CMLS, Cell. Mol. Life Sci. 61 (2004) 1562–1578
1420-682X/04/131562-17
DOI 10.1007/s00018-004-4130-z
© Birkhäuser Verlag, Basel, 2004

CMLS Cellular and Molecular Life Sciences

The ultimate nanoscale mincer: assembly, structure and active sites of the 20S proteasome core

W. Heinemeyer [a,*], P. C. Ramos [b] and R. J. Dohmen [c]

[a] Institute of Biochemistry, University of Stuttgart, Pfaffenwaldring 55, 70569 Stuttgart, (Germany),
e-mail: heinemeyer@po.uni-stuttgart.de
[b] Department of Chemistry and Biochemistry, University of Algarve, Campus de Gambelas, 8000-177 Faro (Portugal),
e-mail: pcramos@ualg.pt
[c] Institute for Genetics, University of Cologne, Zülpicher Strasse 47, 50674 Köln (Germany),
e-mail: j.dohmen@uni-koeln.de

Abstract. 20S proteasomes constitute the proteolytic core of large protease complexes found in all branches of life. Among these, the eukaryotic 26S proteasome ubiquitously poses as a vital final entity in regulated degradation of intracellular proteins. The composition of 20S core particles has been disclosed in detail, facilitated by groundbreaking studies on ancestral prokaryotic 20S proteasomes of low complexity and culminated in the crystal structure determination of the much more complex eukaryotic particles. This article first summarizes insights into the structural organization of the 20S core followed by characterization of its proteolytic activities, which are confined to the central cavity of the particle. In eukaryotes they reside in three different subunit types differing in their preference for cleavage sites in substrates as well as in their importance for the proteasome‘s cellular function. The second part reviews current knowledge on the biogenesis pathways of 20S core particles, which have to ensure not only the fixed subunit arrangement but also activation of proteolytic subunits in a late assembly state.

Key words. Proteasome; 20S core; active sites; proteolysis; Ntn-hydrolases; assembly; Ump1.

Introduction

The 20S proteasome constitutes the proteolytic core particle of larger protein assemblies. In eukaryotes, association with the 19S regulatory ‘cap’ complexes (also called PA700) yields 26S proteasomes, which play vital roles through energy-dependent, selective degradation of polyubiquitylated proteins. The components of the 19S moiety are responsible for recognition, unfolding, de-ubiquitylation and translocation of substrate proteins into the lumen of the core particle, where the substrate chains are finally doomed to degradation into oligopeptides. A more specialized proteasome type, absent from lower eukaryotes but found in species ranging from *Trypanosoma* to mammals, is composed of 11S activator complexes (also called PA26 or PA28) bound to the 20S core. In mammals, this proteasome complex has been implicated in antigen processing. Another recently identified complex found attached to the 20S proteasome is PA200 (in yeast encoded by the *BLM3* gene), an activator that has been implicated in DNA damage repair [1].

This article attempts, first, to give a compressed overview of 20S proteasome structure and of the characteristics of its active sites with an emphasis on their specificities and role in proteolysis. Second, we summarize our current knowledge of 20S proteasome assembly pathways and their interrelation with the maturation of its active sites. Several more specialized and detailed recent reviews that cover related aspects such as proteasome evolution [2], biochemical in vitro studies on proteasome activities [3] and the expanding field of proteasome inhibitors [4] are recommended for further information.

* Corresponding author.

Evolution of 20S proteasome complexity

Proteasomes characterized by a highly conserved overall architecture are to be found in all phylae of life [2]. They appear to be essential components of all eukaryotic cells [5]. Proteasomes are ubiquitously present in archaeons, whereas in eubacteria they seem to be restricted to *Actinomycetales*. Studies on the archaebacterium *Thermoplasma acidophilum* employing selective inhibitors of proteasome activity indicated that it is not essential for viability but required for resistance to high temperature. A genetic study on the actinomycete *Mycobacterium smegmatis* involving the generation of a mutant lacking the proteasome did not reveal a critical function of this protease under the conditions tested, suggesting that as in bacterial species lacking the proteasome, other proteases can take over essential proteolytic functions in this bacterium [6]. Proteasomes found in archaeons and eubacteria are composed usually only of two types of subunits, α and β, which in sequence are related to each other. In most bacteria, these subunits are encoded by proteasome operons. In several archaebacterial genomes; however, two genes encoding β-subunits (β1 and β2) were found [7]. A similar exception among eubacteria is the nocardiaform actinomycete *Rhodococcus* sp. where two operons each encoding a pair of α- and β-subunits (α1 and β1; α2 and β2) have been discovered [8]. Since all β-subunits contain active sites, the prokaryotic proteasomes bear 14 such sites. Another protease that is found in *Escherichia coli* and many other bacteria is the HslV protease (also called ClpQ), a remote relative of the 20S proteasome. It consists of two homo-hexameric rings built from a subunit with sequence similarity (about 20%) to proteasomal β-subunits, but it lacks α-subunits [9–12]. Therefore, proteasomal β-subunits are regarded as the earlier form, from which the inactive α-subunits evolved later [2]. As the proteasome, the HslV protease is a threonine protease [13–15]. Similar to proteasomes, HslV associates with an ATPase complex termed HslU or ClpY [16, 17]. *E. coli* HslV (Heat shock locus V) is induced by heat and involved in the turnover of abnormal proteins [9].

In contrast, eukaryotic 20S proteasomes are composed of seven distinct α- and seven distinct β-subunits. This diversification is proposed to have taken place during a short and early period of eukaroytic evolution [2], which went along with a remarkable reduction of active β-subunits from 2×7 in bacteria to 2×3 in eukaryotes. In lower eukaryotes such as the yeast *Saccharomyces cerevisiae*, the subunits of the 20S proteasome are encoded by 14 individual genes. Another level of complexity has been observed in mammals, where three additional γ-interferon (γ-IFN)-inducible genes encode variants of the three active site subunits (β1i/LMP2, β2i/MECL-1 and β5i/LMP7). Two of these genes are located within the major histocompability locus (MHC). Incorporation of these subunits results in the formation of a proteasome subtype termed the immunoproteasome, which has been implicated in the generation of certain antigenic peptides presented on MHC class I molecules (for review see [18, 19]). In *Drosophila melanogaster*, testis-specific isoforms have been found for six (α3, α4, α6, β2, β4 and β5) of the 14 subunits of the 20S proteasome, suggesting a role of a specific proteasome subtype in spermatogenesis [20]. Genome analysis of the model plant *Arabidopsis thaliana* revealed 23 proteasome genes, 13 of which encode α-subunits, and 10 of which code for β-subunits. By sequence comparison, these subunits can be assigned to the established 14 subfamilies of α- and β-subunits [21]. As for the *Drosophila* testis-specific genes, no information on the functional role of the duplicated genes in *Arabidopsis* is available to date.

Structure

20S proteasomes are defined by a characteristic architecture, a stack of four heptameric rings with two outer α-subunit rings embracing two central head-to-head oriented rings containing catalytic β-subunits. 20S proteasomes showing the simplest organization with only one type of α- and β-subunit were found in most archaebacteria and in some eubacteria (see above). The particle identified in the archaeal species *Thermoplasma acidophilum* in the late eighties served as a prototype not only to elucidate the molecular architecture of 20S proteasomes but also to clarify the nature of their proteolytic mechanism (see below). The seminal ascertainment of its molecular structure by X-ray crystallography [22] showed that the *Thermoplasma* α- and β-subunits have a common fold characterized by a sandwich of two β-sheets each consisting of five strands, surrounded by two α-helixes on each side (fig. 1). The H1 and H2 helixes mediate the interaction of α- and β-rings; H3 and H4 provide contacts between the β-rings. A unique element in the α-subunit is the H0 helix at the N-terminus, which in the precursor of the β-subunit is replaced by a prosequence that is lost during proteasome maturation.

As described in the previous section, eukaryotic proteasomes in comparison to those in prokaryotes are characterized by an increased subunit complexity, in which each ring is composed of seven distinct subunits. The available crystal structures of the yeast [15] (fig. 2) and more recently of the bovine 20S proteasome [23] not only clarified the fixed arrangement of the subunits in the complex, but also confirmed that this topology is conserved from yeast to mammals. Although the general fold seen in the *Thermoplasma* subunits is maintained in all eukaryotic core particle components, some additional structural features acquired by eukaryotic proteasome subunits such as C-terminal extensions and internal loops are

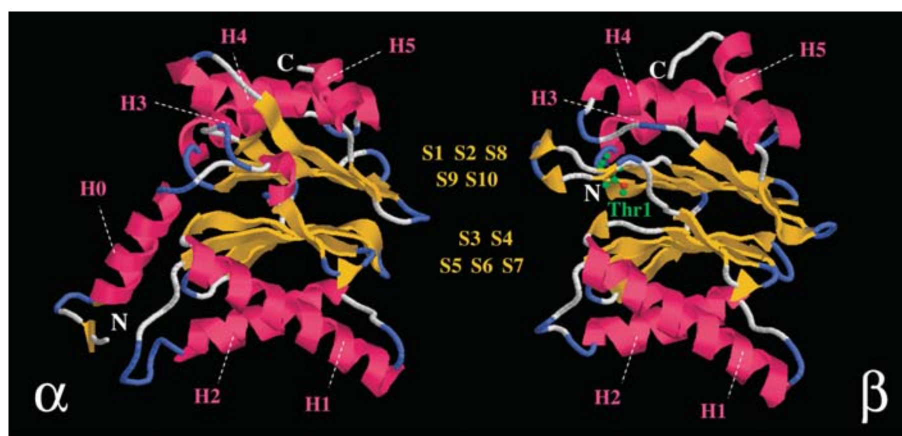

Figure 1. Ribbon diagram of the α-subunit and the β-subunit of the *Thermoplasma acidophilum* proteasome. The two subunits are shown in similar orientation showing the common $\alpha\beta\beta\alpha$ sandwich fold with two β-sheets (yellow, formed by five β-strands each, labelled S1–S10) stacked between two layers of α-helices (purple, labeled H1–H5). The major difference resides in the N-terminal H0 helix of the α-subunit, which is missing in the β-subunit. The N-terminus of the β-subunit is formed by the catalytic threonine (shown in green in ball- and stick-representation with the hydroxyl oxygen highlighted in red), which is followed by the S1 β-strand.

likely to determine the fixed subunit arrangement. Differences between the yeast and bovine 20S particle concern especially these extensions and seem to be related to the ability of the mammalian particle to accommodate either the constitutive or the inducible subunit type in a given location [23].

