Catalytic Mechanism and Assembly of the Proteasome

António J. Marques,[†] R. Palanimurugan,[†] Ana C. Matias,[†,‡,§] Paula C. Ramos,[*,‡,§] and R. Jürgen Dohmen[*,†]

Institute for Genetics, University of Cologne, Zülpicher Strasse 47, D-50674 Cologne, Germany, Department of Chemistry, Biochemistry and Pharmacy, University of Algarve, Faro, Portugal, and Institute for Biotechnology and Bioengineering, Centre for Molecular and Structural Biomedicine, University of Algarve, Faro, Portugal

Received October 8, 2008

Contents

* Corresponding authors. E-mail: pcramos@ualg.pt (P.C.R.), j.dohmen@uni-koeln.de (R.J.D.).
[†] University of Cologne.
[‡] Department of Chemistry, Biochemistry and Pharmacy, University of Algarve.
[§] Institute for Biotechnology and Bioengineering, Centre for Molecular and Structural Biomedicine, University of Algarve.

1. Introduction

Proteins are the main executers of genetic functions. Within a cell, the level of a protein is determined by its synthesis and turnover rates. In the last three decades, it has become increasingly clear that selective proteolysis is a key mechanism not only in cellular quality control but also in protein regulation.[1] A central mediator of selective proteolysis is the proteasome (E.C. 3.4.25.1), a large self-compartmentalized protease complex found in all kingdoms of life. The active sites of these proteases reside within interior chambers, while substrate specificity and entry is controlled by additional factors.[2,3]

In the cytoplasm and in the nucleus of eukaryotic cells, the ubiquitin/proteasome system (UPS) provides the main mechanism for a selective and ATP-dependent degradation of short-lived proteins. Within this system, proteins are covalently modified, mainly on lysine residues, by the attachment of a small (9 kDa) protein, termed ubiquitin because it is ubiquitously found in eukaryotic cells.[1] Ubiquitin modification (ubiquitylation) changes the binding properties of so modified proteins. Ubiquitylation serves numerous functions ranging from the regulation of ribosomes, of DNA repair functions and gene expression, to proteolytic targeting.[4−6] Ubiquitylation of membrane proteins has been implicated in their endosomal targeting which can ultimately lead to their degradation by various peptidases in the lysosome.[7,8] The attachment of certain types of ubiquitin chains (polyubiquitylation), in which one ubiquitin is attached to a lysine residue such as Lys11, Lys29, Lys48, or Lys63 of another ubiquitin, serves as a targeting signal that leads to the degradation by the proteasome.[5,6,9−13] Some substrates, however, are degraded by the proteasome without prior ubiquitylation.[14−16] The UPS is also responsible for the degradation of proteins from the endoplasmic reticulum, which are ubiquitylated upon retrotranslocation into the cytosol.[17,18] The functions of the UPS span from protein quality control and antigen presentation, to regulatory processes involved in cell cycle control, signal transduction,

10.1021/cr8004857 CCC: $71.50
Published on Web 03/06/2009

António Marques studied biochemistry at the University of Algarve, Portugal. In 2003 he was accepted in the International Graduate School in Genetics and Functional Genomics at the University of Cologne, Germany. Here, he received his Ph.D. in 2008 for his studies on the assembly and regulation of the 26S proteasome in *Saccharomyces cerevisiae* working with Prof. Jürgen Dohmen at the Institute for Genetics.

Ana Matias received her degree in biochemistry at the University of Algarve, Portugal, in 2004. She is currently completing her Ph.D. thesis on proteasome assembly in *Saccharomyces cerevisiae* under supervision by Prof. Paula Ramos and Prof. Jürgen Dohmen. Her studies are supported by a fellowship from the Portuguese Foundation for Science and Technology.

Palanimurugan Rangasamy received his Masters in Biochemistry at the University of Madras, India. He then joined Prof. R. Varadarajan at the Molecular Biophysics Unit of the Indian Institute of Science, Bangalore, to study the on-pathway protein aggregation in *Escherichia coli*. In 2001 he was accepted in the International Graduate School in Genetics and Functional Genomics at the University of Cologne. He received his Ph.D. in 2005 for his studies on the proteolytic mechanisms controlling polyamine biosynthesis in *Saccharomyces cerevisiae* working with Prof. Jürgen Dohmen. As a postdoctoral fellow in the Dohmen Laboratory, he is currently exploring multiple co- and post-translational mechanisms that regulate polyamine biosynthesis.

Paula C. Ramos studied biochemistry at the University of Lisbon and then joined the laboratory of Prof. Ricardo Boavida Ferreira at the Institute for Chemical and Biological Technology (ITQB) as a graduate student. In 1995, she received her Ph.D. for biochemical studies on the ubiquitin proteolytic system in plants. In 1996 she joined the group of Dr. Jürgen Dohmen at the University of Düsseldorf as a postdoctoral fellow, where she characterized the proteasome maturation chaperone Ump1 in *Saccharomyces cerevisiae*. Since 1999, she is Professor of biochemistry at the University of Algarve. Her research is focused on proteasome biogenesis.

cell differentiation and apoptosis.[19,20] It is probably not an exaggeration to say that the UPS, in one way or the other, is involved in the control of every major pathway in eukaryotic cells.

Polyubiquitylated proteins are recognized and degraded by a giant proteolytic complex known as the 26S proteasome.[21−24] This protease is composed of a catalytic core particle (CP), also termed the 20S proteasome, which is capped at both ends with 19S regulatory particles (RP).[23,25] The 20S CP has a barrel shape formed by four stacked rings, each of which is composed of seven subunits.[26−31] The active sites are sequestered in the inner chamber of the CP and thus shielded from the intracellular medium. For degradation of a folded polypeptide to occur, it must be recognized, unfolded, and translocated into the CP by the 19S RPs. Substrate unfolding requires ATPase activity which resides in the base of the RP.[3,32]

The UPS is essential for cell viability and malfunctions within this system are implicated in an increasing number of diseases including obesity, neurodegenerative disorders, muscular dystrophies, and cancer.[33−35] On the other hand, the proteasome has become a therapeutic target for the treatment of certain diseases, in particular of cancer.[36−38] Knowledge of the structure, the catalytic activities, and the assembly pathways of the different proteasome complexes existing in cells is essential to understand their function and regulation, as well as to develop strategies and compounds that allow their therapeutic manipulation. In this review, we have attempted to summarize the current knowledge of the structure, enzymatic mechanism, and complexity of proteasomal complexes with an emphasis on the eukaryotic 26S proteasome.

2. Structure and Complexity of Proteasome Core Particles

The proteasome is present ubiquitously in eukaryotes. The discovery of a comparably simple protease, HslV, with homology to the proteasome (Figure 1A), as well as of 20S

R. Jürgen Dohmen studied Biology at the Heinrich Heine University in Düsseldorf (HHUD) and received his Ph.D. in 1989 for cloning and characterization of a yeast glucoamylase working with Prof. Cornelis P. Hollenberg. He then joined the laboratory of Prof. Alexander Varshavsky at M.I.T to study intracellular proteolysis in *Saccharomyces cerevisiae*. In 1992 he moved with the Varshavsky laboratory to Caltech. From 1994−1999 he headed an independent research group in a priority program funded by the German federal government at the HHUD. In 2000 he moved to the University of Cologne as a professor of genetics. His research is concerned with ubiquitin- and SUMO-dependent proteolytic targeting mechanisms, and the proteasome.

proteasomes in archeons and eubacteria indicated that this protease type has an early evolutionary origin.[39−43]

2.1. Archaebacterial Proteasomes

20S proteasomes composed of one or two different α subunits, and one or two different β subunits are found in various archaeons. Generally, with one known exception, all β subunits of archaebacterial proteasomes are catalytically active. The exception is the crenarchaeote *Aeropyrum pernix*, which encodes both an active and an apparently inactive β subunit lacking the characteristic active site residues described in section 3.1[44] The first crystal structure of a 20S proteasome was reported for the one derived from the archaeon *Thermoplasma acidophilum*.[45] This ∼700 kDa complex is composed of only two different types of subunits (Figure 1B). The α subunit is composed of 233 amino acid residues, the β subunit of 211 residues.[46] The sequence and structural similarity between these two subunits indicated that their genes were derived from a common ancestor (Figure 2). All known 20S proteasome subunits found in other species can be related to the α or the β subunit of the *Thermoplasma* proteasome.[46] Structural analysis of this proteasome produced in *Escherichia coli* revealed that it is composed of four stacked rings, each made of seven subunits, which are in an $\alpha_7\beta_7\beta_7\alpha_7$ arrangement (Figure 1B). The resulting hollow cylinder is 15 nm long and has a diameter of 11 nm.[45] The two inner β rings form a central chamber with a volume of ∼84 nm^3, which compares to the volume of a folded ∼70 kDa protein.[3] This chamber contains 14 active sites, one provided by each β subunit, as was revealed by structural analysis of the proteasome bound to the inhibitor Acetyl-Leu-Leu-norleucinal (Ac-LLnL-al, calpain inhibitor I).[45] The two outer α rings and the neighboring β rings form antechambers with a volume of ∼60 nm^3, which may allow larger stretches of a polypeptide to be taken up by a proteasome before or while it is degraded into small peptides.[3,47] The structure revealed a narrow opening into the antechamber located at the center of the α ring (Figure 1B), which was shown to be the site of substrate entry.[46,48] The N-terminal segment of the α subunits, which seal the entry port in the eukaryotic proteasome (see below), were not detectable in the crystal structure of the *Thermoplasma* proteasome suggesting that they are structurally disordered.[45]

For *Archaeoglobus fulgidus*, assembly intermediates as well as the mature 20S proteasome have been subjected to a structural analysis.[49] The overall structure of the *A. fulgidus* proteasome is very similar to that from *T. acidophilum*, including the absence of a clear electron density for the N-terminal segments of the α subunits. This observation, together with additional experimental evidence, led to the proposal that archaeal proteasomes lack a regulatory gating such as has been observed for eukaryotic proteasomes.[49] Other biochemical and structural studies, however, indicate that, although prokaryotic proteasomes appear to have α subunits with disordered or flexible N-termini allowing the entry of small peptides, activator complexes are required to fully open the gate to enable degradation of proteins (see section 4).[50,51]

2.2. Eubacterial Proteasomes

In eubacteria, the first proteasome to be studied was isolated from the actinomycete *Rhodococcus erythropolis*.[43] This protease is built of two distinct α (α1 and α2) and two different β (β1 and β2) subunits. Different combinations of these α-type and β-type subunits resulted in proteasomes with slightly different kinetic parameters.[52] Crystal structure analysis of recombinant proteasomes composed only of α1 and β1 subunits showed that the *Rhodococcus* proteasome has a general architecture very similar to the one from *Thermoplasma*.[53] The contact regions between the α subunits, however, are substantially smaller in the *Rhodococcus* proteasome, which is probably a reason why these subunits do not form rings in the absence of β subunits. Instead the assembly of *Rhodococcus* proteasomes follows an unsual path (see section 5). As in the *Thermoplasma* proteasome, the N-terminal residues of the α subunits are not visible in the crystal structure of the *Rhodococcus* proteasome suggesting that these residues are disordered or mobile.[53]

The proteasome of another actinomycete, *Mycobacterium tuberculosis*, is composed of only one type of α and one type of β subunits, which are 65% identical to those of *Rhodococcus*. As for the archaeal proteasome structures, the N-terminal residues of the α subunits in the *M. tuberculosis* proteasome were disordered. Nevertheless, cryo-electron microscopy as well as negative-staining electron microscopy analyses suggested that the α rings of these particles are largely closed by the disordered N-terminal segments.[54,55] Together with biochemical data, these results indicated that the mycobacterial proteasome requires regulatory partners to open the gate. Inactivation of the proteasome genes (*prcA* and *prcB*) in *Mycobacterium smegmatis*, which form an operon together with a small ORF (*prcS*) encoding a ubiquitin-like protein that is conjugated to proteasome substrates,[56] resulted in viable strains with no apparent mutant phenotype.[57] Studies on *M. tuberculosis*, however, revealed that the orthologous prcA and prcB proteins, as well as ATPases presumed to cooperate with it, are important for nitric oxide resistance and hence for survival in the host cells.[58,59] These results identified bacterial proteasomes as potential drug targets for the therapy of tuberculosis.[60]

The analysis of many eubacterial genomes indicated that the 20S proteasome is only found in a subgroup of *Actinomycetes* suggesting that this branch of Gram-positive bacteria

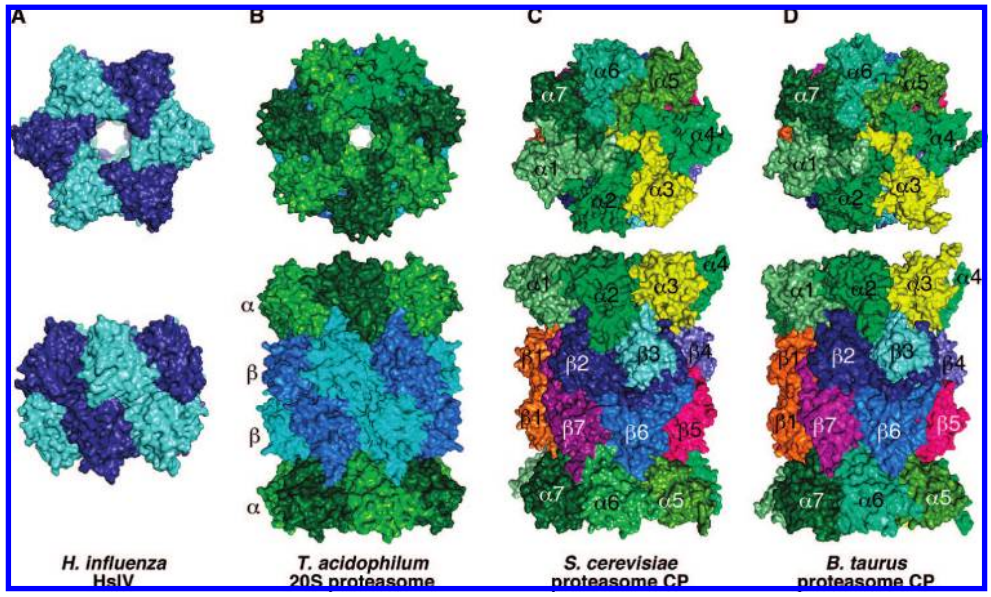

Figure 1. Structure and complexity of pro- and eukaryotic proteasomes. Surface representation of the crystal structures of (A) HslV from *Haemophilus influenza* (PDB code 1G3I), proteasomes from (B) *T. acidophilum* (PDB code 1PMA), (C) *S. cerevisiae* (PDB code 2F16), and (D) *Bos taurus* (PDB code 1IRU). The identical β type subunits of HslV and the *Thermoplasma* proteasome are each shown in two different shades of blue. The identical α subunits of the *Thermoplasma* proteasome are shown in different shades of green. The 14 individual subunits of the yeast and bovine proteasomes are displayed in distinct colors. Shown are top (upper panels) and side views (lower panels) of the particles. The figure was prepared using PyMOL (http://pymol.org).

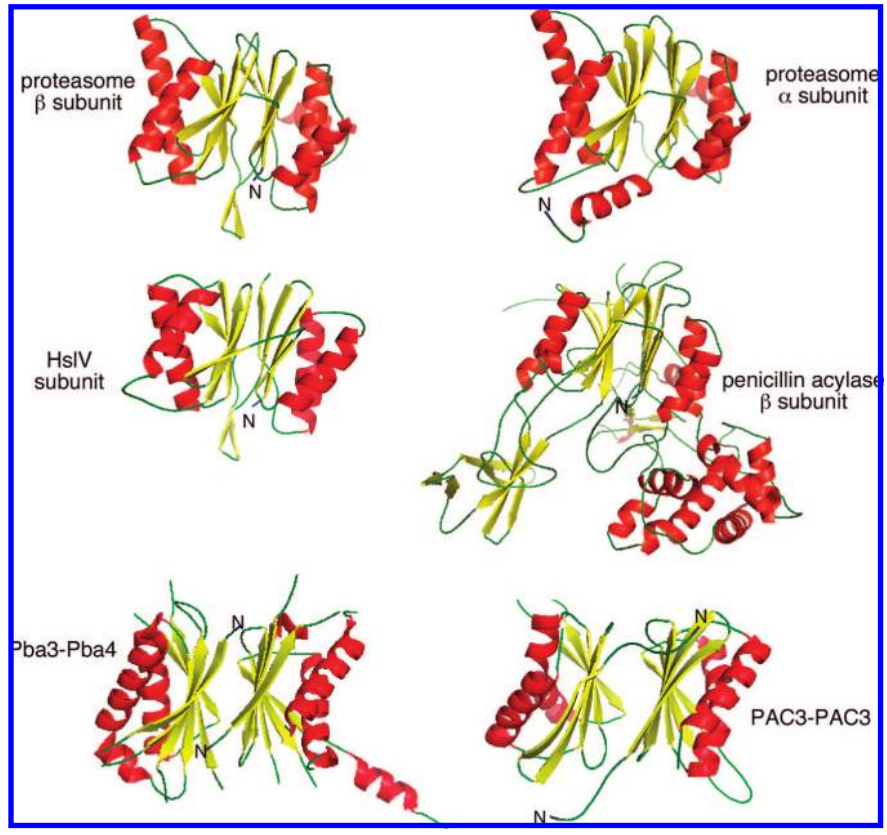

Figure 2. Structures of proteasomal subunits and their structural relatives. Shown are ribbon representations revealing the characteristic $\alpha\beta\beta\alpha$ sandwich fold of the *T. acidophilum proteasome* α and β subunits (PDB code 1PMA), the HslV subunit (PDB code 1G3I), the β subunit of penicillin acylase from *E. coli* (PDB code 1PNK), the proteasome chaperone Pba3-Pba4 from *S. cerevisiae* (PDB code 2Z5B), and the homodimer of its human homologue PAC3 (PDB code 2Z5E). The positions of N-termini are indicated. The figure was prepared using PyMOL.

has acquired the proteasomal genes by horizontal transfer from eukaryotes.[44,61] Recent data from shotgun proteomics and community genomic analysis, suggesting the presence of proteasomes in the Gram-negative bacterium *Leptospirillum* group II, challenge the notion above.[62] A full characterization of the genome of these bacteria confirming the presence of the proteasome genes in *Leptospirillum*, however, is still missing.[63]

Many eubacteria, including *Escherichia coli*, instead of the proteasome, harbor a protease, HslV/ClpQ, which is composed of subunits homologous in sequence to pro- and eukaryotic proteasomes.[39,44,64] HslV moreover shares the catalytic mechanism with the 20S proteasome.[40,64,65] While the 20S proteasome is composed of four heptameric rings (see above), HslV is composed of two hexameric rings (Figure 1A). Its subunits have a nearly identical fold as proteasome subunits except that the latter have an additional C-terminal α helix (Figure 2). The smaller size of the HslV polypeptide may underlie the fact that HslV rings bear only six subunits in contrast to the rings in the proteosome, which are characterized by a seven subunit arrangement.[64] Similar to the 20S proteasome, which associates with ATPase complexes, HslV forms an ATP-dependent protease by associating with homohexameric HslU/ClpY ATPase complexes.[64,66−68] HslUV has been implicated in the degradation of abnormal proteins.[69,70]

Neither the proteasome nor HslUV appear to be essential in bacteria.[57,70] Indeed several bacterial genomes even lack genes for both proteases.[44] Surprisingly, HslUV encoding genes were found in various eukaryotes.[44,71,72] Based upon phylogenetic analyses it was suggested that these genes may have been obtained by endosymbiosis from the proteobacterial ancestor that gave rise to eukaryotic mitochondria.[72]

2.3. Eukaryotic Proteasomes

Unlike in bacteria, proteasomes are generally essential for viability of eukaryotic cells.[73] In contrast to the low subunit complexity of prokaryotic proteasomes, eukaryotic proteasome CPs are composed of 14 distinct subunits, seven of the α type and seven of the β type, which form rings composed of seven different subunits (α1−7 or β1−7). In bakers' yeast (*Saccharomyces cerevisiae*), these subunits are encoded by 14 distinct genes, 13 of which are essential for viability.[74] Only the *PRE9* gene encoding the α3 subunit can be deleted without loss of viability, apparently because two α4 subunits can be incorporated into the α rings of such mutants.[75,76] The crystal structures of the proteasomes from *S. cerevisiae* and *Bos taurus* revealed that, despite the increased subunit complexity, the overall architecture of eukaryotic 20S proteasomes is very similar to that of the more simple prokaryotic counterparts (Figure 1).[45,77,78]

An important functional difference to bacterial proteasomes is that only three of the seven β subunits (β1, β2, and β5) in the eukaryotic proteasome are catalytically active.[77,79] Due to structural differences between these subunits, they display distinct cleavage specificities (discussed in section 3.2). As a consequence of the reduction to three active subunits per β ring, eukaryotic proteasome core particles bear only six active sites.[77] In mammals, a set of alternative active site subunits are induced upon immune stress. Incorporation of these alternative subunits (β1i, β2i, and β5i) results in the formation of the so-called "immunoproteasome", which favors the generation of certain antigenic peptides to be presented by Major Histocompatibility Complex (MHC) class I molecules due to a different cleavage specificity compared to the housekeeping proteasome (described in section 3.2 and discussed in detail in various reviews[80−87]). It should be noted, however, that the immunoproteasome is a proteasome subtype that is not essential for the generation of antigens by the proteasome. The standard (sometimes called constitutive) proteasome is capable of generating antigenic peptides as well. The relative amounts of standard or immunoproteasomes, and of "mixed" proteasomes, in which both interferon-induced subunits and standard β1, β2, or β5 are present, varies between cell types and tissues.[88] The structure of the bovine 20S CP revealed subtle differences to the yeast proteasome, in particular when the β subunits are compared. It was suggested that some of these differences allow for accommodation of either the standard or the inducible subunits.[78]

Another specialized proteasome subtype that was recently discovered has been termed "thymoproteasome" because it contains an alternative subunit (β5t) that is specifically expressed in the thymus.[89] It is preferentially combined with β1i and β2i, and appears to be important for the selection of $CD8^+$ T cells.[90] Aside from these specialized active site subunits found in mammals, genes encoding other alternative 20S proteasome subunits are found in other organisms as well. The model plant *Arabidopsis thaliania* for example encodes 13 α subunits and 10 β subunits.[91] The physiological roles of the alternative subunits remain unclear. Another example is *Drosophila melanogaster*, which expresses testis-specific isoforms of three α subunits and three β subunits.[92] Homozygous inactivation of the testis-specific gene for α6 causes male sterility indicating that a specialized proteasome subtype is essential for spermatogenesis.[93]

Other important structural features of eukaryotic proteasomes that distinguish them from their prokaryotic counterparts include a complete closure of the α rings (Figure 1C,D). The N-terminal domains of their subunits provide a plug to the gate through which substrates have to enter the core particle.[77] As discussed in section 4, opening of this gate is regulated by activators or regulatory particles. The mature eukaryotic core particle is moreover characterized by C-terminal extensions of the β2 and β7 subunits, which appear to be important for its assembly, stability, and activity (Figure 1).[94] Other structural differences concern transient features such as propeptides of β subunits and dedicated assembly chaperones, which are important during the assembly of eukaryotic proteasomes (discussed in section 5.2).

