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Review

# Adaptive modification and flexibility of the proteasome system in response to proteasome inhibition

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**Abstract**

The highly conserved ubiquitin–proteasome system is the principal machinery for extralysosomal protein degradation in eukaryotic cells. The 26S proteasome, a large multicatalytic multisubunit protease that processes cell proteins by limited and controlled proteolysis, constitutes the central proteolytic component of the ubiquitin–proteasome system. By processing cell proteins essential for development, differentiation, proliferation, cell cycling, apoptosis, gene transcription, signal transduction, senescence, and inflammatory and stress response, the 26S proteasome plays a key role in the regulation and maintenance of basic cellular processes. Various synthetic and biologic inhibitors with different inhibitory profiles towards the proteolytic activities of the 26S proteasome have been identified recently. Such proteasome inhibitors induce apoptosis and cell cycle arrest preferentially in neoplastic cells. Based on these findings proteasome inhibitors became useful in cancer therapy. However, under the pressure of continuous proteasome inhibition, eukaryotic cells can develop complex adaptive mechanisms to subvert the lethal attack of proteasome inhibitors. Such mechanisms include the adaptive modification of the proteasome system with increased expression, enhanced proteolytic activity and altered subcomplex assembly and subunit composition of proteasomes as well as the expression of a giant oligomeric protease complex, tripeptidyl peptidase II, which partially compensates for impaired proteasome function. Here we review the adaptive mechanisms developed by eukaryotic cells in response to proteasome inhibition. These mechanisms reveal enormous flexibility of the proteasome system and may have implications in cancer biology and therapy.


*Keywords:* Proteasome; Proteasome inhibition; Proteasome inhibitor; Tripeptidyl peptidase II; Adaptive modification; Apoptosis resistance

## 1. Introduction

The ubiquitin–proteasome system has been identified as the cell's major tool for extralysosomal cytosolic and nuclear protein degradation, and the subtle characterization of its multiple biological functions has pointed out a new conceptional framework for understanding the regulation of basic cellular processes by controlled and limited proteolysis of cell proteins [1–6]. The 26S proteasome, a large multicatalytic multisubunit protease complex, constitutes the central proteolytic machinery of the ubiquitin–proteasome system and is responsible for the degradation and proteolytic processing of cell proteins essential for the regulation of development, differentiation, proliferation, cell cycling, apoptosis, gene transcription, signal transduction, senescence, antigen presentation, immune activation, and inflammatory and stress response, thereby governing basic cellular processes [[7,8,12,13]; Table 1].

Cell proteins destined to undergo processing by entering the ubiquitin–proteasome pathway must be targeted for recognition and subsequent degradation by the 26S proteasome by covalent attachments of multiple monomers of the 76 amino acid polypeptide ubiquitin. This process, called ubiquitination, takes place in a multistep reaction and requires three classes of enzymes (Fig. 1A): ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin–protein ligases (E3). E1 activate ubiquitin by forming a high-energy thiol ester bond between an E1 active site-located cysteine residue and the C-terminal glycine residue of ubiquitin in a reaction that requires the hydrolysis of ATP. This activated ubiquitin moiety is then transferred to E2 via the formation of an additional thiol

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Table 1
Characteristics of the 26S proteasome and TPP II

| | 26S Proteasome | TPP II |
|---|---|---|
| Molecular mass | ~2000 kDa (20S proteasome: ~700 kDa) | >1000 kDa |
| Subunit composition | 20S catalytic core complex capped at both ends by 19S or 11S regulatory complexes | Oligomeric assembly of identical 138 kDa subunits |
| Peptidase activities | Main activities: trypsin-like, chymotrypsin-like, caspase-like; additional activities: cleavage after branched chain amino acids, cleavage between small neutral amino acids | Cleavage of tripeptides from the free N-terminus of oligopeptides; preference for hydrophobic amino acids in P1 position |
| Substrates | Polyubiquitinated proteins; Antigenic proteins (preferentially by immunoproteasomes) | Oligopeptides, antigenic peptides, cholecystokinin |
| Type of enzyme | Threonine protease | Serine peptidase of the subtilisin type |
| Subcellular localization | Cytosol (extralysosomal), nucleus | Cytosol (extralysosomal), inner cytoplasmic membrane |
| Occurrence | Eukaryotes; in bacteria and archaea: 20S proteasome; homologues in bacteria and archaea: HsIV, ClpP, Lon | Eukaryotes; homologues in archaea: tricorn protease |

ester bond, and finally transferred to E3 which catalyze the covalent attachment of ubiquitin to the target protein by the formation of isopeptide bonds. Multiple cycles of ubiquitination finally result in the synthesis and attachment of polyubiquitin chains that serve as a recognition signal for the degradation of the target protein by the 26S proteasome [1,3].