The overall structure of the 20S core, as already visualized by electron microscopy, resembles a barrel with dimensions of 15 nm in length and 11 nm in diameter. The molecular resolution allows to distinguish three inner cavities with a diameter of approximately 5 nm. A central proteolytic chamber is formed by two face to face-oriented β-rings and is separated by approximately 3-nm-wide β-annuli from two antechambers formed by the other side of the β-ring and an α-ring (fig. 2C, D). In eukaryotes, access to the antechambers is possible only after reorganization of the N-terminal H0 helixes of the α-type subunits, which in the crystal structure conformation of the yeast and bovine proteasome were found to form a seal by interdigitating side chain interactions (fig. 2B). This structure corresponds to the 'latent' state of the 20S proteasome obtained by certain purification procedures [3]. Crucial for the reorganization, i.e. activation, is the N-terminus of the α3-subunit, since deletion of this stretch yields crystal structures with an open channel, where all the α-subunit N-termini point upward and surround a pore wide enough to let a peptide chain or even a loop of an extended polypeptide pass through [24]. Association of the 20S core particle with activator complexes is thought to trigger channel opening. This gating mechanism was structurally proven in a crystallized complex of the 11S activator from *Trypanosoma brucei* with the yeast core particle [25]. In analogy, binding of the 19S cap is likely to induce gating as well [24]. The 19S base ATPase Rpt2 seems to be a key element in this mechanism, as deduced from studies using combinations of open channel mutants and *rpt2* mutants [26]. Most likely, however, channel opening by the 11S and 19S regulators is achieved by different mechanisms, implicated by their different symmetry (sevenfold versus sixfold) and the lack of any sequence similarities between their constituents.

Active sites

The catalytic mechanism of the proteasome

Proteolytically, active β-type subunits in proteasomes are members of an enzyme family designated as Ntn-hydrolases. Common to this family is the ability to hydrolyze amide bonds, but only proteasomal β-subunits cleave peptide bonds. All Ntn-hydrolases are made as inactive precursors and are converted to an active form by an autocatalytic internal cleavage, which exposes a threonine, serine or cysteine residue as the new N-terminus. In the matured protein this amino acid acts as 'single residue active site' with its hydroxyl or sulfhydryl side chain providing the nucleophile and the free amino group acting as the general base for the hydrolysis reaction. In the proteasome, a threonine (Thr1) invariably serves as the N-terminal nucleophile. The precursors of the proteasomal β-subunits that gain proteolytic activity by autolytic processing and exposure of Thr1 bear propeptides of different length and unrelated sequence, but with a conserved glycine (Gly-1) preceding Thr1. As in all Ntn-hydrolases, this autolysis reaction requires the same residues that form the mature active site and thus relies on a similar mechanism. In the autolysis reaction, where no N-terminal amino group is available as proton acceptor, a water molecule is predicted to mediate the nucleophilic addition of the Thr1-Oγ to the carbonyl atom of Gly-1 in

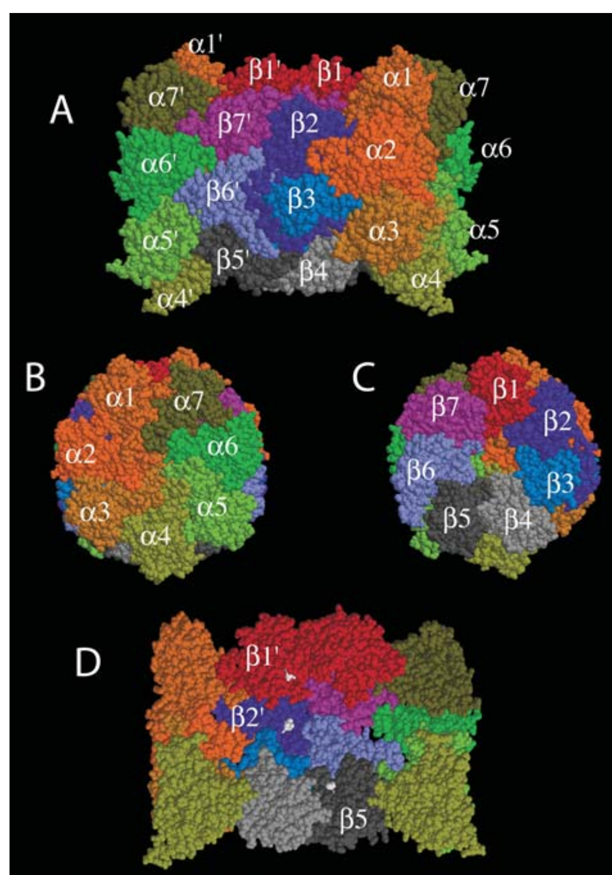

Figure 2. Different views of the *Saccharomyces cerevisiae* 20S proteasome structure in space-filling mode. (*A*) Slightly tilt side view showing the overall subunit arrangement, which results in a C2 symmetry of the particle. (*B*) View onto an α-ring. A central pore of this ring as entrance for substrate polypeptide chains is missing in this crystal structure conformation due to tight interactions of the seven α-subunit N-termini. These interactions are abrogated after binding of activator complexes. (*C*) View onto the β-ring of a half proteasome cut between the two β-rings. The β-annulus is visible as central pore. (*D*) Side view into the lumen of the 20S particle after removal of three subunits per ring. The three types of active β-subunits are labelled, and the catalytic Thr1 residues in the active site pockets are highlighted in white.

the preceding peptide bond [27]. Initiation of the proteolysis reaction carried out by the mature subunit may similarly involve a water molecule that helps in proton shuttling to the N-terminal amino group. Some highly conserved residues, Asp17 (Glu17 in the *Thermoplasma* β-subunit), Lys33, Ser129, Asp166 and Ser169, surround Thr1 in proteasomal Ntn-proteases (fig. 3). Their essential function in autolytic and proteolytic catalysis lies in constituting a charge relay system that is required to polarize atoms in the participating substrate as well as in the enzyme itself, for example to enhance the nucleophilic character of the Thr1 hydroxyl group by delocalizing its proton. A direct role of the Lys33 ε-amino group as proton acceptor, as initially proposed [22], is now commonly excluded because of its positive charge at neutral pH.

Active sites in the eukaryotic proteasome

The nature of the proteasome's catalytic mechanism was unraveled both by crystallography and by mutagenesis of the ancestral 20S proteasome from *Thermoplasma*. In the crystal structure, a co-crystallized peptide aldehyde inhibitor of the proteasome made contact with its aldehyde group to the Thr1 hydroxyl group of the β-subunit [22]. In parallel, extensive site-directed mutagenesis studies with the *Thermoplasma* β-subunit identified Thr1 to be essential for autolytic and proteolytic function [28]. Using baker's yeast as model organism, the same two approaches led to the identification of active subunits in the eukaryotic core particle and verified predictions derived from available yeast and mammalian β-type subunit primary sequences. According to these sequence data, only members from three out of the seven branches of β-type subunits were assumed to be proteolytically active, because they contain all the above-mentioned conserved residues besides an N-terminal propeptide in the precursor that is missing in the matured purified protein. Indeed, the three yeast candidate subunits β1/Pre3, β2/Pup1 and β5/Pre2 were found to bind the aldehyde inhibitor in the crystal structure [15], and concurrently, mutagenesis of Thr1 to alanine in the same three subunits each individually led to loss of one of the long known 'classical' proteasomal activities against small chromogenic or fluorogenic peptide substrates. Thus, it was possible to assign the post-glutamyl splitting (originally termed peptidyl-glutamyl peptide hydrolyzing, PGPH) activity to β1/Pre3, the trypsin-like activity to β2/Pup1 and the chymotrypsin-like activity to β5/Pre2 [29, 30], which nicely reconciled early biochemical studies that had ascribed these different peptidase activities to independent catalytic sites and had led to the concept of the proteasome as a multicatalytic protease (for a recent review on this biochemical work written by two of the main contributors see [3]).

Some disagreement with the restriction of only three catalytic subunits of the Ntn-hydrolase type in all eukaryotic 20S core particles was put forward and is still blazing up. At first, the early extensive biochemical studies argued for more proteasomal peptidase activities because the rather complex inhibitor response patterns for the numerous known peptide substrates was incompatible with only three catalytic centers, and indeed, some radiolabelled inhibitory compounds bound to subunits other than β1, β2 and β5 [3]. Until now, only one of the putative additional peptidase activities with a preference for peptide bonds C-terminal to branched-chain amino acids could unambiguously be attributed to the β1/Pre3 active center in the yeast system [31]. On the other hand, additional proteolytic centers using a different catalytic mechanism were postulated based on structural observations. One such non-conventional active site has been ascribed to the β-annulus [15]. This structure is characterized by a clus-

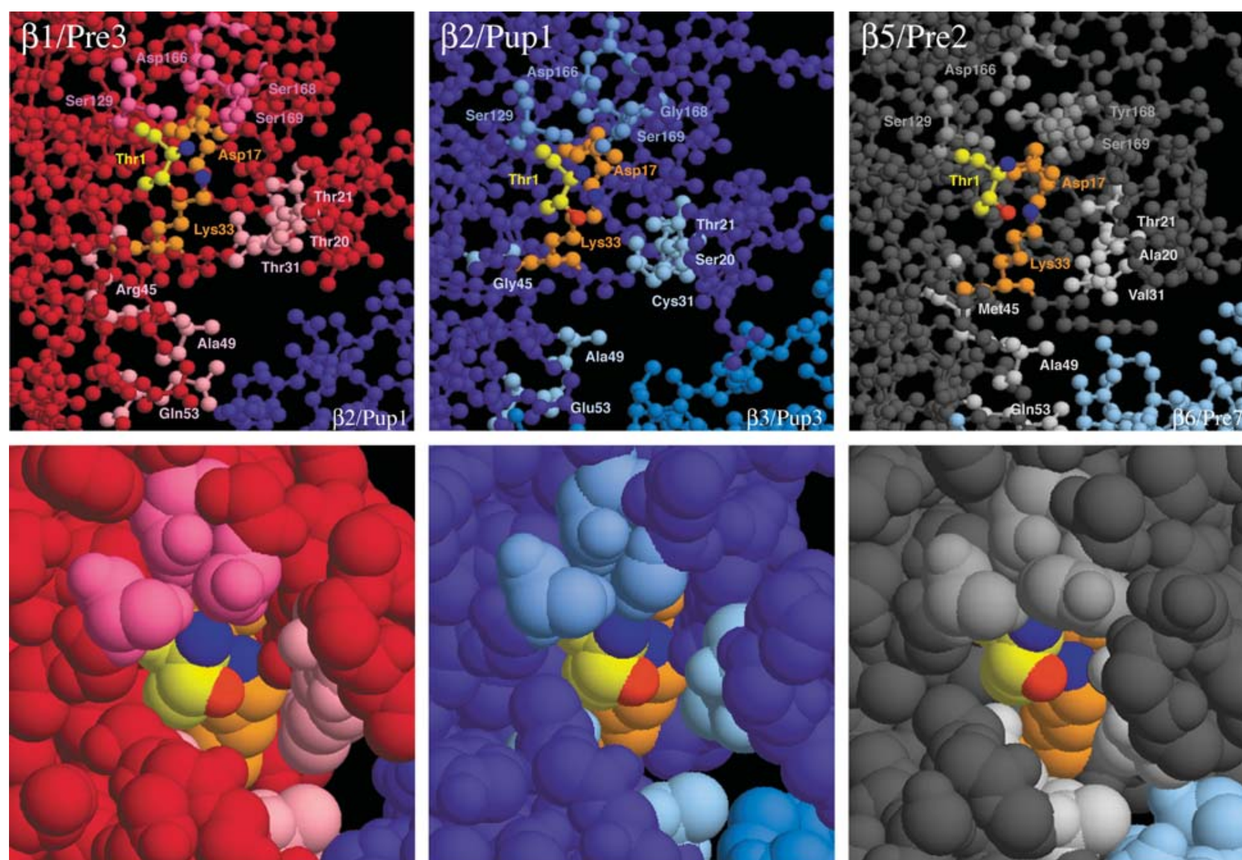

Figure 3. The structures of the three types of active sites in the *Saccharomyces cerevisiae* 20S proteasome. Upper row, ball-and-stick representations; lower row, space-filling representations of the same parts visible above. The views are from similar perspectives to show the similarities among the active sites. Coloring of the subunits is according to figure 2, except for those residues which are labelled in the upper row and contribute to the formation and catalytic function of the active site pocket: The hydroxyl oxygen of Thr1 is red and the nitrogen of its free amino group is dark blue; other parts are yellow. Asp17 and Lys33 are colored orange except the ε-amino group of Lys33, which is also dark blue. The conserved residues Ser129, Asp166 and Ser169 (as well as the variable residue 168) are shown in slightly brighter tone of the subunit color. Together with Lys33 and Asp17, these conserved residues contribute to the charge relay system surrounding Thr1. The variable residues at positions 20, 21, 31, 45, 49 and 53 are each colored in a still brighter tone and form the surface of the substrate binding pocket.