3. Activity of 20S Proteasomes

3.1. The Active Sites - N-Terminal Nucleophiles

Structural analyses of the 20S proteasome identified its active sites and provided an understanding of the catalytic mechanism. The active sites reside in the β subunits. Isolated β subunits, however, do not display proteolytic activity.[95] The cocrystal structure of the *Thermoplasma* proteasome with the inhibitor Ac-LLnL-al revealed that the N-terminal threonine of each of its 14 β subunits is covalently bound to the inhibitor.[45] Together with mutational analyses, these observations identified this Thr residue as a central component of the active site.[79] This finding characterized the proteasome as a novel type of protease, in which the N-terminal Thr of a mature β subunit provides a hydroxyl group that performs the nucleophilic attack onto the carbonyl carbon of a peptide bond. Based upon this property, the active β subunits of the proteasome are members of the Ntn (N-terminal nucleophile) hydrolases.[96] The overall fold of these Ntn-hydrolases can be related to that of proteasome subunits, despite a lack of apparent sequence similarity (Figure 2).[96] Ntn-hydrolases are synthesized as inactive precursors, which, upon autocatalytic cleavage, expose an N-terminal amino acid residue with a nucleophilic side chain (threonine, serine, or cysteine). Penicillin acylase for example possesses a serine

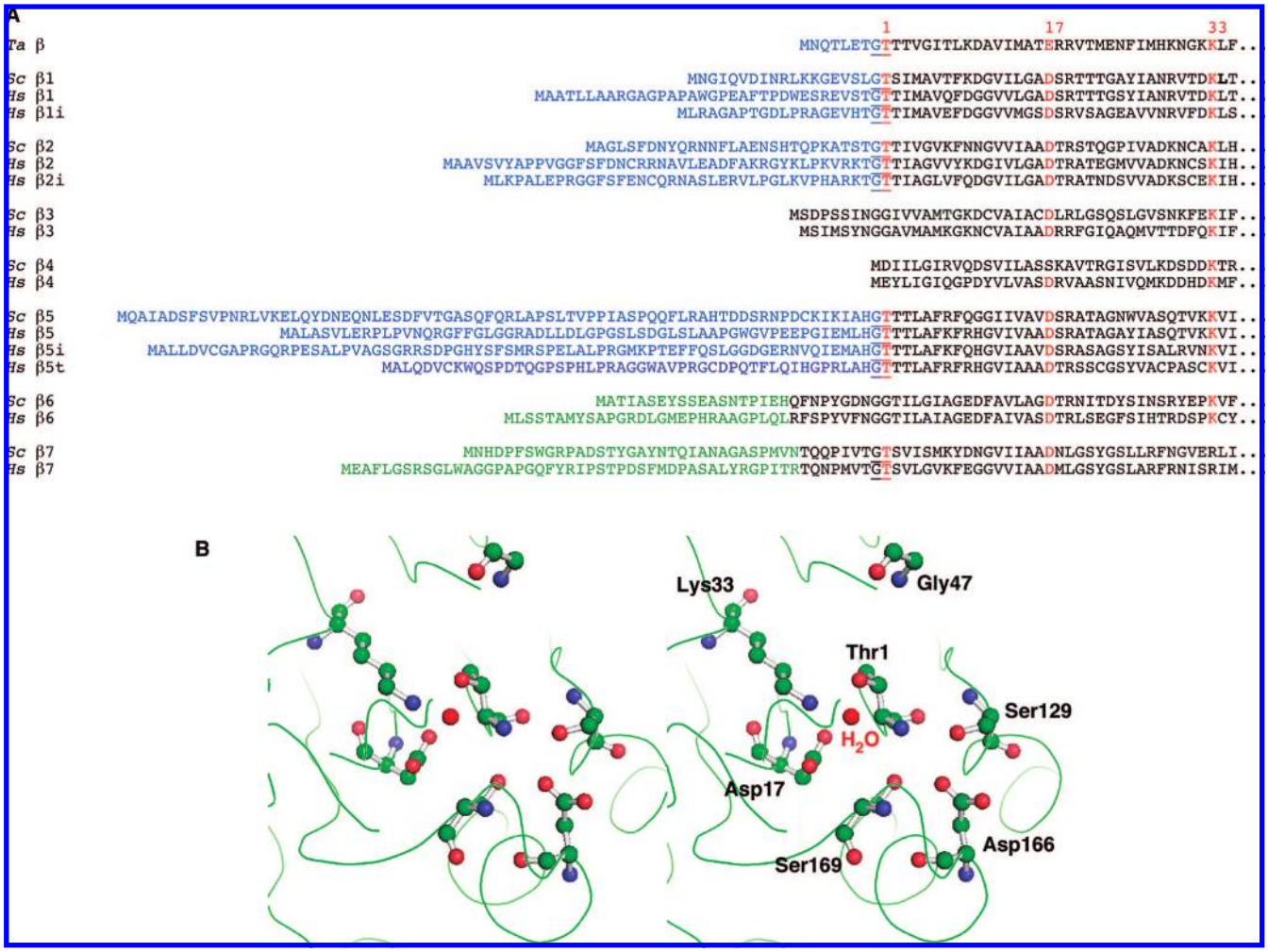

Figure 3. Primary and tertiary structures of the catalytic centers of β subunits. (A) Alignment of the amino acid sequences of the N-terminal regions of β subunit precursors from *T. acidophilum* (*Ta*), *S. cerevisiae* (*Sc*), and *H. sapiens* (*Hs*). Propeptide sequences that are absent from the mature subunits are shown in blue for active subunits and in green for inactive subunits. Conserved residues from the catalytic sites are highlighted in red. For *H. sapiens*, aside from the subunits of the constitutive proteasome, the corresponding subunits of the immunoproteasome (βxi) and the thymoproteasome (β5t) are listed. The conserved processing sites Gly-Thr (GT) are underlined. (B) Shown is a stereo representation of the proteolytic center of an active β subunit (β1) of the yeast 20S proteasome. The main residues are Thr1 (catalytic residue), Asp17 and Lys33. The Thr1N is hydrogen-bonded to Ser129-Oγ, Asp166-O, and Ser169-Oγ and the Thr-Oγ is hydrogen-bonded to Lys33-Nζ. A water molecule close to the Thr1-Oγ, Thr1-N, Gly47-N, and Ser129-Oγ, which is necessary for both autolysis and proteolysis, is shown in red. The figure was prepared using PyMOL.

residue,[97] and glutamine 5-phosphoribosyl-1-pyrophosphate amidotransferase a cysteine residue at the N-terminus.[98] The N-terminal residue of such an enzyme is the active site, wherein the side chain hydroxyl (or sulfhydryl) acts as the catalytic nucleophile and the free amino group as the base for the hydrolysis reaction.[96,99] For the *Thermoplasma* proteasome it was investigated whether the active site Thr1 residue could be replaced by Ser or Cys.[79,100] In a Thr1Ser mutant proteasome, β subunit maturation was reduced to about 50%. When the Thr1Ser subunit was expressed without propeptide, the activity of the resulting proteasome toward fluorogenic peptides was similar to that of wild-type proteasome. The activity toward proteins or a library of decapeptides, however, was significantly reduced for the Thr1Ser mutant.[101] A Thr1Cys was very efficient in subunit maturation but was inactive toward fluorogenic peptides.[100] In conclusion, although Ser can substitute for Thr in the active site of the proteasome during proteolysis, and Cys is compatible with autocatalytic processing, only Thr is fully functional in both processes.[100]

Aside from the N-terminal Thr, structural and mutational studies on proteasomes from various species identified the highly conserved residues Asp17 (Glu17 in the *Thermoplasma* β subunit) and Lys33 (numbering according to the *Thermoplasma* proteasome) as the most important residues of the catalytic centers (Figure 3).[45,77−79,102−104] The Lys33-Nζ atom is hydrogen bonded to Thr1-Oγ. Lys33 is a highly conserved residue among β subunits and essential for catalytic activity.[100,102−105] Other residues close to the active site Thr1 also appear to be required for structural integrity of the proteolytic site. The atoms Ser129-Oγ, Asp166-O, and Ser169-Oγ stabilize the conformation of Thr1 via hydrogen bonds.[45,79] Another critical feature of the active center detected in high resolution structures of the proteasome is a nucleophilic water molecule in the vicinity of Thr1-Oγ, Thr1-N, Ser129-Oγ, and Gly47-N atoms. This water molecule is involved both in intramolecular autolysis and substrate proteolysis (Figure 3).[77,106]

3.1.1. Maturation of Active Sites - Intramolecular Autolysis

Similar to many other proteases,[107] the active β subunits of the proteasome are synthesized as precursor polypeptides containing N-terminal propeptides.[100,103,105] Upon cleavage of the propeptide, the catalytic Thr1 is exposed and the β subunit thereby activated.[45,79,100] In eukaryotes, full sets of the conserved residues characteristic of the catalytic center (Thr1, Asp17, and Lys33) are only present in β1, β2, and β5 (Figure 3). As shown by mutational analysis of the yeast proteasome, activation of these catalytic subunits occurs via intramolecular autolysis.[104,106,108] Subunits β3 and β6 have a Gly residue and β4 an Asp or Glu residue instead of Thr in the predicted +1 position. β7 has an Arg residue instead of Lys33 (Figure 3). Consistent with this lack of critical residues, the latter four subunits are inactive. They are found in the mature proteasome either in an unprocessed form (as is the case for β3 and β4) or in a processed form (as is the case for β6 and β7). The maturation of β subunits occurs during assembly when two half-proteasome precursor complexes dimerize.[103] The N-termini of mature β6 and β7 are Gln-9 (or Arg-9 in humans) and Thr-8, respectively.[77,78,106,109] In contrast to autolysis of β1, β2, and β5, processing of β6 and β7 is apparently due to proteolytic action of the neighboring active subunits.[104] A similar processing is observed in a yeast proteasome carrying a Thr1Ala mutation in β1. This subunit, although unable to perform autolysis, is processed by other subunits to yield Leu-9 as the N-terminal residue.[106] Propeptide shortening was also observed for a catalytically impaired mutant version of LMP2 (a mammalian interferon-induced β1 subunit).[105]

When active β subunits devoid of their propeptides were synthesized in yeast either by expressing them fused to ubiquitin, which is cleaved off rapidly by cellular deubiquitylating enzymes, or with a Met residue directly preceding Thr1, which is cleaved by methionine aminopeptidase, the resulting mature subunits turned out to be acetylated at the N-terminal Thr to varying degrees. For sterical reasons, the acetyl group cannot be removed by autolysis. Therefore acetylation leads to an inactivation of β subunits.[102,110] Yeast cells expressing such mutant β subunits, however, recover the respective proteasomal activtity when N^{α}-acetyltransferase is inactivated in addition.[110] These results demonstrate the importance of a free α-amino group at the N-terminus for activity, as well as a role of the propeptides in protecting unincorporated subunits from inactivation.

The propeptides of the active β subunits are highly divergent in size as well as in sequence among the eukaryotes except for a glycine residue (Gly-1) preceding the catalytic Thr1 (Figure 3). This Gly-1 residue is important for efficient autolysis.[100] Analysis of the crystal structure of a yeast 20S proteasome bearing a Thr1Ala mutation in subunit β1 allowed modeling of the autolysis site, since the structures of the wild-type and mutant subunits were nearly identical when superimposed. This model provided insights into the details of the autolysis mechanism. According to this model, the propeptide adopts the conformation of a γ turn extending from Leu-2 to Thr1.[106] In this conformation, the hydroxyl group of Thr1 appears to be appropriately positioned for a nucleophilic attack onto the Gly-1 carbonyl carbon atom located at the inner side of the γ turn. During the autocatalytic cleavage, the N-terminal amino group is not yet available as a nucleophile. Instead, a water molecule, present in the active site, is thought to act as a general base and to deprotonate the hydroxyl group of Thr1 to enhance its nucleophilicity (Figure 4). The nucleophilic attack onto the carbonyl carbon of Gly-1 by Thr1Oγ results in a tetrahedral oxazolidine intermediate. The decay of this transient intermediate shifts the scissile bond from an amide into an ester bond intermediate (N−O acyl shift).[106] During this process, a protonated water molecule acts as proton donor to the amido nitrogen of the Thr1 residue. Finally, the ester intermediate collapses into a free N-terminal Thr1 residue and a free carboxy terminus at the Gly-1 of the propeptide with the water molecule incorporated into the resulting products (Figure 4).[106]

3.1.2. Catalytic Mechanism - Proteolysis

During proteolysis, a polypeptide substrate docks at the Thr1 site, probably via hydrogen bonds with a pattern similar to the inhibitor Ac-LLnL-al.[49,77] The hydroxyl group of Thr1 needs to be activated by a proton acceptor. Lys33 could be considered to serve this function in the proteolytic reaction, but at neutral pH it is very likely charged and fully engaged in a salt bridge with Asp17 and several hydrogen bonds.[77,106] The role of charged Lys33 is more likely to lower the pK_a of the amino group of Thr1 in a way that this group functions as a proton acceptor in proteolysis.[77] The Thr1-Oγ atom reacts with peptide bonds of substrates (or with electrophilic functional groups of inhibitors). A water molecule present in the neighborhood of the active site is thought to mediate proton transfer between Thr1-Oγ and Thr1-N during substrate binding, resulting in the formation of an acyl-ester intermediate (Figure 4). A water molecule subsequently mediates hydrolysis of the acyl-ester bond thereby regenerating Thr1-Oγ for another reaction.[77]

3.2. Specificity and Cooperativity of Active Sites

3.2.1. Substrate Binding Pockets and Cleavage Specificity

Proteasomal peptidase activities have been classified according to the residues (P1 position) after which they cleave the amide bond of small artificial oligopeptide substrates to release a fluorogenic leaving group such as 7-amino-4-methylcoumarin (AMC) or β-naphthylamine (NA). Prokaryotic proteasomes, like the complexes from *Thermoplasma* and *Rhodococcus*, harbor only one type of β subunit and hence only have one type of activity, which is termed chymotrypsin-like because cleavage occurs preferentially after bulky hydrophobic residues.[43,111] Eukaryotic proteasomes display two additional activities, termed trypsin-like because cleavage occurs after basic residues, and peptidylglutamyl peptide hydrolyzing (PGPH), postacidic, or caspase-like because cleavage occurs after acidic residues. Sequence comparison of β subunits from pro- and eukaryotic proteasomes as well as a structural analysis of the yeast proteasome in the presence of an active site inhibitor identified β1, β2, and β5 as the active subunits in eukaryotic proteasomes.[77,79] Inactivation of key residues in these subunits by site-directed mutagenesis established that β1 mediates the postacidic, β2 the tryptic, and β5 the chymotryptic activities.[104,108]

Studies in which the cleavage pattern of the proteasome on real proteins was determined indicated that the active sites are much less specific with regard to the recognition of certain amino acid residues in the P1 position than the classification based upon the fluorogenic peptide substrates would suggest.[112] Complex cleavage patterns of protein substrates are consistent with a broad specificity of the

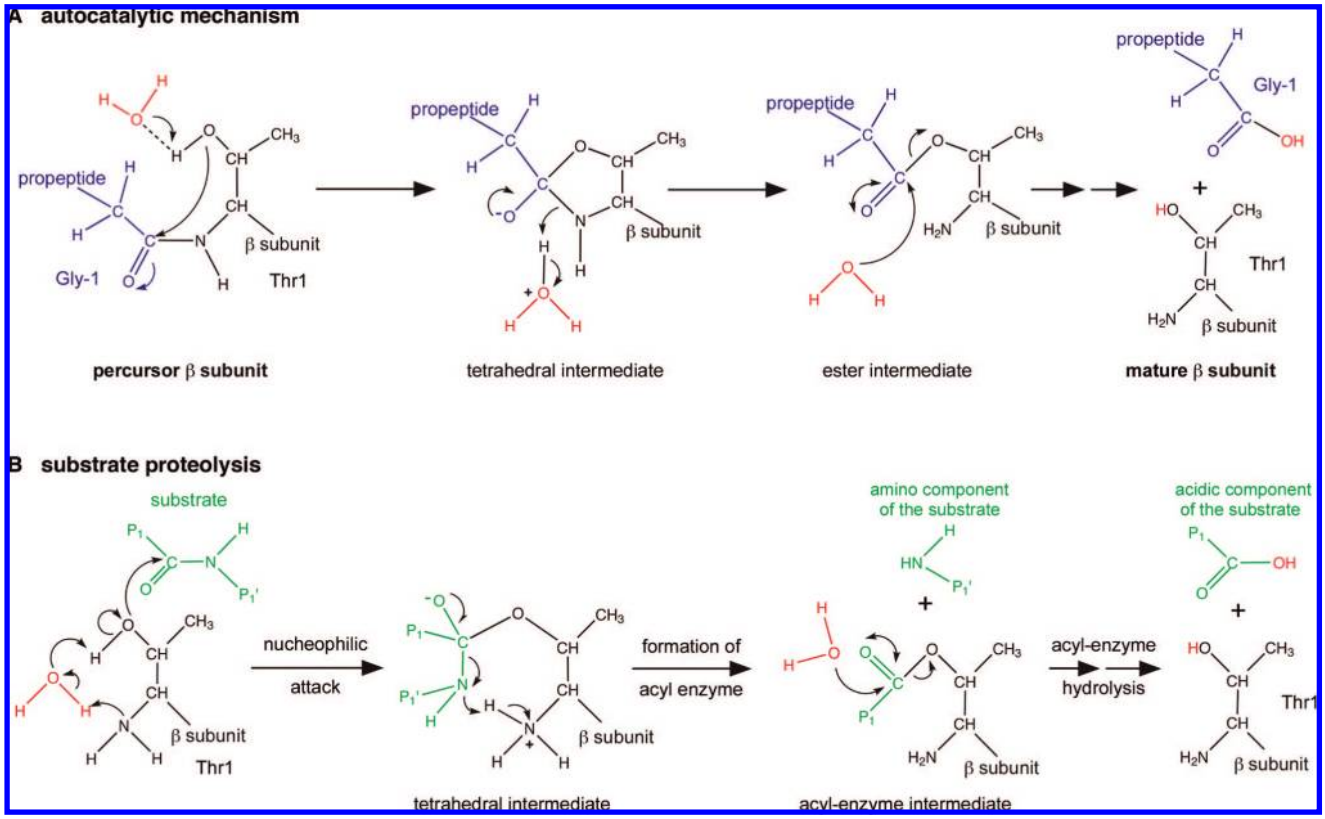

Figure 4. Autocatalytic and proteolytic mechanisms of the 20S proteasome. (A) A schematic representation of the main steps leading to β subunit processing and activation are shown. The propeptide part is shown in blue, and the rest of the subunit is in black. A water molecule is shown in red. (B) Shown is the mechanism leading to substrate peptide bond hydrolysis by the N-terminal Thr residue of a proteasomal active β subunit. The substrate is shown in green; the mature β subunit in black. Details of both reactions are described in the main text.

proteasomal active sites and indicate that substrate residues other than those at P1 are of relevance as well. Selective inhibition of β5 (chymotrypsin-like site) or its inactivation by mutation resulted in a dramatic reduction in protein degradation rates in vitro and in vivo.[104,108,113] By contrast, inactivation of β1 and β2 had comparably little effects in vivo.[104,108] A recent study that employed distinct site-specific inhibitors, however, indicated that an inhibition of multiple active sites is required to strongly inhibit the degradation of proteins in HeLa cells.[114]

Structural inspection of the substrate binding pockets in the yeast proteasome explains the different specificities of the active sites against the chromogenic peptidic substrates.[77] The pockets names, S*n* or S*n*′, refer to the binding sites of substrate amino acid residues either before (P*n*) or after (P*n*′) the peptide bond to be cleaved (P1−P1′). The number *n* defines the distance to the cleavage site.[115] The binding pockets in the yeast proteasome were originally characterized by structural analysis of the proteasome bound to the inhibitor Ac-LLnL-al. The norleucine residue (position P1) was covalently bound to the Thr-Oγ of the β1, β2, and β5 subunits while its side chain projected into the S1 pocket. The P2 residue (leucine side chain) was exposed to the solvent not touching the subunit, and P3 (the last leucine) was in contact with the neighboring β subunit.[77]

An important determinant of specificity in the binding channel of the active subunits is the S1 pocket. A position that largely determines the characters of the different S1 pockets is residue 45, which is at the bottom of the pocket (Figure 5). The basic character of the β1 pocket is provided by Arg45, which favors cleavage after an acidic residue in the P1 position, consistent with a postacidic activity. In higher resolution structures, a bicarbonate ion was found captured in the β1 S1 pocket, which has the potential to neutralize the charge of Arg45 if S1 is occupied by an amino acid residue with a neutral side chain, which is for example the case for Ac-LLnL-al (Figure 6A).[106] This property is consistent with an activity of this site toward substrates with branched side chain amino acids in the P1 position.[116] In β2, a small Gly residue in position 45 confers a wide S1 pocket suitable for basic P1 residues, which is consistent with a trypsin-like activity. The S1 pocket of β5 has an apolar character due to Met45, which explains its chymotrypsin-like peptidase activity (Figure 5). It should be stressed that the S1 pocket alone is insufficient to determine the binding probability of a polypeptide stretch in a substrate or an inhibitor to a particular active subunit. A stretch of amino acids residues flanking the cleavage point, ranging from P5 up to P5′, appears to be important for the selection of the cleavage site by a specific β subunit.[112] Recent structural analyses of proteasomes bound to peptide vinyl sulfone inhibitors indicated that, in particular, residues in the P3 and P4 position are critical determinants in active site selection.[117]

The immunoproteasome, as a consequence of the incorporation of interferon-γ-induced active subunits, has altered peptide cleavage specificity in comparison to the constitutive proteasome.[118−120] The original data have been partly discrepant and a matter of some debate, which was likely to due to heterogeneity of proteasomes with varying contents of immunosubunits and constitutive subunits depending on the tissues used for isolation.[88,120−123] It is clear that the immunoproteasome has reduced postacidic activity and

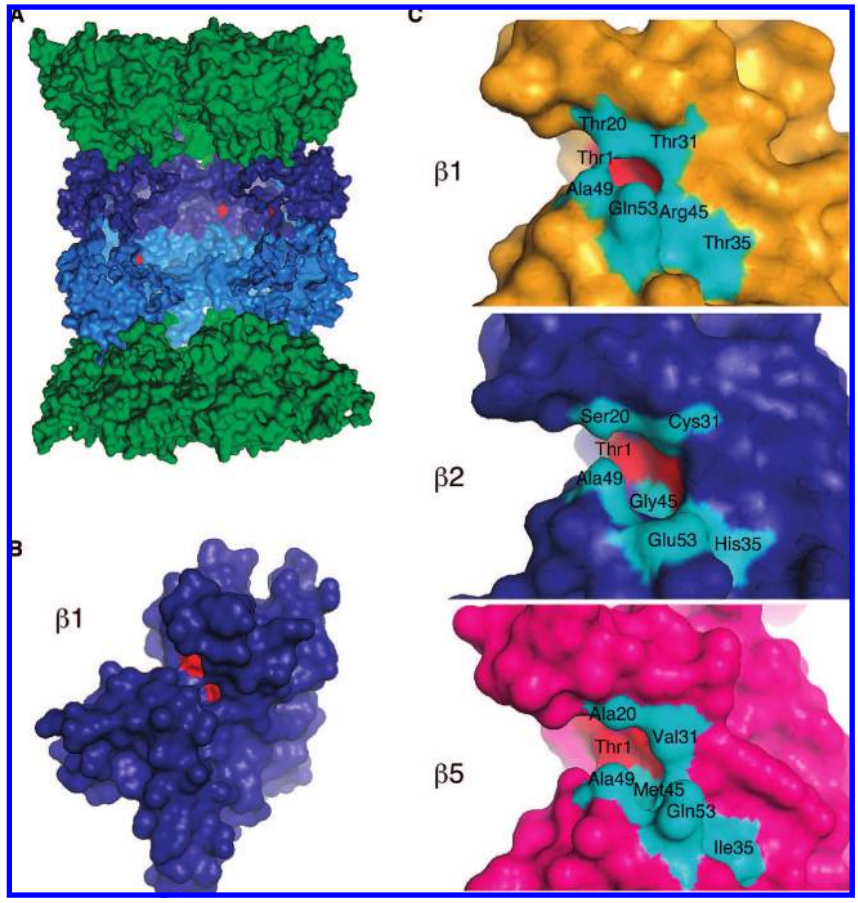

Figure 5. Localization and structure of substrate binding S1 pockets of the yeast proteasome. (A) *S. cerevisiae* 20S proteasome shown in a surface representation with a cut open view of the catalytic chamber. (B) Magnification of the β1 subunit showing the localization of the active residues in a cleft. (C) Shown are the S1 pockets of the three active site subunits. The pocket forming residues are indicated. In (A−C), the three main residues (Thr1, Asp17, and Lys33) are shown in red. The figure was prepared using PyMOL.

increased cleavage after branched and aromatic residues.[85,88,121] The immunosubunit that exhibits the most obvious structural differences to its housekeeping counterpart is β1i/LMP2. The S1 pocket of β1i is likely to be more apolar than the constitutive β1 active site with Arg45 replaced by Leu45. This and other differences are consistent with the reduction of postacidic activity and an increase of chymotryptic activity of the immunoproteasome. Modeling of β2i/MECL-1 and β5i/LMP7, in contrast, did not indicate substantial differences in the properties of their S1 pockets.[77,78] Nonetheless, β2i and β5i expression was shown to change cleavage patterns of the proteasome and to be specifically required for the generation of certain antigens.[88,124−126]

3.2.2. Substrate Cleavage and Cooperativity of Active Sites

An important aspect of the proteasome's function is the generation of peptides, which are trimmed down further by cytosolic peptidases into amino acids reusable for new protein synthesis.[127] This function is particularly important under starvation conditions. Peptides produced by the proteasome in vertebrates moreover serve an important function as antigens to be presented on the surface of, for example, an infected cell.[84,85] In other cases the substrates are not degraded to completion but instead processed to yield biologically active proteins, such as the p50 subunit of the transcription factor NF-κB, in a process called regulated ubiquitin/proteasome-dependent processing (RUP).[128−130] Incomplete digestion appears to be due to tightly folded domains that are resistant to degradation and/or to sequence stretches such as Gly-Ala repeats that do not allow processive unfolding.[131,132]

What determines the nature of the breakdown products of a protein once it has entered the CP? Analyses of peptides resulting from the action of *Thermoplasma* or eukaryotic 20S proteasomes on various denatured or natively unfolded protein substrates revealed that they varied in length between 2 and 30 amino acids residues, with a similar average length of 7−8 amino acids.[112,133−137] Considering that eukaryotic proteasomes carry only six active sites per chamber while the *Thermoplasma* proteasomes bear 14, the similarity in product sizes was striking and suggested that criteria other than the distances between active sites determine the product length. This assumption was corroborated by the finding that further reduction of the numbers of active sites in eukaryotic proteasomes by inactivation did not change the average length of peptides compared to wild-type proteasomes.[112,135] It was demonstrated for the *Thermoplasma* proteasome that, in contrast to conventional proteases such as trypsin, it degrades a protein substrate in a highly processive manner until the cleavage products are released and another substrate molecule is attacked.[133] The number of cuts in a polypeptide and the time needed to degrade it increases with the length of the substrate.[134] These and other studies with eukaryotic proteasomes indicated that cleavage product length is not determined by the number, specificity, or arrangement of the active sites, but that processive cleavage continues until

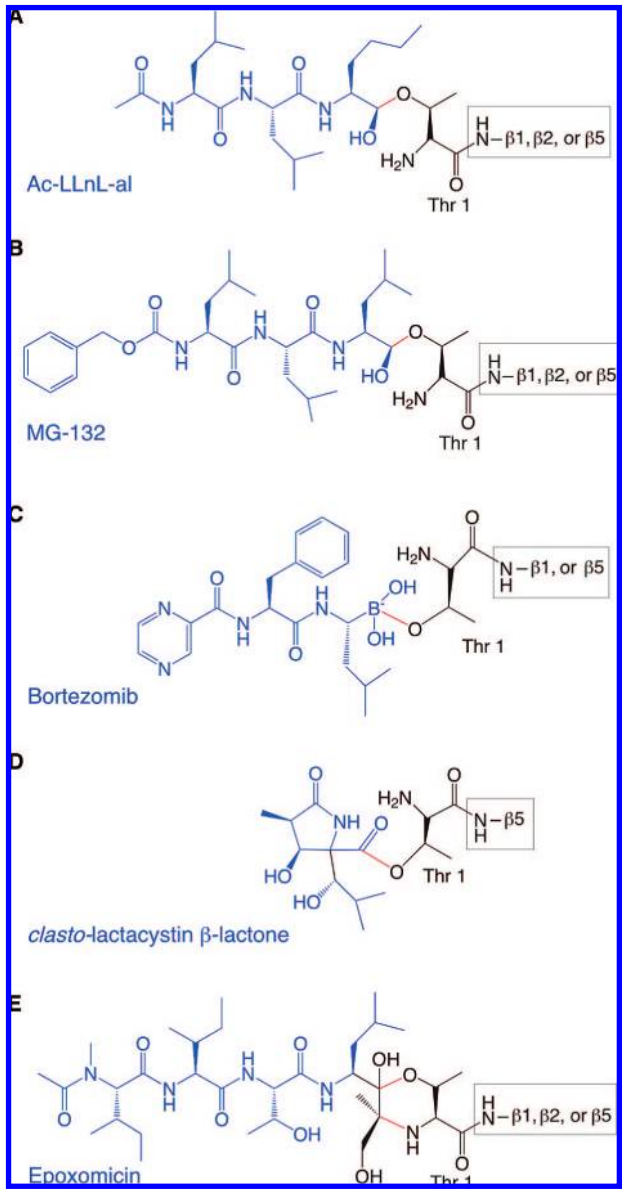

Figure 6. (A−E) Structures of selected inhibitors bound to a proteasome active site. The proteasome subunits that are targeted by each of the inhibitors are shown in boxes to the right. The inhibitors are shown in blue, the N-terminus of an active site subunit is in black, and the bond between the two is in red.

products are sufficiently small to diffuse out through the proteasome's axial gates.[135,137]

The 2-fold symmetry of the proteasomes, which provides two possible entrances and two exits to the catalytic chamber, raised additional questions. Is there a unidirectional flow with substrates entering at one end and the products leaving at the other, or can both events occur simultaneously at both ends? These questions were addressed by immobilizing 20S proteasomes either in end-on or side-on orientation. The end-on orientation resulted in a blocking of one of the proteasomes gates, while in the side-on orientation both gates remained accessible. With this experimental setup, it could be shown that CPs with two accessible gates processed two substrates per degradation cycle with a remarkable positive cooperativity in substrate binding, while CPs with one available gate processed only one substrate molecule.[138] These data indicated that entry of a first substrate molecule facilitates uptake of a second one, presumably by opening of the axial channel at the other end. This concept is consistent with another study demonstrating a stimulation of gate opening by the binding of hydrophobic peptides to noncatalytic sites in the proteasome.[139]

Another study indicated that acidic peptides inhibited the chymotrypsin-like activity. This led to the intriguing idea of a bite-chew mechanism, in which, after an initial cut by the chymotryptic site (bite), engagement of the postacidic site (chew) leads to a transient inhibition of the chymotryptic site.[140] Later studies, however, revealed that inhibition of the chymotryptic site by acidic peptides is independent of an occupancy of the postacidic site indicating that they act by binding to a noncatalytic site.[139,141,142] This led to the proposal of a refined model (two-site modifier model), according to which a substrate or intermediate can bind to an active site as well as to a noncatalytic modifier site, with the latter event resulting in inhibition of the chymotryptic site.[141] It was also observed that occupancy of the postacidic active sites stimulates the trypsin-like activity of the proteasome.[139] Another allosteric affect of active site engagement was observed recently during studies on the in vitro assembly of yeast 26S proteasomes. Occupation of active sites by inhibitors reduced the dissociation of 19S RPs from the CP.[143] Together these studies indicated that the presence of substrates or their breakdown products in the catalytic chamber of the proteasome can have allosteric effects on certain active sites, the gate of the CP and its interaction with the RP, with the latter two effects possibly linked to each other.

