Review

The Ubiquitin-Proteasome System and Its Role in Inflammatory and Autoimmune Diseases

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Protein degradation through the ubiquitin-proteasome system is the major pathway of non-lysosomal proteolysis of intracellular proteins. It plays important roles in a variety of fundamental cellular processes such as regulation of cell cycle progression, division, development and differentiation, apoptosis, cell trafficking, and modulation of the immune and inflammatory responses. The central element of this system is the covalent linkage of ubiquitin to targeted proteins, which are then recognized by the 26S proteasome, an adenosine triphosphate-dependent, multi-catalytic protease. Damaged, oxidized, or misfolded proteins as well as regulatory proteins that control many critical cellular functions are among the targets of this degradation process. Aberration of this system leads to the dysregulation of cellular homeostasis and the development of multiple diseases. In this review, we described the basic biochemistry and molecular biology of the ubiquitin-proteasome system, and its complex role in the development of inflammatory and autoimmune diseases. In addition, therapies and potential therapeutic targets related to the ubiquitin-proteasome system are discussed as well. *Cellular & Molecular Immunology*. 2006;3(4): 255-261.

Key Words: ubiquitin, proteasome, protein degradation, inflammation, autoimmune disease

Introduction

The 2004 Nobel Prize in Chemistry was awarded to a group of scientists for the discovery of the function of ubiquitin (1-4). Ubiquitin is part of the ubiquitin-proteasome system (UPS) that is responsible for the degradation of more than 80% of normal and abnormal intracellular proteins. At the heart of this system is the 26S proteasome, a dynamic multisubunit proteolytic complex with a molecular weight of 700 kD that, in eukaryotes, functions as the key enzyme for non-lysosomal protein degradation. Proteasomal degradation removes denatured, misfolded, damaged or improperly translated proteins from cells and as well as regulating the level of proteins such as cyclins and transcription factors. The products resulting from this enzymatic degradation have different sequences, lengths and biological functions (5). It has been shown that this is a highly regulated, tightly controlled, yet complex system that is central to normal cellular homeostasis including cell cycle regulation, DNA repair, sodium channel function, regulation of immune and inflammatory responses and cellular response to stress (6-8). Derangements of the UPS can lead to many disorders, including malignancies, neurodegenerative diseases and possible systemic autoimmunity (9). Better understanding of the UPS process and identification of the components involved in the degradation of key regulatory proteins has lead to the development of mechanism-based therapeutics in various diseases (10-12). A growing body of evidence suggests that targeting the proteasomal pathway is an attractive approach to treat inflammatory and autoimmune diseases (13-15).

Ubiquitin and ubiquitination

Ubiquitin is a 76-residue protein that is highly evolutionarily conserved in all eukaryotes (16, 17). Selective attachment of ubiquitin to proteins is the initial signal for targeted protein degradation. The linkage of ubiquitin to the target protein is through a branched isopeptide bond between the ubiquitin carboxyl-terminal glycine and an internal lysine on the substrate. Additional ubiquitin moieties are sequentially added to each other to form a polyubiquitin chain that functions as the recognition signal for a downstream proteasome in the UPS. Modification of target proteins by ubiquitin or an ubiquitin-like protein remodels the surface of

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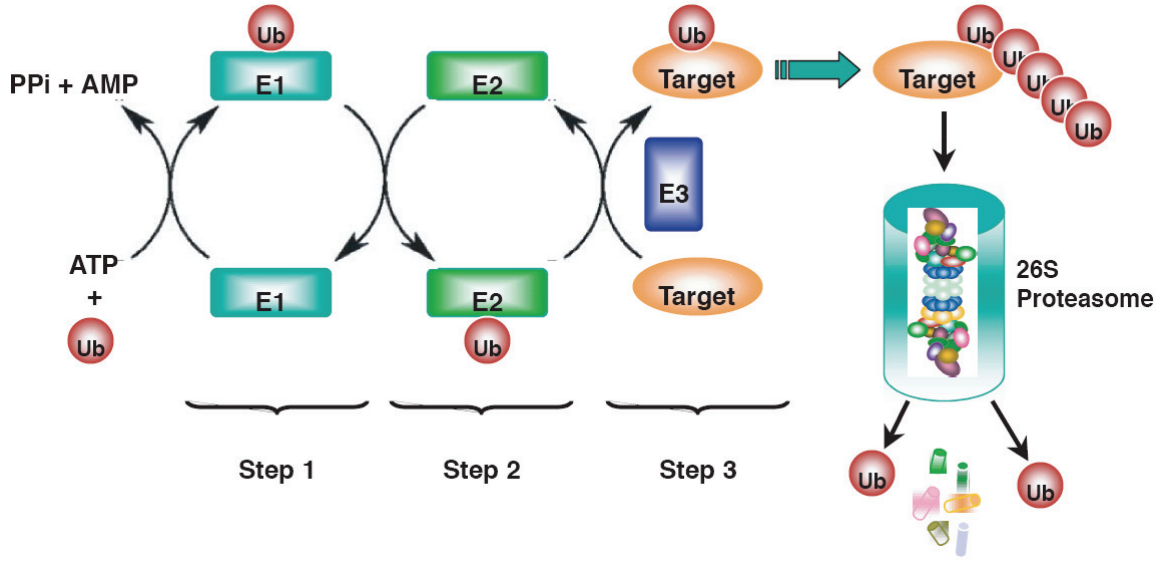