The proteolytic activities of the 26S proteasome occur in a barrel-shaped 20S catalytic core complex composed of four axially stacked rings (Fig. 1B). Each outer ring consists of seven different non-proteolytic α subunits that allow conformational flexibility and substrate translocation into the central cavity of the 20S complex; the two inner rings are formed by seven different but related β subunits, giving the complex the general stoichiometry of $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ [5, 6, 9]. Only β1, β2, and β5 subunits harbor proteolytic sites formed by N-terminal threonine residues that face the central cavity of the 20S complex [5,6]. Based on their specificity toward oligopeptidyl substrates, β1, β2, and β5 subunits have been defined to possess caspase-like, trypsin-like and chymotrypsin-like peptidase activity, respectively [10,11]. During de novo biogenesis and assembly of the 20S complex, the constitutively expressed β1, β2, and β5 subunits can be replaced by IFN-γ-inducible homologous counterparts, the so-called immunosubunits β1i (LMP2), β2i (MECL1), and β5i (LMP7), leading to altered proteasomal cleavage site preference and increased proteasomal production of antigenic peptides for MHC class I presentation [12,13]. Both 20S species, containing either the constitutive β1, β2, and β5 subunits or the IFN-γ-inducible β1i, β2i, and β5i immunosubunits, are incapable of degrading ubiquitin-conjugated and folded substrate proteins [14]. However, they require for this task 19S (PA700) or 11S (PA28) regulatory complexes bound to the α rings and capping the 20S complex at both ends, leading to the assembly of 19S–20S–19S (26S proteasome) or 11S–20S–11S proteasome holoparticles [5,6]. The 19S complex exhibits a sophisticated multisubunit assembly consisting of six ATPase and at least eight non-ATPase subunits, and is required for recognition, deubiquitination, unfolding and translocation of substrate proteins before their proteolytic degradation within the central cavity of the 20S complex [15–18]. The 11S complex, also termed proteasome activator PA28, is an IFN-γ-inducible ring-shaped heptameric assembly composed of three α and four β subunits [19–22]. IFN-γ-inducible PA28αβ attaches to the outer α rings of the 20S complex and largely enhances proteasomal production of antigenic peptides for MHC class I presentation independently of the presence of immunosubunits within the 20S complex. Besides PA28αβ, a second kind of PA28, termed PA28γ, exists. PA28γ is a heptameric assembly of γ subunits structurally and functionally related to PA28αβ, but not inducible by IFN-γ [12,13,23–25].

Mammalian cells usually harbor a heterogeneous population of 20S complexes, which contain either the constitutive proteolytic β1, β2, and β5 subunits or the IFN-γ-inducible β1i, β2i, and β5i immunosubunits or a subunit composition intermediate between constitutive and immuno 20S complexes. Such 20S complexes can be further divided into subtypes that differ with regard to their enzymatic properties, subcellular localization and tissue distribution [26,27]. Similar to the diversity of 20S complexes, proteasome holoparticles exhibit three major species with different regulatory complex assemblies and enzymatic characteristics: 19S–20S–19S, PA28–20S-PA28, and 19S–20S–PA28, the latter referred to as hybrid proteasomes [28,29]. Except for the IFN-γ-induced expression of β1i, β2i, and β5i immunosubunits and PA28 that results in abundant biogenesis of immunoproteasomes, mechanisms that regulate subunit composition, proteolytic activity and the cellular repertoire and amount of proteasome species under steady-state or stress conditions are still rather unclear.

