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## **UBIQUITIN-PROTEASOME SYSTEM IN THE CENTRAL NERVOUS SYSTEM**

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### **1. PROTEIN DEGRADATION**

In order to maintain cellular homeostasis all cells must continually degrade proteins, with proteolysis occurring in a manner that is both highly specific and highly regulated. The proteins to be degraded by intracellular proteolytic pathways include short-lived, long-lived, misfolded, and damaged proteins (2–4). The targeting of each of these different types of proteins for proteolysis is generally achieved by the presence of a targeting motif. Established targeting motifs include a single amino acid residue (i.e. the N-end rule) (5), an amino acid sequence (i.e. PEST sequences) (6), or exposure of a hydrophobic domain (7). Post translational modifications such as phosphorylation and oxidation are also known to increase the targeting of proteins for degradation, with the resulting increase in protein turnover believed in part to be mediated by alterations in the tertiary protein structure. These modifications in protein structure likely promote the exposure of amino acid sequences and/or hydrophobic domains necessary for

protein targeting (7–9). Proteins can also be modified by ubiquitin (Ub) or Ub-like proteins, which appear to dramatically alter protein turnover (10,11). The specificity of protein degradation is necessary to prevent aberrant or unwanted proteolysis, and without such regulation cells would be unlikely to survive for any prolonged period due to the inability to maintain basic aspects of cell homeostasis. Similarly, without effective proteolytic pathways cells would rapidly accumulate unwanted and potentially toxic proteins.

The two principle intracellular proteolytic pathways are the proteasomal and lysosomal system. Proteasome-mediated protein degradation consists of an ATP-dependent (26S) and ATP-independent (20S) form of proteolysis. Meanwhile, the lysosomal proteolytic pathway can also be manifest in several forms including the endosomal-lysosomal pathway and macroautophagy. Together, the proteasomal and lysosomal pathways account for more than 90% of intracellular proteolysis (12,13).

The focus of this book is the proteasomal proteolytic pathway, which can be fully distinguished from lysosomal proteolysis based on several important features. Proteasome-mediated protein degradation occurs at neutral pH, does not require intracellular compartmentalization, occurs within a specialized protein complex, preferentially degrades short-lived proteins, and breaks down proteins to generate peptides not individual amino acids (14,15). Increasing evidence suggests that the proteasome plays an important role in a wide variety cellular processes including inflammation, proliferation, cytoskeletal regulation, and cell signaling (16,17). Numerous studies now also demonstrate a role for the proteasome in a wide range of neurophysiological as well as neuropathological processes, highlighting the significance for understanding the basis and regulation of proteasome-mediated protein degradation in the central nervous system (CNS).

## 2. THE UBIQUITIN SYSTEM

Ub plays a critical role in 26S proteasome-mediated protein degradation, targeting proteins to be degraded by the 26S proteasome in an ATP-dependent manner (18,19). The Ub protein is small (76 amino acids) and is present in all eukaryotic cells. In addition to its well established role in targeting proteins for degradation, a number of studies are now indicate that Ub may have a role in cellular events other than proteolysis. One of the unique aspects of Ub is that it is encoded and expressed as multimeric repeats (polyubiquitin) and also as single Ub encoding sequences (20,21). Interestingly, the single Ub encoding sequences can be fused in frame with a carboxyterminal extension protein (CEP). In humans there are two different ribosomal proteins L40 and S27a that can be fused to individual monomeric Ub encoding genes (22,23). Each of these Ub-fusions appears to play a critical role in ribosome biogenesis. These data suggest the potential existence of an important link between protein synthesis and protein degradation (23). The multimeric Ub products are modified post-translationally by cleavage events that generate monomeric Ub, while Ub-fusion proteins can yield monomeric Ub following cleavage by carboxyterminal Ub hydrolases (24,25).

There are now a number of Ub-like proteins that may have functions similar to Ub. Some of the best examples of these proteins include small ubiquitin-like modifier (SUMO) (10) and neural precursor cell-expressed developmentally down-regulated (NEDD8) (26). While each of these Ub-like proteins attaches to protein substrates via interactions through their carboxyl termini (like Ub), it appears that these Ub-like proteins are unable to form chains or higher order structures. Ub-like proteins are known to colocalize with Ub inclusions, and possibly modulate the degradation of Ub-modified proteins (11). These data suggest a role for Ub-like proteins in regulating Ub-mediated proteolysis and highlight the importance of developing a greater understanding these proteins play in both physiological and pathological processes (26).

The linkage of Ub to target proteins is mediated by isopeptide bonds between the C-terminal glycine residue of Ub and the amino group of lysine residues on target proteins (27). Following placement of the initial Ub onto the target protein, the establishment of a polyubiquitin chain can be rapidly achieved via the sequential addition of mono-Ub to the lysine residue of substrate bound Ub. The placement of Ub onto protein substrate, and development of polyubiquitin chain, requires a number of specific proteins to work together in a coordinated and complex manner (28). In the first step, the E1 enzyme activates Ub in an ATP-dependent reaction that produces a high-energy E1-thiol-ester Ub intermediate, that is then rapidly transferred to a subsequent enzyme termed E2 (Figure 1). The E2 enzymes catalyze the covalent attachment of Ub to target proteins, or the transfer of activated Ub to an E3 molecule in order to form an E3-Ub intermediate (Figure 1). The E3 enzymes are protein ligases, and are