Figure 1. Schematic of the ubiquitin-proteasome system. Ubiquitin (Ub) is added sequentially to targeted substrates in three sequential steps requiring three enzymes, E1, E2 and E3. Step 1: Ubiquination begins with the adenosine triphosphate (ATP)-dependent activation of ubiquitin by the ubiquitin-activating enzyme E1; Step 2: Ubiquitin is then transferred to one of several forms of E2, or ubiquitin conjugating enzyme; Step 3: The addition of ubiquitin to the protein substrate is catalyzed by one of many E3s. Polyubiquinated proteins are recognized and degraded by the 26S proteasome. The cleavage produces small peptides and reusable free ubiquitin.

target proteins, affecting their stability, activity, interactions with other proteins, and subcellular localization (18, 19).

The ubiquitination of protein is carried out in three sequential steps involving three enzymes designated E1, E2 and E3 (Figure 1). E1 and E2 enzymes prepare the ubiquitin chain that is then attached to proteins by the E3 enzyme. Ubiquination begins with the adenosine triphosphate (ATP)-dependent activation of ubiquitin by the ubiquitin-activating enzyme E1. Ubiquitin is attached to an internal E1 Cys residue *via* an intermediate thiol ester generating E1-S~ubiquitin. Ubiquitin is then transferred to one of several forms of E2, or ubiquitin conjugating enzyme. In step three, the addition of ubiquitin to the protein substrate is catalyzed by one of many E3s - a diverse group of proteins with distinct motifs (20).

The high specificity and selectivity of the UPS system lies in the diversity of different ubiquitin-protein ligase E3s that can recognize a specific substrate (7). One of the most important recognition patterns is a "destabilizing" N-terminal amino acid such as arginine and lysine. These unique N-terminal residues can determine the half-life of an intracellular protein and has been defined as the N-end rule. In addition to its substrates, the activity of the ubiquitin system itself can be modulated by numerous factors such as thyroid hormones, glucocorticoid steroids, cytokines, and proteins expressed in malignant cells such as proteolysis-inducing factor (PIF). Interestingly, several factors, including interferon γ (IFN-γ) not only regulate the modification of substrate, such as the IκB family of proteins, but also modify the components of the enzymatic machinery of the ubiquitin system, such as the E3 ubiquitin ligase Itch, and the proteasome complex (21). Similarly, the protein kinase C and tyrosine kinase pathways are involved in both the modification of UPS substrates and phosphorylation of E1 and E2, thereby ensuring their activities are carried out efficiently.

Proteasome

20S proteasome

The ubiquinated substrates targeted for proteolysis are recognized by proteasomes – the central element of the UPS. In the proteasome complex, the core protease (CP) is the barrel-shaped 20S complex (20S proteasome) (Figure 2). It consists of four stacked rings each with 7 distinct subunits, stacked one on top of each other, that are responsible for the proteolytic activity of the proteasome. There are two identical outer α rings and two inner β rings. The outer 2 α-rings have no known function, whereas the β rings contain multiple catalytic sites. In eukaryotes, two of these sites on the β rings are chymotrypsin-like (CTL), two of these sites are trypsin-like (TL) and two are caspase-like. Essentially all known synthetic and natural proteasome inhibitors act on the CTL sites (22).

26S proteasome

The 26S proteasome is composed of 2 subcomplexes – one 20S proteasome and two 19S regulatory particles (RP, also known as PA700) – which cap the ends of the 20S complex (Figure 2). The 19S RP controls the recognition of the ubiquinated proteins, the ATP-dependent unfolding and the