The identification and use of selective synthetic and biologic inhibitors of proteasomal proteolytic activities [30–32] (Table 2) have principally contributed to the identification of essential functions of the 26S proteasome in various processes and pathways of eukaryotic cells [7,33]. In particular, inhibition of proteasomal activities by proteasome inhibitors induces apoptosis preferentially in rapidly proliferating and neoplastic cells [7,34–36]. These findings have recently paved the way for the use of proteasome inhibitors in cancer therapy [37–39]. Moreover, proteasome inhibitors have been shown to suppress survival, activation, proliferation and cytokine synthesis of mouse and human T cells [30–44] and

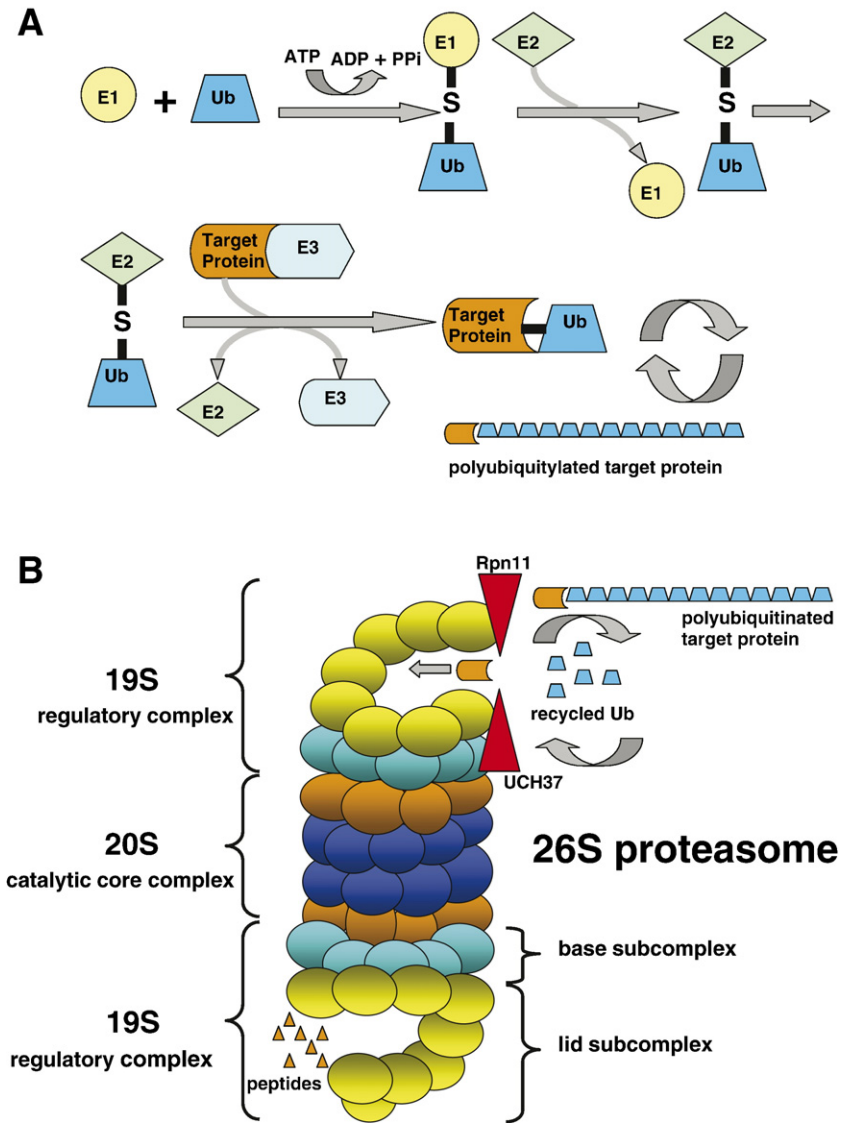


Fig. 1. The ubiquitin–proteasome pathway (schematic). (A) Attachment of ubiquitin (Ub) to the target protein requires three major enzymatic steps. Ubiquitin-activating enzyme (E1) activates ubiquitin by forming a high energy thiol esther bond between an E1 active site-located cystine residue and the C-terminal glycine residue of ubiquitin. This reaction requires energy provided by the hydrolysis of ATP and forms an activated ubiquitin moiety that is transferred and bound by an additional thiol ester bond to ubiquitin-conjugating enzyme (E2), which serves as a carrier protein. Ubiquitin–protein ligase (E3) catalyzes the covalent attachment of ubiquitin to the target protein by the formation of isopeptide bonds. Multiple cycles of ubiquitination finally result in the synthesis and attachment of polyubiquitin chains that serve as a recognition signal for the degradation of the target protein by the 26S proteasome. (B) The 26S proteasome consists of the barrel-shaped 20S catalytic core complex and two 19S regulatory complexes capping the 20S complex at both ends. The 20S complex is composed of four axially stacked rings. Each outer ring consists of non-proteolytic α subunits (orange). Each of the two inner rings are formed by seven proteolytic β subunits (blue) and three of them, β1, β2, and β5 are constitutively expressed proteolytic subunits that can be replaced by the IFN-γ-inducible immunosubunits β1i, β2i, and β5i. The 19S complex consists of the base and lid subcomplex. The base subcomplex contains six non-redundant ATPases of the AAA superfamily (green). The lid subcomplex (yellow) contains at least eight subunits including de-ubiquitinating enzymes and receptors for ubiquitinated proteins. The polyubiquitinated target protein enters the 19S regulatory complex, is recognized, deubiquitinated, unfolded, and translocated into the central cavity of the 20S catalytic core complex, where it is degraded by different hydrolytic activities. Ubiquitin is recycled by ubiquitin carboxy terminal hydrolases, i.e. Rpn11 and UCH37 which are associated with the lid and the base, respectively. Peptides as a product of degradation are released by diffusion.

human dendritic cells [45,46], and studies in rodents suggest that proteasome inhibitors can be used as immunosuppressive agents for the treatment of deregulated T cell-mediated immune responses, including those that contribute to the pathogenesis of polyarthritis, psoriasis, allograft rejection and graft-versus-host disease [41,43,47,48]. Finally, recent studies reveal that proteasome inhibitors can be used for the treatment of arterial restenosis [49] and for the activation of a protective antioxidant defense response in cardiovascular endothelial disorders [50].

Several recent studies have demonstrated complex mechanisms of adaptive modification of the proteasome system and the extralysosomal protease tripeptidylpeptidase II (TPP II) in response to severe cellular stress, such as short-term or continuous inhibition of proteasomal proteolytic activities. Dependent on the distinct proteasome inhibitors and cells types used, these studies

have led to different and even controversial results, showing either an increase or a decrease of proteasome activity, the latter accompanied by increased expression and activity of TPP II as a compensatory mechanism. Herein we review mechanisms of adaptive modification of the proteasome and the related protease TPP II in response to inhibition of proteasome activity in eukaryotic cells, pointing out some novel aspects of compensatory flexibility of highly organized extralysosomal cytosolic protease complexes such as the proteasome and TPP II.