Knowledge of the cleavage patterns is important to understand how the proteasome contributes to the generation of peptides presented by MHC class I molecules on the surface of vertebrate cells. These 8−10 residue oligopeptides can signal an infected or cancer state of a cell to cytotoxic T-lymphocytes. Many peptides released by the housekeeping proteasome, however, are smaller than eight residues. The immunoproteasome makes fewer cleavages and produces longer peptides.[144] A release of longer peptides may also be promoted by the attachment of the interferon-induced alternative regulatory particle PA28.[145] Peptides produced by the proteasome often require further processing by additional peptidases.[144,146,147] Based upon experimental data, algorithms have been developed to predict the probability of the generation of such peptides.[148−154]

3.3. Proteasome Inhibitors

The proteasome is a key molecule in the degradation of proteins that control the cell division cycle and apoptosis, and is therefore an interesting target for therapeutic agents that inhibit cell proliferation in diseases such as cancer.[37] The discovery of inhibitors produced as metabolites by microorganisms and the design of synthetic inhibitor molecules was important for the development of both pharmacologically applicable drugs and of research tools to study the in vivo functions of the proteasome.[155] The design of specific inhibitors with clinical applicability became an important challenge because some inhibitors displayed cytotoxicity not suitable for pharmacological applications. Others were insufficiently specific and inhibited also other proteases such as calpains and cathepsins. At present, there are several proteasome inhibitors in clinical trials. One was approved by the U.S. Food and Drug Administration (FDA) for treatment of multiple myeloma (see below). The determination of various structures of the yeast proteasome bound to different inhibitors provided insight into their specificity and the binding modes (Figure 6). These studies provided

valuable information for a rational design of new compounds with improved specificity and affinity for the distinct active sites. The proteasome inhibitors are grouped into several classes according to their chemical properties. Several recent reviews were devoted exclusively to this subject.[36,115,155−159] Principal properties of some of the most prominent proteasome inhibitors are described below.

3.3.1. Peptide Aldehydes

The first compounds showing proteasome inhibition capacity were peptide aldehydes.[160] Originally identified as reversible serine and cysteine protease inhibitors, certain peptide aldehydes also bind to the N-terminal active site threonine residues in the proteasome (Figure 6A,B). Other proteases such as calpains and cathepsins are also sensitive to such compounds. Cocrystallization with AcLLnL-al (calpain inhibitor I) led to the identification of the active sites in the *Thermoplasma* and *S. cerevisiae* proteasomes.[45,77] In the eukaryotic proteasome, AcLLnL-al binds with highest affinity to β5, the subunit carrying chymotryptic activity, and with lower affinity to the other active subunits β1 and β2 (Figure 6A).[115] MG132 is probably the most commonly used synthetic proteasome inhibitor in experimental research and is a peptide aldehyde similar to the AcLLnL-al but bearing a benzyloxycarbonyl group before the first Leu residue (Figure 6B). It is more potent against the proteasome than AcLLnL-al and more selective.[128,161] A natural inhibitor of this class, fellutamide B, isolated from the marine fungus *Penicillium fellutanum*, has an extended β-hydroxy aliphatic tail that adopts distinct conformations at each of the three different active sites.[162] The inhibitors of this class form hemiacetal bonds between the aldehyde group and the hydroxy group of Thr1 of the active subunits (Figure 6A,B).[77,162]

3.3.2. Boronic Acid Inhibitors

The replacement of the highly reactive pharmacophore of the peptide aldehydes by a boronic acid functional group allowed the generation of a new class of inhibitors. Bortezomib (formerly called VELCADE or PS341) is a dipeptidyl boronic acid that reversibly inhibits the chymotryptic activity of the proteasome and to a lesser extent the postacidic activity (Figure 6C).[163] It shows high selectivity toward the proteasome relative to serine and cysteine proteases. Due to its antitumor properties in a tumor cell line screen,[164] bortezomib was the first proteasome inhibitor to enter clinical tests in patients, yielding positive results in the treatment of multiple myeloma and non-Hodgkin lymphoma. Since 2003, bortezomib is approved by the FDA.[156] Bortezomib also binds the β1i and β5i subunits of the immunoproteasome.[165] Analysis of the structure of the yeast proteasome complexed with bortezomib showed that all sites are occupied by the inhibitor, which was likely due to the high concentration of bortezomib used to soak the crystals. The inhibition is mediated by the boron atom that binds covalently to the nucleophilic oxygen of Thr1.[166] CEP-18770 is a novel orally active proteasome inhibitor suitable for the treatment of multiple myeloma and other malignancies responsive to proteasome inhibition.[167] CEP-18770 showed potency against the chymotrypsin-like proteasome activity comparable to bortezomib, but also weakly inhibited the tryptic and postacidic activities.[168]

3.3.3. β-Lactones

Lactacystin is a natural proteasome inhibitor isolated from *Streptomyces lactacystinaeus* that inhibits all three proteolytic activities, but with different efficiencies.[169] The active compound is *clasto*-lactacystin β-lactone, which is formed upon hydrolysis of lactacystin in aqueous solution.[170] Lactacystin first attracted attention because of its potential to induce differentiation of neuroblastoma cells and to inhibit cell cycle progression in an osteosarcoma cell line.[171,172] Lactacystin irreversibly modifies Thr1 of the β5 subunit through an ester bond (Figure 6D).[77,169] As revealed by the crystal structure of the proteasome complexed with this inhibitor, the dimethyl group of lactacystin mimics a valine or a leucine side chain and interacts with Met45 located at the bottom of the S1 pocket of the β5 subunit. In contrast, the S1 pocket of the active sites of β1 and β2 is not appropriate to bind lactacystin.[77] A synthetic analog of lactacystin, PS-519, was successfully tested in a phase I clinical trial.[173] Salinosporamide A (NPI-0052) is structurally related to *clasto*-lactacystin β-lactone. Here the isopropyl group is substituted by a cyclohexene ring, and a chloroethyl group is in place of the methyl group. These substituents enhance potency of the inhibitor both in vitro and in vivo.[174] This natural compound isolated from the marine actinomycete *Salinispora tropica* irreversibly inhibits the chymotryptic activity and to a lower extent the tryptic activity.[175,176] Salinosporamide A is in clinical trials as an anticancer drug.[175]

3.3.4. Epoxyketones

Another class of proteasome inhibitors comprises epoxyketones. Epoxomicin, a natural product isolated from an *Actinomycetes* strain, binds potently and irreversibly to the active subunits of the proteasome.[177] It is a highly specific inhibitor of the proteasome without any known inhibitory effects on other proteases. The crystal structure of the inhibitor bound to proteasome revealed the formation of a morpholino ring between Thr1-Oγ and the epoxy group of epoxomicin (Figure 6E).[178] Eponemycin is another natural inhibitor belonging to this class that binds particularly well to β1i and β5i but inhibits all active sites of the proteasome to some degree.[179] Carfilzomib is the only inhibitor of this class reported to be in clinical trials.[180] It binds irreversibly and inhibits the chymotryptic activity of the proteasome and the immunoproteasome.[181]

3.3.5. Vinyl Sulfones

A class of inhibitors represented by synthetic products including NLVS (3-nitro-4-hydroxy-5-iodophenylacetate-Leu-Leu-Leu-VS) and ZLVS (benzyloxycarbonyl (Z)-Leu-Leu-Leu-VS) is characterized by a vinyl sulfone (VS) moiety. These molecules are less reactive than the peptide aldehydes but also bind to the proteasomes in an irreversible manner.[182] Libraries of vinyl sulfone inhibitors were used to systematically investigate the importance of residues preceding the cleavage site.[183] Structural analysis of general and subunit-specific proteasome inhibitors derived from these studies revealed important contributions of residues in the P3 and P4 position for active site selectivity.[117]

3.3.6. Vinyl Ketones

The class of inhibitors belonging to the macrocyclic vinyl ketones is represented by two natural products: syringolin A (SylA) and glidobactin A (GlbA). These drugs were shown to block proliferation of malignant cells and induce apoptosis, consistent with an inhibition of the proteasome.[184] SylA is secreted by the plant pathogenic bacterium *Pseudomonas syringae* and irreversibly inhibits the proteasome. It blocks chymotryptic activity at low concentrations and the other two activities at higher concentrations. It is very specific since other proteases tested were not affected.[184] The crystal structure of the proteasome treated with SylA revealed a new binding mechanism. The hydroxy group of the Thr1 performs a Michael type 1,4-addition to the α,β-unsaturated carbonyl group (vinyl ketone moiety) in the 12-membered ring of the SylA, resulting in an ether bond that produces irreversible inhibition.[184] GlbA, isolated from a strain related to human pathogens from the order *Burkholderiales*, has a behavior identical to SylA except that GlbA does not bind to the postacidic active site.[184]

3.3.7. Cyclic Peptides

Finally, a class of cyclic peptides (TMC-95 and its analogues) should be mentioned, which are the only known proteasome inhibitors that do not modify the threonine residues in the active sites. TMC-95 is a compound isolated from *Apiospora montagnei* that selectively blocks the three proteolytic activities of the proteasome in a reversible manner. This inhibitor, the structure of which involves a heterocyclic ring system, binds noncovalently to the active β subunits of the proteasome by interacting with main chain atoms of conserved residues via hydrogen bonds.[178]

3.3.8. Targeting of Cancer Cells

It is not yet entirely clear why proteasome inhibition is more toxic to tumor cells than to normal cells. One mechanism might be that a failure to degrade IκB blocks the activation of NF-κB and thereby the expression of genes required for proliferation and adhesion of myeloma cells.[158] Various cell cycle regulators are substrates of the proteasome. Inhibition of their timed turnover can inhibit the proliferation of cancer cells. Stabilization of pro-apoptotic factors such as Bik or Bim upon proteasome inhibition can contribute to induction of apoptosis.[185,186] Proteasome inhibitors seem to work especially well when applied in combination with conventional chemotherapy probably because inhibition of the proteasome may interfere with the ability of a cancer cell to cope with the inhibitory mechanisms caused by the chemotherapy.[158,187−189]

4. Regulatory Particles − Proteasome Activators

Structural analyses have revealed that, at least in eukaryotic 20S proteasomes, the entry point for substrates is closed by N-terminal residues of the α subunits.[77] This observation was consistent with the requirement of regulatory particles for the in vivo function of proteasomes.[190] Aside from several ATP-independent activators of the proteasome (PA28α,β, PA28γ, and PA200), the major activator in eukaryotic cells is a ~700 kDa ATP-dependent complex that has a sedimentation coefficient of ~19S, and was hence termed PA700 (proteasome activator of 700 kDa) or 19S regulatory particle (RP).[190−193] The 19S RP is an essential cofactor of the 20S CP in the degradation of ubiquitylated proteins. A related ATP-dependent activator of the proteasome from archaeons, termed PAN (proteasome- associated nucleotidase), has been instrumental in studies on the mechanism of proteasome activation.[194]

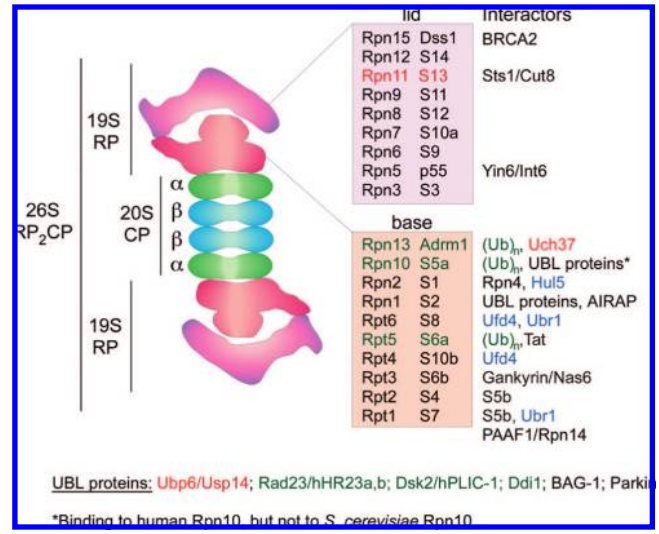

Figure 7. 26S proteasome and its interactions with other proteins. Shown is a schematic representation of the 26S proteasome, which is composed of a 20S core particle (CP) and two 19S regulatory particles (RP). The subunit composition (systematic and alternative names) of the 19S RP subcomplexes lid and base as well as important interactors are shown on the right. Polypeptides with deubiquitylating activity are printed in red. Subunits mediating direct or indirect binding of ubiquitin chains are printed in green. Interacting ubiquitin ligases are printed in blue. Rpt, regulatory particle ATPase; Rpn, regulatory particle non-ATPase.

4.1. 19S Regulatory Particle/PA700

Similar to the 20S proteasome, the 19S RP is a conserved structure present in all eukaryotes and is the only proteasome activator that is essential for viability. The 19S RP attaches to one or both ends of the 20S CP. The complex with two activators (RP_2CP) is commonly referred to as the 26S proteasome, although its actual sedimentation coefficient is ~30S.[195] To simplify a discussion related to this important complex, a unified nomenclature for its subunits has been established.[196] Of the 19 subunits, which are currently defined as components of the 19S RP, 15 are essential in *S. cerevisiae*, the nonessential ones being Rpn9, Rpn10, Rpn13, and Rpn15 (Figure 7). Based upon biochemical and structural properties, this complex can be subdivided into two subcomplexes, the base and the lid.[197]

The base subcomplex, aside from opening the gate into the 20S CP, is assumed to provide substrate interaction sites as well as to unfold and translocate proteins into the CP. The base subcomplex comprises six ~50 kDa subunits that, based upon sequence similarity, belong to the so-called family of AAA ("triple A") (ATPases Associated to a variety of cellular Activities) proteins.[198−201] This family of proteins is characterized by a highly conserved nucleotide binding module of ~230 amino acids, which is present in each of the six Rpt (Regulatory particle ATPase) subunits of the base. While many prokaryotic self-compartmentalized proteases including the proteasome are activated by homohexameric AAA complexes such ClpA, ClpX, HslU or PAN, the six Rpt subunits are assumed to form a heteromeric ring structure, which is attached to the α ring of the 20S CP.[202−204]

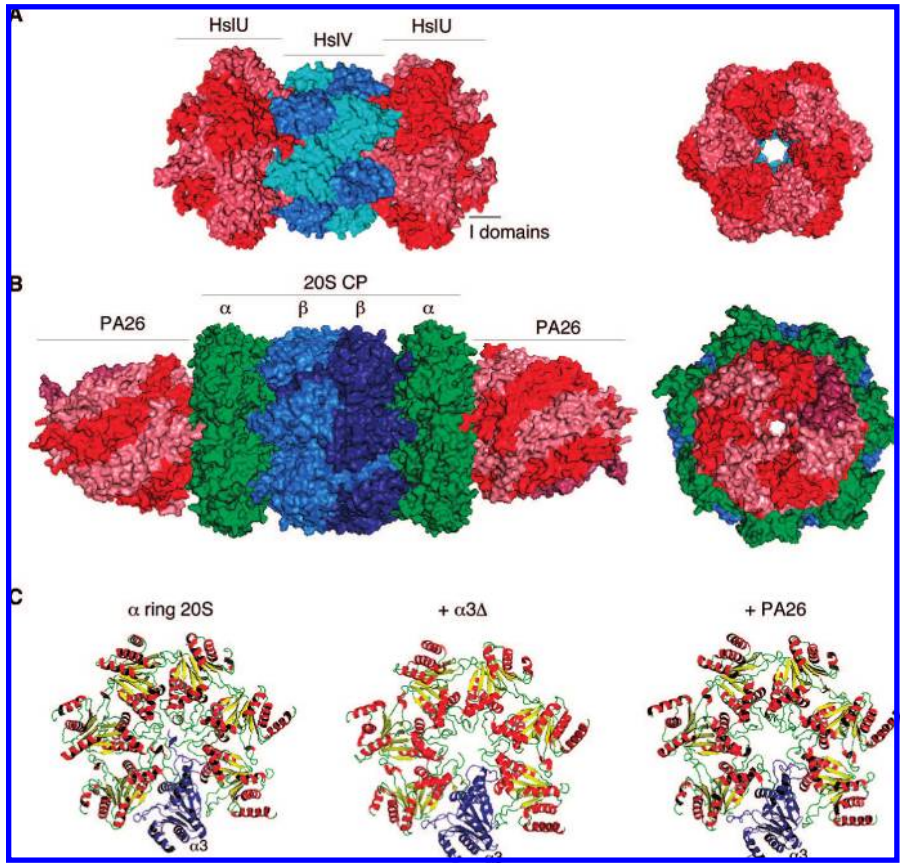

Figure 8. Association of activators with proteolytic core particles. (A) Surface representation of the crystal structure of *H. influenza* HslUV (PDB code 1G3I) in a side (left) and top view (right). The latter shows the axial channel through the protease. The identical six HslV and six HslU subunits are shown in different shades of blue and red, respectively. (B) Structure of the *T. brucei* PA26 in a complex with the *S. cerevisiae* 20S CP (PDB code 1FNT). The identical seven PA26 subunits are shown in different shades of red. The α rings of the proteasome are shown in green, and the *β* rings are in blue. (C) Shown are ribbon diagrams of different configurations of the *S. cerevisiae* proteasomal α rings. Left, uncomplexed wild-type; middle, uncomplexed α3Δ*N* mutant; right, wild-type complexed with PA26. The figure was prepared using PyMOL.

The only available crystal structure of an ATP-dependent activator of a proteasome-related protease is that of HslU/ClpY, the activator of HslV (Figure 8A).[64,66−68] Similar to activators of the proteasome, HslU uses C-terminal domains to make a stable contact with the proteolytic core particle formed by six HslV subunits. C-terminal helices of HslU intercalate between the HslV subunits transmitting conformational changes to the active site regions. Internal ∼130 residue domains (termed intermediate domains) extend outward from the complex.[67] It has been suggested that the proteasomal ATPase subunits are structurally similar to HslU, but have sequences related to the intermediate domain in HslU fused to their N-termini.[64]

Aside from the heterohexameric ATPase units, the base subcomplex of the 19S RP contains the four Rpn (Regulatory particle non-ATPase) subunits Rpn1, Rpn2, Rpn10, and Rpn13 (Figure 7). Two of these, Rpn1 and Rpn2, are the largest subunits (∼100 kDa) of the 26S proteasome. The repeat containing domains of these subunits were proposed to form an α helical solenoid resulting in an overall toroid structure.[205] This proposal is consistent with atomic force microcopy (AFM) images.[206] Biochemical and AFM data suggested that Rpn1 and Rpn2 form a stack of two toroids which is attached to the 20S CP α rings with the center of the toroid aligned with the axial channel of the proteasome.[206] Based upon these data, a speculative model was proposed, in which the Rpt ring encircles the Rpn1-Rpn2 stack, covering the remainder of the 20S surface.[206] Determination of high resolution structures will be required to determine the exact positioning of subunits within the regulatory particle.

The lid subcomplex is composed of nine Rpn subunits (Figure 7), some of which are indicative of a structural relation to the COP9 (Constitutive Photomorphogenesis 9) signalosome (CSN) and the translation initiation complex eIF3.[197] As in the CSN, six subunits (Rpn3, Rpn5, Rpn6, Rpn7, Rpn9, and Rpn12) share a PCI domain (proteasome, CSN, eIF3) at their C-terminus, and two have an MPN (Mpr1/Pad1 N-terminal) domain.[197,207,208] It has been suggested that TPR-like repeats preceding the PCI domains, together with a helical repeat structure in an N-terminal segment of the PCI domain, form an α solenoid. This structure might serve as a binding domain important for intracomplex interactions providing a scaffold within the lid complex and/or for the recruitment of cellular ligands.[208] The MPN domain of Rpn11 provides a metalloprotease (zinc-dependent) activity, which removes ubiquitin from substrates.[209−211] The MPN domain of Rpn8 is similar to that of Rpn11, but lacks residues that would be essential for metalloprotease activity. Aside from its deubiquitylating activity, the function of the lid in protein degradation is poorly understood. It appears to be essential for the degradation of ubiquitylated proteins, while proteasomes only capped with the base subcomplex have been shown to degrade peptides as well as casein.[197] One possibility is that, similar to the CSN, the main activity of which is deneddylation,[212,213] the lid's main

function might be to control degradation by deubiquitylation. In addition, the lid may provide sites for the interaction with other proteins (Figure 7).