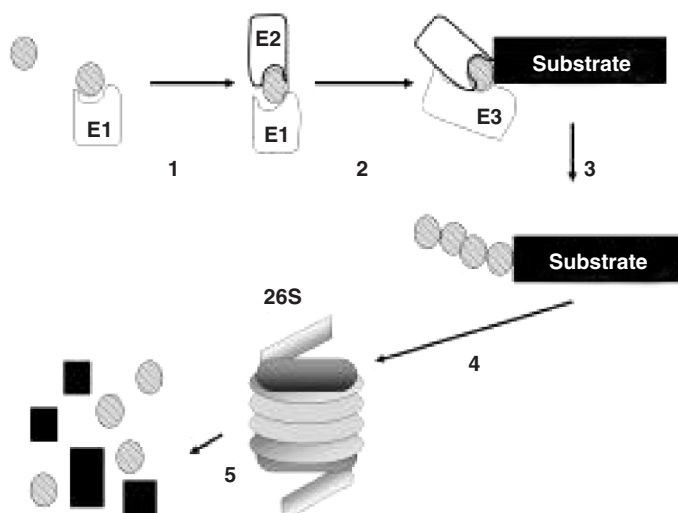


Figure 1. Ubiquitination of target proteins. (1) The E1 recognizes Ub (ovals) and transfers Ub to E2 (1) the E3 recognizes E2-Ub and then conjugates Ub to substrate protein (3) the process continues to add up poly-Ub chain on the substrate protein (4) poly-Ub-substrate is recognized by 26S proteasome and degraded (5) Substrate protein is cleaved into short peptides and Ub is released for recycling.

responsible for the transfer of Ub to the specific protein substrates. Most of the E3 proteins can be placed into two categories: homologous to the E6-AP-C terminus (HECT) (28,29) or the really interesting new gene (RING) (28,30). The HECT E3 proteins accept Ub from E2 enzymes by forming an additional high energy thioester bond between an active site cysteine and Ub, with the Ub subsequently transferred to the ligase bound substrate. In contrast, the RING E3 ligases serve primarily as a bridge to bring the E2-Ub complex and the protein substrate into closer proximity.

The specificity and complexity of protein ubiquitination becomes evident when looking at the number of genes expressed for each class of enzymes involved in the process. In humans there are two isoforms of E1, more than 50 E2, at least 1000 E3 proteins (31). The large number of E3 ligases is believed to contribute to the specificity and selectivity of protein ubiquitination, with individual E3 ligases exhibiting cell type specific expression and highly specific protein substrate selectivity. Mutation of the Ub pathway, in particular mutation of E3 ligase, may play a particularly important role in neurodegenerative events that selectively affect individual neuron populations.

### 3. UBIQUITIN AND THE PROTEASOME

It is important to point out that Ub-mediated protein degradation by the proteasome is the result of the 26S proteasome complex and not the 20S proteasome. The 26S proteasome complex has cap-like structure that contains several specialized proteins which aid in the recognition and recruitment of ubiquitinated proteins (Figure 1). It is likely that the increased hydrophobicity conferred by the polyubiquitin chain is what causes the proteins to be recognized by the 26S proteasome. In such a scenario, Ub would serve as a more important targeting mechanism for proteins that have a well preserved tertiary structure, or as a modifier for proteins that are intended to have an extremely short half-life. The 20S proteasome complex, which is several times more abundant than the 26S proteasome complex, degrades a vast array of proteins in an Ub- and ATP-independent manner. In particular the 20S proteasome is responsible for degrading most mildly oxidized proteins. Recent studies have confirmed that oxidized protein degradation by the proteasome is Ub-conjugation independent (32).

### 4. PROTEASOME AND THE BRAIN

The proteasome is a large intracellular protease composed of multiple subunits that exists in the cytosol and nucleus, and is well conserved from yeast to mammals in both structure and function. The proteasome was first observed in 1968 by J. R. Harries (33), and soon after a number of laboratories reported similar results. Recent studies indicate that in archaea and some bacteria (*actinomycetales*) there is a 20S proteasome possessing four stacked rings instead of the two stacked rings found in the *E. coli* (34), suggesting the proteasome has undergone some evolutionary change but is present even in the most ancient life forms. During the 1970-80s ATP/ubiquitin dependent proteolysis was documented in a cell-free lysate system from rabbit reticulocytes (35), although at that time ubiquitin dependent proteolysis

had not yet been identified as being mediated by the proteasome. During 1970-1980s the proteasome was termed as “multicatalytic proteinase complex”, “macropain”, “prosome”, or “macropain” (36,37). Subsequent research identified the proteasome as the protease responsible for ubiquitin dependent protein degradation, indicating the presence of the so-called ubiquitin/proteasome pathway (UPP) (38,39). After two decades research in this area, it is clear now that proteasome has many different structural isoforms and is involved in a number of diverse tasks, including antigen presentation, stress response, cell proliferation and apoptosis.

The CNS is a highly complex system composed of both mitotic cells (astrocytes, microglia) and postmitotic cells (neurons). The functions of UPP in the CNS are not as defined as compared to other systems, such as the immune system. Studies in Alzheimer’s disease (AD) and other age-related neurodegenerative disorders have provided evidence that the function of the proteasome is impaired and may contribute to both neuropathology and neuron death (40-42). The dysfunction of the proteasome may also lead to the dysfunction of specific organelles including mitochondria, and potentially generate crosstalk with the lysosome system (15,43,44). Developing a better understanding of the proteasome system in the CNS is likely to aid in the development of therapeutic interventions for neurodegenerative disorders as well as normal brain aging.