## 2. The TPP II connection

Tripeptidyl peptidase II (TPP II; E.C. 3.4.14.10.) is a large >1000 kDa cytosolic serine peptidase of the subtilisin type that is built up of an oligomeric assembly of identical 138 kDa subunits [51,52]; Table 1]. TPP II cleaves tripeptides from the free $NH_2$-terminus of oligopeptides generated by different endopeptidases [51,53–56], and its proposed physiologic function is to participate in cytosolic protein degradation in concert with and downstream of different endopeptidases [55,56] and the proteasome [57–60]. A cytoplasmic membrane-bound isoform of TPP II has been identified to inactivate the neuropeptide cholecystokinin in rat cerebral neurons [61], revealing versatility of the enzyme. Besides its rather poorly defined function in general cytosolic protein degradation, TPP II has recently been identified as an endo- and exoproteolytic peptidase that processes peptides of more than 15 amino acids for MHC class I presentation that have been released from the proteasome or are resistant to proteasomal processing [57–60,62], albeit TPP II is unable to fully compensate for proteasomal MHC class I peptide production when proteasome activity is inhibited [63,64]. However, the precise tasks and roles of TPP II in eukaryotic cells remain rather elusive until now, but most interestingly, TPP II seems to have a further close functional relationship to the proteasome. In this regard there came first clues from an intriguing study conducted by Ploegh and co-workers, who demonstrated the existence of a proteolytic system that can compensate for loss of proteasome function [65]. In this study, mouse EL-4 T cell lymphoma cells were continuously grown in the presence of lethal concentrations of the proteasome inhibitor NLVS that initially led to apoptosis and growth inhibition of the cells. After 2–3 weeks of culturing in the presence of NLVS, a population of EL-4 cells grew out and exhibited dramatically reduced proteolytic activity of proteasomes and concomitant increased activity towards the hydrolysis of AAF-AMC, a fluorogenic oligopeptidyl substrate hydrolysed by TPP II, but also by proteasomes [54,65,66]. However, this increased AAF-AMC hydrolysing activity was detected in cytosolic protein fractions corresponding to higher molecular masses than those of 20S proteasomes, indicating the activity of a giant protease such as TPP II. This protease apparently compensated for loss of proteasomal proteolytic activity in NLVS-adapted EL-4 cells because proteasome-specific substrate proteins were still processed in the absence of proteasomal activity, and AAF-AMC hydrolysing activity, but not proteasomal proteolytic activity was essential for survival and growth of the cells [65]. In a subsequent study by Niedermann and co-workers, this AAF-AMC hydrolysing protease was isolated from cytosolic fractions of EL-4 cells adapted to the proteasome inhibitor lactacystin. Analysis by nano-electrospray tandem mass spectroscopy unambiguously revealed that it was in fact TPP II [51]. Subsequently, TPP II has been demonstrated to compensate in EL-4 cells for basic and specific proteasome functions, such as degradation of polyubiquitinated proteins and generation of MHC class I peptide ligands. This compensatory function could be observed in EL-4 cells with increased activity of TPP II due to adaptation to the proteasome inhibitor NLVS, and in NLVS-treated EL-4 cells with increased expression and activity of TPP II due to transfectional overexpression of TPP II [67]. Conversely, EL-4 cells adapted to NLVS retain considerable sensitivity to various highly specific proteasome inhibitors with regard to MHC class I peptide generation, accumulation of polyubiquitinated proteins, degradation of the proteasome-specific substrate protein p53, and cell viability [68]. This clearly demonstrates that residual proteolytic activity of proteasomes remains essential for ubiquitin-dependent extralysosomal cytosolic protein degradation in NLVS-adapted EL-4 cells with increased activity of TPP II, which is apparently unable to completely compensate for proteasome function in these cells.