It should be noted that the 19S RP is a very dynamic structure. Its subunit composition varies with the physiological state of a cell and with the conditions used for purification. The spectrum of proteins that copurify with it varies for example between preparations done in the presence or absence of ATP.[214] The Ubp6 deubiquitylating enzyme that interacts with the Rpn1 subunit is present in preparations of the 26S proteasome in substoichiometric amounts depending of the state of the cell.[215] The 19S RP moreover appears to be involved in processes such as DNA repair and the regulation of transcription independent of its association with the 20S CP.[216−223] Recent studies indicate that free 19S complexes interact with monoubiquitylated proteins such as the transcriptional activator Gal4 via their Rpn1 and Rpt1 subunits.[224] The observed absence of a similar binding for 19S particles attached to the 20S CP would be consistent with the possibility that the ubiquitin binding sites of these subunits might be located at the bottom surface of the base subcomplex, which makes contact with the CP.[206,224]

The stability of the 26S proteasome and of the 19S RP during proteolysis has been controversially discussed in the literature. One study that used a yeast proteasome-based in vitro system suggested that substrate degradation triggers an ATP hydrolysis-dependent dissociation and disassembly of the 19S RP, which was thought to promote the release of degradation products. It was proposed that dissociation of the 19S RP from the CP is a part of the mechanism of protein degradation.[225] Another group that used purified mammalian proteasomes for in vitro degradation experiments, in contrast, used various assays to demonstrate that the 26S proteasomes remained intact during substrate degradation.[226]

4.1.1. Gate Opening, Substrate Unfolding and Translocation into the 20S CP

The center of the α ring of eukaryotic proteasomes is sealed by N-terminal residues of its subunits.[77] The structure of the yeast proteasome indicated that the N-terminal residues of α3 have a particularly important role in plugging the gate as it extends across the center of the ring and makes contact with the N-terminal segments of the other six α subunits (Figure 8).[227,228] Deletion of residues 2−10 of α3 (α3ΔN) led to disordering of the N-terminal segments of the other α subunits and thereby to a constitutively open configuration (Figure 8), which enhances hydrolysis of peptides as well as of native unfolded proteins (casein) 20-fold.[227,228] A similar gate opening was achieved by mutating Asp7 in α3 to Ala.[227] The Asp residue corresponds to Asp9 in the *Thermoplasma* α subunit, and is part of a conserved "YDR" motif. Asp9 of α3 forms a hydrogen bond with the respective Tyr8 in α4, another one with the α3 main chain, and a salt bridge with Arg10 (numberings according to the positions in the *Thermoplasma* proteasome).[227] A similar increase in activity as observed in open gate mutants can be achieved by addition of SDS at low concentration.[229] An interesting observation came from a comparison of the cleavage products resulting from digestion of casein either by wild-type or α3ΔN 20S proteasomes. Wild-type proteasomes produced larger quantities of small peptides (2−3 residues) than did the mutant, while the latter produced increased amounts of longer peptides (20−30 residues). These data suggested that the α3ΔN proteasome generates fewer small peptides because of a more rapid exit of longer peptides from the proteolytic chamber.[228]

The studies described above indicated that regulation of the gate in the 20S CP, which is an essential in vivo function of proteasome activators, provides control of both entry of substrates and exit of peptide products. Recent biochemical and structural studies have provided an understanding of how this control is achieved either by ATP-independent or by ATP-dependent activators. The first insight was obtained by solving the crystal structure of a complex of the yeast 20S CP and the *Trypanosoma brucei* PA26, a protein remotely similar to mammalian PA28α (Figure 8B).[51,230,231] The homoheptameric PA26 complex attaches to the α ring surface of the CP inducing conformational changes in the N-terminal residues of its subunits (Figure 8C). Specifically, the C-terminal tails of PA26 provide binding affinity by inserting into pockets between the α subunits of the 20S proteasome. As a consequence, internal nine residue activation loops of PA26 are pressed against the α subunits' reverse-turn loops resulting in a rotation of the N-terminal segments and thereby in gate opening.[230] Repositioning of Pro17 (numbering according to the *T. acidophilum* proteasome) by the activation loop induces an ordered 7-fold symmetric pore conformation that is stabilized by interactions between the four highly conserved residues Tyr8, Asp9, Pro17, and Tyr26. Interestingly, these residues are also conserved in archaeons, which lack PA26 or related 11S activators. Mutation of these residues in the *T. acidophilum* proteasome, however, impaired protein degradation stimulated by PAN. These and other experiments suggested that the four amino acid cluster, which includes the first two residues of the YDR motif described above, underlies a conserved mechanism of stabilizing an ordered open conformation of the proteasome, which is required for the degradation of folded proteins.[231]

While the requirement to adopt an open conformation through interactions of the above-mentioned cluster is likely to be generally important, the mechanism by which the ATP-dependent activators 19S RP and PAN induces gate opening appears to be different from that of PA26. The latter two activators share a so-called HbYX (hydrophobic-Tyr-X) motif at their very C-terminus. In the 19S RP, this motif is found at the ends of the Rpt1−3 and Rpt5 subunits. Docking of the C-termini either from PAN, Rpt2, or Rpt5 between the α subunits is apparently sufficient to trigger gate opening to allow degradation of peptide substrates as was demonstrated with short peptides mimicking these C-termini.[232,233] The Rpt5 peptide, which cross-links to α4, was in addition shown to enable degradation also of proteins, while the Rpt2 peptide, which cross-links to α7, alone was unable to do so. Both peptides promoted substrate proteolysis synergistically suggesting that CP activation may be a multistate process.[234] Peptides corresponding to the C-terminal residues of PA26 or PA28, in contrast, did not stimulate gate opening, indicating that the mechanism of activation is different for these activators.[235] Deletion of the last residue or mutation of the hydrophobic or Tyr residues in the HbYX motif of PAN peptides prevented gate opening, confirming the importance of these residues.[232] While gate opening can be triggered by the above-mentioned short peptides, PAN or the 19S RP only do so when bound to ATP.[235] This observation indicates that ATP binding induces a conformation of the activators that allows their C-termini to be correctly positioned to fit into the docking sites in the CP α

ring. ATP hydrolysis, however, appears to be neither required for gate opening nor for translocation of unfolded proteins as these processes occur in the presence of ATPγS.[194,235,236]

Degradation of folded and ubiquitylated proteins, in contrast, requires ATP hydrolysis.[235,236] The base of the 19S RP purified from yeast cells displays chaperone activity in promoting protein folding and preventing aggregation.[237] These data are consistent with a role of ATPases in the base as reverse chaperones that unfold substrates, thereby allowing them to enter the 20S CP through its gated entry pore.[238] A similar chaperone activity has been observed for PAN.[239] For the degradation of one SsrA-tagged GFP molecule, PAN consumed ~330 molecules of ATP. For the 19S RP it was shown that ATP hydrolysis is important for a mechanistic coupling of substrate unfolding, deubiquitylation and translocation.[236] In order to understand the contribution of the distinct Rpt subunits in the base of the yeast proteasome, a conserved Lys residue in the Walker A motif of their AAA domain that is critical for ATPase activity was mutated to Ser.[240] Such mutations were lethal for four of the Rpt subunits and severely impaired growth in the case of Rpt1 and Rpt5 indicating that these ATPases are not functionally redundant. Substitution of the same residue to Arg was only lethal for Rpt2. Additional studies with a suppressor of the Rpt2-K229R mutation suggested that ATP binding to Rpt2 may be particulary important for gate opening by the mechanism described above.[137,240] Yeast mutants expressing a proteasome lacking N-terminal segments of two of its α subunits (α3α7ΔN), which increased its ability to degrade casein, were phenotypically normal under favorable growth conditions. These mutants, however, were sensitive to conditions (starvation) under which the activity of the 26S proteasome is downregulated by disassembly of 19S RPs from the CP.[241] These findings therefore provided physiological evidence supporting the idea that sealing the gate is important to prevent nonspecific activity of free 20S CPs.

A recent study showed that, apart from the Rpt subunits, purified Rpn1−Rpn2 complexes, which attach to the center of the CP α ring, can promote hydrolysis of peptides (although only 4-fold) suggesting that this central element in the base may contribute to gating as well.[206]

4.1.2. Recognition of Ubiquitylated Substrates

The essential role of the 26S proteasome in the UPS requires the recognition of ubiquitin-modified protein substrates. Several targeting mechanisms have been elucidated. They involve subunits of the proteasome as well as ubiquitin shuttling systems, both of which bear ubiquitin binding domains (UBD).[242,243] Several subunits of the base subcomplex of the 19S RP have been implicated in ubiquitin binding. The first ubiquitin receptor identified, originally designated "multiubiquitin chain binding protein", was Rpn10.[244−246] This protein is not only a component of the 19S RP, wherein it stabilizes the association of the base with the lid, but to a significant extent is also found as a free subunit.[197,246] Rpn10 specifically binds to ubiquitin in vitro with a preference for chains longer than four ubiquitin moieties.[246] Recognition of ubiquitin chains is mediated by ubiquitin-interaction motifs (UIM), which were first characterized for Rpn10 and later found to be present in a variety of ubiquitin binding proteins.[247,248] While yeast Rpn10 has only one UIM, two UIMs are found in human Rpn10.[247] The latter act cooperatively in the recognition of ubiquitin chains.[247,249] Mutation of the UIM in yeast proteasomes had surprisingly little effect on growth properties and only certain substrates of the UPS were stabilized.[246,250] These data indicated that additional ubiquitin receptors must exist in or at the proteasome. In vitro, Rpt5 could be cross-linked to ubiquitin chains suggesting that this subunit in the base of the 19S RP might be another ubiquitin receptor.[251] This conclusion, however, has not yet been confirmed by a mutational analysis, and a ubiquitin binding domain in Rpt5 has not been identified. Very recently, the Rpn13 subunit of the base has been characterized as a novel type of ubiquitin receptor.[252] In contrast to the UIM domain, the residues in Rpn13 that contact ubiquitin are noncontiguous and distributed over several loops of a domain with structural similarity to pleckstrin. This novel structural motif was therefore termed Pru domain (pleckstrin-like receptor for ubiquitin). Yeast proteasomes, in which the critical residues for the binding of ubiquitin in Rpn10 and Rpn13 were exchanged, have lost most of their capacity to bind ubiquitin-conjugates in vitro suggesting that these two subunits provide the main proteasome-intrinsic ubiquitin chain receptors. Cells expressing this mutant proteasome instead of the wild-type form, however, grew nearly normally and were only slightly more sensitive to amino acid analogues.[252]

Mammalian Rpn10/S5a and Rpn13/ADRM1/GP110 not only bind ubiquitin but also proteins such as Rad23/hHR23A,B, and Disk2/hPLIC1,2 that bear ubiquitin-like (UBL) domains.[252] The latter were identified earlier as proteins that interact both with ubiquitin chains and the proteasome.[253−261] Aside from a UBL domain close to their N-termini, they bear one or two ubiquitin binding domains termed UBA (ubiquitin-associated).[262] The UBA domain has been shown to preferentially bind to ubiquitin chains.[260,263] Based upon these properties it is assumed that these UBL-UBA domain proteins act as shuttling factors that target polyubiquitylated substrates to the proteasome, utilizing the UBA domains to bind the substrate and the UBL domain to dock to the 19S RP.[256,257,264,265] An important aspect of UBL-UBA proteins, aside from the above-mentioned interactions, is that their domains can either "silence" each other intramolecularly, or be used to promote binding to other UBL-UBA, UBL or UIM domain proteins.[266−269] While mammalian S5a/Rpn10 binds UBL-UBA proteins via its second UIM, yeast Rpn10, which lacks this motif, apparently does not do so. Here instead, the shuttling factors bind to the Rpn1 subunit in the base (Figure 7).[270] Genetic evidence obtained in *S. cerevisiae* indicated that UBL-UBA proteins and Rpn10 have a functional overlap in the targeting of ubiquitylated substrates to the proteasome.[256,270,271] In particular strains lacking Rpn10 and Rad23 are severely impaired in ubiquitin-dependent proteolysis.[250,270,272] Surprisingly, yeast cells with mutations in all five known ubiquitin receptors (Rpn10, Rpn13, Rad23, Dsk2, and Ddi1) are viable, suggesting that additional ubiquitin receptors are capable of targeting proteins to the proteasome.[252] One function of multiple ubiquitin receptors might be to increase the affinity for a substrate by simultaneously binding to its ubiquitin chain. Another role of distinct ubiquitin receptors is to provide an additional layer of substrate specificity by recognizing either structural aspects of the ubiquitin tag or of the substrate itself.[250,252,267,269,270]

4.1.3. 19S RP-Linked Deubiquitylating Activity

In the previous paragraph, we discussed the importance and recognition of the ubiquitin tag for proteasomal targeting. Removal of sometimes complex ubiquitin chains by protea-

some-associated deubiquitylating (DUB) activities is required for efficient substrate degradation, probably because these chains would interfere with translocation into the CP.[209,210,236,273] Proteasomal DUBs, in addition, were suggested to provide an "editing" or "checkpoint" function that scrutinizes substrates bound to the proteasome.[274−276]

Several DUB activities are associated with the 19S RP. The DUB activity of the lid subunit Rpn11 has been mentioned earlier. This intrinsic DUB activity mediates an en bloc removal of ubiquitin and appears to be critical for proteasome function. Its activity is coupled to substrate degradation and requires ATP.[209,210,236] Its metalloprotease activity can be inhibited in vitro with Zn chelators such as TPEN or 1,10-phenanthroline.[209,210]

Another DUB activity associated with the 19S RP is provided by Ubp6 in yeast or its orthologue Usp14 in mammalian proteasomes.[273,277,278] Ubp6 is a cysteine protease that interacts with the Rpn1 subunit of the proteasome via its N-terminal UBL domain (Figure 7).[277,278] Ubp6/Usp14 degrades ubiquitin chains from their distal ends.[215] The activity is stimulated by its binding to the proteasome.[277,279] In *S. cerevisiae*, a deletion of the *UBP6* gene is synthetically lethal with an active site mutation of Rpn11 indicating that these two DUB activities have complementary functions.[273]

Ubp6 moreover has an important function in ubiquitin homeostasis. In its absence, some of the substrate-linked ubiquitin is degraded along with the substrate.[280] If ubiquitin levels in the cells are low, expression of the *UBP6* gene is enhanced resulting in a larger fraction of proteasomes being loaded with Ubp6, which in turn increases the efficiency of ubiquitin recycling.[215] Usp14 (ax^J) mutations were found to be the cause of ataxia (ax) in mice.[281] Reduced levels of free ubiquitin were observed in cells from these ax^J mice.[282] Increased expression of Usp14, on the other hand, has been linked to formation of metastases derived from colorectal cancer.[283]

In *S. cerevisiae*, surprisingly, it could be shown that the presence of Ubp6 on the proteasome increases binding of the conserved ubiquitin ligase Hul5, an E4 that is thought to regulate the residence time of substrates at the proteasome by extending their ubiquitin chains.[275] Ubp6 and Hul5 are thought to provide a regulated and balanced proteasome-associated substrate checkpoint.[32,275,276] For Ubp6, this function, aside from its DUB activity, involves a noncatalytic property that delays substrate degradation.[215]

Uch37 is another DUB enzyme of the cysteine protease type that appears to be constitutively associated with the proteasome via the Rpn13 ubiquitin receptor subunit. Orthologues of Uch37 are found in eukaryotes ranging from *Schizosaccharomyces pombe* to humans, but are absent from *S. cerevisiae*.[274,284−287] Similar to Ubp6, Uch37 releases ubiquitin moieties from the distal ends of substrate-attached ubiquitin chains.[274] The activity of Uch37 is stimulated by its binding to the proteasome, which results in a repositioning of an autoinhibitory extension in Uch37.[287] RNAi experiments in human cells indicated that Uch37 and Usp14 have overlapping functions that are important for protein degradation and cell growth.[288] Together these studies on proteasome-associated DUB activities indicated that they have crucial functions in ubiquitin-dependent protein degradation and ubiquitin homeostasis.

4.1.4. Proteins Interacting with the 19S RP

The 19S RP, aside from the discussed unfoldase, gate opening, and deubiquitylating activities, can be viewed as an interaction platform for many proteins that either escort substrates to the proteasome or modulate the function of the 19S RP or the proteasome.[289] These interacting proteins include various ubiquitin ligases such as SCF (SKP1, Cullin, F-box) complexes, anaphase promoting complex, Ubr1, Ufd4, Hul5 and Parkin, as well as ubiquitin conjugating enzymes.[214,268,290−292] As shown for the Ufd4 ligase, interaction of these ligases with the 19S RP can be critical for substrate targeting.[291] Other 19S RP interacting proteins that influence substrate targeting, localization, assembly, or regulation of the proteasome are discussed below.

Gankyrin. Gankyrin is an oncoprotein that is often overexpressed in hepatocellular carcinomas. The name indicates this link ("gann" is the Japanese word for "cancer") as well as the presence of ankyrin repeats, seven of which are present in gankyrin.[293] Ankyrin repeats are ~33 residue domains that fold into two antiparallel α helices followed by a β hairpin, which projects away from the α helices nearly at a right angle.[294] Gankyrin, alias p28, and its yeast homologue Nas6, were earlier suggested to be subunits of the 19S RPs.[295] It is probably more appropriate to classify gankyrin/Nas6 as a proteasome-interacting protein.[214] It interacts with Rpt3 in the base of the 19S RP via the first six of its ankyrin repeats.[293,296] It is thought that gankyrin's interaction with the proteasome and its binding to the Mdm2 ubiquitin ligase underlie its role in enhancing the degradation of the retinoblastoma and p53 tumor suppressors.[297−300] These studies identified gankyrin as a factor that promotes targeting of certain substrates by directly interacting with the proteasome.

Yin6/Int6. The breast cancer associated gene eIF3e/Int6 encodes a PCI domain protein that was shown to interact with the PCI domain containing complexes 19S RP, eIF3a, and CSN.[301−303] The fission yeast Int6 homologue Yin6 appears to be required for normal function of the proteasome. In the absence of Yin6, Rpn5 is not correctly assembled into proteasomes and mislocalized to the cytosol.[301] *S. cerevisiae* Pci8 is a PCI domain protein distantly related to Int6. Shared properties of Pci8 and Int6 are that they interact with eIF3, the CSN and Rpn5.[304−308] A role of Pci8 in proteasome function, similar to what has been described for Yin6, has not yet been reported. Mutants lacking Pci8, however, are phenotypically inconspicuous suggesting at most a minor role of this protein in proteasome function.

Cut8/Sts1. The *S. pombe* Cut8 protein, as well as its *S. cerevisiae* homologue Sts1/Dbf8, have been found to be required for ubiquitin-dependent proteolysis.[309,310] Cut8 was shown to bind to the 26S proteasome and to regulate its nuclear localization. While Sts1 interacted with Rpn11 in a two-hybrid assay,[311] Cut8 interaction with the proteasome was shown to be promoted by ubiquitylation. This modification, which was mediated by the Ubr1 or Rhp18 ubiquitin ligases together with the Rhp6/Rad6 ubiquitin conjugating enzyme, is required for Cut8-dependent nuclear localization of the proteasome in fission yeast.[312] Cut8-dependent distribution of proteasomes apparently also occurs in metozoans as a Cut8 orthologue is required for normal nuclear localization of proteasomes in *Drosophila*.[312]

PAAF1. A 43 kDa protein termed PAAF1 (proteasomal ATPase-associated factor 1) interacts with the 19S RP.[313] Overexpression of PAAF1 in HeLa cells decreased the level

of the 26S proteasome with a concomitant increase in free 19S RP in a dose-dependent manner. These data indicated that PAAF1 binding to proteasomal ATPases interferes with the assembly of 26S proteasomes. RNAi-mediated depletion of PAAF1 enhanced cellular proteasome activity.[313] Together these results suggested that PAAF1 functions as a negative regulator of 26S proteasome assembly. PAAF1 recruitment to the proteasome is promoted by HIV Tat (see below). Rpn14, a yeast relative of PAAF1, has been implicated in the binding of certain ubiquitylated substrates and in stability of the 26S proteasome.[314]

Tat. The human immunodeficiency virus type 1 (HIV-1) transactivator protein, Tat, was reported to interact with the 20S CP as well as with the 19S RP.[315,316] Interaction of Tat with the 20S CP inhibited its activity and prevented its interaction with PA28. This observation suggested that the inhibition of antigen presentation by Tat may be due to interference with PA28 function.[316] While in vitro data suggested that Tat stimulated proteolytic activity of the 26S proteasome,[316] in vivo experiments indicated that Tat recruits PAAF1 to promote dissociation of the 19S RP from the CP.[223] Free 19S RP was shown to act as a coactivator of Tat in promoting transcription from the HIV-1 long terminal repeat.[223]

Rpn4. The yeast transcription factor Rpn4 interacts with the 19S RP and is a substrate of the 26S proteasome.[317,318] Interestingly its degradation appears to involve both ubiquitin-dependent and ubiquitin-independent mechanisms.[319] Rpn4 is a transcription factor that controls the expression of proteasome subunit genes as well as of many other genes involved in various stress responses.[317] Rpn4 is therefore a central player in a regulatory feedback system that detects proteasome function and availability, and controls its abundance.[318,320]

AIRAP. Arsenite-inducible RNA-Associated protein (AIRAP) was identified as a ~19 kDa protein that is induced upon exposure to arsenic in mammalian cells.[321] AIRAP is associated with the 26S proteasome via direct interaction with the Rpn1/S2 subunit. Inactivation of AIRAP leads to an amplification of the accumulation of poly-ubiquitylated proteins caused by the environmental toxin arsenic.[321,322] Binding of AIRAP to the proteasome requires an N-terminal zinc finger domain and is enhanced in the absence of ATP. Interestingly, usually only one molecule of AIRAP is bound to a 26S proteasome. Such AIRAP containing proteasomes have slightly higher peptidase activity compared to the proteasome without AIRAP. Based upon these findings it was suggested that AIRAP may alter the 26S proteasome in a similar manner as PA28, which increases the activity of 19S-CP-PA28 hybrid proteasomes (see below). It was speculated that AIRAP might thereby counteract proteotoxic effects such as those imposed by arsenic by facilitating the transit of substrates that would otherwise interfere with proteasome availability or function.[13] *Caenorhabditis elegans* has only one AIRAP orthologue called AIP-1, which is constitutively expressed but also induced by environmental stress. Its presence is important to cope with proteotoxic stress including treatment with arsenic, but is also required for a normal life span under nonstressed conditions.[323] By contrast, mammals bear two AIRAP paralogues, the arsenite-inducible AIRAP and the constitutively expressed AIRAP-like (AIRAPL) protein. Association of AIRAPL with the proteasome is regulated post-translationally. AIRAPL is associated with the endoplasmic reticulum (ER) suggesting that its function may be linked to ER-associated degradation.[323] AIRAPL and AIP-1 share UIM domains suggesting that these proteins, aside from altering the proteasome's degradation properties, may act as ubiquitin adaptors of the proteasome.[323]

4.2. 11S Regulators (PA28α,β, PA28γ)

The 11S regulator (REG) (also known as PA28) was identified as an alternative activator of the 20S CP.[324,325] It is a heptameric complex composed of two closely related ~28 kDa subunits, PA28α and PA28β, the expression of which is strongly induced by interferon-γ.[326] ATP-independent binding of PA28 to the 20S CP activates cleavage of peptides, whereas it does not enable the degradation of larger or ubiquitylated proteins.[324] Overexpression of PA28α was shown to enhance the presentation of certain viral epitopes.[327] Ablation of PA28α and PA28β in mice caused a deficiency in the processing of certain melanoma tumor antigens,[328] but not a defect in the assembly of immunoproteasomes as was reported earlier.[329]

PA28α forms homoheptamers, the structure of which has been solved.[330] As discussed earlier, the cocrystal structure of the remotely related PA26 activator from *T. brucei* has provided structural insight into the mechanisms of gate opening (Figure 8).[230] The analysis of proteasomal complexes from mammalian cells led to the detection of hybrid proteasomes, in which the 20S CP is capped by the 19S RP enabling recognition and unfolding of ubiquitylated substrates at one end, and with PA28 at the other end.[331] It has been suggested that the role of PA28 in these hybrid proteasomes may be to reduce processivity by allowing the release of longer peptides thereby increasing the probability of the generation of certain antigenic peptides.[230] Hybrid proteasomes were reconstituted in vitro and analyzed for their proteolytic activities.[332,333] When compared to the 26S proteasome, hybrid proteasomes did not produce peptides that were significantly different in length. Instead, differences in the sequences of the peptides that were produced suggested that PA28 changes the cleavage site specificity of the proteasome.[333]

While PA28α,β is predominantly detected in the cytoplasm, another related activator, PA28γ/REGγ is mainly found in the nucleus where it is localized on chromosomes during mitosis.[328,334] PA28γ is a homoheptameric complex, the subunits of which were originally described as K_i, a major autoantigen found in Lupus erythematosus patients.[335,336] Inactivation of PA28γ in mice did not result in a general defect in antigen presentation but only caused defects in the processing of specific antigens.[328,337] Instead, a growth-retardation phenotype and defects in cell cycle progression were observed in these mice.[338] In contrast to PA28α,β, PA28γ has been shown to promote proteasomal degradation of proteins. Strikingly, PA28γ-mediated degradation of cellular proteins such as p53, p21, p16, p14 and steroid receptor coactivator-3, as well as of hepatitis C virus core protein apparently occurs in a ubiquitin- and ATP-independent manner.[16,339−343] PA28γ in vitro induces only the trypsin-like activity of the CP, whereas the chymotryptic and postacidic activities are suppressed.[344] How this activator may promote ATP-independent degradation of proteins is unclear. It has been discussed that PA28γ may promote the entry of internal unstructured domains of substrates into the CP thereby enabling their cleavage.[345]

4.3. PA200/Blm10

The PA200 proteasome activator was identified in mammalian cells as a ~200 kDa nuclear protein that activates proteasomal hydrolysis of peptides, but not of folded proteins.[346] In mammals, PA200 seems to be involved in DNA repair.[346] Upon γ-irradiation, PA200 relocalizes from a uniform nuclear distribution to chromatin yielding a punctate pattern. [346,347] A recent study suggested that upon irradiation PA200 forms hybrid proteasomes with 20S CP and the 19S RP that accumulate on chromatin. The same study correlated the protective effect of PA200 against γ-irradiation with its stimulatory effect on the postacidic activity of the proteasome.[348] Initial reports on bleomycin sensitivity of a yeast strain lacking the 245 kDa yeast homologue of PA200 needed to be revised.[349,350] The sensitivity was observed in a *blm3/ubp3* mutant, while the gene encoding the yeast PA200 (now called Blm10), was apparently cloned as a suppressor of a *blm3/ubp3* mutation. While the latter mutation was reported to cause bleomycin hypersensitivity, no such phenotype has been observed for a *blm10*Δ strain.[351−353] It should be noted therefore that the name Blm10, which indicates a role in bleomycin resistance, is probably misleading for the yeast PA200.

Similar to PA200 in mammals, Blm10 was reported to activate peptide cleavage by the proteasome.[353,354] Sharing ~20% sequence identity, the two proteins are structurally characterized by the presence of a new type of HEAT repeats (named after Huntingtin, Elongation factor 3, subunit A of protein phosphatase 2A, and TOR1 kinase),[355,356] which suggests that these proteins assume a solenoid fold similar to Rpn1 and Rpn2.[205,356] A HEAT repeat encompasses typically 37−50 residues and folds into a hairpin formed by two α helices separated by a sharp turn.[356,357] These hairpins form curved structures with one of the two helices located on the concave side and the other on the convex surface.[356]

Electron microscopic studies of Blm10 or PA200 attached to CPs showed that these activators, similar to PA700 and PA28, associate with the α rings conferring dome-like hollow extensions to the particle. Reduced electron densities in the centers of the α rings indicated that attachment of the PA200-type regulators mediate gate opening.[353,358] As a result of this opening, the axial channel of the proteasome appears to extend into a chamber formed by the activator. While Blm10 seems to contact all seven α subunits and has at most a small opening toward the distal end of the complex, in the PA200-CP complex an opening to the side was observed, as PA200 does not contact the α7 subunit.[353,358]

The physiological role of the yeast Blm10 as an activator remains controversial. Yeast strains lacking Blm10 are phenotypically inconspicuous.[353] The reported activation of peptide cleavage activity by Blm10 is modest (~4-fold increase),[353,354] and was not detected in another study.[359] In a recent paper, it was reported that yeast Blm10 preferentially binds to CPs in the open gate formation. Binding of Blm10 to both ends of a CP in these experiments led to an inhibition of peptide cleavage activity suggesting that Blm10 may act as an inhibitor of untimely activated 20S CP.[360] Other studies found Blm10 in association with proteasome precursor complexes implicating it in proteasome biogenesis (see below).[359,361,362]

5. Proteasome Biogenesis

Another area of proteasome research concerns its assembly and regulation. The assembly pathway of the 20S CP, which in eukaryotes is promoted by several dedicated chaperones, is fairly well understood (see below), while relatively little is known about the assembly of 19S RPs.