ter of negatively charged residues and was implicated in the generation of the N-termini of the inactive β6 and β7 subunits. These subunits possess propeptides that undergo intermediate processing, resulting in short propeptide remnants in the matured particle, which with their N-termini meet at a common point at the β-annulus. However, the hypothesis that processing of the β6 and β7 precursor also occurs at this very point did not prove true, since with the aid of yeast active site mutants the N-terminal shortening of these precursors of inactive subunits could clearly be shown to depend on the activity of the known proteasomal Ntn-hydrolases [32] (see below). The conformation and orientation of the two propeptide remnants seen in the crystal structure thus must be reached only after their trimming by neighboring active sites. The recent report on the bovine 20S proteasome structure revived speculations about an unusual, additional protease site, which the authors propose to rely on the N-terminal threonine (Thr-8) of β7 as nucleophile and a different charge relay system than that characterized for the three established Ntn-protease subunits [23]. This purely hypothetic site would thus again lie close to the β-annulus and would have a peptide binding groove that even extends into the antechamber. Until now, biochemical proof for such active site is lacking. Remarkably, in the yeast proteasome there exists no corresponding structure, and mutagenesis of the N-terminal Thr-8 of the yeast β7/Pre4 subunit excluded at least its participation in any of the classical peptidase activities and had no phenotypic consequences [29]. In summary, a verification of this or any other new proteolytic site is still lacking and would come as a surprise.

The distinguishing specificities of the three known active sites resulting in a preference of cleavage after acidic, basic or large hydrophobic residues at the P1 position in artificial peptide substrates must correlate with the charac-

ters of their S1 pockets, where this substrate residue is bound and cut at its C-terminal side. Structural inspection of the S1 pockets in the yeast proteasome explains these specificities [33] (fig. 3). In the base of the β1/Pre3 pocket, Arg45 can balance the charge of acidic P1 residues, favoring the PGPH activity of β1. However, a bicarbonate ion was found to be captured in the β1/Pre3 pocket, for example in the case of the bound calpain inhibitor I [27], suited to neutralize the positive charge of Arg45 in order to accommodate the hydrophobic norleucinal side chain. This nicely fits with the observation that one of the 'non-classical' peptidase activities preferring amino acids with branched side chains depends on a functional yeast β1/Pre3 subunit [31]. The trypsin-like activity of β2/Pup1 matches with the presence of Glu53 at the bottom of its S1 pocket and an acidic side wall contributed by the β3/Pup3 neighbor subunit. The pocket of β5/Pre2 has an apolar character (Met45 forms the base), explaining its chymotrypsin-like peptidase activity.

This classification of specificities based only on small peptide substrates, however, is oversimplified, since the S1 pocket alone is unlikely to govern the binding probability of a given polypeptide stretch in a substrate to a particular active subunit. Regions adjacent to the cleavage site at P1 must contribute to the selection, which was already apparent for example in the binding mode of the calpain inhibitor I to the yeast proteasome active sites. Here, the residue in P3 makes several contacts with the surface of the respective subunit. A multitude of studies aimed at characterizing preferred cleavage motifs for the proteasome in longer oligopeptides and small proteins (frequently denatured by artificial modifications) clearly established an impact of residues preceding and following a given cleavage site, especially those in P3 and P4. A statistical calculation combining data from several such studies figured out more detailed cleavage motifs, which distributed into 10 classes according to groups of cleaved P1 residues and thus could not be assigned to individual subunits [34]. In parallel, a comprehensive analysis of proteasomal digestion products of a natural protein, enolase-1, took advantage of the power of yeast genetics in order to address true subunit preferences [35]. Wild-type and a set of mutant yeast 20S proteasomes with different inactivated proteolytic sites were compared with regard to the cleavages made in enolase-1, thus establishing both cleavage motifs which are generally preferred by any subunit as well as those which are specific for individual subunits. The detailed statistical evaluation disclosed several positions, ranging from P5 up to P′5 around the actual cleavages, where certain residues or residue characteristics dominate and thus contribute crucially to the subunit-dependent or general affinity (for example proline in P4 and small residues in P′1 are generally favored). But notably, the nature of the P1 residue still is a major determinant, as revealed by the fact that inactivation of β1/Pre3 abolishes almost any cut after acidic residues in enolase-1, and loss of β2/Pup1 activity is correlated with lack of any trypsin-like proteolytic activity. On the other hand, both subunits were suited to cleave as well after several types of uncharged, hydrophobic residues [35].

Interestingly, inactivation of one or even two types of active sites led only to a slight increase in the length distribution of the digestion products from enolase-1 [35], which ranges between 3 and more than 20 amino acids with a mean length of around 8. This argues for a size exclusion affecting the exit of products from the proteasome lumen, meaning that, independent of the number of active sites involved in fragment generation, the chance to diffuse away increases sharply below a given fragment length.

The model substrate enolase-1 was also applied to specify preferred cleavage motifs distinguishing the mammalian proteasome species either harboring the constitutive active β-type subunits β1, β2 and β5 or the γ-interferon inducible set β1i/LMP2, β2i/MECL1 and β5i/LMP7 [36]. Again, this supplemented a huge variety of earlier studies carried out to correlate changes of active site specificities with the ability of the immunoproteasome to produce 'better' MHC class I ligands than the normal proteasome. The qualitative and this time even quantitative analysis of enolase-1 products produced by both proteasome forms clearly confirmed that the replacement of β1 (delta) by the inducible β1i/LMP2 leads to a reduction of cleavages after acidic residues and to more cuts after hydrophobic residues, consistent with the exchange of Arg45 in delta against Met45 in LMP2 [36]. This property of immunoproteasomes seems to be an adaptation to its role in the immune system, the generation of antigenic peptides binding to MHC class I molecules (see below). Because the prediction of potential MHC class I ligands derived from any protein is of great immunological interest, promising efforts are underway to train computer-based neural networks for such predictions on the basis of existing digestion data and MHC class I ligand libraries [37].

Another novel powerful approach to specify motifs in substrates that direct them to individual proteasomal active sites applied positional scanning libraries of vinyl sulfone inhibitors, and measured subunit modification by competition with a radiolabelled, general inhibitor [38]. Although this strategy is limited to residues preceding the P1 position (P2-P4), it allows a systematic and exhaustive analysis by including almost all possible variations in each of these positions and yielded global specificity profiles for individual catalytic subunits of bulk mammalian proteasome populations. Interestingly, this study did not reveal significant differences in P2-P4 specificity profiles for the γ-interferon inducible subunits versus the profiles found for their non-inducible counterparts. Therefore, the different cleavage behavior of normal pro-

teasomes and inducible proteasomes was proposed be a consequence primarily of changes in activities and not of specificities [38].

Cooperativity, redundancy and hierarchy of active sites

A number of genetic and kinetic findings point to diverse interactions between the active sites of the 20S proteasome (summarized in [3]). They imply a conformational flexibility that allows not only positive cooperativity between pairs of active subunits of the same type across the two β-rings but even between different catalytic subunits spatially separated by inactive β-ring members and culminated in an appealing model of a 'bite-and-chew' mechanism [39]. This model proposed a mutual allosteric activation and inhibition of active centers during substrate degradation and was based on the inhibition of the ('biting') chymotrypsin-like site by substrates of the PGPH component and the activation of the ('chewing') PGPH site by substrates of the chymotrypsin-like activity. Meanwhile, a variety of thorough investigations clearly vitiate this model and unanimously favor the existence of one or several non-catalytic sites to which hydrophobic peptides (including the tri- and tetrapeptides commonly used to assay proteasomal activities) can bind and regulate the activity of the catalytic sites [40–42]. The nature and location of such site(s) still remain obscure. One of these studies, however, provides evidence for stimulation of peptidase activities via the gating mechanism residing in the α-ring, which might be coupled to putative non-catalytic, peptide-binding sites [42]. In summary, the complex field addressing possible interactions between active sites currently points to a functional independence of the individual active sites, but certainly not all of the existing data supporting cooperativity will be explained by effects emanating from putative non-catalytic sites. One may be curious whether such sites can be identified and, if so, whether a role under physiological conditions can be approached for them.

As already mentioned, several yeast mutants are available bearing mutationally inactivated variants of one or even two types of proteasomal active site subunits [29, 30, 43]. Thus, there exists a considerable redundancy among the three catalytic centers, in that one type of active center can suffice for yeast cell survival, β1/Pre3 being the exception. Besides arguing against an essential interdependence of active site function during in vivo protein degradation this fact leads to the question of hierarchy among the active sites. This has been addressed in a genetic study employing specifically engineered yeast active site mutants [44]. Effects of individual single and double active site knockouts were compared with regard to growth phenotypes and in vivo degradation rates of test substrates. In this analysis, overlapping effects resulting from proteasome assembly perturbations caused by the inability of mutant subunit precursors to cleave off their propeptides were ruled out by deletion of the propeptide-encoding gene regions and, in cases of essential propeptide functions, by expression of the uncoupled propeptides in trans. A hierarchy could be established with a clear dominance of the β5/Pre2 proteolytic function over the other two, which in turn show a graduation with β2/Pup1 being more important than β1/Pre3. The latter is consistent with the finding that β5/β1 and β2/β1 double mutants are viable, but β5/β2 double mutants are not [29, 44]. Some support for a generalization of this hierarchy comes from numerous studies in the mammalian system. All of the natural proteasome inhibitors found preferentially bind to the β5 subunit (for review see [4]). They all compromise proteasome function substantially, which makes them widely accepted tools to relate the turnover or regulation of a given protein to the proteasomal degradation machinery. In the meantime, some inhibitors have been designed that selectively act on the trypsin-like activity of β2 or the post-acidic activity of β1. Assays to evaluate cell proliferation and protein stability in the presence of an α',β'-epoxyketone derivative directed against the β1 catalytic center [41] revealed no effects and clearly support a dispensable function of this activity also in higher eukaryotes. Therefore, the advantage to have additional catalytic sites in the 20S proteasome besides the dominant β5 site waits to be elucidated by further detailed kinetic studies on protein degradation using mutant or selectively inhibited proteasome species.

