Whereas the CP assembly pathway has been extensively studied, it is largely unclear how the 19S RP is assembled. The recent publication of a series of papers provides some insights into the assembly of the base of the RP (Funakoshi et al., 2009; Kaneko et al., 2009; Le Tallec et al., 2009; Park et al., 2009, Roelofs et al., 2009; Saeki et al., 2009; Thompson et al., 2009). Like the CP assembly pathway, the base assembly is a highly organized process assisted by several dedicated chaperone proteins, including Nas2/p27, Nas6/gankyrin/p28, Rpn14/PAAF1 and Hsm3/S5b. These four chaperone proteins are structurally distinct and interact with specific Rpt subunits. Specifically, Nas2 binds to Rpt5, Nas6 to Rpt3, Rpn14 to Rpt6 and Hsm3 to Rpt1. The base assembly takes place by initial pairing of the Rpt subunits, resulting in three intermediates. The pairing process is facilitated or orchestrated by the chaperone–Rpt interactions. Rpt1 and Rpt2 together with Rpn1 and Hsm3 form a subcomplex called BP1. Rpt5 may associate with BP1 as well. The second subcomplex includes Rpt4, Rpt5 and Nas2, whereas Rpt3 and Rpt6 formed the third subcomplex with Nas6 and Rpn14. Some studies suggest that these three subcomplexes, together with Rpn2 and Rpn13, form the base (Funakoshi et al., 2009; Kaneko et al., 2009; Saeki et al., 2009; Thompson et al., 2009). Other studies, however, argue that the CP may play an important role in the assembly of the base. For example, Kusmierczyk et al. (2008) demonstrated that mutants defective in CP assembly also display defects in RP assembly. Moreover, the C-termini of the Rpt proteins dock into the CP; and deletion of the C-terminal
residue of the Rpt proteins, especially Rpt4 or Rpt6, severely impairs the assembly of the Rpt ring (Smith et al., 2007; Gillette et al., 2008; Rabl et al., 2008; Park et al., 2009; Roelofs et al., 2009). Finley and colleagues propose that the assembly of the Rpt hexameric ring includes sequential steps (Park et al., 2009). A subset of Rpt proteins, including Rpt4, Rpt6 and perhaps Rpt3, assemble into a complex (BP2) directly on the CP. Rpn14 and Nas6 likely guide the components of the BP2 complex to distinct positions on the CP via the interactions between the C-termini of the Rpt proteins and specific CP α subunit pockets. Subsequently, the BP1 complex, which forms independently of the CP, is recruited to BP2 to complete the assembly of the Rpt ring. In addition to the CP, Rpn1 and Rpn2 might perform a scaffolding role in the base assembly since they interact with the CP as well as the Rpt proteins. This model, although supported by some experimental results, cannot explain the existence of free base and RP in the cell (Isuno et al., 2007), which would imply that base assembly is independent of the CP. It is possible that more than one assembly line can be used to generate the Rpt ring and the 19S RP.

The assembly of the lid, which consists of nine subunits, is poorly understood. Recent studies suggest that the lid assembly is also a multi-step process (Isono et al., 2005; Sharon et al., 2006; Fukunaga et al., 2010). Rpn5, 6, 8, 9 and 11 are first assembled to form a core module; then joined by a second module including Rpn3, Rpn7 and Rpn15/Sem1. These two modules are connected by the interaction between Rpn3 and Rpn5. The lid is formed after the final incorporation of Rpn12. The chaperone protein Hsp90 may assist the assembly of the lid in yeast (Imai et al., 2003). The 19S RP is formed when the lid is associated with the base. However, it is not yet clear whether the binding of the lid to the base occurs prior to the docking of the base to the CP or vice versa.

**RP–CP association**

In addition to the CP- and RP-chaperones described above, other auxiliary factors have been suggested to play a role in the association between the RP and the CP. Of particular interest is the protein called extracellular matrix 29 (Ecm29), a member of the family of HEAT-repeat proteins (Kajava et al., 2004). Early studies have shown that Ecm29 interacts with the CP and the RP in yeast cells (Leggett et al., 2002; Kleijnen et al., 2007). Purified 26S proteasomes from Δecm29 cells are relatively unstable and tend to dissociate into RP and CP subcomplexes. These observations suggest that Ecm29 may stabilize the 26S proteasome by tethering the CP and the RP. More recently, Lehmann et al. (2010) suggested that Ecm29 may act as a proteasome-dedicated chaperone that controls the quality of RP–CP assemblies. The authors found that Ecm29 is bound to the RP–CP species in which CP maturation is stalled due to the lack of β3 subunit and that Ecm29 is removed upon the completion of CP maturation. Bim10, another member of the HEAT-repeat protein family, has also been suggested to act at the interface of CP maturation and RP–CP assembly, but likely at different steps from Ecm29 (Fehlker et al., 2003; Marques et al., 2007; Lehmann et al., 2010). The roles of other auxiliary proteins such as Nob1 and Hsp90 in CP–RP assembly are less clear (Tone and Toh, 2002; Fatica et al., 2003; Imai et al., 2003).