## 5. THE 20S PROTEASOME

The proteasome is a large multicatalytic protease (~700 kDa) that comprises up to 1% of total cellular protein content. The barrel-shaped core of the proteasome is known as the 20S proteasome, and consists of 28 individual  $\alpha$ - and  $\beta$ -subunits (18). The 20S proteasome subunits are arranged within four stacked rings, with each ring consisting of either 7 $\alpha$  or 7 $\beta$  subunits. The  $\beta$  subunits comprise the two inner rings of the 20S proteasome, with the outer rings comprised of  $\alpha$ -subunits. The apparent diameter of 20S proteasome is approximately 11nm  $\times$  15nm. The  $\beta$  subunits are responsible for mediating all of the proteolytic activities of the proteasome, while the  $\alpha$ -subunits function in stabilizing the 20S proteasome complex (Figure 2). There are three special  $\beta$  subunits PSMB8, PSMB9 and PSMB10 that are not present in the regular 20S proteasome, which are called inducible subunits (Table 1). The induction of these subunits usually occurs with inflammatory factors such as interferon gamma (INF  $\gamma$ ) (45). Following their expression, inducible subunits replace other  $\beta$  subunits PSMB5, PSMB6, and PSMB7 to form so called “immunoproteasome”. The inducible immunoproteasome subunits are enriched at the endoplasmic reticulum, where they play an important role in generation of MHC class I molecules (46).

The immunoproteasome has been intensively studied for its role in MHC I antigen processing (47), with the functions of the immunoproteasome affected by many factors. For instance, virus infection elevates the level of immunoproteasome (48), but studies indicated that the incorporation of inducible subunits into the proteasome complex may be interrupted by the activities of virus (49). The elevation in immunoproteasome expression after virus infection is INF  $\gamma$ -dependent, with INF  $\alpha$  and other cytokines have no effects (50). Alcohol inhibits the induction of immunoproteasome by IFN- $\gamma$ , and attenuates the

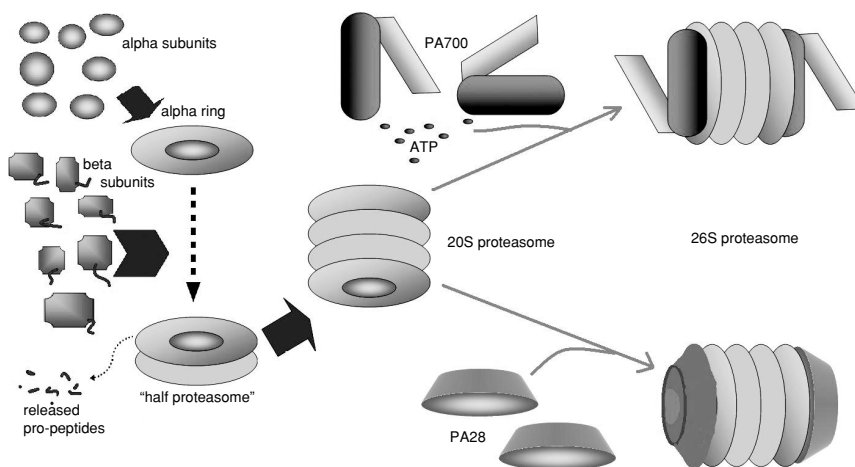


Figure 2. The biogenesis and structure of 20S and 26S proteasome complexes. The  $\alpha$  subunits (ovals) form single  $\alpha$  ring and then the  $\beta$  subunits (squares, the curved line indicate the propeptides) add up to form dual ring ("half proteasome"). Two of such "half proteasome" form 20S proteasome. Two types of 26S proteasomes exist in the cells: PA700 complex adds to 20S proteasome requiring ATP, while PA28 complex adds to 20S proteasome independent of ATP.

catalytic activities of proteasome (51). Interestingly, INF  $\gamma$ -deficient mice have similar basal expression of inducible subunits as compare to normal mice, suggesting that INF  $\gamma$  does not affect the constitutive expression of these subunits (50). The interaction of viral protein with proteasome subunits have been reported, and may interfere with host anti-viral defenses and also contribute to mechanisms of cell transformation (46). Interestingly, the inducible subunits PSMB8 and PSMB9 are increased in mouse model of Huntington's disease (HD), with neuron preferentially exhibiting increased immunoproteasome, suggesting the involvement of immunoproteasome in neurodegeneration (52,53).

An additional 20S proteasome-like protease exist in the mitochondria, and is referred as Lon (54). Lon is encoded by nuclear gene, but is located in the matrix of mitochondria. Lon is necessary to maintain mitochondria homeostasis (55). Studies indicated that the activities and expression are increased correlatively with the biogenesis of mitochondria (56). Different from 20S proteasome, the catalytic activities of Lon are ATP-dependent, and conserved from archae to human. Besides the hydrolysis of proteins and peptides, Lon also binds single stranded DNA, especially in the TG-rich region (57), suggesting a role of Lon in mitochondrial DNA replication and/or mitochondria gene expression. (54) The expression of Lon gene might be regulated by different factors. For example, the expression of Lon declined in aging mice (58), and may be enhanced by hypoxia or ischemia (59). Interestingly, a bacterial Lon protein has chaperone-like activity (60).