5.1. Assembly of Prokaryotic Proteasomes

Prokaryotic proteasomes are the simplest found in nature and most are formed by only two distinct subunits, α and β, which are arranged into four stacked heptameric rings. α subunits from the archaeon *Thermoplasma acidophilum*, when expressed in *E. coli* in the absence of the β subunits, assembled spontaneously mainly into pairs of heptameric rings.[95] In contrast, β subunits expressed in the absence of α subunits remained monomeric and in an unprocessed and proteolytically inactive form. When α and β subunits were coexpressed, fully assembled and functional proteasomes were detected. Their formation occurred both in the presence and absence of the β subunit propeptides.[95] Structural analysis of the proteasome from *Archaeoglobus fulgidus* indicated that the contact areas between α subunits are large enough to enable self-assembly of α subunits. Contact areas between β subunits, in contrast, are much smaller explaining why additional contacts with the α subunits are required to build a β ring.[49] Analysis of the structure of α rings from *A. fulgidus* formed upon expression in *E. coli* revealed that it has nearly the same conformation as α rings in the assembled proteasome, with the exception of the N-terminal regions, which interestingly project away from the surface in a similar way as found in proteasomes complexed with PA26.[49,363] These studies suggested that, in archaeons, α rings represent starting platforms for the formation of higher-ordered intermediates with β subunits. In vitro reconstitution experiments with α and β subunits from *Thermoplasma* as well as from *Methanosarcina thermophila* and *Methanococcus jannaschii* demonstrated that no additional factors are required for the assembly of these archaebacterial proteasomes.[100,364−366]

In the eubacterium *Rhodococcus*, in contrast, no formation of ring structures was observed with individually expressed subunits.[367] The same is true for the proteasome subunits from *Mycobacterium* and *Frankia*.[55,368] The 20S proteasomes from these *actinomycetes* have a substantially smaller contact region between α subunits than the corresponding subunits in the proteasomes from *Thermoplasma*.[53,55] The smaller contact region between α subunits could be the structural reason for a lack of spontaneous α ring assembly. Instead α and β subunits apparently form dimers that subsequently assemble into half-proteasome precursor complexes. The propeptides of the *Rhodococcus* β subunits not only promote subunit folding but also act as an intramolecular assembly chaperones by increasing the β subunit interaction surfaces thereby facilitating oligomerization of $\alpha-\beta$ dimers.[53,367] The propeptides of the mycobacterial β subunits, in contrast, are not required for the assembly of intact proteasome upon coexpression of α and β subunits in *E. coli*.[54,55]

5.2. Assembly of Eukaryotic Proteasomes

The assembly of eukarytic 20S proteasome is far more complex than that of its prokaryotic relatives because each of its four rings is formed by seven distinct subunits, which

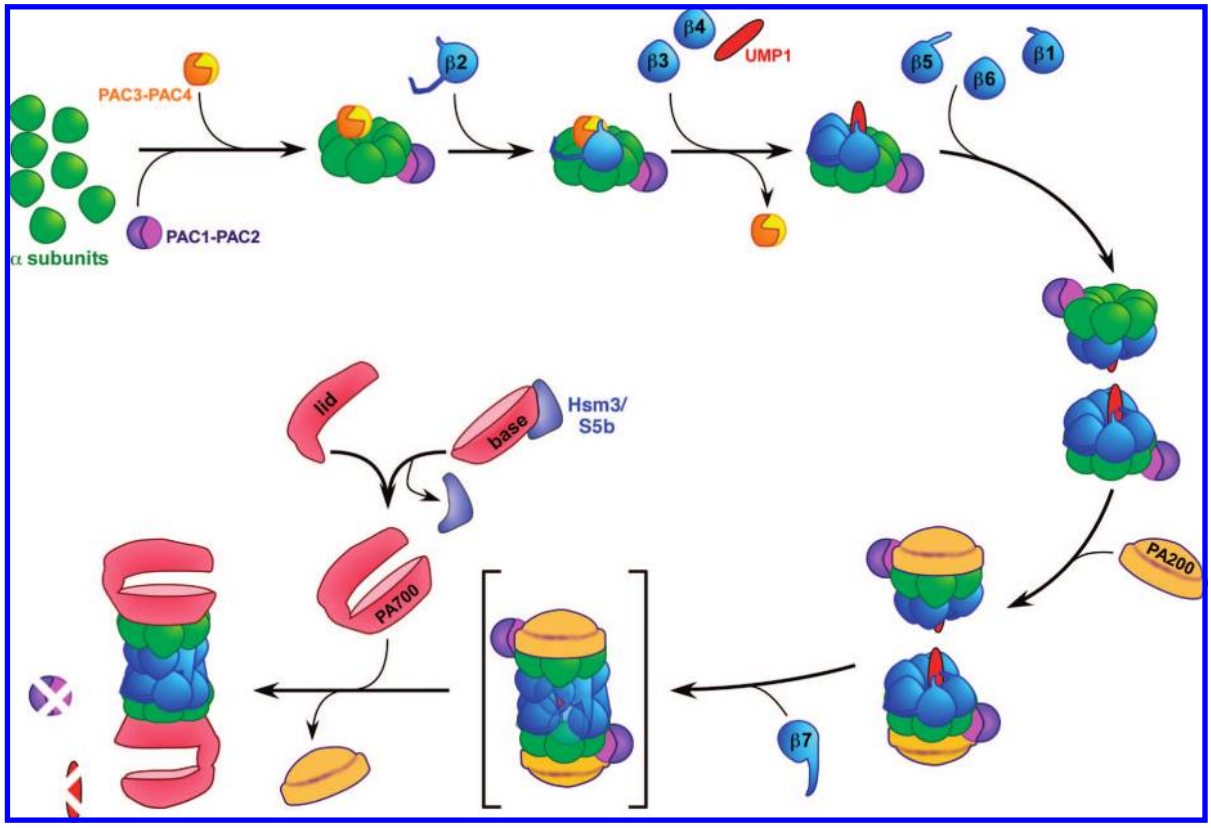

Figure 9. Eukaryotic proteasome assembly pathway. Shown is a schematic model that summarizes the order of events in the assembly of 26S proteasomes and the involvement of dedicated proteasome assembly chaperones and activators. Two heterodimeric chaperone complexes, PAC1-PAC2 and PAC3-PAC4, promote the assembly of the subunits α1−α7 into heptameric rings. PAC1-PAC2 prevent the dimerization of such rings (not shown). The first β subunit to enter the complex is β2, followed by UMP1, β3, and β4. During these steps, PAC3-PAC4 is evicted from the complex. Then, β5, β6, and β1 join the complex to form a half-proteasome precursor complex that only lacks β7. At least in yeast, this complex is found in association with the orthologue of human PA200. Dimerization of such precursor complexes is driven by the binding of β7. Its long C-terminal extension and PA200 stabilize the nascent proteasome, which is short-lived and therefore shown in brackets. The nascent proteasome is activated by autocatalytic maturation of its β subunits. The activated proteasome then degrades the chaperones UMP1 and PAC1-PAC2. While the nascent proteasome is preferentially associated with PA200, the mature 20S proteasome is mostly complexed with 19S RP/PA700. Assembly of the 19S RP is promoted by Hsm3/S5b. Some of the β subunits (shown in blue) are drawn with N-terminal propeptides, which are cleaved upon 20S CP formation, and with C-terminal extensions.

occupy a defined position. As a consequence of this increased complexity, assembly of eukaryotic CPs requires several dedicated chaperones: PAC1-PAC2, PAC3-PAC4, and UMP1 (Figure 9). In addition, the 20S CP has to bind to 19S RPs to form the 26S proteasome. Recent evidence indicates that these subcomplexes influence each others' assembly.

5.2.1. Assembly of α Ring Intermediates

Similar to the pathways described above for archaebacterial proteasomes, the assembly of eukaryotic proteasome CPs is initiated by the formation of α rings. Several eukaryotic α subunits have retained the ability to spontaneously assemble into homomeric rings. The human α7 subunit expressed in *E. coli*, for example, was found to form double ring structures similar to those observed with the Thermoplasma α subunits,[369] while the human subunits α6 or α1 were unable to do so. Curiously, when expressed together with α7, these subunits were incorporated in the double ring assemblies in variable positions.[370] The more complex task to be achieved by the seven distinct eukaryotic α subunits is to find an exact position in an α ring, that is defined by the two neighboring subunits. This task is promoted by dedicated chaperone complexes.

5.2.2. Proteasome Assembly Chaperones PAC1-PAC2 and PAC3-PAC4

PAC1 and PAC2 were first identified in human cells as two polypeptides of 33 and 29 kDa, respectively, that were associated with intermediates occurring early in the proteasome assembly pathway.[371] They were found in complexes with a subset of α subunits but also in intermediates containing all seven α subunits, apparently α rings.[371] PAC1 and PAC2 form heterodimers that bind directly to α5 and α7 and promote the formation of α rings.[371] siRNA-mediated depletion of PAC1 or PAC2 resulted in the accumulation of α ring dimers similar to those observed upon expression of prokaryotic α subunits (see above) indicating that PAC1-PAC2 prevents the dimerization of α rings under normal conditions. These and related findings characterized this heterodimeric protein as a proteasome assembly chaperone (PAC).[371] PAC1-PAC2 remains associated with intermediates until assembly of the proteasome is complete. Once active, the proteasome apparently degrades this chaperone.[371] It remains unclear to which surface of an α ring PAC1-PAC2 is bound and whether it is enclosed in the nascent 20S particle during the assembly process.

Proteins with weak sequence similarity to PAC1 and PAC2 were subsequently identified in *S. cerevisiae* by several

independent approaches and hence received several names, PBA1/POC1 for the PAC1-related protein and PBA2/POC2/ADD66 for the PAC2-related one. Functional analyses characterized Pba1 and Pba2 as orthologues of human PAC1 and PAC2 as they were found in similar assembly intermediates of yeast proteasomes.[362,371−373]

Affinity-tagged PAC1 was used to purify early intermediates in the assembly of proteasomes from human cells. In α ring intermediates containing the PAC1-PAC2 dimer another small 14 kDa protein was identified. This protein, termed PAC3, could be detected in complexes containing β2 and Ump1, but was absent from complexes containing other β subunits.[374] PAC3 was later found to form stoichiometric complexes with another protein termed PAC4.[372] The absence of one of these proteins resulted in the degradation of the other indicating that the heterodimer is likely to be the functional entity in vivo.[372] This chaperone complex is required to achieve normal cellular proteasome activity since siRNA-mediated depletion of PAC3 resulted in a reduction of proteasomal activity and accumulation of ubiquitylated proteins.[375] Biochemical analysis revealed that PAC3 knockdown caused an accumulation of free forms of α subunits and of PAC1-PAC2. Together, these studies identified PAC3-PAC4 as a heterodimeric chaperone that promotes α ring formation (Figure 9). This function is different from that of PAC1-PAC2, depletion of which resulted in the accumulation of α ring dimers containing PAC3. Thus the two chaperone complexes apparently have distinct functions in α ring formation and stabilization.[375]

The latter conclusion is supported by the analysis of related proteins in *S. cerevisiae*. The yeast orthologues of PAC3 and PAC4 were identified in several studies and were termed Pba3/Poc3/Dmp2 and Pba4/Poc4/Dmp1, respectively.[362,372,376−378] Pba3 and Pba4 form stoichiometric complexes with each other. Mutants lacking Pba3-Pba4 are impaired in proteasome function.[372,376−378] Specifically, they display defects in the assembly of proteasomal α rings.[376,377] 20−50% of the proteasomes formed in these mutants lack α3, the only nonessential subunit.[376] Like a mutant lacking the gene (*PRE9*) encoding α3, *pba3*Δ or *pba4*Δ mutants instead incorporate two α4 subunits into an α ring.[376] Similar to their human counterpart, Pba3-Pba4 therefore functions in the proper assembly of α rings.

The following observation suggested that proteasomes lacking α3 might be physiologically relevant entities rather than just defective proteasomes. Yeast strains lacking Pba3-Pba4 or α3 are more resistant to oxidative stress induced by the heavy metal cadmium.[376,379] An "alternative proteasome subtype" lacking α3 thus provides a selective advantage under such stress conditions. N-terminal residues of the α3 subunit are known to seal the entry pore of the α ring in isolated 20S proteasomes.[227] Replacing α3 by α4 is expected to result in a constitutively open channel into the proteasome. In an intriguing model, a constitutively open 20S proteasome would be particularly suited to degrade damaged proteins that occur upon cadmium treatment or oxidative stress in general.[376] It is noteworthy, however, that this effect is not a specific property of open channel mutants, as yeast strains with weak mutations affecting β subunits display hyperresistance to cadmium as well.[108] This raises the possibility that some proteasome mutations are sufficient to elicit an Rpn4-mediated response (see section 5.5) and thereby provide an additional resistance against acute stress. Consistent with the relevance of such a response in *pba3*Δ or *pba4*Δ mutants is the observation that additional deletion of *RPN4* in these strains is lethal.[372,377] It therefore remains to be demonstrated whether a differential regulation of PACs and proteasome subunits indeed provides a physiologically relevant response to such stress conditions via formation of alternative proteasomes.

Biochemical and structural analyses revealed that Pba3-Pba4 binds directly to the α5 subunit.[376,377] This interaction occurs also when α5 is bound to α6 and α7.[376] These findings indicated that a Pba3-Pba4-α5 complex might serve as a starting point of α ring assembly (Figure 9). Pba3 and Pba4 are structurally similar. The αββα sandwich structure of the dimer resembles those of proteasomal subunits (Figure 2).[377] In the crystal structure of the Pba3-Pba4-α5 complex, the α5 structure is nearly identical to that of α5 in the mature 20S.[377] Modeling of Pba3-Pba4 onto an α ring based upon this structure suggested that the chaperone complex is located toward the center of the surface of the ring, on which β subunits will assemble. This position would be incompatible with a binding of β4 to the position it eventually will occupy in the 20S CP. This model is therefore consistent with the observation that Pba3-Pba4 is released before this subunit joins the complex.[374,377] Analysis of a homodimer of human PAC3 revealed that its structure is quite similar to that of yeast Pba3-Pba4 (Figure 2).[377] Even though the experimental evidence indicates that PAC3-PAC4 is the physiological relevant entity,[372,376−378] similarities between the PAC3 homodimer and Pba3-Pba4 crystal structures underscore a conservation of this chaperone complex from yeast to humans.[377] In conclusion the chaperones PAC1-PAC2 and PAC3-PAC4 and their orthologues perform conserved functions in facilitating the formation of α rings and promoting the assembly of β subunits onto these structures by preventing α ring dimerization.[380,381]

Thus far no experimental evidence exists to suggest that formation of prokaryotic proteasomes requires or involves specific assembly chaperones. In a recent report, however, it was suggested, based on sequence comparisons, that a protein encoded in the neighborhood of proteasome genes in bacteria might be an ancestral proteasome chaperone related to eukaryotic PAC2.[382] It will be interesting to see whether this proposed heritage of PAC2 can be sustained by experimental evidence.

5.2.3. Assembly of Half-Proteasome Precursors

The studies described above identified α rings complexed with the chaperones PAC1-PAC2 and PAC3-PAC4 as a platform for the formation of half-proteasome precursor complexes (Figure 9).[371,374] Such intermediates accumulate when β2 subunits are depleted by siRNA.[374] These and other data suggested that β2 is the subunit that starts the assembly of β rings on α ring precursors.[374,377] The order of events in the assembly of β rings has been systematically dissected by knocking down individual β subunits and assembly chaperones in human cells.[374] This procedure resulted in the accumulation of distinct intermediates that were characterized by native gel analysis. Even though it cannot be entirely excluded that some of the complexes identified may be just relatively more stable entities under the chosen experimental conditions rather than true intermediates, these experiments yielded a fairly convincing picture of the distinct steps, many of which are in agreement with previous studies. According to this analysis, β ring assembly starts with the recruitment of β2 and UMP1. Afterward, β3, β4, β5, and β6 join the

complex sequentially. β1 might join the complex before or after β4, β5, or β6. Consistent with observations made during the analysis of yeast proteasome assembly, β7 appears to be the last subunit to join the formation of a half-proteasome precursor complex, an event that is closely linked with 20S CP formation by dimerization of such precursors.[361,362,374]

Consistent with earlier work in yeast, the C-terminal extension of β2, which is wrapped around β3 in the mature proteasome (Figure 1), is essential for the incorporation of β3.[94,374] PAC3-PAC4 probably leaves the complex upon binding of β3 as it is absent in intermediates containing the latter subunit, which accumulate upon depletion of β4.[374] The propeptide of β5 appears to be required for incorporation of β6 in mammalian cells.

The order of events derived from the subunit depletion studies discussed above is in agreement with earlier studies in which 13S complexes containing all α-subunits as well as β2, β3, and β4 were described.[383] Related assembly intermediates containing also the assembly chaperone Pba1-Pba2 have been found in *S. cerevisiae*.[362] Only a subset of these complexes also contained Ump1, which suggested that in yeast this chaperone may be incorporated after the β4 subunit. Earlier intermediates, however, were not classified in this study.[362] The last intermediate that has been detected in the assembly of yeast proteasomes is a half-proteasome precursor containing all α and β subunits except β7, the chaperones Pba1-Pba2 and Ump1, as well as the yeast PA200 relative Blm10.[361,362]

5.2.4. Maturation Factor UMP1

Ump1 was identified in a genetic screen for budding yeast mutants defective in ubiquitin-mediated proteolysis, hence the name.[384] The ~17 kDa Ump1 protein is a component of proteasome precursor complexes containing unprocessed β subunits but was not detectable in mature proteasomes. In a mutant with impaired proteasomal peptidase activity, however, Ump1 was found enclosed within the 20S CP. This observation suggested that Ump1 is present in precursor complexes and is encased in the newly formed CPs. Upon maturation of the active sites, which is linked to precursor complex dimerization, Ump1 is degraded.[103,384] The *ump1*Δ mutant grows poorly and is sensitive to proteotoxic stress due to an impairment of proteasome biogenesis. In the mutant, proteasome precursor complexes accumulate. Assembled proteasomes display an incomplete processing of β subunits. These results identified Ump1 as a proteasome assembly chaperone that promotes dimerization of precursor complexes and maturation of active sites.[384]

Ump1 is a conserved protein among eukaryotes. Its human orthologue, hUMP1 (also called POMP or proteassemblin), has been shown to perform similar functions in proteasome biogenesis as its yeast counterpart.[371,385−388] In contrast to yeast Ump1, hUMP1 appears to be essential for viability as was suggested by siRNA knockdown experiments.[372,374,388] hUMP1 binds directly to several α and β subunits and associates with α rings in vitro.[374,388,389] In line with this finding, it was observed that, in contrast to its yeast orthologue, hUMP1 appears to be essential for the binding of the β2 subunit to α ring precursor complexes, and therefore for the initiation and assembly of β rings (see above).[374] Consistent with this early and essential requirement of hUMP1 in the assembly process, no 20S CPs with unprocessed β subunits similar to those detected in the yeast *ump1*Δ mutant were observed upon its knockdown in human cells.[374]

It was reported recently that hUMP1 binds to membranes and recruits precursor complexes to the endoplasmic reticulum (ER). This study led to the conclusion that proteasome assembly in mammalian cells occurs at the ER.[389] Studies in *S. cerevisiae* suggested that Ump1-containing precursor complexes are imported into the nucleus where the assembly of nuclear proteasomes is completed.[390]

5.2.5. Assembly and Maturation of Proteasome Core Particles

Formation of 20S CPs occurs by dimerization of two half-proteasome precursor complexes containing the chaperones PAC1-PAC2 and UMP1 (Figure 9). Incorporation of the β7 subunit completes the assembly of these precursor complexes and is closely followed by or occurs simultaneaously with complex dimerization.[361,362,374] A C-terminal extension (CTE) of *S. cerevisiae* β7 is important for precursor dimerization to occur efficiently.[94] The two β7 extensions stabilize nascent CPs by inserting into the trans rings at the interface of β1 and β2 (Figure 1C,D). Residues within this extension contact the β1 subunit and contribute to the postacidic site.[77,94] Deletion of the CTE results in a loss of postacidic activity and increased levels of half-proteasome precursor complexes lacking the β7 subunit.[361,391] Other features of β subunits that appear to be important for precursor complex dimerization include the propeptides of β5 and β6 (Figure 3).[362] In the presence of Ump1, both of them are essential for viability.[103,362] Lethality of their deletion, however, can be suppressed either by deletion of the *UMP1* gene or by overexpression of β7.[362,384] It was therefore proposed that Ump1 may provide a checkpoint function that monitors the correct assembly of precursor complexes and prevents dimerization until their assembly is completed, possibly by inhibiting incorporation of β7.[362]

The β7 CTE is important for stabilization of nascent proteasomes although they still form in its absence but with a reduced efficiency. Blm10, which is related to mammalian PA200, appears to contribute to stability of nascent 20S CP since a combination of a *BLM10* deletion with a β7 CTE truncation caused a striking synthetic inhibition of proteasome assembly.[361] This finding indicated that binding of an activator complex to the α ring surface of nascent proteasome has a stabilizing effect, possibly by promoting maturation of the CP (Figure 9). The 19S RP can partially substitute for Blm10 in stabilizing nascent CPs.[361] While Blm10 has been observed in association with proteasome precursor complexes,[359,361,362] mammalian PA200 was not detected in such complexes.[374]

Dimerization of half-proteasome precursor complexes triggers autocatalytic processing of β1, β2, and β5.[103,383] Upon activation of these subunits, Ump1, which is encased during precursor dimerization, as well as the PAC1-PAC2 chaperone are degraded.[371,384] Active site maturation and removal of these chaperones may be accompanied by conformational changes that complete the formation of stable 20S proteasomes. Yeast mutants lacking Ump1 not only accumulated precursor complexes but also proteasomes with incompletely processed β subunits.[384] Upon precursor complex dimerization, Ump1 might help to induce conformational changes in the β subunit that facilitate their autocatalytic processing.[74]

5.3. Assembly of Alternative 20S Proteasomes

Multicellular organisms often express isoforms of the proteasome such as the "immunoproteasome" found in vertebrates. This subtype forms by incorporation of interferon-inducible β subunits, two of which are encoded in the MHC class II region interdigitating with the genes encoding the two subunits of TAP (transporter associated with antigen presentation).[85] As a result of the incorporation of the immunosubunits (β1i, β2i, β5i), the specificity of the peptidase activities in the immunoproteasome is changed in a way that the generation of certain antigenic peptides is promoted (see section 3.2). Cooperative incorporation of all three immunosubunits is favored, although the formation of mixed proteasomes with a combination of interferon-induced and constitutive subunits occurs as well.[88] The assembly of the immunoproteasome differs from that of the constitutive proteasome in some aspects. β1i enters the assembly pathway earlier than the constitutive β1 subunit. The presence of β1i abets the incorporation of β2i.[383,392,393] The presence of β1i and β2i in turn abets incorporation of β5i.[394] The cleavable propeptides of these β subunits play an important role in their cooperative assembly.[394−396] Immunoproteasome assembly is about 4-fold faster than that of constitutive proteasomes.[388] This appears to be promoted by increased synthesis of the maturation factor UMP1, which is induced by interferon.[385,388] In vitro experiments indicated that β5i binds more strongly to hUMP1 than β5, which in large part is due to the β5i propeptide.[388] The faster and cooperative assembly of immunoproteasomes upon immune stress signaled by interferon-γ ensures a rapid expansion of the peptide cleavage repertoire of an infected cell.

PI31 (proteasome inhibitor of 31 kDa) has been implicated in the regulation of the immunoproteasome.[397] PI31 was originally identified because of its capacity to inhibit proteasome activity in vitro and to block activation by PA28 or the 19S RP, suggesting that it might bind to the α ring surface of the 20S CP [398−400] Subsequently, it was proposed that in vivo PI31 may act as a negative regulator of the formation of immunoproteasomes in the absence of infection.[397] This conclusion was based upon experiments that employed overexpression of PI31 in a mouse cell line. PI31 was located predominantly at the cytosolic side of the nuclear envelope and/or ER membrane. Surprisingly, overexpression of PI31 did not result in an overall inhibition of proteasome activity. Instead it caused a specific defect in the maturation of proteasome precursor complex containing immuno-subunits. As a result, PI31 overexpressing cells displayed a defect in the generation of immunoproteasome-dependent antigens.[397] Previous studies have implicated a proline-rich C-terminal domain of PI31 in binding to the proteasome.[400] This region as well as a segment spanning the first 150 residues of PI31 show sequence similarity to stretches within the F-Box protein Fbxo7. The N-terminal domain, which was termed FP because of its presence in these two proteins, assumes a novel α/β fold. This domain mediates homodimerization of either PI31 and Fbxo7 as well as heterodimerization of the two proteins.[401] Whether the latter interaction has a physiological relevance is not known.