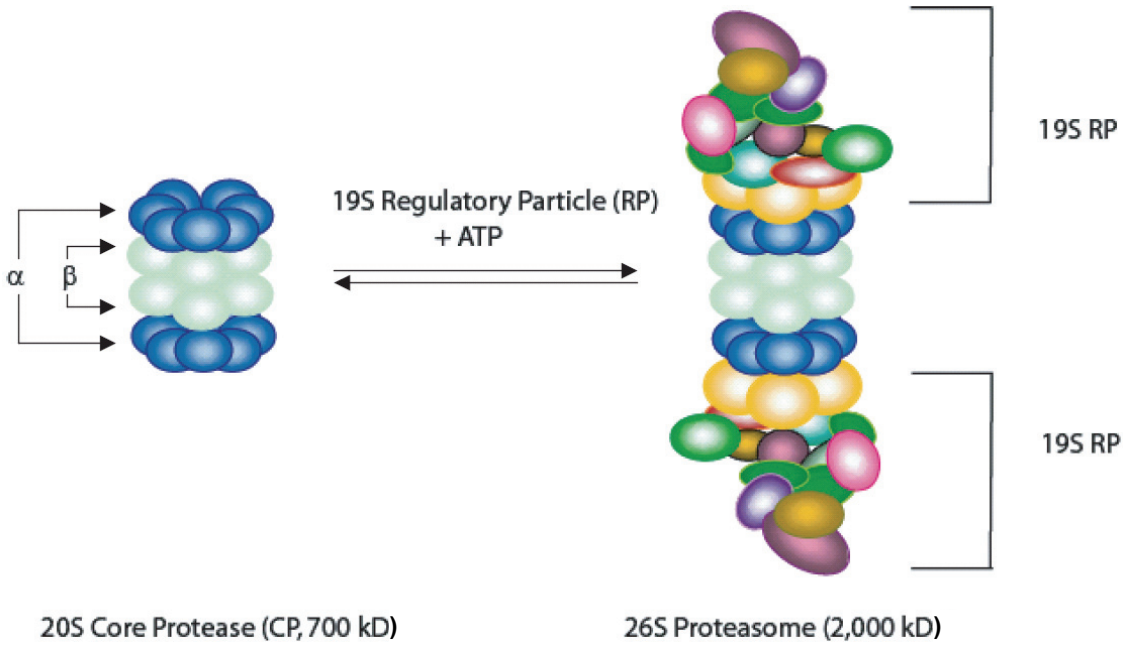

Figure 2. Schematic of the 20S core protease (CP) and the 26S proteasome. The 26S proteasome is composed of the barrel-shaped 20S complex with a molecular weight of about 700 kD capped by two 19S regulatory particles (RP). The molecular weight for this 26S proteasome is about 2000 kD. The 19S regulatory particle recognizes the polyubiquitin tag on targeted substrates and unfolds the substrate to enter the proteolytic chamber. The 20S core particle contains the catalytic sites responsible for the proteolysis.

opening of the channel in the 20S proteasome that allows entry into the proteolytic chamber.

Immunoproteasome

PA28 is an activator of the 20S proteasome and an alternative RP. It is composed of two homologous subunits (α and β) and a separate but related protein termed PA28γ which is also known as the Ki antigen (23). PA28 associates with the 20S proteasome to form a football-like structure, hybrid proteasome (19S RP-20S proteasome-PA28) - a complex referred to as an immunoproteasome due to its enhanced capability to generate major histocompatibility complex (MHC) class I-binding peptides. The expression of PA28 is inducible by IFN-γ, which is different from its constitutively expressed counterparts α and β subunits in 26S proteasome. More recently, it was noted that IFN-γ induces the expression of PA28-α and PA28-β, but not the Ki antigen in human cells, which indicates the PA28 family of proteins may have distinct biological functions (24).

After ubiquinated proteins have been degraded within the CP, the 19S RP removes the polyubiquitin tag from the substrate protein and generates free and reusable ubiquitin. Cleaved peptides generated by proteasome degradation can have different fates. Some are further degraded by cytosolic peptidases. Some can be transported into the endoplasmic reticulum (ER) for binding to MHC class I molecules and cell-surface expression. This latter subset of antigenic peptides may arise from hybrid proteasome that generates peptides trimmed to properly fit the MHC class I binding groves. In this regard, immunoproteasomes have unique value during immune responses.

UPS and inflammatory and autoimmune diseases

The UPS is responsible for the majority of eukaryotic intracellular protein turnover (1, 2). Coordinated UPS function is essential to variety of cellular processes (17, 25). Aberration of the system can lead to the dysregulation of cellular homeostasis and the development of multiple diseases such as malignancy (12, 26-35), neurodegenerative diseases (36), and cardiovascular diseases (37-39). This review, however, will focus on the role of UPS in the immune system and the development of inflammatory and autoimmune diseases.

UPS and the immune system

A prime example for the involvement of UPS in the immune system is MHC class I antigen processing by antigen-presenting cells (APC) (40). It has been well established that the majority of peptide antigens presented on MHC I molecules are generated by the UPS (41). As previously mentioned, PA28, a ring-shaped 11S multimeric complex, mediates this line of function within the UPS (42). It has been demonstrated that PA28 binds to the ends of 20S proteasome and dramatically enhances its capability to hydrolyze oligopeptides, which leads to the generation of small oligopeptides suitable for MHC I presentation (43, 44). More specifically, the proteasome is responsible for

generating the precise C termini of many MHC-presented peptides, whereas aminopeptidases in the cytoplasm and endoplasmic reticulum can trim the N terminus of the extended peptides to their proper size (45, 46). Interestingly, IFN-γ is a key pleiotropic regulatory cytokine within this context (47). It controls an inducible proteolytic cascade, which consists of PA28 and other inducible proteasome subunits, and the activity of aminopeptidases, which lead to increased peptide production for MHC I presentation. In addition, IFN-γ also decreases peptide destruction by down-regulating the expression of a metalloproteinase, thimet oligopeptidase, that actively destroys many antigenic peptides (48, 49).