As subsequently shown by Glas and co-workers, NLVS-adapted EL-4 cells display acquired resistance to apoptosis induced by diverse stimuli, and, compared to non-adapted EL-4 cells, establish rapidly growing tumors when engrafted in mice [69]. This acquired apoptosis resistance of NLVS-adapted EL-4 cells is mainly a consequence of the lack of proteasomal degradation of inhibitor of apoptosis proteins (IAPs), whose integrity contributes to tumor cell apoptosis resistance by the inhibition of caspases, the executioners of apoptosis [69–73]. Additionally, not only decreased proteasomal activity leading to the failure of degradation of IAPs, but also concomitant increased activity of TPP II confers apoptosis resistance and enables hyperproliferation of EL-4 cells [67]. This altered specificity of cytosolic proteolysis with an imbalance of proteasome activity and TPP II activity apparently renders cells apoptosis-resistant and leads to growth advantage of tumor cells, pointing out a fatal consequence of continuous and long-term proteasome inhibition.

## 3. The proteasome self-connection

From the results obtained in EL-4 cells it seemed clear that TPP II partially compensates for proteasome function and allows survival and apoptosis resistance in the scenario of continuous proteasome inhibition, but there is another major player on the stage: the proteasome itself. It was not entirely unexpected that a sophisticated and highly organized protease complex like the proteasome is able to undergo some adaptive modification under the pressure of proteasome inhibition, but it was intriguing to discover that there exists a proteasomal positive autoregulatory feedback mechanism that allows the compensation of insufficient proteasome activity following proteasome inhibition. The first indirect evidence for such a