Presently 7  $\alpha$  subunit genes and 10  $\beta$  subunit genes for the 20S proteasome have been identified in the human genome (Table 1). At least 2 additional  $\beta$  subunits have been described in zebrafish (*Danio rerio*, PSMB11, PSMB12) but have not yet been identified in human (61). PSMA2 and PSMA6 have 2 functional gene copies, while PSMA6 and PSMA7 each has 1 pseudogene copy. The

Table 1. 20S proteasome subunits.

Symbol	GeneID	locus	Alternative symbols	Size**
$\alpha$ subunits				
PSMA1	5682	11p15.1	NU,HC2, PROS30	263 a.a.
PSMA2	5683	7p13	HC3,PSC2	225 a.a.
PSMA3	5684	14q23	HC8,PSC3	248 a.a.
PSMA3P*		14q23.1		
PSMA4	5685	15q24.1	HC9, HsT17706	261 a.a.
PSMA5	5686	1p13	9534, PSC5, ZETA	241 a.a.
PSMA6	5687	14q13	IOTA; p27K;PROS27	246 a.a.
PSMA6'		13q32.2	LOC121906	
PSMA6P		Yq11.21		
PSMA7	5688	20q13.33	C6; HSPC; RC6-1; XAPC7	248 a.a.
PSMA7P		9q22.33		
$\beta$ subunits				
PSMB1	5689	6q27	HC5	241 a.a.
PSMB2	5690	1p34.2	HC7-I	201 a.a.
PSMB3	5691	17q12	9540, HC10-II	205 a.a.
PSMB3P		2q35		
PSMB3P		12q13.13		
PSMB4	5692	1q21	HN3, HsN3, PROS26	264 a.a.
PSMB5	5693	14q11.2	LMPX, MB1, X	263 a.a.
PSMB6	5694	17p13	DELTA, LMPY, Y	239 a.a.
PSMB7	5695	9q34.11-12	9544, Z	277 a.a.
PSMB8	5696	6p21.3	LMP7, RING10, D6S216	276 a.a.
PSMB9	5698	6p21.3	LMP2, RING12	219 a.a.
PSMB10	5699	16q22.1	LMP10, MECL1	273 a.a.
PSMB11	64279	( <i>Danio rerio</i> )		217 a.a.
PSMB12	64280	( <i>Danio rerio</i> )		281 a.a.

\*The mark' indicates the functional isoforms of the gene while the letter "P" indicates the pseudogene.

\*\*Indicates the longest known amino acid sequence.

PSMB3 gene has 2 pseudogene copies. It is not yet clear why and how these extra gene copies developed, although most pseudogenes are believed to result from genomic evolution (23,62). Interestingly, one of the PSMB3 pseudogenes is in the same location as functional PSMB3 on chromosome 14, while another PSMB3 pseudogene is located on chromosome 2. The inducible subunits PSMB8

and PSMB9 are very close to each other on chromosome 6, with the inducible immune subunit PSMB10 located on chromosome 16. For all  $\alpha$  subunits, there is an approximately 30% shared identity in amino acid sequence while the  $\beta$  subunits appear to be much more diverse. Most  $\alpha$  subunits possess a conserved motif in their N-termini that is essential for proteasome assembly (63). Another conserved motif is RP×G where R, P and G refer to arginine, proline and glycine, respectively. This motif is found in the contact region among  $\alpha$  subunits, although its function is still unclear (61). Some  $\alpha$  subunits contain a functional nuclear localization signal that may regulate their nuclear localization (61). Some  $\beta$  subunits have an N-terminus pro-peptide that may work as an internal chaperone to ensure proper folding (64) or to prevent premature activation (65). These pro-peptides are removed during proteasome assembly, in order to expose a threonine that is essential for catalytic activity of most  $\beta$  subunits (66). The functional genes of  $\beta$  subunits are located on different chromosomes (Table 1), and it appears unlikely that their promoter regions share any significant homology. Additionally the activity of the promoters for  $\beta$  subunits is apparently unique, and the exon/intron organization of each  $\beta$  subunit gene lacks any apparent uniformity (67). It's interesting to speculate that cells could coordinate the expression of proteasome subunits in order to fulfill a specific need during normal physiological conditions or in response to stress. For instance, in LMP2 (PSMB9) knock out mice, the expression of PSMA4, PSMB1, PSMB3 are increased while the expression of PSMB5 and PSMB8 are decreased in the brain (Ding and Keller unpublished observation). In the mouse model of HD, the expression of PSMA3, PSMB1, PSMB3 and PSMB6 are selectively up-regulated in the brain. Lastly many proteasome subunit genes change their expression with age (Ding and Keller unpublished observation).

As a multi-subunit complex, the proteasome needs to be assembled from many individual proteins (Figure 2). *In vitro* studies indicate that the  $\alpha$  subunits can form 7-member rings ( $\alpha$ 1-7) by themselves. To form  $\beta$  rings, the presence of  $\alpha$  rings is required. In addition, the  $\alpha$  rings can associate in pairs without  $\beta$  rings (66). The N-termini conserved sequences of  $\alpha$  subunits are important for the assembly of  $\alpha$  rings, with deletion or mutation in these regions preventing the formation of  $\alpha$  rings (63,66). For  $\beta$  subunits the pro-peptides in the N-termini are essential to ring assembly (Figure 2). Yeast studies have shown that without a proper pro-peptide the  $\beta$  subunit can not incorporate into a 20S complex and the cell is unable to survive (68). Interestingly, when full length  $\beta$  subunits are expressed in *E. coli* they accumulated as inactive monomers, while expression of  $\beta$  subunit without a pro-peptide forming aggregates possessing peptidase activity (66). Some studies have shown that phosphorylation might be involved in the incorporation of  $\beta$  subunits into 20S proteasome (69), but the details of this process are not clear. Other reports indicated that during assembly of the 20S proteasome, coupled  $\alpha$  and  $\beta$  rings ( $\alpha$ 1-7 $\beta$ 1-7) might form an intermediate form of mature 20S proteasome (70). One maturation factor, UMP1 was identified originally in yeast, which is necessary in the assembly of the 20S proteasome (70,71). The mammalian homolog of UMP1 is referred as proteasembilin (72), which is regarded as a chaperone, interacting with standard  $\beta$  subunits and inducible  $\beta$  subunits selectively to assemble either the standard proteasome or the immunoproteasome (73). Data indicated that the inter-