In vertebrates, another proteasome subtype is found in the thymus and hence called "thymoproteasome". An alternative subunit, β5t, is specifically expressed in the thymus, and incorporated into proteasomes preferentially together with β1i and β2i. Ablation of the β5t gene in mice resulted in a reduction of MHC class I-restricted $CD8^+$ T cells suggesting a role of thymoproteasomes in their development.[402]

5.4. Assembly of the 26S Proteasome

The 26S proteasome is formed by association of the 20S CP with two 19S RPs. In vitro, this process requires the presence of ATP but not its hydrolysis.[23,25,191,236] The in vivo assembly of the 26S proteasome is less clear, as is the assembly of its 19S regulator. A protein that has been implicated in the assembly or stabilization of 26S proteasomes is Ecm29, which may be a stoichiometric component of 26S proteasomes.[277] This protein binds in vitro both to the CP and the RP. Proteasomes purified in the absence of Ecm29 are less stable in the presence of ATP and dissociate into CP and RP in the absence of ATP. These observations indicate that ATPases and Ecm29 both contribute to the association of these subcomplexes.[277] Another study has suggested that, in mammals, Ecm29 might serve to couple the 26S proteasome to sites of ER-associated degradation and other sites of enhanced proteolysis.[403] Genetic data have implicated the Hsp90 chaperone in the assembly of 26S proteasomes from its subcomplexes.[404]

The assembly pathway of the 19S RP is a largely unexplored area. Subunit interaction maps give only a crude idea of the arrangements of subunits in this complex.[405−408] It has been long assumed that the 19S RP, which indeed has functions independent of the CP (as mentioned above), can assemble independently of the 20S proteasome. Recent evidence obtained with yeast mutants deficient in 20S CP biogenesis, however, suggested that the CP may influence 19S RP assembly or stability.[376] A possible scenario might be that the α ring surface of 20S CP provides a favorable platform for an initiation of 19S base complex assembly. In vitro disassembly of the 26S proteasome yielded the Rpn1 and Rpn2 subunits as the last two subunits to be attached in the center of the α ring surface of the CP.[206] Mutations in Rpn2 lead to a disassembly of the 26S proteasome consistent with a central role of this protein for base stability.[361,409] Whether Rpn1 and Rpn2 or the six ATPase subunits are starting points for the assembly of the 19S RP base subcomplex in vivo, however, is not known.

Analysis of yeast mutants affected in subunits of the 19S RP lid or base indicated that these subcomplexes can assemble independently of each other.[409] Another study identified the yeast Hsm3 protein (apparently an ortholog of human S5b) as a dedicated chaperone of 19S RP assembly.[410] Specifically, Hsm3 binds to the C-terminus of the Rpt1 subunit and promotes base complex formation. These properties of Hsm3/S5b are compatible with the possibility that it provides a checkpoint function in 26S proteasome assembly by preventing docking of the base to the CP, a process for which the Rpt1 C terminus is critical,[232] untill assembly of the 19S RP is complete (Figure 9).[410]

5.5. Regulation of Proteasome Gene Expression

Proteasomes are fairly abundant in eukaryotic cells, with ~800,000 molecules estimated to be present in a mouse fibroblast cell.[411] Proteasome expression can be upregulated when the cells are under proteotoxic stress.[317,318,320,412−414] In yeast, this regulation is mediated by the transcription factor Rpn4 (see above). Orthologues of Rpn4 appear to be absent from higher eukaryotes, but the transcription factors Nrf2 in humans or the related SKN-1 in *C. elegans*, which are

regulators of stress responses, similarly control the upregulation of proteasome levels upon treatment with proteasome inhibitor.[414,415] Similar to Rpn4, the abundance of Nrf2 itself is controlled via ubiquitin-dependent degradation by the proteasome providing a regulatory feedback loop.[415,416]

6. Conclusions and Perspectives

The proteasome is a central player in cellular regulation and protein quality control and has therefore been intensely studied. A variety of specific inhibitors of the proteasome have been developed that have promising potential in the treatment of diseases. The basic enzymatic and structural properties of the catalytic 20S CP as well as the pathways leading to its assembly are fairly well understood. The intracellular activation and regulation of this giant protease is very complex and involves various activators, which control substrate entry into the CP. These activators themselves, especially the 19S RP, interact with a large number of proteins that mediate control of assembly, substrate recognition, and deubiquitylation. In particular, this area of research offers extensive room for new and exciting discoveries to come.

7. Acknowledgments

We thank Katrin Fischer and Brigitte Kisters for advice in using structure analysis software, Custódia Fonseca and Américo Lemos for helpful discussions, and anonymous reviewers for helpful comments. Ana Matias is supported by a predoctoral fellowship from the Fundação para a Ciência e Tecnologia (FCT). Work in the authors' laboratories is supported by grants from the FCT (to P.C.R.) and by the Deutsche Forschungsgemeinschaft (to R.J.D.).

8. References

(1) Hershko, A.; Ciechanover, A.; Varshavsky, A. *Nat. Med.* **2000**, *6*, 1073.
(2) Rubin, D. M.; Finley, D. *Curr. Biol.* **1995**, *5*, 854.
(3) Baumeister, W.; Walz, J.; Zuhl, F.; Seemuller, E. *Cell* **1998**, *92*, 367.
(4) Spence, J.; Gali, R. R.; Dittmar, G.; Sherman, F.; Karin, M.; Finley, D. *Cell* **2000**, *102*, 67.
(5) Pickart, C. M. *Trends Biochem. Sci.* **2000**, *25*, 544.
(6) Weissman, A. M. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 169.
(7) Dupre, S.; Urban-Grimal, D.; Haguenauer-Tsapis, R. *Biochim. Biophys. Acta* **2004**, *1695*, 89.
(8) Mukhopadhyay, D.; Riezman, H. *Science* **2007**, *315*, 201.
(9) Chau, V.; Tobias, J. W.; Bachmair, A.; Marriott, D.; Ecker, D. J.; Gonda, D. K.; Varshavsky, A. *Science* **1989**, *243*, 1576.
(10) Jin, L.; Williamson, A.; Banerjee, S.; Philipp, I.; Rape, M. *Cell* **2008**, *133*, 653.
(11) Johnson, E. S.; Ma, P. C.; Ota, I. M.; Varshavsky, A. *J. Biol. Chem.* **1995**, *270*, 17442.
(12) Baboshina, O. V.; Haas, A. L. *J. Biol. Chem.* **1996**, *271*, 2823.
(13) Saeki, Y.; Kudo, T.; Sone, T.; Kikuchi, Y.; Yokosawa, H.; Toh, E. A.; Tanaka, K. *EMBO J. 28*, **2009**.
(14) Orlowski, M.; Wilk, S. *Arch. Biochem. Biophys.* **2003**, *415*, 1.
(15) Hoyt, M. A.; Coffino, P. *Cell. Mol. Life Sci.* **2004**, *61*, 1596.
(16) Mao, I.; Liu, J.; Li, X.; Luo, H. *Cell. Mol. Life Sci.* **2008**, *65*, 3971.
(17) Hiller, M. M.; Finger, A.; Schweiger, M.; Wolf, D. H. *Science* **1996**, *273*, 1725.
(18) Meusser, B.; Hirsch, C.; Jarosch, E.; Sommer, T. *Nat. Cell Biol.* **2005**, *7*, 766.
(19) Hershko, A.; Ciechanover, A. *Annu. Rev. Biochem.* **1998**, *67*, 425.
(20) Ciechanover, A. *EMBO J.* **1998**, *17*, 7151.
(21) Rose, I. A.; Warms, J. V.; Hershko, A. *J. Biol. Chem.* **1979**, *254*, 8135.
(22) DeMartino, G. N.; Goldberg, A. L. *J. Biol. Chem.* **1979**, *254*, 3712.
(23) Hough, R.; Pratt, G.; Rechsteiner, M. *J. Biol. Chem.* **1987**, *262*, 8303.
(24) Arrigo, A. P.; Tanaka, K.; Goldberg, A. L.; Welch, W. J. *Nature* **1988**, *331*, 192.
(25) Eytan, E.; Ganoth, D.; Armon, T.; Hershko, A. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 7751.
(26) Hase, J.; Kobashi, K.; Nakai, N.; Mitsui, K.; Iwata, K.; Takadera, T. *Biochim. Biophys. Acta* **1980**, *611*, 205.
(27) Kleinschmidt, J. A.; Hugle, B.; Grund, C.; Franke, W. W. *Eur. J. Cell Biol.* **1983**, *32*, 143.
(28) Kopp, F.; Steiner, R.; Dahlmann, B.; Kuehn, L.; Reinauer, H. *Biochim. Biophys. Acta* **1986**, *872*, 253.
(29) Tanaka, K.; Yoshimura, T.; Ichihara, A.; Kameyama, K.; Takagi, T. *J. Biol. Chem.* **1986**, *261*, 15204.
(30) Falkenburg, P. E.; Haass, C.; Kloetzel, P. M.; Niedel, B.; Kopp, F.; Kuehn, L.; Dahlmann, B. *Nature* **1988**, *331*, 190.
(31) Baumeister, W.; Dahlmann, B.; Hegerl, R.; Kopp, F.; Kuehn, L.; Pfeifer, G. *FEBS Lett.* **1988**, *241*, 239.
(32) Hanna, J.; Finley, D. *FEBS Lett.* **2007**, *581*, 2854.
(33) Wing, S. S. *BMC Biochem.* **2008**, *9*Suppl 1, S6.
(34) Sakamoto, K.; Sato, Y.; Shinka, T.; Sei, M.; Nomura, I.; Umeno, M.; Ewis, A. A.; Nakahori, Y. *Obesity* **2009** (Published online 22 January 2009) DOI:, 10.1038/oby.2008.612.
(35) Ciechanover, A. *Biochem. Soc. Trans.* **2003**, *31*, 474.
(36) Adams, J. *Nat. Rev. Cancer* **2004**, *4*, 349.
(37) Goldberg, A. L. *Biochem. Soc. Trans.* **2007**, *35*, 12.
(38) Voorhees, P. M.; Dees, E. C.; O'Neil, B.; Orlowski, R. Z. *Clin. Cancer Res.* **2003**, *9*, 6316.
(39) Chuang, S. E.; Burland, V.; Plunkett, G., 3rd; Daniels, D. L.; Blattner, F. R. *Gene* **1993**, *134*, 1.
(40) Rohrwild, M.; Coux, O.; Huang, H. C.; Moerschell, R. P.; Yoo, S. J.; Seol, J. H.; Chung, C. H.; Goldberg, A. L. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 5808.
(41) Rohrwild, M.; Pfeifer, G.; Santarius, U.; Muller, S. A.; Huang, H. C.; Engel, A.; Baumeister, W.; Goldberg, A. L. *Nat. Struct. Biol.* **1997**, *4*, 133.
(42) Dahlmann, B.; Kopp, F.; Kuehn, L.; Niedel, B.; Pfeifer, G.; Hegerl, R.; Baumeister, W. *FEBS Lett.* **1989**, *251*, 125.
(43) Tamura, T.; Nagy, I.; Lupas, A.; Lottspeich, F.; Cejka, Z.; Schoofs, G.; Tanaka, K.; De Mot, R.; Baumeister, W. *Curr. Biol.* **1995**, *5*, 766.
(44) Gille, C.; Goede, A.; Schloetelburg, C.; Preissner, R.; Kloetzel, P. M.; Gobel, U. B.; Frommel, C. *J. Mol. Biol.* **2003**, *326*, 1437.
(45) Lowe, J.; Stock, D.; Jap, B.; Zwickl, P.; Baumeister, W.; Huber, R. *Science* **1995**, *268*, 533.
(46) Zwickl, P.; Grziwa, A.; Puhler, G.; Dahlmann, B.; Lottspeich, F.; Baumeister, W. *Biochemistry* **1992**, *31*, 964.
(47) Sharon, M.; Witt, S.; Felderer, K.; Rockel, B.; Baumeister, W.; Robinson, C. V. *J. Biol. Chem.* **2006**, *281*, 9569.
(48) Wenzel, T.; Baumeister, W. *Nat. Struct. Biol.* **1995**, *2*, 199.
(49) Groll, M.; Brandstetter, H.; Bartunik, H.; Bourenkow, G.; Huber, R. *J. Mol. Biol.* **2003**, *327*, 75.
(50) Benaroudj, N.; Zwickl, P.; Seemuller, E.; Baumeister, W.; Goldberg, A. L. *Mol. Cell* **2003**, *11*, 69.
(51) Forster, A.; Masters, E. I.; Whitby, F. G.; Robinson, H.; Hill, C. P. *Mol. Cell* **2005**, *18*, 589.
(52) Zuhl, F.; Tamura, T.; Dolenc, I.; Cejka, Z.; Nagy, I.; De Mot, R.; Baumeister, W. *FEBS Lett.* **1997**, *400*, 83.
(53) Kwon, Y. D.; Nagy, I.; Adams, P. D.; Baumeister, W.; Jap, B. K. *J. Mol. Biol.* **2004**, *335*, 233.
(54) Hu, G.; Lin, G.; Wang, M.; Dick, L.; Xu, R. M.; Nathan, C.; Li, H. *Mol. Microbiol.* **2006**, *59*, 1417.
(55) Lin, G.; Hu, G.; Tsu, C.; Kunes, Y. Z.; Li, H.; Dick, L.; Parsons, T.; Li, P.; Chen, Z.; Zwickl, P.; Weich, N.; Nathan, C. *Mol. Microbiol.* **2006**, *59*, 1405.
(56) Pearce, M. J.; Mintseris, J.; Ferreyra, J.; Gygi, S. P.; Darwin, K. H. *Science* **2008**, *322*, 1104.
(57) Knipfer, N.; Shrader, T. E. *Mol. Microbiol.* **1997**, *25*, 375.
(58) Darwin, K. H.; Ehrt, S.; Gutierrez-Ramos, J. C.; Weich, N.; Nathan, C. F. *Science* **2003**, *302*, 1963.
(59) Gandotra, S.; Schnappinger, D.; Monteleone, M.; Hillen, W.; Ehrt, S. *Nat. Med.* **2007**, *13*, 1515.
(60) Butler, S. M.; Festa, R. A.; Pearce, M. J.; Darwin, K. H. *Mol. Microbiol.* **2006**, *60*, 553.
(61) Lupas, A.; Zuhl, F.; Tamura, T.; Wolf, S.; Nagy, I.; De Mot, R.; Baumeister, W. *Mol. Biol. Rep.* **1997**, *24*, 125.
(62) Ram, R. J.; Verberkmoes, N. C.; Thelen, M. P.; Tyson, G. W.; Baker, B. J.; Blake, R. C., 2nd; Shah, M.; Hettich, R. L.; Banfield, J. F. *Science* **2005**, *308*, 1915.
(63) De Mot, R. *Trends Microbiol.* **2007**, *15*, 335.
(64) Bochtler, M.; Ditzel, L.; Groll, M.; Huber, R. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 6070.
(65) Yoo, S. J.; Shim, Y. K.; Seong, I. S.; Seol, J. H.; Kang, M. S.; Chung, C. H. *FEBS Lett.* **1997**, *412*, 57.
(66) Kessel, M.; Wu, W.; Gottesman, S.; Kocsis, E.; Steven, A. C.; Maurizi, M. R. *FEBS Lett.* **1996**, *398*, 274.
(67) Sousa, M. C.; Trame, C. B.; Tsuruta, H.; Wilbanks, S. M.; Reddy, V. S.; McKay, D. B. *Cell* **2000**, *103*, 633.

(68) Wang, J.; Song, J. J.; Franklin, M. C.; Kamtekar, S.; Im, Y. J.; Rho, S. H.; Seong, I. S.; Lee, C. S.; Chung, C. H.; Eom, S. H. *Structure* **2001**, *9*, 177.
(69) Missiakas, D.; Schwager, F.; Betton, J. M.; Georgopoulos, C.; Raina, S. *EMBO J.* **1996**, *15*, 6899.
(70) Kanemori, M.; Nishihara, K.; Yanagi, H.; Yura, T. *J. Bacteriol.* **1997**, *179*, 7219.
(71) Couvreur, B.; Wattiez, R.; Bollen, A.; Falmagne, P.; Le Ray, D.; Dujardin, J. C. *Mol. Biol. Evol.* **2002**, *19*, 2110.
(72) Ruiz-Gonzalez, M. X.; Marin, I. *J. Mol. Evol.* **2006**, *63*, 504.
(73) Heinemeyer, W.; Kleinschmidt, J. A.; Saidowsky, J.; Escher, C.; Wolf, D. H. *EMBO J.* **1991**, *10*, 555.
(74) Heinemeyer, W.; Ramos, P. C.; Dohmen, R. J. *Cell. Mol. Life Sci.* **2004**, *61*, 1562.
(75) Emori, Y.; Tsukahara, T.; Kawasaki, H.; Ishiura, S.; Sugita, H.; Suzuki, K. *Mol. Cell. Biol.* **1991**, *11*, 344.
(76) Velichutina, I.; Connerly, P. L.; Arendt, C. S.; Li, X.; Hochstrasser, M. *EMBO J.* **2004**, *23*, 500.
(77) Groll, M.; Ditzel, L.; Lowe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463.
(78) Unno, M.; Mizushima, T.; Morimoto, Y.; Tomisugi, Y.; Tanaka, K.; Yasuoka, N.; Tsukihara, T. *Structure* **2002**, *10*, 609.
(79) Seemuller, E.; Lupas, A.; Stock, D.; Lowe, J.; Huber, R.; Baumeister, W. *Science* **1995**, *268*, 579.
(80) Kelly, A.; Powis, S. H.; Glynne, R.; Radley, E.; Beck, S.; Trowsdale, J. *Nature* **1991**, *353*, 667.
(81) Pamer, E.; Cresswell, P. *Annu. Rev. Immunol.* **1998**, *16*, 323.
(82) Rock, K. L.; Goldberg, A. L. *Annu. Rev. Immunol.* **1999**, *17*, 739.
(83) Groettrup, M.; Khan, S.; Schwarz, K.; Schmidtke, G. *Biochimie* **2001**, *83*, 367.
(84) Goldberg, A. L.; Cascio, P.; Saric, T.; Rock, K. L. *Mol. Immunol.* **2002**, *39*, 147.
(85) Kloetzel, P. M. *Nat. Immunol.* **2004**, *5*, 661.
(86) Strehl, B.; Seifert, U.; Kruger, E.; Heink, S.; Kuckelkorn, U.; Kloetzel, P. M. *Immunol. Rev.* **2005**, *207*, 19.
(87) Borissenko, L.; Groll, M. *Biol. Chem.* **2007**, *388*, 947.
(88) Orlowski, M.; Wilk, S. *Arch. Biochem. Biophys.* **2000**, *383*, 1.
(89) Murata, S.; Takahama, Y.; Tanaka, K. *Curr. Opin. Immunol.* **2008**, *20*, 192.
(90) Murata, S.; Sasaki, K.; Kishimoto, T.; Niwa, S.; Hayashi, H.; Takahama, Y.; Tanaka, K. *Science* **2007**, *316*, 1349.
(91) Fu, H.; Doelling, J. H.; Arendt, C. S.; Hochstrasser, M.; Vierstra, R. D. *Genetics* **1998**, *149*, 677.
(92) Ma, J.; Katz, E.; Belote, J. M. *Insect. Mol. Biol.* **2002**, *11*, 627.
(93) Zhong, L.; Belote, J. M. *Development* **2007**, *134*, 3517.
(94) Ramos, P. C.; Marques, A. J.; London, M. K.; Dohmen, R. J. *J. Biol. Chem.* **2004**, *279*, 14323.
(95) Zwickl, P.; Kleinz, J.; Baumeister, W. *Nat. Struct. Biol.* **1994**, *1*, 765.
(96) Brannigan, J. A.; Dodson, G.; Duggleby, H. J.; Moody, P. C.; Smith, J. L.; Tomchick, D. R.; Murzin, A. G. *Nature* **1995**, *378*, 416.
(97) Duggleby, H. J.; Tolley, S. P.; Hill, C. P.; Dodson, E. J.; Dodson, G.; Moody, P. C. *Nature* **1995**, *373*, 264.
(98) Smith, J. L.; Zaluzec, E. J.; Wery, J. P.; Niu, L.; Switzer, R. L.; Zalkin, H.; Satow, Y. *Science* **1994**, *264*, 1427.
(99) Oinonen, C.; Rouvinen, J. *Protein Sci.* **2000**, *9*, 2329.
(100) Seemuller, E.; Lupas, A.; Baumeister, W. *Nature* **1996**, *382*, 468.
(101) Kisselev, A. F.; Songyang, Z.; Goldberg, A. L. *J. Biol. Chem.* **2000**, *275*, 14831.
(102) Groll, M.; Heinemeyer, W.; Jager, S.; Ullrich, T.; Bochtler, M.; Wolf, D. H.; Huber, R. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10976.
(103) Chen, P.; Hochstrasser, M. *Cell* **1996**, *86*, 961.
(104) Heinemeyer, W.; Fischer, M.; Krimmer, T.; Stachon, U.; Wolf, D. H. *J. Biol. Chem.* **1997**, *272*, 25200.
(105) Schmidtke, G.; Kraft, R.; Kostka, S.; Henklein, P.; Frommel, C.; Lowe, J.; Huber, R.; Kloetzel, P. M.; Schmidt, M. *EMBO J.* **1996**, *15*, 6887.
(106) Ditzel, L.; Huber, R.; Mann, K.; Heinemeyer, W.; Wolf, D. H.; Groll, M. *J. Mol. Biol.* **1998**, *279*, 1187.
(107) Khan, A. R.; James, M. N. *Protein Sci.* **1998**, *7*, 815.
(108) Arendt, C. S.; Hochstrasser, M. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 7156.
(109) Lee, L. W.; Moomaw, C. R.; Orth, K.; McGuire, M. J.; DeMartino, G. N.; Slaughter, C. A. *Biochim. Biophys. Acta* **1990**, *1037*, 178.
(110) Arendt, C. S.; Hochstrasser, M. *EMBO J.* **1999**, *18*, 3575.
(111) Dahlmann, B.; Kuehn, L.; Grziwa, A.; Zwickl, P.; Baumeister, W. *Eur. J. Biochem.* **1992**, *208*, 789.
(112) Nussbaum, A. K.; Dick, T. P.; Keilholz, W.; Schirle, M.; Stevanovic, S.; Dietz, K.; Heinemeyer, W.; Groll, M.; Wolf, D. H.; Huber, R.; Rammensee, H. G.; Schild, H. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 12504.
(113) Rock, K. L.; Gramm, C.; Rothstein, L.; Clark, K.; Stein, R.; Dick, L.; Hwang, D.; Goldberg, A. L. *Cell* **1994**, *78*, 761.
(114) Kisselev, A. F.; Callard, A.; Goldberg, A. L. *J. Biol. Chem.* **2006**, *281*, 8582.
(115) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.
(116) Dick, T. P.; Nussbaum, A. K.; Deeg, M.; Heinemeyer, W.; Groll, M.; Schirle, M.; Keilholz, W.; Stevanovic, S.; Wolf, D. H.; Huber, R.; Rammensee, H. G.; Schild, H. *J. Biol. Chem.* **1998**, *273*, 25637.
(117) Groll, M.; Nazif, T.; Huber, R.; Bogyo, M. *Chem. Biol.* **2002**, *9*, 655.
(118) Driscoll, J.; Brown, M. G.; Finley, D.; Monaco, J. J. *Nature* **1993**, *365*, 262.
(119) Gaczynska, M.; Rock, K. L.; Goldberg, A. L. *Nature* **1993**, *365*, 264.
(120) Boes, B.; Hengel, H.; Ruppert, T.; Multhaup, G.; Koszinowski, U. H.; Kloetzel, P. M. *J. Exp. Med.* **1994**, *179*, 901.
(121) Gaczynska, M.; Rock, K. L.; Spies, T.; Goldberg, A. L. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 9213.
(122) Gaczynska, M.; Goldberg, A. L.; Tanaka, K.; Hendil, K. B.; Rock, K. L. *J. Biol. Chem.* **1996**, *271*, 17275.
(123) Ustrell, V.; Pratt, G.; Rechsteiner, M. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 584.
(124) Kuckelkorn, U.; Frentzel, S.; Kraft, R.; Kostka, S.; Groettrup, M.; Kloetzel, P. M. *Eur. J. Immunol.* **1995**, *25*, 2605.
(125) Cerundolo, V.; Kelly, A.; Elliott, T.; Trowsdale, J.; Townsend, A. *Eur. J. Immunol.* **1995**, *25*, 554.
(126) Sibille, C.; Gould, K. G.; Willard-Gallo, K.; Thomson, S.; Rivett, A. J.; Powis, S.; Butcher, G. W.; De Baetselier, P. *Curr. Biol.* **1995**, *5*, 923.
(127) Vabulas, R. M.; Hartl, F. U. *Science* **2005**, *310*, 1960.
(128) Palombella, V. J.; Rando, O. J.; Goldberg, A. L.; Maniatis, T. *Cell* **1994**, *78*, 773.
(129) Hoppe, T.; Matuschewski, K.; Rape, M.; Schlenker, S.; Ulrich, H. D.; Jentsch, S. *Cell* **2000**, *102*, 577.
(130) Rape, M.; Jentsch, S. *Biochim. Biophys. Acta* **2004**, *1695*, 209.
(131) Piwko, W.; Jentsch, S. *Nat. Struct. Mol. Biol.* **2006**, *13*, 691.
(132) Hoyt, M. A.; Zich, J.; Takeuchi, J.; Zhang, M.; Govaerts, C.; Coffino, P. *EMBO J.* **2006**, *25*, 1720.
(133) Akopian, T. N.; Kisselev, A. F.; Goldberg, A. L. *J. Biol. Chem.* **1997**, *272*, 1791.
(134) Kisselev, A. F.; Akopian, T. N.; Goldberg, A. L. *J. Biol. Chem.* **1998**, *273*, 1982.
(135) Kisselev, A. F.; Akopian, T. N.; Woo, K. M.; Goldberg, A. L. *J. Biol. Chem.* **1999**, *274*, 3363.
(136) Emmerich, N. P.; Nussbaum, A. K.; Stevanovic, S.; Priemer, M.; Toes, R. E.; Rammensee, H. G.; Schild, H. *J. Biol. Chem.* **2000**, *275*, 21140.
(137) Kohler, A.; Cascio, P.; Leggett, D. S.; Woo, K. M.; Goldberg, A. L.; Finley, D. *Mol. Cell* **2001**, *7*, 1143.
(138) Hutschenreiter, S.; Tinazli, A.; Model, K.; Tampe, R., *EMBO J.* **2004**, *23*, 2488.
(139) Kisselev, A. F.; Kaganovich, D.; Goldberg, A. L. *J. Biol. Chem.* **2002**, *277*, 22260.
(140) Kisselev, A. F.; Akopian, T. N.; Castillo, V.; Goldberg, A. L. *Mol. Cell* **1999**, *4*, 395.
(141) Schmidtke, G.; Emch, S.; Groettrup, M.; Holzhutter, H. G. *J. Biol. Chem.* **2000**, *275*, 22056.
(142) Myung, J.; Kim, K. B.; Lindsten, K.; Dantuma, N. P.; Crews, C. M. *Mol. Cell* **2001**, *7*, 411.
(143) Kleijnen, M. F.; Roelofs, J.; Park, S.; Hathaway, N. A.; Glickman, M.; King, R. W.; Finley, D. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1180.
(144) Cascio, P.; Hilton, C.; Kisselev, A. F.; Rock, K. L.; Goldberg, A. L. *EMBO J.* **2001**, *20*, 2357.
(145) Tanahashi, N.; Kawahara, H.; Murakami, Y.; Tanaka, K. *Mol. Biol. Rep.* **1999**, *26*, 3.
(146) Stoltze, L.; Schirle, M.; Schwarz, G.; Schroter, C.; Thompson, M. W.; Hersh, L. B.; Kalbacher, H.; Stevanovic, S.; Rammensee, H. G.; Schild, H. *Nat. Immunol.* **2000**, *1*, 413.
(147) Komlosh, A.; Momburg, F.; Weinschenk, T.; Emmerich, N.; Schild, H.; Nadav, E.; Shaked, I.; Reiss, Y. *J. Biol. Chem.* **2001**, *276*, 30050.
(148) Nussbaum, A. K.; Kuttler, C.; Hadeler, K. P.; Rammensee, H. G.; Schild, H. *Immunogenetics* **2001**, *53*, 87.
(149) Saxova, P.; Buus, S.; Brunak, S.; Kesmir, C. *Int. Immunol.* **2003**, *15*, 781.
(150) Hakenberg, J.; Nussbaum, A. K.; Schild, H.; Rammensee, H. G.; Kuttler, C.; Holzhutter, H. G.; Kloetzel, P. M.; Kaufmann, S. H.; Mollenkopf, H. J. *Appl. Bioinf.* **2003**, *2*, 155.
(151) Tenzer, S.; Peters, B.; Bulik, S.; Schoor, O.; Lemmel, C.; Schatz, M. M.; Kloetzel, P. M.; Rammensee, H. G.; Schild, H.; Holzhutter, H. G. *Cell. Mol. Life Sci.* **2005**, *62*, 1025.
(152) Kuttler, C.; Nussbaum, A. K.; Dick, T. P.; Rammensee, H. G.; Schild, H.; Hadeler, K. P. *J. Mol. Biol.* **2000**, *298*, 417.
(153) Ginodi, I.; Vider-Shalit, T.; Tsaban, L.; Louzoun, Y. *Bioinformatics* **2008**, *24*, 477.