Assembly of 20S proteasomes

Thanks to the outstanding studies on the structure and function of the proteasomes from *Thermoplasma* and yeast, and more recently from cow, we have detailed knowledge of the conserved architecture of this protease (see above). What do we know about how this complex structure is generated from its components? Again, much of the available information stems from studies on simpler models. The relatively low complexity with respect to subunit composition of the 20S proteasomes from archaeons and eubacteria has allowed recapitulation of their assembly both in vivo and in vitro, taking advantage of *E. coli* expression systems. Since *E. coli* is lacking a proteasome, it proved to be an ideal host to produce heterologous proteasomes or their subunits in large quantities without interfering endogenous activity (see below).

Assembly and maturation of archaebacterial proteasomes

Co-expression of proteasomal α- and β-subunits of the archaebacterium *Thermoplasma* in *E. coli* yielded mature

and active 20S proteasomes (fig. 4A). α-subunits expressed in the absence of β-subunits assembled mainly into pairs of heptameric rings. Only a minor fraction of single heptameric rings could be detected [45]. Purified α-rings had no proteolytic activity. The N-terminal sequences of the α-subunits have no equivalent in the otherwise homologous β-subunits. Deletion of the first 34 residues abrogated the ability of α-subunits to form heptameric rings. Similarly, a mutation of Glu25 to Pro precluded ring formation, indicating that an α-helix close to the N-terminus of α-subunits is required for subunit assembly [45]. β-subunits expressed in the absence of α-subunits, in contrast, remained monomeric. They remained proteolytically inactive, and no processing of the propeptides occurred. Assembly of 20S proteasomes could also be recapitulated in vitro by mixing α- and β-subunits subjected to low pH treatment to disassemble aggregates followed by dialysis at neutral pH. In such experiments, 15% of the β-subunits could be recovered as part of assembled proteasomes. Only one-third of the assembled β-subunits were processed, indicating that processing is not a prerequisite for assembly [46]. These authors observed, in addition, that the fraction of processed assembled β-subunits does not increase with time, suggesting that β-subunit processing must occur before a subunit reaches its final fold within the structure of the proteasome. This conclusion is corroborated by the observation that upon mixing of active β-subunits with inactive β-subunits (e.g., the Lys33Ala mutant) processing of the inactive subunit occurs. Such processing in trans can only be envisioned in a state where the subunits have not yielded their final fold within the complex or assuming a high conformational flexibility. The presence of the β-subunits' propeptide, which is comparably short in *Thermoplasma* (eight residues), is not required for assembly of proteasomes [46, 47].

Similar to the above, in vitro assembly was observed with proteasomal α- and β-subunits of two other archaebacteria, *Methanosarcina thermophila* and *Methanococcus jannaschii*, indicating that no additional factors are essential for the assembly of these proteasomes [48, 49]. In both cases the formation of ringlike structures was detected when α-subunits were expressed alone in *E. coli*, whereas β-subunits did not self-assemble into distinct complexes and remained inactive even when expressed without the propeptides. Altogether, these studies suggested that the formation of higher-order intermediates during the assembly of archaebacterial proteasomes is driven by assembly of α-subunits. As of yet, however, in no case have rings of α-subunits been shown to be intermediates in the in vivo assembly of archaebacterial proteasomes. Whether α-rings really are assembly intermediates in vivo depends on the kinetics of the formation of the α/α-homodimer versus that of the α/β-heterodimer. It is indeed possible that the assembly of proteasomes from α/β-heterodimers is much faster than the formation of α-rings, in which case the assembly process of archaebacterial proteasomes would be similar to those in eubacteria (see below).

Assembly of eubacterial proteasomes

The first eubacterial proteasome to be studied was that of the nocardioform actinomycete *Rhodococcus* sp. Proteasomes purified from this bacterium are composed of two different α- and two different β-subunits [8]. These subunits are encoded by two related operons. Since the two operons differ markedly in G+C content, it was concluded that one of them was likely obtained by horizontal gene transfer rather than by gene duplication [50]. As for the *Thermoplasma* proteasome, active *Rhodococcus* proteasome could be reconstituted using an *E. coli* expression system [51]. All combinations of subunits ($\alpha1\beta1$, $\alpha1\beta2$, $\alpha2\beta1$ and $\alpha1\beta2$) yielded active proteasome either in vivo in *E. coli* or in vitro. In contrast to the *Thermoplasma* system, no formation of ring structures was observed with individually expressed α-subunits. Only when α- and β-subunits were mixed formation of ring structures was to be observed. In vitro the assembly proceeded via a half-proteasome intermediate apparently consisting of seven α- and seven unprocessed β-subunits. Processing of the latter occurred only when two such half-proteasomes joined to form the holoproteasome [51]. The formation of a short-lived intermediate termed 'preholoproteasome' was inferred from in vitro assembly studies with an inactive mutant version of the β-subunit (βK33A) [52]. As judged by electron microscopy, this processing-incompetent variant was able to form stable structures similar to mature 20S proteasomes. Therefore, the inability to remove the propeptides of *Rhodococcus* β-subunits does not interfere with the formation of stable holoproteasomes, but in this case both the central cavity as well as the two antechambers were nearly filled by the 14 propeptides. These propeptides together constitute almost 100 kDa of polypeptide (14×7 kDa) which, according to calculations, would not fit into the approximately 84 nm^3 central cavity that cannot hold more than ~ 70 kDa of folded protein [52]. After activation of the catalytic sites by autocatalytic cleavage between Gly-1 and Thr1, the propeptides are processively degraded down to small peptides that can be released from the structure [52].

20S proteasomes in other actinomycete genera such as *Mycobacterium, Streptomyces* and *Frankia* are composed of only one type of α- and one type of β-subunit, which are encoded, respectively, by the prcA and prcB genes that are organized in operons [6, 53, 54]. In these operons, the prcB and prcA genes are preceded by short conserved open reading frames (ORFs) (termed prcS or ORF7) encoding short proteins ranging from 63 to 72 amino acid residues in length. The presence of these conserved ORFs in proteasome operons might point to a role in protea-

some biogenesis. The function of these small proteins, however, remains enigmatic, as attempts to detect these proteins as constituents of the respective proteasomes were unsuccessful [54]. Mixing of the two types of *Frankia* proteasomal subunits expressed in *E. coli* resulted in in vitro assembly, followed by processing of the 52-residue propeptide of the β-subunits and thereby the formation of proteolytically active 20S proteasomes. As observed with the *Rhodococcus* subunits, no formation of distinct complexes was to be detected for individually incubated *Frankia* α- or β-subunits. In support of the observed similarity of the assembly processes underlying the formation of *Rhodococcus* and *Frankia* proteasomes, it could be shown that α-subunits of the former and β-subunits of the latter, and vice versa, assembled into proteolytically active chimeric proteasomes [54].

In summary, the studied cases of eubacterial proteasomes have shown that neither α-subunits nor β-subunits alone are able to form ringlike structures. Only when both are present are assembly intermediates composed of seven-membered rings detectable. The only intermediates that are stable enough to be detected when wild-type subunits are mixed or co-expressed are half-proteasome precursor complexes (fig. 4B). Zühl et al. ([51]) concluded that formation of the *Rhodococcus* proteasome is likely to involve the assembly of half-proteasomes from α/β-heterodimers. Half-proteasomes are proteolytically inactive even when they are formed with β-subunits lacking the propeptide. The presence of these propeptides is not essential for the formation of active *Rhodococcus* proteasomes, but their absence ($\beta\Delta$pro) strongly reduced the efficiency of assembly. Supply of the propeptide *in trans* largely restored the formation of active proteasomes from α1 and β1Δpro subunits [51]. The addition of the propeptide in trans, interestingly, accelerated the formation of holoproteasomes from half-proteasome precursors to the extent that the latter where hardly detectable. This result demonstrated that the propeptide of *Rhodococcus* β1 promotes assembly of the pre-holoproteasome from two precursor complexes. The processing of β-subunits appears to be a slow and rate-limiting step in the assembly of holoproteasome from its precursors [51].

Biogenesis of 20S proteasomes in eukaryotes

'Prosomes' were described as 19S ribonucleoprotein (RNP) particles that were thought to be involved in regulation of messenger RNA (mRNA) translation [55]. Subsequently it was shown that prosomes and the 'multicatalytic protease complex' identified by Wilk and Orlowski ([56]) and characterized by Hough et al. ([57]) are identical [58, 59]. The term 'proteasome' was proposed as a unifying name [59]. Structural analyses established that the eukaryotic 20S proteasome does not contain RNA and is very similar in its overall organization to the archaebacterial 'urproteasome' [22, 33]. As described above, however, eukaryotic proteasomes are characterized by a more complex subunit composition when compared to their bacterial counterparts. They are composed of seven different α- and seven different β-subunits, all of which occupy defined positions within the 20S particle. Only five of the latter are expressed with N-terminal propeptides that are cleaved off upon their maturation, and only three of them yield catalytic sites. Owing to the increased complexity, the assembly of eukaryotic proteasomes is by far more complicated than of those in prokaryotes, as it has to integrate a multitude of interactions between the individual subunits.

Not much is known about the early steps in the assembly of subunits in eukaryotes. Similar to the subunits of the archaebacterial proteasomes (see above), the human subunit α7/C8 when expressed in *E. coli* has been shown to spontaneously form double ringlike structures [60]. The two neighboring subunits α6/HsPros30 and α1/HsPros 27, in contrast, were unable to form ringlike structures when expressed by themselves. They were, however, incorporated in such assemblies when co-expressed with α7/HsC8. These assemblies were characterized by a high variation of subunit positioning. The latter observation suggested that at least not all α-subunits contain the information for their correct positioning within a ring of subunits but instead probably require additional guidance through their interaction with β-subunits [61]. This conclusion argues against a model involving preassembled α-rings as early intermediates in eukaryotic proteasome assembly, unless one considers subsequent β-subunit-driven replacements of α-subunits in order to yield the final correct positioning of α-subunits. Nonetheless, the propensity of α-subunits to assemble into ring structures is corroborated by studies on α5 and α6 subunits of *Trypanosoma brucei* expressed in *E. coli* [62, 63]. In these experiments, the α5 subunit yielded complexes ranging from 190 to 800 kDa. Inspection of the latter by electron microscopy revealed that they represent cylindrical particles apparently formed by up to four stacked heptameric rings. The significance of this finding with respect to natural proteasome assembly is unclear, as it is difficult to envision the nature of the underlying subunit interactions considering the asymmetric nature of the surfaces of a single ring of α-subunits. Where would be the beginning and where the end of such a stack of rings if both sides are capable of binding to the next?