UPS plays a significant role in the regulation of both T cell receptor (TCR) and costimulatory CD28 signaling through the action of ubiquitin ligases of the Cbl family (50). CD28 costimulation results in the ubiquitination and degradation of Cbl-β, which eliminates the negative regulators and allows the expression of proinflammatory cytokines and their receptors. However, the most important link between the UPS and inflammation is related to NF-κB. NF-κB is a master regulator of many inflammatory cytokine genes, and its activation is mediated through the UPS. NF-κB is actively inhibited when bound to IκB. NF-κB activation follows the degradation of IκB, which is dependent on ubiquination of IκB followed by proteasomal degradation. Hence, alterations in the UPS would have profound effects on immune responses including the regulation of an array of inflammatory cytokines. In addition, proteasome has been shown to regulate inflammatory pathway by controlling the function of macrophages (51).

It has also been reported that the deubiquitinating enzyme CYLD positively regulates proximal T cell receptor signaling in thymocytes by selectively binding to and deubiquitinating the active form of the kinase Lck. Due to cell-autonomous defect in T cell development, CYLD-deficient mice had substantially fewer mature $CD4^+$ and $CD8^+$ single-positive thymocytes and peripheral T cells (52).

Autoimmunity to components of UPS

Autoantibodies to the α and β subunits of 20S proteasome, PA28 and other components of the UPS have been detected in systemic lupus erythematosus (SLE), primary Sjögren's syndrome, myositis and other autoimmune diseases (53, 54). Autoantibodies to Ki antigen were detected in 6-21% of patients with SLE. The antibodies were associated with certain clinical features such as sicca syndrome [xerostomia (dry mouth), xerophthalmia (dry eyes), and lymphocytic infiltration of the exocrine glands], persistent arthritis, pericarditis, positive anti-Sm antibody and skin involvement in lupus (54, 55). The functional relevance of these auto-antibodies remains to be determined. However, it has been shown that autoantibodies against 20S proteasome have the capability to block proteasome activation by PA28 *in vitro*, which indicates that autoantibodies may have a regulatory role in proteasome function, and antibody targeting of the interaction between proteasome subunits represents a novel mechanism of proteasome inhibition (56, 57).

Increased levels of circulating proteasomes has also been observed in autoimmune myositis, SLE, primary Sjögren's syndrome, rheumatoid arthritis (RA) and other autoimmune diseases (58). In addition, circulating proteasome levels correlate with disease activity in RA and SLE patients. Hence, it has the potential to serve as a biomarker for cellular damage and disease activity in these patients (57).

UPS and inflammatory and autoimmune diseases

The UPS is involved in the development of inflammatory and autoimmune diseases through multiple pathways, including MHC-mediated antigen presentation, cytokine and cell cycle regulation, and apoptosis (59).

1) Inflammatory arthritis. NF-κB regulates multiple critical cytokines involved in the pathogenesis of RA (60, 61). In the peptoglycan/polysaccharide-induced inflammatory arthritis model, a proteasome inhibitor improved the arthritis score by suppressing the activation of NF-κB, reducing the expression of cell adhesion molecules and IL-6. In addition, proteasome inhibition may regulate the development of inflammatory arthritis by controlling angiogenesis (62).

2) Psoriasis. Psoriasis is one of the prototypical T cell-mediated diseases, and its development is related to the activation of NF-κB. Administration of a proteasome inhibitor reduced the size of psoriatic lesions in human skin explants grafted onto mice. The treatment also resulted in reduced superantigen-mediated T-cell activation, attenuated cell adhesion molecule expression, decreased expression of T-cell activation markers that were significantly elevated during the disease process (63).

3) Allergy and asthma. Abnormal activation of type 2 helper T cells (Th2) results in asthmatic and allergic symptoms (64). E3 ubiquitin ligase Itch plays a critical role in maintaining immune tolerance mediated through Th2 cells both *in vitro* and *in vivo*. Itch deficient mice failed to block the development of airway inflammation in an allergic model (65). Consistent with these findings, encouraging therapeutic effects were observed in a rodent model of allergen-induced asthma (66).