action occurring between the C-termini of  $\beta$  subunits and proteasemlin aid in the formation of four-ring-complex from two-ring-complex (half proteasome) (73). In addition, HSC73 is another chaperone that specifically involved in the formation of immunoproteasome (74). HSC73 appears to aid in holding two half-proteasomes together and Hsp90 co-precipitates with the pro-proteasome suggesting a role for Hsp90 in the assembly of proteasome (74,75). In summary, it is presently accepted that 20S proteasome biogenesis occurs as followed:  $\alpha$  subunits form the  $\alpha$ -rings and the  $\beta$  subunits then associate with one  $\alpha$ -ring (Figure 2). The pro-peptides of  $\beta$  subunits are then cleaved to form a complete  $\beta$  ring on  $\alpha$  ring, resulting in the formation of a "half proteasome":  $\alpha 1-7\beta 1-7$ . Finally two of such "half proteasome" associate together to form the regular 20S proteasome complex:  $\alpha 1-7\beta 1-7\beta 1-7\alpha 1-7$  (Figure 2) (76). The 20S proteasome complex is far more abundant than the 26S complex, with both 20S and 26S proteasomes outnumbered by developing proteasomes and free subunits.

The 20S proteasome has three principle peptidase activities: chymotrypsin-like activity (cleavage after big hydrophobic residues), trypsin-like activity (cleavage after basic residues), and caspase-like activity (postglutamyl activity, cleavage after acidic residues) (18). These activities are all executed within the inner chamber of the  $\beta$  subunit ring. Purified 20S proteasome can degrade many peptides in ubiquitin- and ATP-independent manner (18,76). The free 20S proteasome particles are present in the cells (69), and some reports indicated that the 20S proteasome degrades oxidized, misfolded proteins, and peptides *in vivo* (77). For example, oxidized hemoglobin is rapidly degraded after ATP depletion in reticulocytes (78), and IK $\kappa$ B  $\alpha$  is selectively degraded by 20S proteasome (79). In fresh extracts 20S proteasome is resistant to heat (up to 55°C), fatty acids, and denaturing agents such as guanidine and SDS (80). Interestingly these treatments have been demonstrated to even enhance the catalytic activities of 20S proteasome(80). Since these treatments would be expected to induce the conformational change of proteins leading to unfolding or denaturing, it is possible that these treatments might aid the entry of misfolded proteins into the 20S proteasome complex. In the living cells, oxidative stressors like H<sub>2</sub>O<sub>2</sub> treatment do not significantly change the activity of 20S proteasome while the function of 26S proteasome is dramatically decreased (see below).

As mentioned above, 20S proteasome complexes are relatively stable, resistant to certain level of heat, detergent as SDS, and oxidative stress. Research indicates that most proteasome complexes may last throughout cell cycle, and in post-mitotic cells like neurons a 20S proteasome complex might last for years. When necessary, it is presumed that proteasome complexes are degraded by lysosome system (81), while additional evidence suggests that caspases may degrade proteasome subunits especially during apoptosis (82).

## 6. THE 26S PROTEASOME

The 26S proteasome is the principle mechanism for the degradation of ubiquitinated proteins. Generally the term of 26S proteasome refers to a 20S proteasome associated with PA28 or PA700 activator (18). The PA700 complex is a V-shaped complex responsible for the recognizing, binding and unfolding the

ubiquitinated proteins and then delivering them to the hydrolytic sites of 20S (Figure 1). PA700 activator, which is also termed the 19S complex, can combine with 20S proteasome core on one or both  $\alpha$  rings in the presence of ATP and greatly enhances the proteasome hydrolysis activity. PA700 can be dissociated from the 20S proteasome core under ATP depletion, and it appears that in the cell PA700 continuously shuttles on and off 20S proteasome complex in response to the environmental stress. PA28 is an alternative cap for proteasome, and is a bell-shaped complex, which binds the 20S proteasome core independent of ATP (Figure 2). Apparently PA28 has a weaker association with 20S proteasome than PA700, and may be released from 20S proteasome readily following exposure to low level ionic conditions. PA28 has been found as a free complex, but the potential function of the free PA28 complex is not clear (83,84). Other factors that have been demonstrated to interact with the proteasome include protein kinases, (85) isopeptidases, (86) heat shock proteins (HSP) and EF-1 $\alpha$  (87).