*Drosophil*a α-subunit α2/DM25 can assemble into mouse proteasomes by replacing the corresponding subunit MC3. The ability to assemble into mouse proteasomes is lost when an N-terminal segment is deleted [64]. These findings suggest that the role of the N-terminal α-helix of α-type subunits is preserved between archaeons (see above) and eukaryotes. As for the former, it

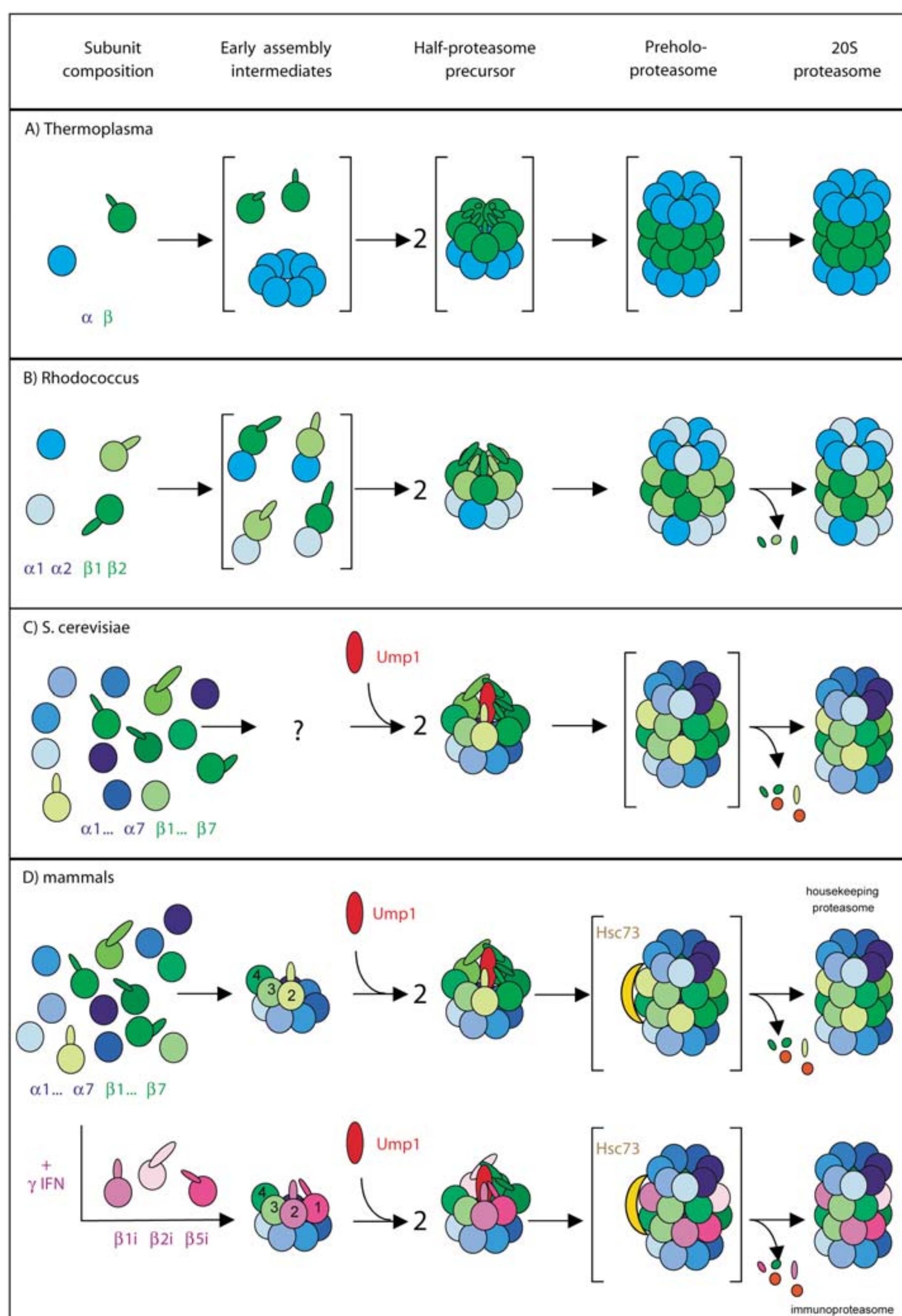

Figure 4. Models of the 20S proteasome biogenesis in different organisms. The models are represented in five stages: free subunits, early assembly intermediates, half-proteasome precursors, preholoproteasome and the mature 20S proteasome. The intermediates in brackets are too unstable to be detected in vivo. Colors: α-subunits, various shades of blue; β-subunits, shades of green; β-subunits induced by γ-interferon (γ-IFN), shades of purple; factor underpinning maturation of the proteasome Ump1, red; heat shock protein Hsc73, yellow. The β-subunits' propeptides are represented as extensions. (*A*) In the archaeon *Thermoplasma acidophilum*, proteasomes are composed of only one type of α-subunit and one type of β-subunit. The former are capable of forming ring structures in vitro. (*B*) The actinomycete *Rhodococcus* sp. 20S proteasome is made from two distinct α- and two different β-subunits, and probably assembles via α/β-dimer intermediates. (*C*) The level of subunit complexity of the eukaryote 20S proteasome reached the maximum with seven different α- and seven distinct β-subunits. The early steps in the assembly of the *Saccharomyces cerevisiae* 20S proteasome are still unrevealed. The first intermediate to be detected corresponds to a half-proteasome that is composed of one α-ring, and a ring containing unprocessed β-subunits, plus Ump1. (*D*) In mammals, two distinct types of proteasomes, the housekeeping and the immunoproteasomes, differ in their three active subunits. Those found in the immunoproteasomes (β1i, β2i and β5i) are induced by γ-IFN. An early assembly intermediate of the housekeeping proteasome is composed of an α-ring and subunits β2, β3 and β4. Upon γ-IFN induction, besides β3 and β4, the immunosubunits β1i and β2i are found in the corresponding early assembly intermediate of the immunoproteasome. Completion of the half-proteasome precursor is followed by dimerization, yielding a preholoproteasome. Hsc73 was detected in preparations of these intermediates. As shown for the yeast proteasome, preholoproteasomes mature by processing of β-subunits and subsequent degradation of Ump1.

is unclear whether eukaryotic proteasomes assembly proceeds via rings of α-subunits as an early intermediate, or whether assembly is initiated by α/β-interactions. It is interesting to note that of the fourteen 20S proteasome subunits in the yeast *S. cerevisiae*, the only non-essential subunit is an α-subunit, α3/Pre9/Y13 [65]. In the *pre9*-Δ mutants this subunit is apparently replaced by α4/Pre6 which is therefore present twice per α-ring [Velichutina, I., Connerly, P. L. et al., EMBO J.; 23, 500–510].

Putative early intermediates in the proteasome assembly pathway, such as dimers or ring structures, appear to be either very short-lived or inhomogeneous in vivo as they have not been identified and characterized as of yet. The analysis of mammalian and yeast proteasome assembly revealed the occurrence of distinct and already more complex intermediates [66–70]. One of these intermediates appears to be a half-proteasome precursor complex composed of one α-ring and a ring composed of unprocessed β-subunits as well as proteasome maturation factor Ump1 (fig. 4C, D, and E). Biochemical analyses of these proteolytically inactive complexes revealed that they sediment at 13–15S and are 300–350 kDa by gel filtration. The discovery that certain antibodies against α7 and β1i specifically immunoprecipitated proteasomal precursor complexes but failed to bring down mature proteasomes enabled a detailed analysis of the subunit composition of mouse proteasome assembly intermediates [68]. These studies revealed that on the way to formation of the half-proteasome precursor complexes, another distinct intermediate is stable enough to be detected. This incomplete precursor complex is composed of all seven α-subunits and subunits β2, β3 and β4 (fig. 4D). These data are inconsistent with a model that mammalian half-proteasomes are assembled from seven individual α/β-dimers, which would be similar to what has been suggested for the assembly of *Rhodococcus* proteasomes (see above). Interestingly, the three β-subunits detected in the mammalian proteasome assembly intermediate are direct neighbors within the same β-ring of the fully assembled 20S proteasome. A long C-terminal extension of β2 wraps around β3 and thus may contribute to the stability of the assembly intermediate. Across the two β-rings within the 20S structure, the only contact is between the two β4 subunits. Because the dimerization of half-proteasome precursor complexes is probably mediated by multiple interactions between the two β-rings, the early intermediates containing only β2, β3 and β4 are probably unable to dimerize and therefore may be somewhat more long-lived structures. Addition of some if not all the remaining β-subunits (β1, β5, β6 and β7) that complete the assembly of half-proteasome precursor complexes may then be required to enable dimerization of precursor complexes into short-lived processing competent preholoproteasomes. Interestingly, the most prominent connection between the two β-rings is mediated by subunit β7. Its long C-terminal extension inserts into a channel established between β1- and β2-subunits in the opposing ring. This interaction appears to be important for efficient dimerization, as a deletion of the β7 C-terminal extension impairs this process [Ramos, P. C., Marques, A. et al., J. Biol. Chem. 279, 14323–14330].

One interesting study described the isolation of a putative human preholoproteasome [71]. This complex was reported to sediment at 16S, to have a molecular weight of around 650 kDa and was found to be in association with hsc73. Processing of subunit $\beta 1/\delta$ or β1i/LMP2 was reported to occur in these '16 S complexes' in vitro. However no concomitant appearance of proteolytic activity was detected, leading to the conclusion that additional factors are required for activation [71]. As these studies used a stably transfected human B-cell line T2 expressing β1i/LMP2 but lacking β5i/LMP7, it remains to be established whether these complexes represent natural processing intermediates that are stable enough to be detectable in other cell types, and whether they contain all the subunits found in the 20S proteasome.

According to pulse chase experiments that followed the fate of proteasomal subunits in mouse cells, depending on the cell line used, the formation of active proteasomes containing processed β-subunits is completed after 2 h [67] or takes several hours [66, 68]. The maturation of proteasomes in yeast cells is by and large completed after 30–40 min [69, 70].

Coupling of active site generation to the completion of 20S proteasome assembly

The details of autocatalytic processing of active β-subunits have been described above. Aside of the active β-subunits, in eukaryotes there are two other β-subunits (β6 and β7) synthesized in precursor form with N-terminal propeptides. These propeptides are processed by neighboring active subunits. A detailed study on the processing of proβ7/Pre4 in yeast active site mutants led to the 'nearest neighbor model', according to which the propeptide of Pre4 receives its final shortening by the active site that is closest by [29]. A similar observation was made in mammalian T2 cells expressing β1i/LMP2. In these cells, incompletely processed LMP2 was observed in particular when an active site mutant version of it was expressed. Based on this finding the authors proposed an 'ordered two-step mechanism' for active site generation that involves a peptide shortening event in trans and a cis-autocatalytic second cleavage to generate the N-terminal threonine [72]. More recent data obtained in yeast with active site double mutants, however, suggest that this model is not generally applicable [44]. The observed intermediates may in part be explained by the absence of β5/LMP7 in the T2 cell system used [72]. The propeptide of LMP7 has recently been shown to be important for efficient mat-

uration of LMP2 and MECL-1 [73, 74]. In conclusion, the propeptides of inactive β-subunit precursors, as well as of inefficiently cleaved active subunits, that occur in the 'chamber of doom' appear to be treated just as any other invader. They are cleaved and shortened as much as possible by any active site they come too close to.

A central question to understanding the biogenesis of functional proteasomes was what triggers their activation. As outlined in the previous section, no peptide cleaving activity was found to be linked to proteasome precursor complexes from yeast or mammals. Consistent with this was the observation that the active site subunits in these precursor complexes are in the inactive propeptide-bearing form. These studies led to the idea that active site maturation occurs following the dimerization of two half-proteasome precursor complexes [67–69, 71]. This was elegantly supported by studies employing a yeast mutant analysis demonstrating that formation of the active site capable of autocatalytic processing of β5 depends on the juxtaposition of Proβ5 and β4 on opposite sides of the two halves of the proteasome [69]. That an active site formation from two subunits meeting at the halfproteasome interface is only part of the story of proteasome activation became clear with the discovery of maturation factor Ump1 (see below).