4) Other inflammatory and autoimmune diseases. Sero-negative spondyloarthropathies (SpA) are a group of diseases characterized by, but not limited to, axial joint inflammation. Ankylosing spondylitis (AS) is the prototypical SpA. Most patients with AS carry the MHC class I HLA-B27 gene, and so much research effort has been directed at understanding the role of this gene in the disease pathogenesis. Much interest has been focused on determining the origin and nature of the peptides being presented by HLA-B27 and the cell surface expression of misfolded HLA-B27, two areas in which the UPS is known to play a role. The UPS is involved in the regulation or induction of apoptosis. Apoptosis has been implicated in both experimental models and clinical SLE. In mature, activated lymphocytes, the proteasome inhibitor lactacystin induces DNA fragmentation and apoptosis in a dose-dependent fashion, indicating that proteasome suppresses apoptosis in these cells. Altered clearance of autoantigens is thought to allow for targeting by

the immune system and the development of autoimmunity. The involvement of UPS in regulating the levels of Ku70 and other autoantigens has been reported (67-69).

Proteasome inhibitors as novel therapeutic agents

An array of synthetic and natural inhibitors of the proteolytic sites on the 20S proteasome have been developed both as research tools, and more importantly, as therapeutic agents (9, 13, 70-72). Currently there are several inhibitors either approved or in clinical trials for the treatment of multiple cancers and strokes (71, 73). These inhibitors have the capability of entering cells and blocking protein degradation by the ubiquitine-proteasome pathway. They have also proven to be valuable tools for investigating the basic intracellular function of proteasomes and have facilitated the discovery of numerous novel regulatory functions of UPS.

Proteasome inhibitors represent a novel class of anticancer drug (74). Preclinical studies have demonstrated that bortezomib, a dipeptidyl boronic acid, that is a selective and potent inhibitor of the 26S proteasome, decreases proliferation, induces apoptosis, enhances the activity of chemotherapy and radiation, and reverses chemoresistance in a variety of hematologic and solid malignancy models both *in vitro* and *in vivo* (75). It has been shown that bortezomib inhibits PKR-like endoplasmic reticulum kinase (PERK), and sensitizes pancreatic cancer cells to endoplasmic reticulum stress-mediated apoptosis (33, 76). Bortezomib was the first proteasome inhibitor to enter clinical trials. Recently, bortezomib received Food and Drug Administration (FDA) approval for the use of multiple myeloma and is being evaluated for the treatment of solid tumors (33, 34, 74, 77). The antineoplastic effects of bortezomib have been attributed, at least in part, to inhibition of IκB degradation leading to inactivation of the pivotal pro-survival transcription factor, NF-κB (74).

In addition to anti-tumor effects, proteasome inhibitors also have direct impact on the inflammatory pathway. With the help of a proteasome inhibitor, IκB was the first substrate of UPS identified (78). Through regulation of IκB degradation, the UPS controls the activity of NF-κB, which activates the expression of many genes encoding key inflammatory mediators such as cytokines (TNF and IL-1), leukocyte adhesion molecules (ICAM, VCAM) (79), and enzymes (cyclooxygenase, nitric oxide synthetase) (71). In preclinical animal models, selective proteasome inhibitors effectively suppress inflammatory arthritis and other inflammatory conditions (80-84). In recent years, numerous novel drugs for the treatment of rheumatologic diseases have been successfully developed. The main approach has been to target cytokines, immune cells and their activation pathways (85). Hence proteasome inhibitors could represent a novel class of drugs through regulation of catabolism of pro-inflammatory proteins. On the other hand, proteasome inhibition stabilizes short-lived cyclooxygenase II, a critical enzyme involved in synthesis of inflammatory prostaglandins in the neuronal cell line, so their use could potentially exacerbates inflammation (86).

Conclusion

The UPS is a complex system that controls many important aspects of cell function. The covalent linkage of ubiquitin to protein substrates can selectively and specifically alter their fate through proteolytic and non-proteolytic pathways. Loss of normal homeostasis though many mechanisms, including stress or infection, can lead to aberrant cellular function and diseases. Increase in ubiquitinated products has been noted in a variety of pathophysiologic states associated with increased oxidative stress such as neurodegenerative diseases and coronary atherogenesis (39, 87). So far, dysregulation of the UPS has been linked to the pathogenesis of a variety of inherited and acquired diseases such as cancer, diabetes, stroke, graft injection, Alzheimer's disease, amyotropic lateral sclerosis, multiple sclerosis, asthma, inflammatory bowl disease, autoimmune thyroiditis, inflammatory arthritis and SLE. The involvement of the UPS in the pathogenesis of inflammatory and autoimmune diseases is mediated through various mechanisms. Multiple lines of evidence have indicated UPS has the potential to be an exciting novel therapeutic target for the treatment of inflammatory and autoimmune diseases.