**Proteasome-interacting proteins**

Besides chaperones, a number of proteins are loosely associated with the proteasome at lower abundance compared with the canonical proteasome subunits (Verma et al., 2000; Xie and Varshavsky, 2000, 2002; You and Pickart, 2001; Chen and Madura, 2002; Leggett et al., 2002; Crosas et al., 2006; Guerrero et al., 2006; Hanna et al., 2007). These proteins, called proteasome-interacting proteins (PIPs), include several ubiquitin ligases. Recent studies have shown that the physical interaction between the proteasome and the ubiquitin ligases is functionally important. For example, disruption of the interaction between the Ufd4 ubiquitin ligase and the proteasome inhibits the degradation of Ufd4 substrates even though it has no effect on substrate ubiquitylation (Xie and Varshavsky, 2000, 2002). It is likely that Ufd4 acts as a carrier to deliver the substrates to the proteasome as well as a ubiquitin ligase to catalyze substrate ubiquitylation. In support of this argument, simultaneous loss of Ufd4 and Rad23, a protein known to deliver ubiquitylated substrates to the proteasome, exhibits a synthetic inhibitory effect on the degradation of Rad4, a nucleotide excision repair protein (Ju and Xie, 2006). The association of ubiquitin ligases with the proteasome may also allow substrate ubiquitylation to occur in the vicinity of the proteasome, thereby facilitating the capture and processing of the substrates by the proteasome. Indeed, the proteasome-associated Hul5 ubiquitin ligase is able to extend polyubiquitin chains at the proteasome in Saccharomyces cerevisiae (Crosas et al., 2006). Interestingly, the polyubiquitin chains generated by Hul5 are subject to disassembly by a proteasome-associated deubiquitylating enzyme named Ubp6 (USP14 in mammals; Crosas et al., 2006). Unlike the Rpn11 subunit, which facilitates substrate degradation through its deubiquitylating activity (Verma et al., 2002; Yao and Cohen, 2002), Ubp6 negatively regulate proteasomal degradation (Hanna et al., 2006). Higher eukaryotes have a third proteasomal deubiquitylating enzyme, Uch37, which appears to inhibit proteasomal degradation as well (Lam et al., 1997). Note that the fission yeast Schizosaccharomyces pombe has an orthologue of Uch37, named Uch2 (Stone et al., 2004). Ubp6 and Uch37 may serve as ‘proof-reading’ enzymes to remove ubiquitin chains from proteins that are mistakenly encountered and ubiquitylated by the ubiquitin system, thereby setting the ‘innocent’ proteins free from the proteasome. The binding of ubiquitylating and deubiquitylating enzymes to the proteasome suggests that the proteasome is not simply a ‘garbage disposal’, but plays a role in specifying the substrates that ought to be degraded.

The association of PIPs with the proteasomes can change in response to stresses. For example, Hanna et al. (2007) revealed that ubiquitin stress alters the proteasome composition. They found that the loading of Ubp6 to the proteasome increases in response to ubiquitin depletion. As a result, the cellular ubiquitin pool bounce back via recycling ubiquitin from ubiquitin conjugates by the deubiquitylating activity of Ubp6. Another example for the alternation of proteasome composition upon environment...
stress is the increased association of AIRAP (arsenite-inducible RNA-associated protein) with the proteasome in response to arsenic (Stanhill et al., 2006). Interestingly, AIRAP-containing proteasomes hydrolyze model substrates such as succ-LLVY-AMC faster than regular proteasomes, suggesting that AIRAP enhances the proteasome activity. The underlying mechanism, however, is not yet clear. Because of the diversity of PIPs and their dynamic interactions with the proteasome, it is reasonable to speculate that the cell may have a large pool of functionally distinct proteasomes, and that the proteasome populations may change in response to cellular stress via recruiting different PIPs. In fact, even the 195 RP can be replaced by the PA28 heteroheptamer in response to interferon-$
abla$ (Rock and Goldberg, 1999). Thus, the 26S proteasome should be considered a dynamic complex.

Feedback regulation of proteasome gene expression

The proteasome can be regulated at different levels. In addition to the assembly pathways and the interactions with PIPs, regulation of proteasome gene expression is another important mechanism that controls proteasome homeostasis.