(154) Mishto, M.; Luciani, F.; Holzhutter, H. G.; Bellavista, E.; Santoro, A.; Textoris-Taube, K.; Franceschi, C.; Kloetzel, P. M.; Zaikin, A. *J. Mol. Biol.* **2008**, *377*, 1607.
(155) Goldberg, A. L.; Rock, K. *Nat. Med.* **2002**, *8*, 338.
(156) Richardson, P. G.; Mitsiades, C.; Hideshima, T.; Anderson, K. C. *Annu. Rev. Med.* **2006**, *57*, 33.
(157) Voorhees, P. M.; Orlowski, R. Z. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 189.
(158) Orlowski, R. Z.; Kuhn, D. J. *Clin. Cancer Res.* **2008**, *14*, 1649.
(159) Kisselev, A. F. *Chem. Biol.* **2008**, *15*, 419.
(160) Vinitsky, A.; Michaud, C.; Powers, J. C.; Orlowski, M. *Biochemistry* **1992**, *31*, 9421.
(161) Tsubuki, S.; Saito, Y.; Tomioka, M.; Ito, H.; Kawashima, S. *J. Biochem.* **1996**, *119*, 572.
(162) Hines, J.; Groll, M.; Fahnestock, M.; Crews, C. M. *Chem. Biol.* **2008**, *15*, 501.
(163) Berkers, C. R.; Verdoes, M.; Lichtman, E.; Fiebiger, E.; Kessler, B. M.; Anderson, K. C.; Ploegh, H. L.; Ovaa, H.; Galardy, P. J. *Nat. Methods* **2005**, *2*, 357.
(164) Adams, J.; Palombella, V. J.; Sausville, E. A.; Johnson, J.; Destree, A.; Lazarus, D. D.; Maas, J.; Pien, C. S.; Prakash, S.; Elliott, P. J. *Cancer Res.* **1999**, *59*, 2615.
(165) Altun, M.; Galardy, P. J.; Shringarpure, R.; Hideshima, T.; LeBlanc, R.; Anderson, K. C.; Ploegh, H. L.; Kessler, B. M. *Cancer Res.* **2005**, *65*, 7896.
(166) Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. *Structure* **2006**, *14*, 451.
(167) Dorsey, B. D.; Iqbal, M.; Chatterjee, S.; Menta, E.; Bernardini, R.; Bernareggi, A.; Cassara, P. G.; D'Arasmo, G.; Ferretti, E.; De Munari, S.; Oliva, A.; Pezzoni, G.; Allievi, C.; Strepponi, I.; Ruggeri, B.; Ator, M. A.; Williams, M.; Mallamo, J. P. *J. Med. Chem.* **2008**, *51*, 1068.
(168) Piva, R.; Ruggeri, B.; Williams, M.; Costa, G.; Tamagno, I.; Ferrero, D.; Giai, V.; Coscia, M.; Peola, S.; Massaia, M.; Pezzoni, G.; Allievi, C.; Pescalli, N.; Cassin, M.; di Giovine, S.; Nicoli, P.; de Feudis, P.; Strepponi, I.; Roato, I.; Ferracini, R.; Bussolati, B.; Camussi, G.; Jones-Bolin, S.; Hunter, K.; Zhao, H.; Neri, A.; Palumbo, A.; Berkers, C.; Ovaa, H.; Bernareggi, A.; Inghirami, G. *Blood* **2008**, *111*, 2765.
(169) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726.
(170) Dick, L. R.; Cruikshank, A. A.; Grenier, L.; Melandri, F. D.; Nunes, S. L.; Stein, R. L. *J. Biol. Chem.* **1996**, *271*, 7273.
(171) Omura, S.; Ikeda, H.; Tanaka, H. *J Antibiot. (Tokyo)* **1991**, *44*, 560.
(172) Fenteany, G.; Standaert, R. F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 3358.
(173) Shah, I. M.; Lees, K. R.; Pien, C. P.; Elliott, P. J. *Br. J. Clin. Pharmacol.* **2002**, *54*, 269.
(174) Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T. H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W. F.; Neuteboom, S. T.; Lam, K. S.; Palladino, M. A.; Potts, B. C. *J. Med. Chem.* **2005**, *48*, 3684.
(175) Chauhan, D.; Catley, L.; Li, G.; Podar, K.; Hideshima, T.; Velankar, M.; Mitsiades, C.; Mitsiades, N.; Yasui, H.; Letai, A.; Ovaa, H.; Berkers, C.; Nicholson, B.; Chao, T. H.; Neuteboom, S. T.; Richardson, P.; Palladino, M. A.; Anderson, K. C. *Cancer Cell* **2005**, *8*, 407.
(176) Groll, M.; Huber, R.; Potts, B. C. *J. Am. Chem. Soc.* **2006**, *128*, 5136.
(177) Meng, L.; Mohan, R.; Kwok, B. H.; Elofsson, M.; Sin, N.; Crews, C. M. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10403.
(178) Groll, M.; Koguchi, Y.; Huber, R.; Kohno, J. *J. Mol. Biol.* **2001**, *311*, 543.
(179) Meng, L.; Kwok, B. H.; Sin, N.; Crews, C. M. *Cancer Res.* **1999**, *59*, 2798.
(180) Demo, S. D.; Kirk, C. J.; Aujay, M. A.; Buchholz, T. J.; Dajee, M.; Ho, M. N.; Jiang, J.; Laidig, G. J.; Lewis, E. R.; Parlati, F.; Shenk, K. D.; Smyth, M. S.; Sun, C. M.; Vallone, M. K.; Woo, T. M.; Molineaux, C. J.; Bennett, M. K. *Cancer Res.* **2007**, *67*, 6383.
(181) Kuhn, D. J.; Chen, Q.; Voorhees, P. M.; Strader, J. S.; Shenk, K. D.; Sun, C. M.; Demo, S. D.; Bennett, M. K.; van Leeuwen, F. W.; Chanan-Khan, A. A.; Orlowski, R. Z. *Blood* **2007**, *110*, 3281.
(182) Bogyo, M.; McMaster, J. S.; Gaczynska, M.; Tortorella, D.; Goldberg, A. L.; Ploegh, H. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 6629.
(183) Nazif, T.; Bogyo, M. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2967.
(184) Groll, M.; Schellenberg, B.; Bachmann, A. S.; Archer, C. R.; Huber, R.; Powell, T. K.; Lindow, S.; Kaiser, M.; Dudler, R. *Nature* **2008**, *452*, 755.
(185) Nikrad, M.; Johnson, T.; Puthalalath, H.; Coultas, L.; Adams, J.; Kraft, A. S. *Mol. Cancer Ther.* **2005**, *4*, 443.
(186) Zhu, H.; Zhang, L.; Dong, F.; Guo, W.; Wu, S.; Teraishi, F.; Davis, J. J.; Chiao, P. J.; Fang, B. *Oncogene* **2005**, *24*, 4993.
(187) Crawford, L. J.; Walker, B.; Irvine, A. E. *Front. Biosci.* **2008**, *13*, 4285.
(188) Roccaro, A. M.; Leleu, X.; Sacco, A.; Jia, X.; Melhem, M.; Moreau, A. S.; Ngo, H. T.; Runnels, J.; Azab, A.; Azab, F.; Burwick, N.; Farag, M.; Treon, S. P.; Palladino, M. A.; Hideshima, T.; Chauhan, D.; Anderson, K. C.; Ghobrial, I. M. *Blood* **2008**, *111*, 4752.
(189) Chauhan, D.; Hideshima, T.; Anderson, K. C. *Adv. Exp. Med. Biol.* **2008**, *615*, 251.
(190) Larsen, C. N.; Finley, D. *Cell* **1997**, *91*, 431.
(191) Chu-Ping, M.; Vu, J. H.; Proske, R. J.; Slaughter, C. A.; DeMartino, G. N. *J. Biol. Chem.* **1994**, *269*, 3539.
(192) Peters, J. M.; Franke, W. W.; Kleinschmidt, J. A. *J. Biol. Chem.* **1994**, *269*, 7709.
(193) Coux, O.; Tanaka, K.; Goldberg, A. L. *Annu. Rev. Biochem.* **1996**, *65*, 801.
(194) Smith, D. M.; Benaroudj, N.; Goldberg, A. *J. Struct. Biol.* **2006**, *156*, 72.
(195) Yoshimura, T.; Kameyama, K.; Takagi, T.; Ikai, A.; Tokunaga, F.; Koide, T.; Tanahashi, N.; Tamura, T.; Cejka, Z.; Baumeister, W.; et al. *J. Struct. Biol.* **1993**, *111*, 200.
(196) Finley, D.; Tanaka, K.; Mann, C.; Feldmann, H.; Hochstrasser, M.; Vierstra, R.; Johnston, S.; Hampton, R.; Haber, J.; McCusker, J.; Silver, P.; Frontali, L.; Thorsness, P.; Varshavsky, A.; Byers, B.; Madura, K.; Reed, S. I.; Wolf, D.; Jentsch, S.; Sommer, T.; Baumeister, W.; Goldberg, A.; Fried, V.; Rubin, D. M.; Toh-e, A.; et al. *Trends Biochem. Sci.* **1998**, *23*, 244.
(197) Glickman, M. H.; Rubin, D. M.; Coux, O.; Wefes, I.; Pfeifer, G.; Cjeka, Z.; Baumeister, W.; Fried, V. A.; Finley, D. *Cell* **1998**, *94*, 615.
(198) Dubiel, W.; Ferrell, K.; Pratt, G.; Rechsteiner, M. *J. Biol. Chem.* **1992**, *267*, 22699.
(199) Zwickl, P.; Baumeister, W. *Nat. Cell Biol.* **1999**, *1*, E97.
(200) Beyer, A. *Protein Sci.* **1997**, *6*, 2043.
(201) Confalonieri, F.; Duguet, M. *BioEssays* **1995**, *17*, 639.
(202) Zwickl, P.; Baumeister, W.; Steven, A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 242.
(203) Vale, R. D. *J. Cell Biol.* **2000**, *150*, F13.
(204) Lupas, A. N.; Martin, J. *Curr. Opin. Struct. Biol.* **2002**, *12*, 746.
(205) Kajava, A. V. *J. Biol. Chem.* **2002**, *277*, 49791.
(206) Rosenzweig, R.; Osmulski, P. A.; Gaczynska, M.; Glickman, M. H. *Nat. Struct. Mol. Biol.* **2008**, *15*, 573.
(207) Hofmann, K.; Bucher, P. *Trends Biochem. Sci.* **1998**, *23*, 204.
(208) Scheel, H.; Hofmann, K. *BMC Bioinf.* **2005**, *6*, 71.
(209) Verma, R.; Aravind, L.; Oania, R.; McDonald, W. H.; Yates, J. R., 3rd; Koonin, E. V.; Deshaies, R. J. *Science* **2002**, *298*, 611.
(210) Yao, T.; Cohen, R. E. *Nature* **2002**, *419*, 403.
(211) Maytal-Kivity, V.; Reis, N.; Hofmann, K.; Glickman, M. H. *BMC Biochem.* **2002**, *3*, 28.
(212) Cope, G. A.; Suh, G. S.; Aravind, L.; Schwarz, S. E.; Zipursky, S. L.; Koonin, E. V.; Deshaies, R. J. *Science* **2002**, *298*, 608.
(213) Bech-Otschir, D.; Seeger, M.; Dubiel, W. *J. Cell Sci.* **2002**, *115*, 467.
(214) Verma, R.; Chen, S.; Feldman, R.; Schieltz, D.; Yates, J.; Dohmen, J.; Deshaies, R. J. *Mol. Biol. Cell* **2000**, *11*, 3425.
(215) Hanna, J.; Hathaway, N. A.; Tone, Y.; Crosas, B.; Elsasser, S.; Kirkpatrick, D. S.; Leggett, D. S.; Gygi, S. P.; King, R. W.; Finley, D. *Cell* **2006**, *127*, 99.
(216) Russell, S. J.; Reed, S. H.; Huang, W.; Friedberg, E. C.; Johnston, S. A. *Mol. Cell* **1999**, *3*, 687.
(217) Russell, S. J.; Johnston, S. A. *J. Biol. Chem.* **2001**, *276*, 9825.
(218) Gillette, T. G.; Huang, W.; Russell, S. J.; Reed, S. H.; Johnston, S. A.; Friedberg, E. C. *Genes Dev.* **2001**, *15*, 1528.
(219) Ferdous, A.; Kodadek, T.; Johnston, S. A. *Biochemistry* **2002**, *41*, 12798.
(220) Ezhkova, E.; Tansey, W. P. *Mol. Cell* **2004**, *13*, 435.
(221) Lee, D.; Ezhkova, E.; Li, B.; Pattenden, S. G.; Tansey, W. P.; Workman, J. L. *Cell* **2005**, *123*, 423.
(222) Sulahian, R.; Sikder, D.; Johnston, S. A.; Kodadek, T. *Nucleic Acids Res.* **2006**, *34*, 1351.
(223) Lassot, I.; Latreille, D.; Rousset, E.; Sourisseau, M.; Linares, L. K.; Chable-Bessia, C.; Coux, O.; Benkirane, M.; Kiernan, R. E. *Mol. Cell* **2007**, *25*, 369.
(224) Archer, C. T.; Burdine, L.; Liu, B.; Ferdous, A.; Johnston, S. A.; Kodadek, T. *J. Biol. Chem.* **2008**, *283*, 21789.
(225) Babbitt, S. E.; Kiss, A.; Deffenbaugh, A. E.; Chang, Y. H.; Bailly, E.; Erdjument-Bromage, H.; Tempst, P.; Buranda, T.; Sklar, L. A.; Baumler, J.; Gogol, E.; Skowyra, D. *Cell* **2005**, *121*, 553.
(226) Kriegenburg, F.; Seeger, M.; Saeki, Y.; Tanaka, K.; Lauridsen, A. M.; Hartmann-Petersen, R.; Hendil, K. B. *Cell* **2008**, *135*, 355.
(227) Groll, M.; Bajorek, M.; Kohler, A.; Moroder, L.; Rubin, D. M.; Huber, R.; Glickman, M. H.; Finley, D. *Nat. Struct. Biol.* **2000**, *7*, 1062.
(228) Kohler, A.; Bajorek, M.; Groll, M.; Moroder, L.; Rubin, D. M.; Huber, R.; Glickman, M. H.; Finley, D. *Biochimie* **2001**, *83*, 325.
(229) Tanaka, K.; Yoshimura, T.; Ichihara, A. *J. Biochem.* **1989**, *106*, 495.

(230) Whitby, F. G.; Masters, E. I.; Kramer, L.; Knowlton, J. R.; Yao, Y.; Wang, C. C.; Hill, C. P. *Nature* **2000**, *408*, 115.
(231) Forster, A.; Hill, C. P. *Trends Cell Biol.* **2003**, *13*, 550.
(232) Smith, D. M.; Chang, S. C.; Park, S.; Finley, D.; Cheng, Y.; Goldberg, A. L. *Mol. Cell* **2007**, *27*, 731.
(233) Rabl, J.; Smith, D. M.; Yu, Y.; Chang, S. C.; Goldberg, A. L.; Cheng, Y. *Mol. Cell* **2008**, *30*, 360.
(234) Gillette, T. G.; Kumar, B.; Thompson, D.; Slaughter, C. A.; Demartino, G. N. *J. Biol. Chem. 283*, **2008**.
(235) Smith, D. M.; Kafri, G.; Cheng, Y.; Ng, D.; Walz, T.; Goldberg, A. L. *Mol. Cell* **2005**, *20*, 687.
(236) Liu, C. W.; Li, X.; Thompson, D.; Wooding, K.; Chang, T. L.; Tang, Z.; Yu, H.; Thomas, P. J.; DeMartino, G. N. *Mol. Cell* **2006**, *24*, 39.
(237) Braun, B. C.; Glickman, M.; Kraft, R.; Dahlmann, B.; Kloetzel, P. M.; Finley, D.; Schmidt, M. *Nat. Cell Biol.* **1999**, *1*, 221.
(238) Liu, C. W.; Millen, L.; Roman, T. B.; Xiong, H.; Gilbert, H. F.; Noiva, R.; DeMartino, G. N.; Thomas, P. J. *J. Biol. Chem.* **2002**, *277*, 26815.
(239) Benaroudj, N.; Goldberg, A. L. *Nat. Cell Biol.* **2000**, *2*, 833.
(240) Rubin, D. M.; Glickman, M. H.; Larsen, C. N.; Dhruvakumar, S.; Finley, D. *EMBO J.* **1998**, *17*, 4909.
(241) Bajorek, M.; Finley, D.; Glickman, M. H. *Curr. Biol.* **2003**, *13*, 1140.
(242) Hicke, L.; Schubert, H. L.; Hill, C. P. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 610.
(243) Di Fiore, P. P.; Polo, S.; Hofmann, K. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 491.
(244) Deveraux, Q.; Ustrell, V.; Pickart, C.; Rechsteiner, M. *J. Biol. Chem.* **1994**, *269*, 7059.
(245) van Nocker, S.; Deveraux, Q.; Rechsteiner, M.; Vierstra, R. D. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 856.
(246) van Nocker, S.; Sadis, S.; Rubin, D. M.; Glickman, M.; Fu, H.; Coux, O.; Wefes, I.; Finley, D.; Vierstra, R. D. *Mol. Cell. Biol.* **1996**, *16*, 6020.
(247) Young, P.; Deveraux, Q.; Beal, R. E.; Pickart, C. M.; Rechsteiner, M. *J. Biol. Chem.* **1998**, *273*, 5461.
(248) Hofmann, K.; Falquet, L. *Trends Biochem. Sci.* **2001**, *26*, 347.
(249) Wang, Q.; Young, P.; Walters, K. J. *J. Mol. Biol.* **2005**, *348*, 727.
(250) Verma, R.; Oania, R.; Graumann, J.; Deshaies, R. J. *Cell* **2004**, *118*, 99.
(251) Lam, Y. A.; Lawson, T. G.; Velayutham, M.; Zweier, J. L.; Pickart, C. M. *Nature* **2002**, *416*, 763.
(252) Husnjak, K.; Elsasser, S.; Zhang, N.; Chen, X.; Randles, L.; Shi, Y.; Hofmann, K.; Walters, K. J.; Finley, D.; Dikic, I. *Nature* **2008**, *453*, 481.
(253) Schauber, C.; Chen, L.; Tongaonkar, P.; Vega, I.; Lambertson, D.; Potts, W.; Madura, K. *Nature* **1998**, *391*, 715.
(254) Ortolan, T. G.; Tongaonkar, P.; Lambertson, D.; Chen, L.; Schauber, C.; Madura, K. *Nat. Cell Biol.* **2000**, *2*, 601.
(255) Chen, L.; Shinde, U.; Ortolan, T. G.; Madura, K. *EMBO Rep.* **2001**, *2*, 933.
(256) Chen, L.; Madura, K. *Mol. Cell. Biol.* **2002**, *22*, 4902.
(257) Kleijnen, M. F.; Shih, A. H.; Zhou, P.; Kumar, S.; Soccio, R. E.; Kedersha, N. L.; Gill, G.; Howley, P. M. *Mol. Cell* **2000**, *6*, 409.
(258) Kleijnen, M. F.; Alarcon, R. M.; Howley, P. M. *Mol. Biol. Cell* **2003**, *14*, 3868.
(259) Bertolaet, B. L.; Clarke, D. J.; Wolff, M.; Watson, M. H.; Henze, M.; Divita, G.; Reed, S. I. *Nat. Struct. Biol.* **2001**, *8*, 417.
(260) Wilkinson, C. R.; Seeger, M.; Hartmann-Petersen, R.; Stone, M.; Wallace, M.; Semple, C.; Gordon, C. *Nat. Cell Biol.* **2001**, *3*, 939.
(261) Rao, H.; Sastry, A. *J. Biol. Chem.* **2002**, *277*, 11691.
(262) Hofmann, K.; Bucher, P. *Trends Biochem. Sci.* **1996**, *21*, 172.
(263) Kang, Y.; Vossler, R. A.; Diaz-Martinez, L. A.; Winter, N. S.; Clarke, D. J.; Walters, K. J. *J. Mol. Biol.* **2006**, *356*, 1027.
(264) Funakoshi, M.; Sasaki, T.; Nishimoto, T.; Kobayashi, H. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 745.
(265) Madura, K. *Trends Biochem. Sci.* **2004**, *29*, 637.
(266) Kim, I.; Mi, K.; Rao, H. *Mol. Biol. Cell* **2004**, *15*, 3357.
(267) Kang, Y.; Zhang, N.; Koepp, D. M.; Walters, K. J. *J. Mol. Biol.* **2007**, *365*, 1093.
(268) Goh, A. M.; Walters, K. J.; Elsasser, S.; Verma, R.; Deshaies, R. J.; Finley, D.; Howley, P. M. *BMC Biochem.* **2008**, *9*, 4.
(269) Richly, H.; Rape, M.; Braun, S.; Rumpf, S.; Hoege, C.; Jentsch, S. *Cell* **2005**, *120*, 73.
(270) Elsasser, S.; Chandler-Militello, D.; Muller, B.; Hanna, J.; Finley, D. *J. Biol. Chem.* **2004**, *279*, 26817.
(271) Saeki, Y.; Saitoh, A.; Toh-e, A.; Yokosawa, H. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 986.
(272) Lambertson, D.; Chen, L.; Madura, K. *Genetics* **1999**, *153*, 69.
(273) Guterman, A.; Glickman, M. H. *J. Biol. Chem.* **2004**, *279*, 1729.
(274) Lam, Y. A.; Xu, W.; DeMartino, G. N.; Cohen, R. E. *Nature* **1997**, *385*, 737.
(275) Crosas, B.; Hanna, J.; Kirkpatrick, D. S.; Zhang, D. P.; Tone, Y.; Hathaway, N. A.; Buecker, C.; Leggett, D. S.; Schmidt, M.; King, R. W.; Gygi, S. P.; Finley, D. *Cell* **2006**, *127*, 1401.
(276) Kraut, D. A.; Prakash, S.; Matouschek, A. *Trends Cell Biol.* **2007**, *17*, 419.
(277) Leggett, D. S.; Hanna, J.; Borodovsky, A.; Crosas, B.; Schmidt, M.; Baker, R. T.; Walz, T.; Ploegh, H.; Finley, D. *Mol. Cell* **2002**, *10*, 495.
(278) Chernova, T. A.; Allen, K. D.; Wesoloski, L. M.; Shanks, J. R.; Chernoff, Y. O.; Wilkinson, K. D. *J. Biol. Chem.* **2003**, *278*, 52102.
(279) Borodovsky, A.; Kessler, B. M.; Casagrande, R.; Overkleeft, H. S.; Wilkinson, K. D.; Ploegh, H. L. *EMBO J.* **2001**, *20*, 5187.
(280) Hanna, J.; Leggett, D. S.; Finley, D. *Mol. Cell. Biol.* **2003**, *23*, 9251.
(281) Wilson, S. M.; Bhattacharyya, B.; Rachel, R. A.; Coppola, V.; Tessarollo, L.; Householder, D. B.; Fletcher, C. F.; Miller, R. J.; Copeland, N. G.; Jenkins, N. A. *Nat. Genet.* **2002**, *32*, 420.
(282) Anderson, C.; Crimmins, S.; Wilson, J. A.; Korbel, G. A.; Ploegh, H. L.; Wilson, S. M. *J. Neurochem.* **2005**, *95*, 724.
(283) Shinji, S.; Naito, Z.; Ishiwata, S.; Ishiwata, T.; Tanaka, N.; Furukawa, K.; Suzuki, H.; Seya, T.; Matsuda, A.; Katsuta, M.; Tajiri, T. *Oncol. Rep.* **2006**, *15*, 539.
(284) Stone, M.; Hartmann-Petersen, R.; Seeger, M.; Bech-Otschir, D.; Wallace, M.; Gordon, C. *J. Mol. Biol.* **2004**, *344*, 697.
(285) Hamazaki, J.; Iemura, S.; Natsume, T.; Yashiroda, H.; Tanaka, K.; Murata, S. *EMBO J.* **2006**, *25*, 4524.
(286) Qiu, X. B.; Ouyang, S. Y.; Li, C. J.; Miao, S.; Wang, L.; Goldberg, A. L. *EMBO J.* **2006**, *25*, 5742.
(287) Yao, T.; Song, L.; Xu, W.; DeMartino, G. N.; Florens, L.; Swanson, S. K.; Washburn, M. P.; Conaway, R. C.; Conaway, J. W.; Cohen, R. E. *Nat. Cell Biol.* **2006**, *8*, 994.
(288) Koulich, E.; Li, X.; Demartino, G. N. *Mol. Biol. Cell* **2008**, *19*, 1072.
(289) Ferrell, K.; Wilkinson, C. R.; Dubiel, W.; Gordon, C. *Trends Biochem. Sci.* **2000**, *25*, 83.
(290) Xie, Y.; Varshavsky, A. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 2497.
(291) Xie, Y.; Varshavsky, A. *Nat. Cell Biol.* **2002**, *4*, 1003.
(292) Demartino, G. N.; Gillette, T. G. *Cell* **2007**, *129*, 659.
(293) Nakamura, Y.; Nakano, K.; Umehara, T.; Kimura, M.; Hayashizaki, Y.; Tanaka, A.; Horikoshi, M.; Padmanabhan, B.; Yokoyama, S. *Structure* **2007**, *15*, 179.
(294) Sedgwick, S. G.; Smerdon, S. J. *Trends Biochem. Sci.* **1999**, *24*, 311.
(295) Hori, T.; Kato, S.; Saeki, M.; DeMartino, G. N.; Slaughter, C. A.; Takeuchi, J.; Toh-e, A.; Tanaka, K. *Gene* **1998**, *216*, 113.
(296) Whitby, F. G.; Hill, C. P. *Structure* **2007**, *15*, 137.
(297) Higashitsuji, H.; Itoh, K.; Nagao, T.; Dawson, S.; Nonoguchi, K.; Kido, T.; Mayer, R. J.; Arii, S.; Fujita, J. *Nat. Med.* **2000**, *6*, 96.
(298) Dawson, S.; Apcher, S.; Mee, M.; Higashitsuji, H.; Baker, R.; Uhle, S.; Dubiel, W.; Fujita, J.; Mayer, R. J. *J. Biol. Chem.* **2002**, *277*, 10893.
(299) Higashitsuji, H.; Higashitsuji, H.; Itoh, K.; Sakurai, T.; Nagao, T.; Sumitomo, Y.; Masuda, T.; Dawson, S.; Shimada, Y.; Mayer, R. J.; Fujita, J. *Cancer Cell* **2005**, *8*, 75.
(300) Dawson, S.; Higashitsuji, H.; Wilkinson, A. J.; Fujita, J.; Mayer, R. J. *Trends Cell Biol.* **2006**, *16*, 229.
(301) Yen, H. C.; Gordon, C.; Chang, E. C. *Cell* **2003**, *112*, 207.
(302) Yen, H. C.; Chang, E. C. *Cell Cycle* **2003**, *2*, 81.
(303) von Arnim, A. G.; Chamovitz, D. A. *Curr. Biol.* **2003**, *13*, R323.
(304) Shalev, A.; Valasek, L.; Pise-Masison, C. A.; Radonovich, M.; Phan, L.; Clayton, J.; He, H.; Brady, J. N.; Hinnebusch, A. G.; Asano, K. *J. Biol. Chem.* **2001**, *276*, 34948.
(305) Maytal-Kivity, V.; Piran, R.; Pick, E.; Hofmann, K.; Glickman, M. H. *EMBO Rep.* **2002**, *3*, 1215.
(306) Wee, S.; Hetfeld, B.; Dubiel, W.; Wolf, D. A. *BMC Genet.* **2002**, *3*, 15.
(307) Krogan, N. J.; Cagney, G.; Yu, H.; Zhong, G.; Guo, X.; Ignatchenko, A.; Li, J.; Pu, S.; Datta, N.; Tikuisis, A. P.; Punna, T.; Peregrin-Alvarez, J. M.; Shales, M.; Zhang, X.; Davey, M.; Robinson, M. D.; Paccanaro, A.; Bray, J. E.; Sheung, A.; Beattie, B.; Richards, D. P.; Canadien, V.; Lalev, A.; Mena, F.; Wong, P.; Starostine, A.; Canete, M. M.; Vlasblom, J.; Wu, S.; Orsi, C.; Collins, S. R.; Chandran, S.; Haw, R.; Rilstone, J. J.; Gandi, K.; Thompson, N. J.; Musso, G.; St Onge, P.; Ghanny, S.; Lam, M. H.; Butland, G.; Altaf-Ul, A. M.; Kanaya, S.; Shilatifard, A.; O'Shea, E.; Weissman, J. S.; Ingles, C. J.; Hughes, T. R.; Parkinson, J.; Gerstein, M.; Wodak, S. J.; Emili, A.; Greenblatt, J. F. *Nature* **2006**, *440*, 637.
(308) Gavin, A. C.; Aloy, P.; Grandi, P.; Krause, R.; Boesche, M.; Marzioch, M.; Rau, C.; Jensen, L. J.; Bastuck, S.; Dumpelfeld, B.; Edelmann, A.; Heurtier, M. A.; Hoffman, V.; Hoefert, C.; Klein, K.; Hudak, M.; Michon, A. M.; Schelder, M.; Schirle, M.; Remor, M.; Rudi, T.; Hooper, S.; Bauer, A.; Bouwmeester, T.; Casari, G.; Drewes, G.; Neubauer, G.; Rick, J. M.; Kuster, B.; Bork, P.; Russell, R. B.; Superti-Furga, G. *Nature* **2006**, *440*, 631.
(309) Tatebe, H.; Yanagida, M. *Curr. Biol.* **2000**, *10*, 1329.