References

1. Hershko A. The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. Cell Death Differ. 2005;12:1191-1197.
2. Ciechanover A. Intracellular protein degradation: from a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. Cell Death Differ. 2005;12:1178-1190.
3. Rose I. Ubiquitin at Fox Chase. Cell Death Differ. 2005;12: 1198-1201.
4. Ciechanover A, Iwai K. The ubiquitin system: from basic mechanisms to the patient bed. IUBMB Life. 2004;56:193-201.
5. Bogyo M, Shin S, McMaster JS, Ploegh HL. Substrate binding and sequence preference of the proteasome revealed by active-site-directed affinity probes. Chem Biol. 1998;5:307-320.
6. Ciechanover A. The ubiquitin proteolytic system: from a vague idea, through basic mechanisms, and onto human diseases and drug targeting. Neurology. 2006;66:S7-19.
7. Ciechanover A. The ubiquitin-proteasome pathway: on protein death and cell life. EMBO J. 1998;17:7151-7160.
8. Malik B, Price SR, Mitch WE, Yue Q, Eaton DC. Regulation of epithelial sodium channels by the ubiquitin-proteasome proteolytic pathway. Am J Physiol Renal Physiol. 2006;290: F1285-1294.
9. Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin-proteasome system. Nat Rev Drug Discov. 2006;5: 596-613.
10. Mitsiades CS, Mitsiades N, Hideshima T, Richardson PG, Anderson KC. Proteasome inhibitors as therapeutics. Essays Biochem. 2005;41:205-218.
11. Hideshima T, Richardson P, Chauhan D, et al. The proteasome

inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res. 2001;61:3071-3076.
12. Hideshima T, Bradner JE, Chauhan D, Anderson KC. Intracellular protein degradation and its therapeutic implications. Clin Cancer Res. 2005;11:8530-8533.
13. Goldberg AL, Rock K. Not just research tools--proteasome inhibitors offer therapeutic promise. Nat Med. 2002;8:338-340.
14. Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol. 2006;17:1807-1819.
15. Ciechanover A. The ubiquitin proteolytic system and pathogenesis of human diseases: a novel platform for mechanism-based drug targeting. Biochem Soc Trans. 2003;31:474-481.
16. Nandi D, Tahiliani P, Kumar A, Chandu D. The ubiquitin-proteasome system. J Biosci. 2006;31:137-155.
17. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998;67:425-479.
18. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. Nature. 2003;426:895-899.
19. Welchman RL, Gordon C, Mayer RJ. Ubiquitin and ubiquitin-like proteins as multifunctional signals. Nat Rev Mol Cell Biol. 2005;6:599-609.
20. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell. 1994;79:13-21.
21. Yang C, Zhou W, Jeon MS, et al. Negative regulation of the E3 ubiquitin ligase itch *via* Fyn-mediated tyrosine phosphorylation. Mol Cell. 2006;21:135-141.
22. Adams J. The proteasome: structure, function, and role in the cell. Cancer Treat Rev. 2003;29 Suppl 1:3-9.
23. Nikaido T, Shimada K, Shibata M, et al. Cloning and nucleotide sequence of cDNA for Ki antigen, a highly conserved nuclear protein detected with sera from patients with systemic lupus erythematosus. Clin Exp Immunol. 1990;79:209-214.
24. Tanahashi N, Yokota K, Ahn JY, et al. Molecular properties of the proteasome activator PA28 family proteins and γ-interferon regulation. Genes Cells. 1997;2:195-211.
25. Kaiser P, Tagwerker C. Is this protein ubiquitinated? Methods Enzymol. 2005;399:243-248.
26. Moon RT. Wnt/β-catenin pathway. Sci STKE. 2005;2005:cm1.
27. Adams J, Palombella VJ, Elliott PJ. Proteasome inhibition: a new strategy in cancer treatment. Invest New Drugs. 2000;18: 109-121.
28. Spano JP, Bay JO, Blay JY, Rixe O. Proteasome inhibition: a new approach for the treatment of malignancies. Bull Cancer. 2005;92:E61-66, 945-952.
29. Leonard JP, Furman RR, Coleman M. Proteasome inhibition with bortezomib: A new therapeutic strategy for non-Hodgkin's lymphoma. Int J Cancer. 2006;119:971-979.
30. Richardson PG, Mitsiades C, Hideshima T, Anderson KC. Proteasome inhibition in the treatment of cancer. Cell Cycle. 2005;4:290-296.
31. Dou QP, Smith DM, Daniel KG, Kazi A. Interruption of tumor cell cycle progression through proteasome inhibition: implications for cancer therapy. Prog Cell Cycle Res. 2003;5: 441-446.
32. Vlahakis SR, Badley AD. Influence of proteasome inhibitors on apoptosis. Curr Opin Clin Nutr Metab Care. 2006;9:42-47.
33. Nawrocki ST, Carew JS, Pino MS, et al. Bortezomib sensitizes pancreatic cancer cells to endoplasmic reticulum stress-mediated apoptosis. Cancer Res. 2005;65:11658-11666.
34. Papandreou CN, Logothetis CJ. Bortezomib as a potential treatment for prostate cancer. Cancer Res. 2004;64:5036-5043.
35. Mani A, Gelmann EP. The ubiquitin-proteasome pathway and its role in cancer. J Clin Oncol. 2005;23:4776-4789.
36. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron. 2003;40:427-446.
37. Zolk O, Schenke C, Sarikas A. The ubiquitin-proteasome system: Focus on the heart. Cardiovasc Res. 2006;70:410-421.
38. Herrmann J, Ciechanover A, Lerman LO, Lerman A. The ubiquitin-proteasome system in cardiovascular diseases-a hypothesis extended. Cardiovasc Res. 2004;61:11-21.
39. Versari D, Herrmann J, Gossl M, et al. Dysregulation of the ubiquitin-proteasome system in human carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 2006; in press.
40. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. Annu Rev Immunol. 1999;17:739-779.
41. Rock KL, Gramm C, Rothstein L, et al. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell. 1994;78:761-771.
42. Cascio P, Call M, Petre BM, Walz T, Goldberg AL. Properties of the hybrid form of the 26S proteasome containing both 19S and PA28 complexes. EMBO J. 2002;21:2636-2645.
43. Cascio P, Hilton C, Kisselev AF, Rock KL, Goldberg AL. 26S proteasomes and immunoproteasomes produce mainly N-extended versions of an antigenic peptide. EMBO J. 2001;20: 2357-2366.
44. Saric T, Chang SC, Hattori A, et al. An IFN-γ-induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. Nat Immunol. 2002;3:1169-1176.
45. Mo XY, Cascio P, Lemerise K, Goldberg AL, Rock K. Distinct proteolytic processes generate the C and N termini of MHC class I-binding peptides. J Immunol. 1999;163:5851-5859.
46. Strehl B, Seifert U, Kruger E, Heink S, Kuckelkorn U, Kloetzel PM. Interferon-γ, the functional plasticity of the ubiquitin-proteasome system, and MHC class I antigen processing. Immunol Rev. 2005;207:19-30.
47. Goldberg AL, Cascio P, Saric T, Rock KL. The importance of the proteasome and subsequent proteolytic steps in the generation of antigenic peptides. Mol Immunol. 2002;39:147-164.
48. York IA, Goldberg AL, Mo XY, Rock KL. Proteolysis and class I major histocompatibility complex antigen presentation. Immunol Rev. 1999;172:49-66.
49. Saveanu L, Carroll O, Hassainya Y, van Endert P. Complexity, contradictions, and conundrums: studying post-proteasomal proteolysis in HLA class I antigen presentation. Immunol Rev. 2005;207:42-59.
50. Zhang J, Bardos T, Li D, et al. Cutting edge: regulation of T cell activation threshold by CD28 costimulation through targeting Cbl-β for ubiquitination. J Immunol. 2002;169:2236-2240.
51. Qureshi N, Vogel SN, Van Way C, 3rd, Papasian CJ, Qureshi AA, Morrison DC. The proteasome: a central regulator of inflammation and macrophage function. Immunol Res. 2005;31: 243-260.
52. Reiley WW, Zhang M, Jin W, et al. Regulation of T cell development by the deubiquitinating enzyme CYLD. Nat Immunol. 2006;7:411-417.
53. Kaneda K, Takasaki Y, Takeuchi K, et al. Autoimmune response to proteins of proliferating cell nuclear antigen multiprotein complexes in patients with connective tissue diseases. J Rheumatol. 2004;31:2142-2150.
54. Matsushita M, Takasaki Y, Takeuchi K, Yamada H, Matsudaira R, Hashimoto H. Autoimmune response to proteasome activator 28α in patients with connective tissue diseases. J Rheumatol.