What is the role of the β-subunits' propeptides in proteasome biogenesis? Of the three active subunits of the yeast 20S proteasome only β5 is synthesized with a propeptide whose presence is essential for viability. As is outlined in the next section, this propeptide is essential for proper execution of Ump1's function in proteasome maturation. Deletion of the propeptides of β1 has very little effect on yeast cells. In contrast, deletion of the propeptide of β2 results in significant growth impairment [43, 44], but its role does not appear to depend on Ump1 [R. J. D., unpublished results]. One important role of the propeptides in precursors of active subunits appears to be to protect the subunits from inactivation due to acetylation of the N-terminal threonine residue until the catalytic chamber has been sealed off by formation of the 20S proteasome from two precursor complexes [43, 44]. A propeptide deletion analysis was meanwhile extended to the three inactive subunits that are either partially processed (β6 and β7) or, in the case of β3/Pup3, have only a short, unprocessable N-terminal extension. Whereas for β3 and β7 no effect on cell growth was detectable upon complete propeptide removal (up to the position +1 where Thr1 of active subunits would be located), the most C-terminal part of the β6 propeptide (close to position +1) turned out to be indispensable for cell survival. Interestingly, this essential propeptide function was again only seen in the presence of Ump1 [S. Iyappan, and W. H., unpublished results]. Propeptides appear not to be essential for determining the positioning of β-subunits within a β-ring [72, 74]. As discussed below, however, propeptides play an important role in the coordinated assembly of γ-IFN-induced β-subunits, leading to the formation of immunoproteasomes.

In summary, aside from keeping active β-subunits in a dormant and protected state, propeptides appear to have a role in chaperoning efficient subunit folding or assembly, the latter by mediating interactions with maturation factor Ump1 (see below) as well as potentially with other subunits.

Proteasome maturation factor Ump1

Work in the yeast *Saccharomyces cerevisiae* has established the role of a dedicated chaperone termed Ump1 that underpins the maturation of the proteasome [70]; reviewed in [75]. Loss of function *ump1* mutants were isolated in a screen that selects for cells defective in ubiquitin/proteasome-mediated proteolysis, leading to the original designation of the mutant. *ump1* null mutants are viable but are hypersensitive to various stresses such as heat or treatment with heavy metals, and are impaired in the degradation of any known proteasome substrate that was tested [70, 76]. Biochemical analysis revealed that Ump1 is present in 15S half-proteasome precursor complexes, but is absent from 20S and 26S proteasomes (fig. 4C, D). As shown by pulse chase analysis, Ump1 is an extremely short-lived protein, and its degradation that coincides with the maturation of β-subunits requires a functional 20S proteasome. In a mutant (*pre1-1*/β4) affected in the catalytic activity of the 20S proteasome, Ump1 was drastically stabilized and detectable in the 20S structures that may resemble preholoproteasomes. Experiments involving trypsin treatment and antibody detection showed that Ump1 is enclosed within the 20S structure in these mutants (fig. 5A). In the *ump1*-Δ mutant, proteasome assembly and maturation is strongly impaired. The formation of 20S structures from two half-proteasome precursors appears to be less efficient in *ump1*-Δ, and the maturation of the three active site subunits β1, β2 and β5 is drastically reduced. The detrimental effects of the *ump1* mutation appear to be compensated in part by increased expression of proteasomes [70]. A surprising result was that *ump1* null mutations suppressed the lethality of the deletion of β5/Pre2 propeptide. It had been shown previously that the β5 propeptide is essential for viability of yeast cells, and it was concluded that this peptide acts as an intramolecular chaperone that is required for incorporation of β5 into proteasomes [69]. The observation that in the absence of Ump1 the propeptide of β5 becomes dispensable suggested a different model (illustrated in fig. 5A) in which the propeptide of β5 is not required for incorporation of β5, but in which Ump1 and this propeptide mutually induce conformational or positional changes of each other upon dimerization of halfproteasome precursor complexes. According to this model, in the absence of the β5 propeptide, Ump1 remains in a position or conforma-

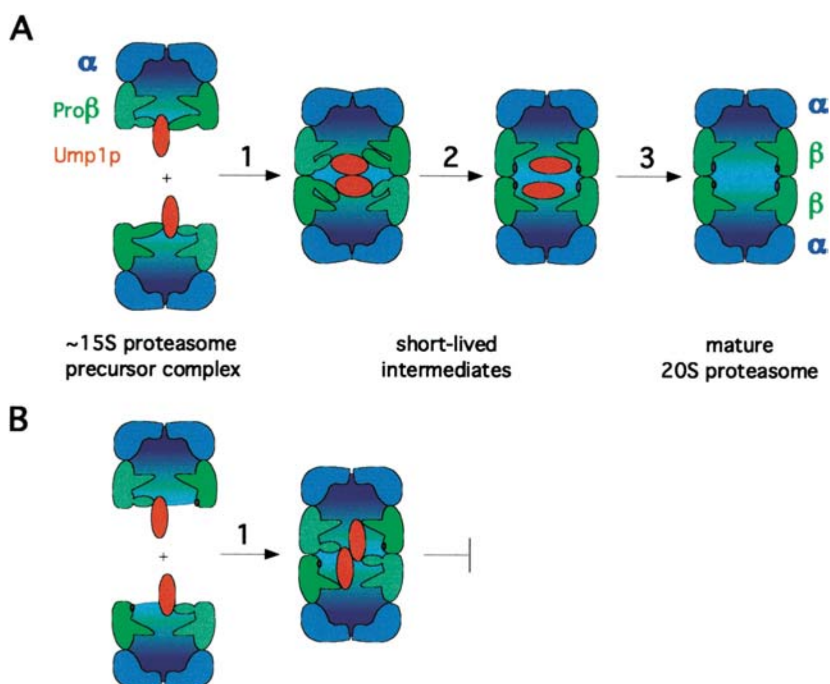

Figure 5. Model illustrating the role of Ump1 and β-subunit propeptides in activation of the proteasome. (*A*) In wild-type cells, Ump1 and unprocessed β-subunit precursors are detetected in 15S half-proteasome precursor complexes. Upon dimerization of these precursors (step 1), Ump1 becomes encased, leading to a mutual induced conformational and/or positional shift of Ump1 and propeptides. These Ump1-mediated conformational changes of propeptides trigger their autocatalytic processing (step 2). The activated proteasome then degrades Ump1 as its first substrate (step 3). (*B*) In a yeast mutant lacking the propeptide of β5 (Pre2-Δpro), Ump1 remains in a position that blocks subsequent steps in proteasome maturation, explaining why the lethality of this mutation is only observed in the presence of Ump1.

tion that is incompatible with subsequent maturation steps or function of the proteasome (fig. 5B). It is noteworthy in this context that of the five eukaryotic β-subunits that are synthesized in the precursor form, β5 is the one that carries the longest propeptide by far (75 residues in the case of yeast β5/Pre2), and this very propeptide is the only one that is essential for viability. This observation is consistent with an essential role of the β5 propeptide in execution of Ump1 function. Interestingly, when one compares orthologous β-subunits, the propeptides stand out as far less conserved than the rest of these polypeptides. Similarly, Ump1, whose orthologues appear to be present in all eukaryotes [77, M. London, J. Höckendorff and R. J. D, unpublished results] is far less conserved (~ 22% identity between *S. cerevisiae* and human UMP1) than the subunits of the mature proteasome (generally more than 50% identity). As no functional homologues of Ump1 have been identified in prokaryotes to date, these data suggest that Ump1 might be an invention of eukaryotes that coincided with the development of seven distinct β-subunits, only five of which are synthesized with propeptides.

In the absence of Ump1, 20S proteasomes are not only formed with reduced efficiency, but they are impaired in β-subunit maturation and hence in catalytic activity. These data suggested that Ump1 has a dual role in proteasome maturation, first in that it helps keeping the half-proteasome precursor complex in a conformation that is best suited for dimerization, and subsequently that it is required for triggering the maturation of active sites within the 20S complex. The detection of Ump1 protein in mouse and human proteasome precursor complexes in three independent studies suggested that its role in proteasome maturation, as has been characterized for *S. cerevisiae*, is conserved from yeast to humans [70, 77–79]. Surprisingly, the same protein, now under the name of KCNA4B, was more recently claimed to be a β-subunit of voltage-gated K^+ channel KCNA10 in humans [80]. Aside of two-hybrid interactions and in vitro binding, these authors observed, upon co-injection of KCNA10 and KCNA4B (alias UMP1) mRNAs into *Xenopus* oocytes, a 2.8-fold higher KCNA10 current when compared to cells injected only with KCNA10 RNA. If confirmed, these studies raised the possibility that UMP1 may serve multiple functions in vertebrate cells.

Proteasome assembly and import into the cell nucleus

An interesting question is how proteasomes in the nucleus are generated. Are they assembled in the nucleus or are they imported as a whole or in precursor form? A recent

study on the nuclear import of proteasomes in yeast led to the conclusion that proteasome precursor complexes containing Ump1 and unprocessed β-subunits are imported into the cell nucleus via a pathway in which so called classical nuclear localization signals (cNLSs) are the targeting signals [81]. These signals are recognized by a heterodimer formed by importin/karyopherin α (Srp1 in yeast) and importin/karyopherin β (Kap95 in yeast). Proteasomal precursor complexes were found to be in association with the importin α/β complex and to accumulate in certain mutants deficient in cNLS-dependent nuclear import [81]. This led the authors to propose that transfer to the nucleus is a necessary step in the biogenesis of 20S proteasomes, at least for the majority of them. In another study, two new proteins, Nob1 and Pno1, were reported to be required for maturation of 20S proteasomes in yeast [82]. Nob1 was originally isolated due to its interaction with the Rpn12 subunit of the 19S regulator of the proteasome, Pno1 (partner of Nob1) due to its interaction with Nob1. It was proposed that Nob1 and Pno1 mediate the assembly of half-proteasome precursor complexes with 19S regulator complexes in the nucleus [82]. This interpretation, however, is controversial, as Nob1 was proposed instead to be an endonuclease involved in the maturation of the 18S ribosomal RNA (rRNA) component of the 40S ribosomal subunit in another report [83]. Consistent with the latter report, the results described in reference [82] were not reproducible in our hands [C. Glanemann and R. J. D., unpublished results]. Future studies will have to clarify the role of additional assembly and maturation factors and of the 19S regulator in the generation of functional 20S proteasome core particles. Recent work has implicated the yeast Blm3 protein complex, which is remotely related to PA200 in mammalian cells [1], in the regulation of proteasome maturation [84]. The presence of Blm3 appears to inhibit the generation of active proteasomes from Ump1-containing precursor complexes.

Assembly of immunoproteasomes

As described above, vertebrates synthesize a specialized proteasome subtype implicated in the generation of class I antigenic peptides. This immunoproteasome is distinguished from housekeeping proteasomes by an exchange of the three active β-subunits by γ-IFN-induced isoforms. The incorporation of the induced subunits results in an altered cleavage specificity rendering the immunoproteasome more active towards cleavage after basic and hydrophobic residues. It is thought that this change of specificity favors the generation of peptides that are suitable for binding to MHC class I antigen-presenting molecules (reviewed in [18, 19, 85]).