mechanism was provided by a study in human MM.1S multiple myeloma cells treated with the proteasome inhibitor bortezomib. Gene expression profiles obtained by oligonucleotide-microarray analysis revealed a 4- to 64-fold up-regulation of transcription of proteasomal α and β subunits and subunits of the 19S regulatory complex after 8 h exposure of the cells to bortezomib [74]. These results demonstrated that short-term proteasome inhibition induces increased transcription of proteasome genes. In addition, increased protein expression of proteasomal subunits was demonstrated in *Drosophila* S2 cells with impaired proteasomal chymotrypsin-like peptidase activity as a result of targeted β5 subunit expression by RNA interference. Such cells treated for 4 days with double-stranded RNA blocking expression of proteasomal β5 subunit displayed increased expression of non-targeted subunits of the 20S complex (α2 and α7) and the 19S regulatory complex (Rpt2, Rpt6 and Rpn12), remained viable and retained partial viability in the presence of otherwise lethal concentrations of the proteasome inhibitors lactacystin, epoxomicin and bortezomib [75]. However, proteasomal β5 subunit-located chymotrypsin-like peptidase activity has been attributed to constitute the main proteolytic site of proteasomal protein degradation in mammalian cells [76]. From the results obtained in *Drosophila* S2 cells it could be speculated that isolated and short-term inhibition of this central proteasomal peptidase activity leads to increased expression of non-targeted proteasomal subunits, probably to substitute for the lack of chymotrypsin-like peptidase activity and to ensure viability. Subsequently, a study by Krüger and co-workers demonstrated transient and concerted up-regulation of most 26S proteasome subunit mRNAs, increased protein expression of proteasomal subunits β1, α6 and Rpt1, and increased de novo biogenesis of proteasomes accompanied by enhanced expression of the proteasome maturation protein POMP in rat vascular smooth muscle cells exposed for 6–8 h to the proteasome inhibitors c-lactacystin and MG132, which target proteasomal chymotrypsin-like peptidase activity [77]. These results have been complemented by a more recent study demonstrating increased mRNA levels and protein expression of proteasomal α and β subunits in murine neocortical neurons treated for 18 h with subtoxic concentrations of the proteasome inhibitor MG-132 [78]. In addition, a very recent study by Wójcik and co-workers demonstrated up-regulation of proteasomal 20S complexes and α7 and Rpt5 subunits in C-26 murine colon adenocarcinoma cells treated for 24 h and 48 h with subtoxic concentrations of the proteasome inhibitor bortezomib [79].

Thus, for the first time it was clear that mammalian cells respond to short-term and subtoxic inhibition of proteasomal proteolytic activity with a coordinated up-regulation of proteasome subunits at both the transcriptional and translational level, and compensate proteasome inhibition by de novo formation of proteasomes. But how do mammalian cells respond to long-term and toxic inhibition of proteasomal proteolytic activity? As discussed above, results from studies in murine EL-4 T cell lymphoma cells revealed compensatory increased proteolytic activity and up-regulation of TPPII in such a condition, but strikingly, recent studies in cells other than EL-4 cells have demonstrated increased expression and activity of proteasomes in response to long-term and toxic inhibition of proteasomal proteolytic activity. In particular, human A431 squamous carcinoma cells exposed for at least 2 months to lethal concentrations of the proteasome inhibitor epoxomicin could be adapted to grow in the presence of lethal and gradually increasing concentrations of epoxomicin. These cells exhibited increased protein expression of proteasomal β subunits accompanied by markedly increased proteasomal chymotrypsin-like peptidase activity, apparently to compensate continuous proteasome inhibition [80]. In contrast to their non-adapted counterparts, epoxomicin-adapted A431 cells displayed resistance to apoptosis induced by high concentrations of different proteasome inhibitors and the cytostatic drug doxorubicin [80]. These findings suggest that increased proteasome activity and β subunit expression in response to continuous proteasome inhibition was the major mechanism for the establishment of apoptosis resistance of the epoxomicin-adapted A431 cells. Finally, a recent study in human Namalwa Burkitt lymphoma cells adapted to grow in the presence of lethal concentrations of bortezomib demonstrated the occurrence of increased biogenesis and expression, altered subunit composition and increased proteolytic activity of proteasomes, and interestingly, markedly decreased expression and activity of TPP II [81]. After continuous exposure to lethal concentrations of bortezomib for at least 4 weeks, the cells grew out and exhibited increased de novo biogenesis of proteasomes accompanied by increased expression of the proteasome maturation protein POMP and non-proteolytic α and β subunits. The cells abundantly expressed 19S–20S–19S proteasomes, and completely abrogated expression of β1i, β2i, and β5i immunosubunits and PA28 in favor of increased expression of constitutive proteolytic β1, β2, and β5 subunits and 19S regulatory complexes. These alterations of proteasomal expression and subunit composition are accompanied by an increase in proteasomal caspase-like, trypsin-like and chymotrypsin-like peptidase activities, not inhibitable by high doses of proteasome inhibitors. Most notably, this adaptive modification of the proteasome system in Namalwa cells enables hyperproliferation and confers resistance to apoptosis induced by diverse stimuli, most likely due to de novo expression of anti-apoptotic protein Hsp27 and the loss of ability to stabilize and accumulate pro-apoptotic protein p53 [81]. Thus, increased biogenesis and expression, altered subunit composition and increased proteolytic activity of proteasomes constitute an adaptive mechanism of the proteasome system in response to the lethal challenge of continuous proteasome inhibition. This mechanism induced by continuous proteasome inhibition apparently establishes hyperproliferation and apoptosis resistance of tumor cells, as also observed in NLVS-adapted EL-4 cells, which, however, showed up-regulation of TPP II and down-regulation of the proteasome system.