2004;31:252-259.
55. Cavazzana I, Franceschini F, Vassalini C, et al. Clinical and serological features of 35 patients with anti-Ki autoantibodies. Lupus. 2005;14:837-841.
56. Brychcy M, Kuckelkorn U, Hausdorf G, et al. Anti-20S proteasome autoantibodies inhibit proteasome stimulation by proteasome activator PA28. Arthritis Rheum. 2006;54:2175-2183.
57. Mountz JD. Significance of increased circulating proteasome in autoimmune disease. J Rheumatol. 2002;29:2027-2030.
58. Egerer K, Kuckelkorn U, Rudolph PE, et al. Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases. J Rheumatol. 2002;29:2045-2052.
59. Colmegna I, Sainz B, Jr., Garry RF, Espinoza LR. The proteasome and its implications in rheumatology. J Rheumatol. 2005;32:1192-1198.
60. Firestein GS. NF-κB: Holy Grail for rheumatoid arthritis? Arthritis Rheum. 2004;50:2381-2386.
61. Agarwal S, Deschner J, Long P, et al. Role of NF-κB transcription factors in antiinflammatory and proinflammatory actions of mechanical signals. Arthritis Rheum. 2004;50:3541-3548.
62. Elliott PJ, Zollner TM, Boehncke WH. Proteasome inhibition: a new anti-inflammatory strategy. J Mol Med. 2003;81:235-245.
63. Zollner TM, Podda M, Pien C, Elliott PJ, Kaufmann R, Boehncke WH. Proteasome inhibition reduces superantigen-mediated T cell activation and the severity of psoriasis in a SCID-hu model. J Clin Invest. 2002;109:671-679.
64. Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. Nat Immunol. 2002;3:715-720.
65. Venuprasad K, Elly C, Gao M, et al. Convergence of Itch-induced ubiquitination with MEKK1-JNK signaling in Th2 tolerance and airway inflammation. J Clin Invest. 2006;116:1117-1126.
66. Elliott PJ, Pien CS, McCormack TA, Chapman ID, Adams J. Proteasome inhibition: A novel mechanism to combat asthma. J Allergy Clin Immunol. 1999;104:294-300.
67. Wang J, Dong X, Reeves WH. A model for Ku heterodimer assembly and interaction with DNA. Implications for the function of Ku antigen. J Biol Chem. 1998;273:31068-31074.
68. Wang J, Satoh M, Pierani A, et al. Assembly and DNA binding of recombinant Ku (p70/p80) autoantigen defined by a novel monoclonal antibody specific for p70/p80 heterodimers. J Cell Sci. 1994;107(Pt 11):3223-3233.
69. Gama V, Yoshida T, Gomez JA, et al. Involvement of the ubiquitin pathway in decreasing Ku70 levels in response to drug-induced apoptosis. Exp Cell Res. 2006;312:488-499.
70. Gaczynska M, Osmulski PA. Small-molecule inhibitors of proteasome activity. Methods Mol Biol. 2005;301:3-22.
71. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. Chem Biol. 2001;8:739-758.
72. Tsukamoto S, Yokosawa H. Natural products inhibiting the ubiquitin-proteasome proteolytic pathway, a target for drug development. Curr Med Chem. 2006;13:745-754.
73. Kisselev AF, Callard A, Goldberg AL. Importance of the different proteolytic sites of the proteasome and the efficacy of inhibitors varies with the protein substrate. J Biol Chem. 2006;281:8582-8590.
74. Richardson PG, Mitsiades C, Hideshima T, Anderson KC. Bortezomib: proteasome inhibition as an effective anticancer therapy. Annu Rev Med. 2006;57:33-47.
75. Adams J. The development of proteasome inhibitors as anticancer drugs. Cancer Cell. 2004;5:417-421.
76. Nawrocki ST, Carew JS, Dunner K, Jr., et al. Bortezomib inhibits PKR-like endoplasmic reticulum (ER) kinase and induces apoptosis *via* ER stress in human pancreatic cancer cells. Cancer Res. 2005;65:11510-11519.
77. Papandreou CN, Daliani DD, Nix D, et al. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. J Clin Oncol. 2004;22:2108-2121.
78. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. Cell. 1994;78:773-785.
79. Pahl HL. Activators and target genes of Rel/NF-κB transcription factors. Oncogene. 1999;18:6853-6866.
80. Palombella VJ, Conner EM, Fuseler JW, et al. Role of the proteasome and NF-κB in streptococcal cell wall-induced polyarthritis. Proc Natl Acad Sci U S A. 1998;95:15671-15676.
81. Meng L, Mohan R, Kwok BH, Elofsson M, Sin N, Crews CM. Epoxomicin, a potent and selective proteasome inhibitor, exhibits *in vivo* antiinflammatory activity. Proc Natl Acad Sci U S A. 1999;96:10403-10408.
82. Grisham MB, Palombella VJ, Elliott PJ, et al. Inhibition of NF-κB activation *in vitro* and *in vivo*: role of 26S proteasome. Methods Enzymol. 1999;300:345-363.
83. Bo Kim K, Fonseca FN, Crews CM. Development and characterization of proteasome inhibitors. Methods Enzymol. 2005;399:585-609.
84. Crews CM. Feeding the machine: mechanisms of proteasome-catalyzed degradation of ubiquitinated proteins. Curr Opin Chem Biol. 2003;7:534-539.
85. Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med. 2004;350:2167-2179.
86. Rockwell P, Yuan H, Magnusson R, Figueiredo-Pereira ME. Proteasome inhibition in neuronal cells induces a proinflammatory response manifested by upregulation of cyclooxygenase-2, its accumulation as ubiquitin conjugates, and production of the prostaglandin PGE(2). Arch Biochem Biophys. 2000;374:325-333.
87. Layfield R, Lowe J, Bedford L. The ubiquitin-proteasome system and neurodegenerative disorders. Essays Biochem. 2005;41:157-171.