The 26S proteasome is composed of 33 distinct subunits each encoded by a different gene. It was a difficult task to understand how the proteasome genes, in such a large number, are regulated. The central question was whether the proteasome genes are coordinately regulated by a common transcription factor or they are activated by different transcription factors. Early biochemical studies have shown that the proteasome subunits are nearly stoichiometrically present in yeast. The proteasome is a heteroheptamer in which proteasome homeostasis is regulated by a negative feedback circuit (Figure 2). On the one hand, Rpn4 induces the proteasome; on the other hand, Rnp4 is rapidly degraded by the assembled/active proteasome. The Rpn4-proteasome feedback loop provides an efficient and sensitive means to gauge proteasome abundance in the cell (Ju et al., 2010; Wang et al., 2010).

A question naturally arising from the discovery of the Rpn4-proteasome negative feedback circuit is whether the proteasome genes in higher eukaryotes are regulated by a similar feedback mechanism. So far, bioinformatics analyses have found Rpn4 sequence homologs and conserved PACE motifs only in Hemiascomycetes (Gasch et al., 2004; Mannhaupt and Feldmann, 2007). However, the proteasome genes in higher eukaryotes are coordinately regulated by a feedback mechanism (Wójcik and DeMartino, 2002; Lundgren et al., 2003; Meiners et al., 2003; Lee et al., 2004; Kraft et al., 2006; Xu et al., 2008; Sato et al., 2009; Radhakrishnan et al., 2010). For example, knockdown of individual proteasome subunits by RNAi results in up-regulation of non-targeted subunits in Drosophila cells; and the feedback response is dependent on the 5'-untranslated regions of the proteasome genes (Wójcik and DeMartino, 2002; Lundgren et al., 2003). In addition, the suppression of proteasome activity by proteasome inhibitors causes up-regulation of proteasome genes in mammalian cells (Meiners et al., 2003; Lee et al., 2004; Kraft et al., 2006; Xu et al., 2008; Radhakrishnan et al., 2010). These experimental observations strongly suggest that there is a functional homolog of Rpn4 in higher eukaryotes.

Nrf2 (nuclear factor erythroid-derived 2-related factor 2) may be an Rpn4 counterpart in mammalian cells (Kraft et al., 2006). Nrf2 is a cap ‘n’ collar-basic leucine zipper transcription factor that activates its target genes through a common cis-acting element named ARE (antioxidant response element; Moi et al., 1994). Previous studies have shown that Nrf2 is the most potent transcription factor in inducing the expression of antioxidant enzyme genes when the cell is under oxidative and electrophilic stress (Kensler et al., 2007; Nguyen et al., 2009). Kensler et al. (2003) found that administration of antioxidant 3H-1,2-dithiole-3-thione, which is known to activate Nrf2, enhances the expression of proteasome genes in the liver of wild-type, but not Nrf2-/- mice. This observation suggests that the proteasome genes are induced by Nrf2 in stress response. The same research group further demonstrated that the Nrf2-dependent induction of proteasome subunit PSMB5 by 3'-methylicholanthrene requires the AREs of the PSMB5 promoter in murine neuroblastoma Neuro2A cells (Kwik and Kensler, 2006). Enhanced expression of proteasome genes by Nrf2 was also detected in human fibroblasts and colon cancer cells (Arlt et al., 2009; Kapeta et al., 2010). Like Rpn4, Nrf2 is very short-lived and degraded by the proteasome. The cellular Nrf2 level

Figure 2 Negative feedback regulation of proteasome homeostasis. The transcription factor Rpn4 in yeast (A) and the transcription factors Nrf1 and Nrf2 in mammals (B) induce the expression of proteasome genes. The assembled/active proteasome in turn rapidly degrades the transcription factors.
implications of feedback regulation of proteasome gene expression