The 'immunosubunits' (β1i, β2i and β5i) are highly homologous to their housekeeping counterparts. They are synthesized, however, with propeptides that are highly dissimilar to those of the housekeeping subunits. Several studies have demonstrated that these propeptides are critical determinants of a cooperative assembly of immunosubunits during de novo biogenesis of proteasomes. The order of events appears to be different from those for the incorporation of housekeeping β-subunits. Upon γ-IFN induction, early proteasome precursor complexes contain β1i/LMP2, β2i/MECL-1, β3 and β4 (fig. 4D). Precursor complexes of housekeeping proteasomes were shown instead to contain β2, β3 and β4 (see above). The observation that β1 assembles late into housekeeping proteasomes whereas β1i is incorporated early during the formation of immunoproteasomes suggests that incorporation of β1i is a key step in the assembly of the latter proteasome subtype [68]. This notion is supported by the observation that efficient incorporation of β2i/MECL-1 depends on the presence of β1i/LMP2 [86]. β5i/LMP7 was shown to be incorporated preferentially over β5/X into precursor complexes containing β1i and β2i. The propeptide of β5i was shown elegantly to be responsible for determining this preference. Swapping of the propeptides between β5 and β5i reversed the incorporation preference of these subunits [87]. Similarly, it was shown that attaching the propeptide of β2/Z to β2i/MECL-1 favors incorporation of this chimeric subunit into proteasome precursor complexes bearing the housekeeping β-subunits [88].

In conclusion, the cooperative incorporation of either housekeeping or inducible active β-subunits occurs preferentially over an assembly of 'mixed proteasome subtypes' [89], due at least in part to a guiding function of the subtype-specific propeptides. Since the interaction of several active β-subunits with the maturation factor Ump1 is dependent on their propeptides [70, M. London, J. Höckendorff and R. J. D., unpublished results] it is conceivable that such interactions are different and mutually exclusive for the propeptides of housekeeping and inducible subunits. Transcription of the mammalian proteasome maturation factor gene UMP1 interestingly is induced twofold by γ-IFN, suggesting that the same maturation factor may be involved in the generation of both the housekeeping and the immunoproteasome [77].

Concluding remarks

As we have tried to summarize in this review, our current knowledge of the structure and function of the 20S is far advanced. In addition, key steps in the assembly and activation of the 20S proteasome are well understood, although many details to reach a complete picture of this process are still missing. It will be a challenge for the future to fill in the gaps, and to extend our knowledge to the structure, assembly and activation of the 26S proteasome, which remains far less understood.

1 Ustrell V., Hoffman L., Pratt G. and Rechsteiner M. (2002) PA200, a nuclear proteasome activator involved in DNA repair. EMBO J. **21:** 3516–3525
2 Volker C. and Lupas A. N. (2002) Molecular evolution of proteasomes. Curr. Top. Microbiol. Immunol. **268:** 1–22
3 Orlowski M. and Wilk S. (2000) Catalytic activities of the 20 S proteasome, a multicatalytic proteinase complex. Arch. Biochem. Biophys. **383:** 1–16
4 Bogyo M. and Wang E. W. (2002) Proteasome inhibitors: complex tools for a complex enzyme. Curr. Top. Microbiol. Immunol. **268:** 185–208
5 Voges D., Zwickl P. and Baumeister W. (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu. Rev. Biochem. **68:** 1015–1068
6 Knipfer N. and Shrader T. E. (1997) Inactivation of the 20S proteasome in Mycobacterium smegmatis. Mol. Microbiol. **25:** 375–383
7 Zwickl P., Goldberg A. and Baumeister W (2000) Proteasomes in prokaryotes, In: Proteasomes: The World of Regulatory Proteolysis, Hilt W. and Wolf D.H. Eurekah.com/Landes Bioscience, Georgetown, TX. pp. 9–20, (Eds)
8 Tamura T., Nagy I., Lupas A., Lottspeich F., Cejka Z., Schoofs G. et al.. (1995) The first characterization of a eubacterial proteasome: the 20S complex of *Rhodococcus*. Curr. Biol. **5:** 766–774
9 Missiakas D., Schwager F., Betton J. M., Georgopoulos C. and Raina S. (1996) Identification and characterization of HsIV HsIU (ClpQ ClpY) proteins involved in overall proteolysis of misfolded proteins in *Escherichia coli*. EMBO J. **15:** 6899–6909
10 Kessel M., Wu W., Gottesman S., Kocsis E., Steven A. C. and Maurizi M. R. (1996) Six-fold rotational symmetry of ClpQ, the *E. coli* homolog of the 20S proteasome, and its ATP-dependent activator, ClpY. FEBS Lett. **398:** 274–278
11 Yoo S. J., Seol J. H., Shin D. H., Rohrwild M., Kang M. S., Tanaka K. et al. (1996) Purification and characterization of the heat shock proteins HslV and HslU that form a new ATP-dependent protease in *Escherichia coli*. J. Biol. Chem. **271:** 14035–14040
12 Rohrwild M., Coux O., Huang H. C., Moerschell R. P., Yoo S. J., Seol J. H. et al. (1996) HslV-HslU: A novel ATP-dependent protease complex in *Escherichia coli* related to the eukaryotic proteasome. Proc. Natl. Acad. Sci. USA **93:** 5808–5813
13 Yoo S. J., Shim Y. K., Seong I. S., Seol J. H., Kang M. S. and Chung C. H. (1997) Mutagenesis of two N-terminal Thr and five Ser residues in HslV, the proteolytic component of the ATP-dependent HslVU protease. FEBS Lett. **412:** 57–60
14 Bogyo M., McMaster J. S., Gaczynska M., Tortorella D., Goldberg A. L. and Ploegh H. (1997) Covalent modification of the active site threonine of proteasomal beta subunits and the *Escherichia coli* homolog HslV by a new class of inhibitors. Proc. Natl. Acad. Sci. USA **94:** 6629–6634
15 Bochtler M., Ditzel L., Groll M. and Huber R. (1997) Crystal structure of heat shock locus V (HslV) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:** 6070–6074
16 Sousa M. C., Trame C. B., Tsuruta H., Wilbanks S. M., Reddy V. S. and McKay D. B. (2000) Crystal and solution structures of an HslUV protease-chaperone complex. Cell **103:** 633–643
17 Bochtler M., Hartmann C., Song H. K., Bourenkov G. P., Bartunik H. D. and Huber R. (2000) The structures of HsIU and the ATP-dependent protease HsIU-HsIV. Nature **403:** 800–805
18 Pamer E. and Cresswell P. (1998) Mechanisms of MHC class I-restricted antigen processing. Annu. Rev. Immunol. **16:** 323–358
19 Rock K. L. and Goldberg A. L. (1999) Degradation of cell proteins and the generation of MHC class I-presented peptides. Annu. Rev. Immunol. **17:** 739–779
20 Ma J., Katz E. and Belote J. M. (2002) Expression of proteasome subunit isoforms during spermatogenesis in *Drosophila melanogaster*. Insect Mol. Biol. **11:** 627–639
21 Fu H., Doelling J. H., Arendt C. S., Hochstrasser M. and Vierstra R. D. (1998) Molecular organization of the 20S proteasome gene family from *Arabidopsis thaliana*. Genetics **149:** 677–692
22 Löwe J., Stock D., Jap B., Zwickl P., Baumeister W. and Huber R. (1995) Crystal structure of the 20S proteasome from the archae on *T. acidophilum* at 3.4 Å resolution. Science **268:** 533–539
23 Unno M., Mizushima T., Morimoto Y., Tomisugi Y., Tanaka K., Yasuoka N. et al. (2002) The structure of the mammalian 20S proteasome at 2.75 Å resolution. Structure **10:** 609–618
24 Groll M., Bajorek M., Kohler A., Moroder L., Rubin D. M., Huber R. et al. (2000) A gated channel into the proteasome core particle. Nat. Struct. Biol. **7:** 1062– 1067
25 Hill C. P., Masters E. I. and Whitby F. G. (2002) The 11S regulators of 20S proteasome activity. Curr. Top. Microbiol. Immunol. **268:** 73–89
26 Kohler A., Cascio P., Leggett D. S., Woo K. M., Goldberg A. L. and Finley D. (2001) The axial channel of the proteasome core particle is gated by the Rpt2 ATPase and controls both substrate entry and product release. Mol. Cell **7:** 1143–1152
27 Ditzel L., Huber R., Mann K., Heinemeyer W., Wolf D. H. and Groll M. (1998) Conformational constraints for protein self-cleavage in the proteasome. J. Mol. Biol. **279:** 1187–1191
28 Seemüller E., Lupas A., Stock D., Löwe J., Huber R. and Baumeister W. (1995) Proteasome from *Thermoplasma acidophilum*: a threonine protease. Science **268:** 579–582
29 Heinemeyer W., Fischer M., Krimmer T., Stachon U. and Wolf D. H. (1997) The active sites of the eukaryotic 20 S proteasome and their involvement in subunit precursor processing. J. Biol. Chem. **272:** 25200–26209
30 Arendt C. S. and Hochstrasser M. (1997) Identification of the yeast 20S proteasome catalytic centers and subunit interactions required for active-site formation. Proc. Natl. Acad. Sci. USA **94:** 7156–7161
31 Dick T. P., Nussbaum A. K., Deeg M., Heinemeyer W., Groll M., Schirle M. et al. (1998) Contribution of proteasomal beta-subunits to the cleavage of peptide substrates analyzed with yeast mutants. J. Biol. Chem. **273:** 25637–25646
32 Groll M., Heinemeyer W., Jäger S., Ullrich T., Bochtler M., Wolf D. H. et al. (1999) The catalytic sites of 20S proteasomes and their role in subunit maturation: A mutational and crystallographic study. Proc. Natl. Acad. Sci. USA **96:** 10976–10983
33 Groll M., Ditzel L., Löwe J., Stock D., Bochtler M., Bartunik H. D. et al. (1997) Structure of 20S proteasome from yeast at 2.4 Å resolution. Nature **386:** 463–471
34 Holzhütter H. G., Frömmel C. and Kloetzel P.-M. (1999) A theoretical approach towards the identification of cleavage-determining amino acid motifs of the 20 S proteasome. J. Mol. Biol. **286:** 1251–1265
35 Nussbaum A. K., Dick T. P., Keilholz W., Schirle M., Stevanovic S., Dietz K. et al. (1998) Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1. Proc. Natl. Acad. Sci. USA **95:** 12504–12509
36 Toes R. E., Nussbaum A. K., Degermann S., Schirle M., Emmerich N. P., Kraft M. et al. (2001) Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. J. Exp. Med. **194:** 1–12
37 Kesmir C., Nussbaum A. K., Schild H., Detours V. and Brunak S. (2002) Prediction of proteasome cleavage motifs by neural networks. Protein Eng. **15:** 287–296
38 Nazif T. and Bogyo M. (2001) Global analysis of proteasomal substrate specificity using positional-scanning libraries of covalent inhibitors. Proc. Natl. Acad. Sci. USA **98:** 2967–2972
39 Kisselev A. F., Akopian T. N., Castillo V. and Goldberg A. L. (1999) Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. Mol. Cell **4:** 395–402
40 Schmidtke G., Emch S., Groettrup M. and Holzhütter H. G. (2000) Evidence for the existence of a non-catalytic modifier