## 4. Conclusions

It has become evident that the proteasome as a sophisticated and highly organized protease complex can undergo subtle

Table 2
Characteristics of Proteasome Inhibitors

| Proteasome inhibitor | Origin/source | Peptidase activities targeted | Use/comments | Reference |
|---|---|---|---|---|
| Lactacystin | *Streptomyces spec.*, irreversible inhibitor | All peptidase activities | Basic research, animal models | [85–87] |
| Epoxomicin | Peptide α′,β′-epoxyketone, *actinomycetes* strain No. Q996-17 | Chymotrypsin-like | Basic research, anti-inflammatory activity, inhibition of NFκB, antitumor activity (B16 murine melanom), modulation of antigen presentation | [88–90] |
| Gliotoxin | Epipolythiodioxo-piperazine, fungal metabolite from *Aspergillus fumigatus* | Chymotrypsin-like | Inhibition of NFêB in Hodgkin lymphoma Sternberg Reed cells, antimalaria therapy study: apoptosis of *P. falciparum* | [91,92] |
| TMC | Natural product from *Apiospora montagnei,* reversible inhibitor | All peptidase activities | Basic research | [93] |
| Epigallocatechin-gallate (EGCG) | Green tea polyphenol, reversible inhibitor | Chymotrypsin-like | Anticancer properties, NFκB inhibition | [94,95] |
| Bortezomib (Velcade®, PS-341) | Dipeptidyl boronic acid, reversible inhibitor, Millenium Pharmaceuticals | Chymotrypsin-like | Clinical use, multiple myeloma, solid tumors, non-Hodgkin lymphoma, murine xenograft models | [96–98] |
| Salinosporamide A (NPI-0052) and B (NPI-0047) | *Salinospora,* strain CNB-392, irreversible inhibitor, Nereus Pharmaceuticals | All peptidase activities | Chronic lymphatic leukemia, multiple myeloma | [99–101] |
| NLVS | Peptide vinyl sulfone | All peptidase activities | Basic research | [65,67–69,102] |
| MG132 | Z-Leu–Leu–Leu–aldehyde, peptide–aldehyd, reversible inhibitor MyoGenetics | Chymotrypsin-like | Basic research, modulation of antigen presentation | [103–105] |
| MG262 | Boronate analogue of MG132, Z-Leu–Leu–Leu-B(OH)2, more potent, reversible inhibitor | Chymotrypsin-like | Basic research | [68] |
| MG115 | N-CBZ–Leu–Leu–norvalinal, Z-LLnV, reversible inhibitor | Chymotrypsin-like | Basic research | [104] |
| ALLN | N-acetyl-leucyl-leucyl-norleucinal (ALLN), reversible inhibitor | Chymotrypsin-like | Basic research | [106] |

modifications in response to inhibition of its activity. Such modifications include down-regulation of proteasome activity and compensatory up-regulation of TPP II activity as well as increased de novo biogenesis and expression, altered subunit composition and increased proteolytic activity of the proteasome system. These modifications apparently constitute adaptive feedback mechanisms to allow eukaryotic cells to survive the lethal effects of proteasome inhibition and to establish a hyperproliferative and apoptosis-resistant phenotype. Furthermore, it can be speculated that higher eukaryotic cells harbor yet unknown sensory mechanisms that regulate levels of proteasome and TPP II expression and modes of proteasome subunit composition in response to proteasome inhibition. In yeast, however, up-regulation of proteasome subunits in response to proteasome inhibition can be explained by the activity of the proteasome-specific transcription factor Rpn4 whose ortholog has not been identified in the genome of higher eukaryotes [82–84]. The fact that mammalian cells respond in a similar manner to proteasome inhibition suggests that a functional ortholog of Rpn4 must exist and awaits discovery. Proteasome inhibitors have recently entered clinical trials for the treatment of various cancers (Table 2). In view of the adaptive and compensatory mechanisms developed by the proteasome system in response to its inhibition, proteasome inhibitors should be used carefully and, as far as possible, in combination with functionally unrelated cytostatic drugs in cancer therapy.

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