PA700 has 6 subunits with ATPase activities, and 15 subunits that lack ATPase activities (Table 2 & Table 3). Four of the ATPase subunits (PSMC1, PSMC2, PSMC4, PSMC5) form a tetramer ring as the core of PA700 (88), but the functions of non-ATPase subunits are not yet clear. Mutation of these subunits leads to the accumulation of ubiquitinated proteins (89), suggesting that these subunits play a role in recognizing, binding and/or delivering ubiquitinated proteins to 20S proteasome core. Besides ATP the association of PA700 to 20S proteasome is regulated by a 300 KD modulator. Interestingly this modulator contains two ATPase subunits of PA700 (90). These data raise the possibility that regulator subunits may have multiple functions, potentially even proteasome

Table 2. 26S proteasome regulatory PA700 subunits (ATPases).

GDB symbol	GeneID	locus	Alternative symbols	Size**
PA700 ATPase subunits				
PSMC1	5700	14q32.11	P26S4, S4, p56	440aa
PSMC2	5701	7q22.1-3	MSS1, S7	433aa
PSMC2*		3q22.1	LOC402142	
PSMC3	5702	11p12-13	TBP1	491aa
PSMC3P		9p22.1		
PSMC4	5704	19q13.11-13	MIP224, S6, TBP7	418aa
PSMC5	5705	17q23-25	S8, SUG1, TBP10, TRIP1, p45	406aa
PSMC6	5706	14q22.1	CADP44, P44, SUG2, p42	389aa
PSMC6P		8q11.23		
PSMC6P		12q14.3		

\*The mark ' indicates the functional isoforms of the gene, while the letter "P" indicates the pseudogene.

\*\*Indicates the longest known amino acid sequence.

Table 3. 26S proteasome regulatory PA700 subunits (non-ATPases).

Symbol	GeneID	locus	Alternative symbols	Size**
PSMD1	5707	2q37.1	P112, S1	953aa
PSMD2	5708	3q27.3	P97, S2, TRAP2	908aa
PSMD2P*		1q43	LOC266783	
PSMD3	5709	17q21.2	P58, RPN3, S3	534aa
PSMD4	5710	1q21.3	AF, ASF, MCB1, S5A, Rpn10, pUB-R5	377aa
PSMD4P		10q23.33		
PSMD5	5711	9q34.11	S5B	504aa
PSMD6	5712		S10	389aa
PSMD7	5713	16q23-24	MOV34, P40, S12	326aa
PSMD7P		17q24.2	LOC280637 HIP6, HYPF, S14, p13,	
PSMD8	5714	19q13.13	Nin1p	257aa
PSMD8P		chromosome1	LOC276721	
PSMD9	5715	12q24.31-32	p27	223aa
PSMD10	5716	Xq22.3	p28	226aa
PSMD10P1		3q28	LOC280644	
PSMD10P2		20q13.13		
PSMD11	5717	17q12	S9, p44.5	422aa
PSMD12	5718	17q24.3	p55	456aa
PSMD12P		3p14	LOC317753	
PSMD13	5719	11p15.5	HSPC027, p40.5	376aa
PSMD14	10213	2q24.3	PAD1, POH1, rpn11	310aa
PSMD15	54035	21q22.13	PSMD4P (pseudogene)	

\*The letter "P" indicates the pseudogene.

\*\*Indicates the longest known amino acid sequence.

independent functions. PA28 has four subunits (PSME1, PSME2, PSME3, PSME4) that are homologous (Table 4), and may form a hetero-heptametrical complex (91). Interestingly, PA28  $\alpha$  (PSME1) is capable of forming a hexameric ring composed only PA28  $\alpha$ . (91) Presently it is not clear what roles the PSMA3 and PSMA 4 subunits play in protein degradation. PA28 is  $\gamma$ -interferon inducible, required for the antigen processing, and is necessary for the assembly of immunoproteasome (92).

The 21 genes of the PA700 subunits are located on different human chromosomes (Table 2 and Table 3). The 6 ATPase subunits belongs to the same ATPase

family (AAA), with a second functional copy of PSMC2 gene located on a different chromosome, and another two of these ATPase subunits (PSMC3 and PSMC6) having pseudogenes. PSMC6 actually has 2 copies of pseudogenes (Table 2). Pseudogenes exist in non-ATPase subunits as well, including PSMD2, PSMD4, PSMD7, PSMD8, PSMD10 and PSMD12 genes (Table 3). Interestingly, these pseudogenes are located on different chromosomes, with even double pseudogenes present on separate chromosomes. For example, the functional PSMD10 are located on chromosome X while two of its pseudogenes located on chromosome 3 and chromosome 20 (Table 3). With the high preservation of the proteasome system from bacteria to mammals, it would not be surprising if more pseudogenes and functional copies of proteasomal genes are found in the human genome, demonstrating the evolutionary specialization of the proteasome.

The homology among ATPase subunits is significantly higher than that of non-ATPase subunits, although the highest homology is in PA28 subunits (PSME1, PSME2, PSME3, and PSME4) (Table 4). PSME1 and PSME2 genes are composed of 11 exons each, consistent with gene duplication during vertebrate evolution. The intron/exon organization of these genes is highly conserved, with the PSME2 lacking the exon encoding the lysine and glutamic acid-rich KEKE motif. These two genes are closely linked on 14q11.2, within 30~40 kb(93). In fact, this locus is very close (within 1MB) to one of  $\beta$  subunits of 20S proteasome, PSMB5 (Table 4) (94).

Table 4. 26S proteasome regulatory PA28 subunits.