site of peptide hydrolysis by the 20 S proteasome. J. Biol. Chem. **275:** 22056–22063
41 Myung J., Kim K. B., Lindsten K., Dantuma N. P. and Crews C. M. (2001) Lack of proteasome active site allostery as revealed by subunit-specific inhibitors. Mol. Cell **7:** 411–420
42 Kisselev A. F., Kaganovich D. and Goldberg A. L. (2002) Binding of hydrophobic peptides to several non-catalytic sites promotes peptide hydrolysis by all active sites of 20 S proteasomes. Evidence for peptide-induced channel opening in the alpha-rings. J. Biol. Chem. **277:** 22260–22270
43 Arendt C. S. and Hochstrasser M. (1999) Eukaryotic 20S proteasome catalytic subunit propeptides prevent active site inactivation by N-terminal acetylation and promote particle assembly. EMBO J. **18:** 3575–3585
44 Jäger S., Groll M., Huber R., Wolf D. H. and Heinemeyer W. (1999) Proteasome β-type subunits: Unequal roles of propeptides in core particle maturation and a hierarchy of active site function. J. Mol. Biol. **291:** 997–1013
45 Zwickl P., Kleinz J. and Baumeister W. (1994) Critical elements in proteasome assembly. Nat. Struct. Biol. **1:** 765–770
46 Seemüller E., Lupas A. and Baumeister W. (1996) Autocatalytic processing of the 20S proteasome. Nature **382:** 468–471
47 Grziwa A., Maack S., Pühler G., Wiegand G., Baumeister W. and Jaenicke R. (1994) Dissociation and reconstitution of the *Thermoplasma* proteasome. Eur. J. Biochem. **223:** 1061–1067
48 Maupin-Furlow J. A., Aldrich H. C. and Ferry J. G. (1998) Biochemical characterization of the 20S proteasome from the methanoarchaeon *Methanosarcina thermophila.* J. Bacteriol. **180:** 1480–1487
49 Wilson H. L., Ou M. S., Aldrich H. C. and Maupin-Furlow J. (2000) Biochemical and physical properties of the *Methanococcus jannaschii* 20S proteasome and PAN, a homolog of the ATPase (Rpt) subunits of the eucaryal 26S proteasome. J. Bacteriol. **182:** 1680–1692
50 Zühl F., Tamura T., Dolenc I., Cejka Z., Nagy I., De Mot R. and Baumeister W. (1997) Subunit topology of the *Rhodococcus* proteasome. FEBS Lett. **400:** 83–90
51 Zühl F., Seemüller E., Golbik R. and Baumeister W. (1997) Dissecting the assembly pathway of the 20S proteasome. FEBS Lett. **418:** 189–194
52 Mayr J., Seemüller E., Müller S. A., Engel A. and Baumeister W. (1998) Late events in the assembly of 20S proteasomes. J. Struct. Biol. **124:** 179–188
53 Nagy I., Tamura T., Vanderleyden J., Baumeister W. and De Mot R. (1998) The 20S proteasome of *Streptomyces coelicolor.* J. Bacteriol. **180:** 5448–5453
54 Pouch M. N., Cournoyer B. and Baumeister W. (2000) Characterization of the 20S proteasome from the actinomycete *Frankia.* Mol. Microbiol. **35:** 368–377
55 Schmid H. P., Akhayat O., Martins De Sa C., Puvion F., Koehler K. and Scherrer K. (1984) The prosome: an ubiquitous morphologically distinct RNP particle associated with repressed mRNPs and containing specific ScRNA and a characteristic set of proteins. EMBO J. **3:** 29–34
56 Wilk S. and Orlowski M. (1983) Evidence that pituitary cation-sensitive neutral endopeptidase is a multicatalytic protease complex. J. Neurochem. **40:** 842–849
57 Hough R., Pratt G. and Rechsteiner M. (1986) Ubiquitin-lysozyme conjugates. Identification and characterization of an ATP-dependent protease from rabbit reticulocyte lysates. J. Biol. Chem. **261:** 2400–2408
58 Falkenburg P. E., Haass C., Kloetzel P. M., Niedel B., Kopp F., Kuehn L. et al. (1988) *Drosophila* small cytoplasmic 19S ribonucleoprotein is homologous to the rat multicatalytic proteinase. Nature **331:** 190–192
59 Arrigo A. P., Tanaka K., Goldberg A. L. and Welch W. J. (1988) Identity of the 19S 'prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature **331:** 192–194
60 Gerards W. L., Enzlin J., Haner M., Hendriks I. L., Aebi U., Bloemendal H. et al. (1997) The human alpha-type proteasomal subunit HsC8 forms a double ringlike structure, but does not assemble into proteasome-like particles with the beta-type subunits HsDelta or HsBPROS26. J. Biol. Chem. **272:** 10080–10086
61 Gerards W. L., de Jong W. W., Bloemendal H. and Boelens W. (1998) The human proteasomal subunit HsC8 induces ring formation of other alpha-type subunits. J. Mol. Biol. **275:** 113–121
62 Yao Y., Toth C. R., Huang L., Wong M. L., Dias P., Burlingame A. L. et al. (1999) alpha5 subunit in *Trypanosoma brucei* proteasome can self-assemble to form a cylinder of four stacked heptamer rings. Biochem. J. **344:** 349–358
63 Huang L., Shen M., Chernushevich I., Burlingame A. L., Wang C. C. and Robertson C. D. (1999) Identification and isolation of three proteasome subunits and their encoding genes from *Trypanosoma brucei.* Mol. Biochem. Parasitol. **102:** 211–223
64 Seelig A., Multhaup G., Pesold-Hurt B., Beyreuther K. and Kloetzel P. M. (1993) *Drosophila* proteasome Dm25 subunit substitutes the mouse MC3 subunit in hybrid proteasomes. The N-terminal domain is essential for subunit incorporation. J. Biol. Chem. **268:** 25561–25567
65 Emori Y., Tsukahara T., Kawasaki H., Ishiura S., Sugita H. and Suzuki K. (1991) Molecular cloning and functional analysis of three subunits of yeast proteasome. Mol. Cell. Biol. **11:** 344–353
66 Frentzel S., Pesold-Hurt B., Seelig A. and Kloetzel P. M. (1994) 20 S proteasomes are assembled via distinct precursor complexes. Processing of LMP2 and LMP7 proproteins takes place in 13-16 S preproteasome complexes. J. Mol. Biol. **236:** 975–981
67 Yang Y., Früh K., Ahn K. and Peterson P. A. (1995) In vivo assembly of the proteasomal complexes, implications for antigen processing. J. Biol. Chem. **270:** 27687–27694
68 Nandi D., Woodward E., Ginsburg D. B. and Monaco J. J. (1997) Intermediates in the formation of mouse 20S proteasomes: implications for the assembly of precursor beta subunits. EMBO J. **16:** 5363–5375
69 Chen P. and Hochstrasser M. (1996) Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly. Cell **86:** 961–972
70 Ramos P. C., Höckendorff J., Johnson E. S., Varshavsky A. and Dohmen R. J. (1998) Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. Cell **92:** 489–499
71 Schmidtke G., Schmidt M. and Kloetzel P. M. (1997) Maturation of mammalian 20 S proteasome: purification and characterization of 13 S and 16 S proteasome precursor complexes. J. Mol Biol **268:** 95–106
72 Schmidtke G., Kraft R., Kostka S., Henklein P., Frommel C., Löwe J. et al. (1996) Analysis of mammalian 20S proteasome biogenesis: the maturation of beta-subunits is an ordered two-step mechanism involving autocatalysis. EMBO J. **15:** 6887–6898
73 Griffin T. A., Nandi D., Cruz M., Fehling H. J., Kaer L. V., Monaco J. J. et al. (1998) Immunoproteasome assembly: cooperative incorporation of interferon gamma (IFN-gamma)-inducible subunits. J. Exp. Med. **187:** 97–104
74. Schmidt M., Zantopf D., Kraft R., Kostka S., Preissner R. and Kloetzel P. M. (1999) Sequence information within proteasomal prosequences mediates efficient integration of beta-subunits into the 20 S proteasome complex. J. Mol. Biol. **288:** 117–128
75 Maurizi M. R. (1998) Proteasome assembly: biting the hand. Curr. Biol. **8:** R453–456
76 Cooper K. F., Mallory M. J. and Strich R. (1999) Oxidative stress-induced destruction of the yeast C-type cyclin Ume3p requires phosphatidylinositol-specific phospholipase C and the 26S proteasome. Mol. Cell. Biol. **19:** 3338–3348

77 Burri L., Höckendorff J., Boehm U., Klamp T., Dohmen R. J. and Levy F. (2000) Identification and characterization of a mammalian protein interacting with 20S proteasome precursors. Proc. Natl. Acad. Sci. USA **97:** 10348–10353
78 Griffin T. A., Slack J. P., McCluskey T. S., Monaco J. J. and Colbert R. A. (2000) Identification of proteassemblin, a mammalian homologue of the yeast protein, Ump1p, that is required for normal proteasome assembly. Mol. Cell. Biol. Res. Commun. **3:** 212–217
79 Witt E., Zantopf D., Schmidt M., Kraft R., Kloetzel P. M. and Krüger E. (2000) Characterisation of the newly identified human Ump1 homologue POMP and analysis of LMP7(beta 5i) incorporation into 20 S proteasomes. J. Mol. Biol. **301:** 1–9
80 Tian S., Liu W., Wu Y., Rafi H., Segal A. S. and Desir G. V. (2002) Regulation of the voltage-gated K+ channel KCNA10 by KCNA4B, a novel beta-subunit. Am. J. Physiol. Renal Physiol. **283:** F142–149
81 Lehmann A., Janek K., Braun B., Kloetzel P. M. and Enenkel C. (2002) 20 S proteasomes are imported as precursor complexes into the nucleus of yeast. J. Mol. Biol. **317:** 401–413
82 Tone Y. and Toh-e A. (2002) Nob1p is required for biogenesis of the 26S proteasome and degraded upon its maturation in *Saccharomyces cerevisiae.* Genes Dev. **16:** 3142–3157
83 Fatica A., Oeffinger M., Dlakic M. and Tollervey D. (2003) Nob1p is required for cleavage of the 3' end of 18S rRNA. Mol. Cell. Biol. **23:** 1798–1807
84 Fehlker M., Wendler P., Lehmann A. and Enenkel C. (2003) Blm3 is part of nascent proteasomes and is involved in a late stage of nuclear proteasome assembly. EMBO Rep. **4:** 959–963
85 Kloetzel P. M. (2001) Antigen processing by the proteasome. Nat. Rev. Mol. Cell. Biol. **2:** 179–187
86 Groettrup M., Standera S., Stohwasser R. and Kloetzel P. M. (1997) The subunits MECL-1 and LMP2 are mutually required for incorporation into the 20S proteasome. Proc. Natl. Acad. Sci. USA **94:** 8970–8975
87 Kingsbury D. J., Griffin T. A. and Colbert R. A. (2000) Novel propeptide function in 20 S proteasome assembly influences beta subunit composition. J. Biol. Chem. **275:** 24156–24162
88 De M., Jayarapu K., Elenich L., Monaco J. J., Colbert R. A. and Griffin T. A. (2003) Beta 2 subunit propeptides influence cooperative proteasome assembly. J. Biol. Chem. **278:** 6153–6159
89 Dahlmann B., Ruppert T., Kloetzel P. M. and Kuehn L. (2001) Subtypes of 20S proteasomes from skeletal muscle. Biochimie **83:** 295–299

To access this journal online:
http://www.birkhauser.ch