(310) Romero-Perez, L.; Chen, L.; Lambertson, D.; Madura, K. *J. Biol. Chem.* **2007**, *282*, 35574.
(311) Tabb, M. M.; Tongaonkar, P.; Vu, L.; Nomura, M. *Mol. Cell. Biol.* **2000**, *20*, 6062.
(312) Takeda, K.; Yanagida, M. *Cell* **2005**, *122*, 393.
(313) Park, Y.; Hwang, Y. P.; Lee, J. S.; Seo, S. H.; Yoon, S. K.; Yoon, J. B. *Mol. Cell. Biol.* **2005**, *25*, 3842.
(314) Seong, K. M.; Baek, J. H.; Yu, M. H.; Kim, J. *FEBS Lett.* **2007**, *581*, 2567.
(315) Nelbock, P.; Dillon, P. J.; Perkins, A.; Rosen, C. A. *Science* **1990**, *248*, 1650.
(316) Seeger, M.; Ferrell, K.; Frank, R.; Dubiel, W. *J. Biol. Chem.* **1997**, *272*, 8145.
(317) Mannhaupt, G.; Schnall, R.; Karpov, V.; Vetter, I.; Feldmann, H. *FEBS Lett.* **1999**, *450*, 27.
(318) Xie, Y.; Varshavsky, A. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3056.
(319) Ju, D.; Xie, Y. *J. Biol. Chem.* **2004**, *279*, 23851.
(320) Dohmen, R. J.; Willers, I.; Marques, A. J. *Biochim. Biophys. Acta* **2007**, *1773*, 1599.
(321) Sok, J.; Calfon, M.; Lu, J.; Lichtlen, P.; Clark, S. G.; Ron, D. *Cell Stress Chaperones* **2001**, *6*, 6.
(322) Stanhill, A.; Haynes, C. M.; Zhang, Y.; Min, G.; Steele, M. C.; Kalinina, J.; Martinez, E.; Pickart, C. M.; Kong, X. P.; Ron, D. *Mol. Cell* **2006**, *23*, 875.
(323) Yun, C.; Stanhill, A.; Yang, Y.; Zhang, Y.; Haynes, C. M.; Xu, C. F.; Neubert, T. A.; Mor, A.; Philips, M. R.; Ron, D. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7094.
(324) Dubiel, W.; Pratt, G.; Ferrell, K.; Rechsteiner, M. *J. Biol. Chem.* **1992**, *267*, 22369.
(325) Ma, C. P.; Slaughter, C. A.; DeMartino, G. N. *J. Biol. Chem.* **1992**, *267*, 10515.
(326) Rechsteiner, M.; Realini, C.; Ustrell, V. *Biochem. J.* **2000**, *345 Pt 1*, 1.
(327) Groettrup, M.; Soza, A.; Eggers, M.; Kuehn, L.; Dick, T. P.; Schild, H.; Rammensee, H. G.; Koszinowski, U. H.; Kloetzel, P. M. *Nature* **1996**, *381*, 166.
(328) Murata, S.; Udono, H.; Tanahashi, N.; Hamada, N.; Watanabe, K.; Adachi, K.; Yamano, T.; Yui, K.; Kobayashi, N.; Kasahara, M.; Tanaka, K.; Chiba, T. *EMBO J.* **2001**, *20*, 5898.
(329) Preckel, T.; Fung-Leung, W. P.; Cai, Z.; Vitiello, A.; Salter-Cid, L.; Winqvist, O.; Wolfe, T. G.; Von Herrath, M.; Angulo, A.; Ghazal, P.; Lee, J. D.; Fourie, A. M.; Wu, Y.; Pang, J.; Ngo, K.; Peterson, P. A.; Fruh, K.; Yang, Y. *Science* **1999**, *286*, 2162.
(330) Knowlton, J. R.; Johnston, S. C.; Whitby, F. G.; Realini, C.; Zhang, Z.; Rechsteiner, M.; Hill, C. P. *Nature* **1997**, *390*, 639.
(331) Hendil, K. B.; Khan, S.; Tanaka, K. *Biochem. J.* **1998**, *332 (Pt 3)*, 749.
(332) Kopp, F.; Dahlmann, B.; Kuehn, L. *J. Mol. Biol.* **2001**, *313*, 465.
(333) Cascio, P.; Call, M.; Petre, B. M.; Walz, T.; Goldberg, A. L. *EMBO J.* **2002**, *21*, 2636.
(334) Zannini, L.; Lecis, D.; Buscemi, G.; Carlessi, L.; Gasparini, P.; Fontanella, E.; Lisanti, S.; Barton, L.; Delia, D. *Cell Cycle* **2008**, *7*, 504.
(335) Nikaido, T.; Shimada, K.; Nishida, Y.; Lee, R. S.; Pardee, A. B.; Nishizuka, Y. *Exp. Cell Res.* **1989**, *182*, 284.
(336) Tanahashi, N.; Yokota, K.; Ahn, J. Y.; Chung, C. H.; Fujiwara, T.; Takahashi, E.; DeMartino, G. N.; Slaughter, C. A.; Toyonaga, T.; Yamamura, K.; Shimbara, N.; Tanaka, K. *Genes Cells* **1997**, *2*, 195.
(337) Barton, L. F.; Runnels, H. A.; Schell, T. D.; Cho, Y.; Gibbons, R.; Tevethia, S. S.; Deepe, G. S., Jr.; Monaco, J. J. *J. Immunol.* **2004**, *172*, 3948.
(338) Murata, S.; Kawahara, H.; Tohma, S.; Yamamoto, K.; Kasahara, M.; Nabeshima, Y.; Tanaka, K.; Chiba, T. *J. Biol. Chem.* **1999**, *274*, 38211.
(339) Moriishi, K.; Okabayashi, T.; Nakai, K.; Moriya, K.; Koike, K.; Murata, S.; Chiba, T.; Tanaka, K.; Suzuki, R.; Suzuki, T.; Miyamura, T.; Matsuura, Y. *J. Virol.* **2003**, *77*, 10237.
(340) Li, X.; Lonard, D. M.; Jung, S. Y.; Malovannaya, A.; Feng, Q.; Qin, J.; Tsai, S. Y.; Tsai, M. J.; O'Malley, B. W. *Cell* **2006**, *124*, 381.
(341) Li, X.; Amazit, L.; Long, W.; Lonard, D. M.; Monaco, J. J.; O'Malley, B. W. *Mol. Cell* **2007**, *26*, 831.
(342) Chen, X.; Barton, L. F.; Chi, Y.; Clurman, B. E.; Roberts, J. M. *Mol. Cell* **2007**, *26*, 843.
(343) Zhou, Y.; Fang, L.; Du, D.; Zhou, W.; Feng, X.; Chen, J.; Zhang, Z.; Chen, Z. *Acta Biochim. Biophys. Sin. (Shanghai)* **2008**, *40*, 729.
(344) Realini, C.; Jensen, C. C.; Zhang, Z.; Johnston, S. C.; Knowlton, J. R.; Hill, C. P.; Rechsteiner, M. *J. Biol. Chem.* **1997**, *272*, 25483.
(345) Zhou, P. *Cell* **2006**, *124*, 256.
(346) Ustrell, V.; Hoffman, L.; Pratt, G.; Rechsteiner, M. *EMBO J.* **2002**, *21*, 3516.
(347) Blickwedehl, J.; McEvoy, S.; Wong, I.; Kousis, P.; Clements, J.; Elliott, R.; Cresswell, P.; Liang, P.; Bangia, N. *Radiat. Res.* **2007**, *167*, 663.
(348) Blickwedehl, J.; Agarwal, M.; Seong, C.; Pandita, R. K.; Melendy, T.; Sung, P.; Pandita, T. K.; Bangia, N. w. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16165.
(349) Moore, C. W. *J. Bacteriol.* **1991**, *173*, 3605.
(350) Rechsteiner, M.; Hill, C. P. *Trends Cell Biol.* **2005**, *15*, 27.
(351) Febres, D. E.; Pramanik, A.; Caton, M.; Doherty, K.; McKoy, J.; Garcia, E.; Alejo, W.; Moore, C. W. *Cell. Mol. Biol. (Noisy-le-grand)* **2001**, *47*, 1149.
(352) McCullock, S.; Kinard, T.; McCullough, L.; Formosa, T. *J. Mol. Biol.* **2006**, *363*, 660.
(353) Iwanczyk, J.; Sadre-Bazzaz, K.; Ferrell, K.; Kondrashkina, E.; Formosa, T.; Hill, C. P.; Ortega, J. *J. Mol. Biol.* **2006**, *363*, 648.
(354) Schmidt, M.; Haas, W.; Crosas, B.; Santamaria, P. G.; Gygi, S. P.; Walz, T.; Finley, D. *Nat. Struct. Mol. Biol.* **2005**, *12*, 294.
(355) Andrade, M. A.; Bork, P. *Nat. Genet.* **1995**, *11*, 115.
(356) Kajava, A. V.; Gorbea, C.; Ortega, J.; Rechsteiner, M.; Steven, A. C. *J. Struct. Biol.* **2004**, *146*, 425.
(357) Andrade, M. A.; Petosa, C.; O'Donoghue, S. I.; Muller, C. W.; Bork, P. *J. Mol. Biol.* **2001**, *309*, 1.
(358) Ustrell, V.; Pratt, G.; Gorbea, C.; Rechsteiner, M. *Methods Enzymol.* **2005**, *398*, 321.
(359) Fehlker, M.; Wendler, P.; Lehmann, A.; Enenkel, C. *EMBO Rep.* **2003**, *4*, 959.
(360) Lehmann, A.; Jechow, K.; Enenkel, C. *EMBO Rep.* **2008**, *9*, 1237.
(361) Marques, A. J.; Glanemann, C.; Ramos, P. C.; Dohmen, R. J. *J. Biol. Chem.* **2007**, *282*, 34869.
(362) Li, X.; Kusmierczyk, A. R.; Wong, P.; Emili, A.; Hochstrasser, M. *EMBO J.* **2007**, *26*, 2339.
(363) Forster, A.; Whitby, F. G.; Hill, C. P. *EMBO J.* **2003**, *22*, 4356.
(364) Grziwa, A.; Maack, S.; Puhler, G.; Wiegand, G.; Baumeister, W.; Jaenicke, R. *Eur. J. Biochem.* **1994**, *223*, 1061.
(365) Maupin-Furlow, J. A.; Aldrich, H. C.; Ferry, J. G. *J. Bacteriol.* **1998**, *180*, 1480.
(366) Wilson, H. L.; Ou, M. S.; Aldrich, H. C.; Maupin-Furlow, J. *J. Bacteriol.* **2000**, *182*, 1680.
(367) Zuhl, F.; Seemuller, E.; Golbik, R.; Baumeister, W. *FEBS Lett.* **1997**, *418*, 189.
(368) Pouch, M. N.; Cournoyer, B.; Baumeister, W. *Mol. Microbiol.* **2000**, *35*, 368.
(369) Gerards, W. L.; Enzlin, J.; Haner, M.; Hendriks, I. L.; Aebi, U.; Bloemendal, H.; Boelens, W. *J. Biol. Chem.* **1997**, *272*, 10080.
(370) Gerards, W. L.; de Jong, W. W.; Bloemendal, H.; Boelens, W. *J. Mol. Biol.* **1998**, *275*, 113.
(371) Hirano, Y.; Hendil, K. B.; Yashiroda, H.; Iemura, S.; Nagane, R.; Hioki, Y.; Natsume, T.; Tanaka, K.; Murata, S. *Nature* **2005**, *437*, 1381.
(372) Le Tallec, B.; Barrault, M. B.; Courbeyrette, R.; Guerois, R.; Marsolier-Kergoat, M. C.; Peyroche, A. *Mol. Cell* **2007**, *27*, 660.
(373) Scott, C. M.; Kruse, K. B.; Schmidt, B. Z.; Perlmutter, D. H.; McCracken, A. A.; Brodsky, J. L. *Mol. Biol. Cell* **2007**, *18*, 3776.
(374) Hirano, Y.; Kaneko, T.; Okamoto, K.; Bai, M.; Yashiroda, H.; Furuyama, K.; Kato, K.; Tanaka, K.; Murata, S. *EMBO J.* **2008**, *27*, 2204.
(375) Hirano, Y.; Hayashi, H.; Iemura, S.; Hendil, K. B.; Niwa, S.; Kishimoto, T.; Kasahara, M.; Natsume, T.; Tanaka, K.; Murata, S. *Mol. Cell* **2006**, *24*, 977.
(376) Kusmierczyk, A. R.; Kunjappu, M. J.; Funakoshi, M.; Hochstrasser, M. *Nat. Struct. Mol. Biol.* **2008**, *15*, 237.
(377) Yashiroda, H.; Mizushima, T.; Okamoto, K.; Kameyama, T.; Hayashi, H.; Kishimoto, T.; Niwa, S.; Kasahara, M.; Kurimoto, E.; Sakata, E.; Takagi, K.; Suzuki, A.; Hirano, Y.; Murata, S.; Kato, K.; Yamane, T.; Tanaka, K. *Nat. Struct. Mol. Biol.* **2008**, *15*, 228.
(378) Hoyt, M. A.; McDonough, S.; Pimpl, S. A.; Scheel, H.; Hofmann, K.; Coffino, P. *Yeast* **2008**, *25*, 199.
(379) Kusmierczyk, A. R.; Hochstrasser, M. *Biol. Chem.* **2008**, *389*, 1143.
(380) Rosenzweig, R.; Glickman, M. H. *Nat. Struct. Mol. Biol.* **2008**, *15*, 218.
(381) Ramos, P. C.; Dohmen, R. J. *Structure* **2008**, *16*, 1296.
(382) Iyer, L. M.; Burroughs, A. M.; Aravind, L. *Biol. Direct* **2008**, *3*, 45.
(383) Nandi, D.; Woodward, E.; Ginsburg, D. B.; Monaco, J. J. *EMBO J.* **1997**, *16*, 5363.
(384) Ramos, P. C.; Hockendorff, J.; Johnson, E. S.; Varshavsky, A.; Dohmen, R. J. *Cell* **1998**, *92*, 489.
(385) Burri, L.; Hockendorff, J.; Boehm, U.; Klamp, T.; Dohmen, R. J.; Levy, F. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 10348.
(386) Witt, E.; Zantopf, D.; Schmidt, M.; Kraft, R.; Kloetzel, P. M.; Kruger, E. *J. Mol. Biol.* **2000**, *301*, 1.
(387) Griffin, T. A.; Slack, J. P.; McCluskey, T. S.; Monaco, J. J.; Colbert, R. A. *Mol. Cell Biol. Res. Commun.* **2000**, *3*, 212.

(388) Heink, S.; Ludwig, D.; Kloetzel, P. M.; Kruger, E. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9241.
(389) Fricke, B.; Heink, S.; Steffen, J.; Kloetzel, P. M.; Kruger, E. *EMBO Rep.* **2007**, *8*, 1170.
(390) Lehmann, A.; Janek, K.; Braun, B.; Kloetzel, P. M.; Enenkel, C. *J. Mol. Biol.* **2002**, *317*, 401.
(391) Hilt, W.; Enenkel, C.; Gruhler, A.; Singer, T.; Wolf, D. H. *J. Biol. Chem.* **1993**, *268*, 3479.
(392) Groettrup, M.; Standera, S.; Stohwasser, R.; Kloetzel, P. M. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 8970.
(393) Griffin, T. A.; Nandi, D.; Cruz, M.; Fehling, H. J.; Kaer, L. V.; Monaco, J. J.; Colbert, R. A. *J. Exp. Med.* **1998**, *187*, 97.
(394) Kingsbury, D. J.; Griffin, T. A.; Colbert, R. A. *J. Biol. Chem.* **2000**, *275*, 24156.
(395) De, M.; Jayarapu, K.; Elenich, L.; Monaco, J. J.; Colbert, R. A.; Griffin, T. A. *J. Biol. Chem.* **2003**, *278*, 6153.
(396) Schmidt, M.; Zantopf, D.; Kraft, R.; Kostka, S.; Preissner, R.; Kloetzel, P. M. *J. Mol. Biol.* **1999**, *288*, 117.
(397) Zaiss, D. M.; Standera, S.; Kloetzel, P. M.; Sijts, A. J. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 14344.
(398) Chu-Ping, M.; Slaughter, C. A.; DeMartino, G. N. *Biochim. Biophys. Acta* **1992**, *1119*, 303.
(399) Zaiss, D. M.; Standera, S.; Holzhutter, H.; Kloetzel, P.; Sijts, A. J. *FEBS Lett.* **1999**, *457*, 333.
(400) McCutchen-Maloney, S. L.; Matsuda, K.; Shimbara, N.; Binns, D. D.; Tanaka, K.; Slaughter, C. A.; DeMartino, G. N. *J. Biol. Chem.* **2000**, *275*, 18557.
(401) Kirk, R.; Laman, H.; Knowles, P. P.; Murray-Rust, J.; Lomonosov, M.; Meziane el, K.; McDonald, N. Q. *J. Biol. Chem.* **2008**, *283*, 22325.
(402) Murata, S.; Takahama, Y.; Tanaka, K. *Curr. Opin. Immunol.* **2008**.
(403) Gorbea, C.; Goellner, G. M.; Teter, K.; Holmes, R. K.; Rechsteiner, M. *J. Biol. Chem.* **2004**, *279*, 54849.
(404) Imai, J.; Maruya, M.; Yashiroda, H.; Yahara, I.; Tanaka, K. *EMBO J.* **2003**, *22*, 3557.
(405) Gorbea, C.; Taillandier, D.; Rechsteiner, M. *Mol. Biol. Rep.* **1999**, *26*, 15.
(406) Fu, H.; Reis, N.; Lee, Y.; Glickman, M. H.; Vierstra, R. D. *EMBO J.* **2001**, *20*, 7096.
(407) Davy, A.; Bello, P.; Thierry-Mieg, N.; Vaglio, P.; Hitti, J.; Doucette-Stamm, L.; Thierry-Mieg, D.; Reboul, J.; Boulton, S.; Walhout, A. J.; Coux, O.; Vidal, M. *EMBO Rep.* **2001**, *2*, 821.
(408) Sharon, M.; Taverner, T.; Ambroggio, X. I.; Deshaies, R. J.; Robinson, C. V. *PLoS Biol.* **2006**, *4*, e267.
(409) Isono, E.; Nishihara, K.; Saeki, Y.; Yashiroda, H.; Kamata, N.; Ge, L.; Ueda, T.; Kikuchi, Y.; Tanaka, K.; Nakano, A.; Toh-e, A. *Mol. Biol. Cell* **2007**, *18*, 569.
(410) Le Tallec, B.; Barrault, M. B.; Guérois, R.; Peyroche, A. *Mol. Cell* **2009**, *33*, 389.
(411) Princiotta, M. F.; Finzi, D.; Qian, S. B.; Gibbs, J.; Schuchmann, S.; Buttgereit, F.; Bennink, J. R.; Yewdell, J. W. *Immunity* **2003**, *18*, 343.
(412) Meiners, S.; Heyken, D.; Weller, A.; Ludwig, A.; Stangl, K.; Kloetzel, P. M.; Kruger, E. *J. Biol. Chem.* **2003**, *278*, 21517.
(413) London, M. K.; Keck, B. I.; Ramos, P. C.; Dohmen, R. J. *FEBS Lett.* **2004**, *567*, 259.
(414) Kahn, N. W.; Rea, S. L.; Moyle, S.; Kell, A.; Johnson, T. E. *Biochem. J.* **2008**, *409*, 205.
(415) Kraft, D. C.; Deocaris, C. C.; Wadhwa, R.; Rattan, S. I. *Ann. N.Y. Acad. Sci.* **2006**, *1067*, 420.
(416) Kobayashi, A.; Kang, M. I.; Watai, Y.; Tong, K. I.; Shibata, T.; Uchida, K.; Yamamoto, M. *Mol. Cell. Biol.* **2006**, *26*, 221.

CR8004857