The discovery of feedback regulation of proteasome gene expression has several important implications in cancer therapy that targets the proteasome. First, it provides a clue to understand the cause of proteasome overexpression often-detected in cancers (Kumatori et al., 1990; Schwartz and Ciechanover, 1999; Pilarsky et al., 2004; Chen and Madura, 2005; Bazzaro et al., 2006). The feedback induction, which normally occurs only when the proteasome activity is suppressed, may become constitutively active in cancer cells. In support of this hypothesis, Xu et al. (2008) demonstrated that the proteasome genes are less inducible in response to proteasome inhibitors in breast cancer cells than in non-cancerous breast epithelial cells. Second, the feedback mechanism may contribute to drug resistance in cancer therapy. Bortezomib is the only FDA-approved proteasome inhibitor in clinical use. Although this drug has shown promising results in the treatment of multiple myeloma and mantle cell lymphoma, it has limited efficacy in cancers (Milano et al., 2007; Orlowski and Kuhn, 2008). The Bortezomib resistance may involve different mechanisms, depending on the cancer types. For example, the lack of Bax protein, whose stabilization by proteasome inhibitors is assumed to facilitate apoptosis, may play a role in the resistance to Bortezomib in breast cancer cells (Xu et al., 2008). The feedback induction of proteasome gene expression likely compromises the efficacy of Bortezomib, regardless of cancer types. Bortezomib is a reversible inhibitor and is rapidly cleared from the patients’ blood (Papandreou et al., 2004; Schwartz and Davidson, 2004; Orlowski and Kuhn, 2008). The transiently inhibited proteasome activity recovers after drug clearance and drug dissociation from the active site. The recovery is conceivably boosted by new proteasome synthesis induced by the feedback mechanism, thereby reducing the extent and duration of proteasome inhibition. Whereas the compromised efficacy of Bortezomib may still be sufficient to kill myeloma tumor cells, it may not be strong enough to be effective in other cancers, especially solid tumors. Third, the feedback pathway presents a potential target for cancer therapy. An early work by Ju et al. (2004) has already raised the concern that the efficacy of proteasome inhibitors may be compromised by the feedback up-regulation of proteasome genes. Ju et al. have also suggested that simultaneous targeting of the proteasome active sites and the transcription factor of the proteasome genes would be an efficient regimen for the treatment of cancer. This idea originated from the observation that the depletion of Rpn4 displays a strong synthetic growth defect with impairment of proteasome activity (Ju et al., 2004). In line with this prediction, a recent study demonstrated that knockdown of Nrf2 enhances the cytotoxic effect of proteasome inhibitor YU101 in breast and osteosarcoma cancer cell lines (Radhakrishnan et al., 2010). It will be of interest to examine whether this combined treatment is applicable to other types of cancer cells and whether it has a beneficial effect in clinical trials.

It is worthy of note that down-regulation of one of the proteasome genes dramatically reduces the assembled proteasome level and the proteasome activity in the cell (Wang et al., 2008). This finding is of clinical relevance because it provides a potentially important approach to reduce the proteasome activity in cancer cells. To date, proteasome inhibitors attacking the catalytic sites of the proteasome are the only tool to reduce the proteasome activity. However, many cancers are resistant to proteasome inhibitors by a variety of mechanisms. Knockdown of individual proteasome genes may present a promising alternative to proteasome inhibitors in cancer therapy. Indeed, a recent study revealed that knockdown of proteasome subunits by RNA interference impairs cancer cell growth and exhibits a synthetic lethal phenotype when combined with proteasome inhibitors (Chen et al., 2010).

Concluding remarks

The 26S proteasome is a complex protease. Although recent studies have provided some mechanistic insights into the structure and assembly of the 26S proteasome, there are many unanswered questions. For example, how are the substrates delivered to the ATPase ring of the 19S RP? Is there a similar structure as the CC–OB subcomplex of the PAN ATPases in the 19S RP to first encounter the incoming substrates? What is the arrangement
of the subunits in the lid of the 19S RP? How does the lid interplay with the base in processing substrates? The lid has a deubiquity-
ating activity in the Rpn11 subunit, whereas ubiquitylated sub-
strates are recruited to the base by Rpn10 and Rpn13. Rpn1 and Rpt5 have also been suggested to bind ubiquitylated proteins (Finley, 2009). It is unclear how these subunits cooperate to ensure the proper timing for the cleavage of the polyubiquitin chains from the substrates before or during their translocation and degradation. Solving the 19S RP and ultimately the 26S pro-
tesome structures will provide more definitive answers to these questions. The assembly of the 26S proteasome is not yet com-
pletely understood. It remains to be determined whether the lid is preassembled and then transferred to the base, or if it is built on the base. It is also unclear whether the base joins the CP as a preformed subcomplex or assembled on the CP. One should not be surprised if more chaperone proteins are found to participate in the assembly of the 26S proteasome in the near future.

The discovery of feedback regulation of proteasome gene expression not only provides insight into how proteasome homeostasis is regulated, but also raises a concern that feedback up-regulation of proteasome genes may compromise the efficacy of proteasome inhibitors in the treatment of cancers. This problem may be overcome by combined therapy that targets the transcription factor(s) of the proteasome genes together with inhibition of the proteasome activity. It is worthy of note that the proteasome activity can be regulated at different levels. In addition to gene expression and proteasome assembly, post-translational modifications of proteasome subunits such as oxidation, phosphorylation, glycosylation, acetylation, myristoy-
lization and ubiquitylation have been reported to alter the protea-
some activity (Kimura et al., 2003; Zhang et al., 2003; Powell et al., 2005; Zong et al., 2006; Holic et al., 2010). Further investiga-
tion of the biological significance and mechanisms of these modifications will provide more choices for proteasome-targeting cancer therapy.

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Structure, assembly and homeostatic regulation of the 26S proteasome