Symbol	GeneID	locus	Alternative symbols	Size**
PSME1	5720	14q11.2	PA28A, PA28 $\alpha$ , REG $\alpha$	249aa
PSME2	5721	14q11.2	PA28B, PA28 $\beta$ , REG $\beta$	239aa
PSME2*	389312	5q21.1	LOC389312	239aa
PSME2P1		5q21		
PSME2P2		13q13	LOC338099	
PSME2P3		4p14	LOC338096	
PSME2P4		10p12	LOC338098	
PSME2P5		4q32	LOC338095	
PSME2P6		8p21	LOC338097	
PSME3	10197	17q21	Ki, PA28 $\gamma$ , PA28G, REG $\gamma$	267aa
PSME4	23198	2p16.3	PA200	1798aa
others				
PSMF1	9491	20p13	PI31	271aa
p44S10	9861	3p21.1	KIAA0107, SGA-13M,p42A	389aa

\*The mark ' indicates the functional isoforms of the gene, and letter "P" indicates the pseudogene.

\*\*Indicates the longest known amino acid sequence.

Currently it's not clear whether the linkage of proteasome genes indicates a functional coordination of gene expression (Table 5). It's worthy to mention that in yeast the proteasomal genes are dispersed amongst almost all chromosomes and their expression is both constitutive and possibly correlated. It will be interesting in future studies to elucidate the transcriptional patterns of proteasome subunit expression in different paradigms, to determine if these genes exhibit coordinated expression. With microarray technology it was found that the expression of most of the proteasome subunit genes is tightly coordinated upon initiation of transcription (95). Other studies indicate that under certain situations such as DNA damage, up-regulation of proteasome genes is mediated by a single transcriptional factor (RPN4) (96). RPN4 is a transcriptional activator that promotes the expression of most proteasomal subunit genes in yeast. RPN4 is degraded by the proteasome, thus forming an auto-regulatory circuit. Interestingly, RPN4 is degraded by proteasome in at least two ways, ubiquitin-dependent one and ubiquitin-independent one (97).

Table 5. Gene clusters of subunits in human proteasome system.

Gene	Locus	Gene	Locus
PSMB4	1q21	PSMB8	6p21.3
PSMD4	1q21.3	PSMB9	6p21.3
Gene	Locus	Gene	Locus
PSMA7P	9q22.33	PSMC3	11p12-13
PSMD5	9q34.11	PSMA1	11p15.1
PSMB7	9q34.11-12	PSMD13	11p15.5
Gene	Locus	Gene	Locus
PSMB5	14q11.2	PSMC6	14q22.1
PSME1	14q11.2	PSMA3	14q23
PSME2	14q11.2	PSMA3P	14q23.1
PSMA6	14q13		
Gene	Locus	Gene	Locus
PSMB3	17q12	PSMC4	19q13.11-13
PSMD11	17q12	PSMD8	19q13.13
Gene	Locus	Gene	Locus
PSME3	17q21	PSMF1	20p13
PSMD3	17q21.2	PSMD10P2	20q13.13
PSMC5	17q23-25	PSMA7	20q13.33
PSMD7P	17q24.2		
PSMD12	17q24.3		

As mentioned above, a 300KD modulator of 26S proteasome has been reported, which can enhance the function of PA700 on proteasome without affecting the activity of the 20S proteasome (90). Another regulator is PI31, which is believed to be a natural inhibitor of proteasome, is associated with the nuclear envelope/endoplasmic reticulum membrane (98). Recent research indicate that PI31 might act as a modulator of proteasome-induced MHC class I antigen processing (98). Over-expression of PI31 in mouse embryonic cells selectively interferes with the maturation of immunoproteasome precursor complexes, decreased the surface MHC class I levels on IFN  $\gamma$ -treated mouse embryonic cells (98). PAN (proteasome-activating nucleotidase) is a homolog of mammalian 19S complex, expressed in archaeal cells (99). PAN has a molecular weight of ~560kD, possessing high homology to the ATPase subunits in PA700. Besides ATP, PAN can utilize CTP, TTP, GTP, UTP and even ITP to enhance the catalytic activity of proteasome (99). Reports indicate that PAN has chaperone activity to reduce aggregation of denatured proteins and may enhance protein refolding (99). Although ATP is not required, the presence of ATP can increase the efficiency of protein folding by PAN (100). PAN does not promote the degradation of small peptides. Other proteins are also involved in regulating proteasome proteolysis including tripeptidyl peptidase II (TPP II) which plays a critical role in cleaving proteasomal produced peptides into shorter peptides that can then be degraded by aminopeptidases (101).

## 7. PROTEASOME MEDIATED PROTEIN DEGRADATION

Studies indicated that about one-third of newly synthesized proteins have structural errors, and these proteins need to be removed eventually by the proteasome (102-104). Denatured proteins and otherwise misfolded proteins are degraded by proteasome as well. This proteolytic process is strictly regulated. As mentioned above, Ub and Ub-like proteins (SUMO, NEDD8) are the most popular markers for destruction. In fact, the E3 group of Ub ligases is largely responsible for the recognition of proteins with destruction signals, and the E3 may be activated by structural modification such as phosphorylation or allosteric transition. Environmental and intracellular signals can also trigger the degradation of specific proteins (105). The adaptive cellular immune system in mammals is highly dependent on peptides generation, which are made by the proteasome from viruses and other intracellular pathogens. CD8<sup>+</sup> T cells in the adaptive immune system first detect the foreign peptides, and then a clonally restricted receptor is expressed to recognize peptides with 8- to 11-residue, nestling in the groove of major histocompatibility complex class (MHC) I molecules (106). The newly synthesized class I molecules carry viral peptides to the surface of infected cells, where they are recognized by non-self-reactive T cells specific for the given peptide-class I complex. Activated T cells then deliver a cocktail of immune effector molecules that is capable of interfering with viral replication either by brute force (killing the virally infected cell) or by subtle pathway (reprogramming the virally infected cell to disfavor viral replication) (107). Recognizing a single peptide-class I complex on the surface of a target cell thus provides the most efficient approach for regulating T-cell function.

It is uncertain what fraction of the rapidly degraded pool of proteins is short-lived proteins, and what fraction represents defective proteins. A very small fraction of proteasome-generated peptides are presented by MHC class I molecules to T cells. It is important to note that peptides are subject to further trimming by endoplasmic reticulum associated aminopeptidases (108). The relative contributions of errors in folding, translation, and transcription to the defective protein pool are also unclear. Data show that a virus nuclear antigen of Epstein-Barr virus (EBV) has an amino-terminal sequence that disfavors proteasome degradation and also reduces translation of its own message. Together, these features reduce the generation of EBNA1 peptides, enabling cells harboring EBV to escape immune surveillance (109). Other studies showed that ribosome can initiate translation aberrantly, generating unintended translation products that contribute to defective protein pool (110-112).

The proteasome can recognize and degrade a class of substrates that do not require ubiquitin modification (97). Ornithine decarboxylase (ODC) is one of such substrates. ODC catalyzes the initial step in polyamine biosynthesis and is regulated by end products spermidine and spermine, through the regulatory protein antizyme 1 (AZ1) (113). Excess polyamines induce the expression of AZ1, which binds the ODC monomer, dissociating the active ODC homodimer and thereby inhibiting its activity (114). AZ1 binding exposes a C-terminal degradation signal in the ODC protein, resulting the degradation of ODC independent of ubiquitination (115). Further studies indicate that the degradation of ODC can process independent of mammalian AZ1, with the degradation signal present in five amino acids on the C-terminal and Cys441 of ODC (116). Other examples of ubiquitin independent 26S proteasome degradation include p21 and RPN4 (117). The protein p21 is a cyclin-dependent kinase inhibitor and RPN4 is a transcriptional activator of genes encoding subunits of the proteasome. Interestingly RPN4 protein is short-lived and interacts with the Rpn2 subunit of the base of the 19S regulatory particle (117).

## 8. THE PLASTICITY OF PROTEASOME IN THE CNS

Oxidative stress decreases the proteasome peptidase activities in a rapid manner. Treatment with diamide, a potent oxidant, decreased 20S core proteasome activities, de-ubiquitinating activity, and 26S proteasome activities (118). It is suggested that in the CNS the proteasome is progressively inhibited by small accumulations of oxidized and cross-linked proteins, and the impaired proteasome system then promotes further accumulation of oxidized and aggregated proteins. Because the proteasome is composed of multiple proteases, the individual activities of proteasome may be altered differently following oxidative stress. Ethanol administration, which is regarded as a form of oxidative stress, decreases the chymotrypsin-like activity and the trypsin-like activity by 35% to 40%, without affecting the caspase like activity significantly (119). Aged animals have decreased proteasome activity, with the individual peptidase activities differently affected during aging (120).

During the development of glaucoma, the protein levels of proteasome  $\alpha$  subunits increase ~3 folds as determined by Western Blot (121). After the injection

of lipopolysaccharide (122), the inducible subunit LMP7 (PSMB9) shown increased protein level in kidney, heart and lung but not brain (123). Interestingly, those organs (kidney, heart and lung) had decreased weights 3 days after LPS injection (123). After global ischemia the expression of 26S complex subunit PSMD1 was elevated at 12 hours in the dentate gyrus (124). After 24 hours, PSMD1 increased its expression significantly in both the CA1 and dentate gyrus compared with control animals. This alteration in proteasome expression was also associated with the change of transcriptional factor (SEF-2) (124).

Data from our laboratory demonstrate that neural proteasome expression is increased in response to oxidative stress (15) and following the expression of proteins with polyglutamine extension (53). These changes in proteasome expression (increased immunoproteasome expression) were associated with a preservation of proteasome function. However, following an additional stressor (heat stress) the proteasome was unable to increase its activity in neural cells with increased immunoproteasome expression (53). These data suggest that proteasome plasticity in the CNS may have beneficial effects in the short-term, but the long-term effects may be deleterious, based on the fact that the immunoproteasome appears unable to respond to subsequent stressors.

Proteasome plasticity is a relative new concept, and may explain some of the current controversies associated with the role of the proteasome in neurodegenerative disorders. In AD, HD, and Parkinson's disease (PD), neurodegeneration likely requires decades. It is unlikely that the proteasome contributes to neurodegeneration in these disorders by undergoing permanent and dramatic decreases in function. Far more likely, in each of these conditions there is a short-term proteasome inhibition that is followed by intracellular changes that allow the cells to recover proteasome-mediated protein degradation in the short-term. Changes in proteasome expression, proteasome complex function, and proteasome localization are very likely to play a direct role in mediating these beneficial short-term adaptations. However, the long-term and cumulative effects of proteasome alterations may ultimately result in cytotoxicity and neurodegeneration.

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Stefanis, L.; Keller, J.N. (Eds.)

2006, XV, 306 p., Hardcover

ISBN: 978-0-387-28499-6