Structure of the Proteasome

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The ubiquitin-proteasomal system is an essential element of the protein quality control machinery in cells. The central part of this system is the 20S proteasome. The proteasome is a barrel-shaped multienzyme complex, containing several active centers hidden at the inner surface of the hollow cylinder. So, the regulation of the substrate entry toward the inner proteasomal surface is a key control mechanism of the activity of this protease.

This chapter outlines the knowledge on the structure of the subunits of the 20S proteasome, the binding and structure of some proteasomal regulators and inducible proteasomal subunits. Therefore, this chapter imparts the knowledge on proteasomal structure which is required for the understanding of the following chapters.

I. Introduction

In order to maintain the functionality and the viability of a cell, most of the cellular proteins are subjected to a highly regulated turnover. To realize this, proteins that are misfolded, (oxidatively) damaged, or no longer required, have to be recognized and removed.[1–4] Removal of proteins is usually realized via proteolytic degradation. The most important proteolytic intracellular system of the cytosol is the proteasomal system, an evolutionarily very old and distributed machinery that was found to be present even in many of the oldest bacteria, as

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well as in plants and animals. The central part of the proteasomal system is the 20S "core" proteasome, a large multisubunit and multicatalytic protease, as well as several different regulators that can change the activity of the specificity of the "core" particle.

In the following sections, we describe the structure and function of the 20S proteasome and its regulators. For a better differentiation between the variations of the "proteasome," the 20S "core" particle is always referred to as "proteasome," and the other forms, according to the regulators that are attached to that "core."

II. The 20S Proteasome

The 20S proteasome represents the catalytic part of the proteasomal system, a highly regulated group of proteins that perform degradation of damaged or misfolded proteins, regulation of their life spans,[1–6] and "quality control" of newly synthesized proteins[7–12] that are involved in regulation of the cell cycle,[5] gene expression,[13–17] immune responses,[18–23] responses to (oxidative) stress,[24–28] and carcinogenesis.[29–31] Furthermore, the nuclear protein is involved in the maintenance of chromatin and influences DNA repair.[32–34] So an evermore increasing spectrum of cellular functions are related to the proteasomal system.

The term "20S" results from the sedimentation constant of the proteasome "core" particle.[35] The mammalian form of this particle is of a cylindrical structure of about 100×160 Å that contains four homologous rings (two alpha (α-) and two beta (β-)rings, arranged in the sequence α-β-β-α), which are built of seven different subunits each. The three-dimensional structure of the large protease of several organisms has been investigated extensively via X-ray crystallography.[36–41]

Two basic forms of the proteasome are known: the ancestral one that is found in Archaea bacteria like *Thermoplasma acidophilum* and the evolutionarily higher form of yeast, plants, and animals.

As the evolutionary higher form, the ancestral proteasome contains four heptameric rings, arranged in the common α-β-β-α sequence, but in this ancestral proteasome each ring contains seven equal subunits, so only one α- and β-subunit are present (see Fig. 1). It is obvious that from these simple forms of the proteasome a more complex one evolved via the divergence of the single subunits into several homologous ones. So the evolutionary higher form of the 20S proteasome contains 14 different subunits overall (α_1–α_7 and β_1–β_7), showing molecular masses between 20 and 30 kDa (see Table I), summarizing to a molecular weight of some 700 kDa. While the catalytic centers are located in the inner β-rings, the outer α-rings of the proteasome

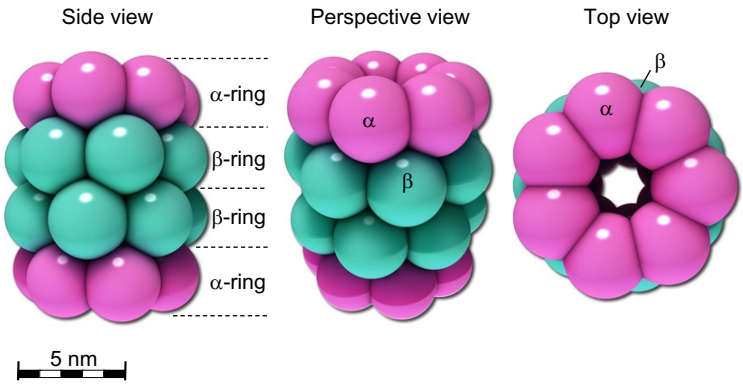

FIG. 1. The structure of the archaea 20S proteasome from *Thermoplasma acidophilum*. This figure shows a basic model of the archaea proteasomal structure. As shown on the left, the proteasome contains four homologous rings in the sequence α-β-β-α. Each ring contains seven identical subunits: the α-ring only α-, the β-ring only β-subunits, as shown in the central image. The right panel shows the arrangement of the α-subunits in a vertical view onto an α-ring.

are responsible for the regulation of substrate entrance to the inner proteolytic chamber, as well as for recognition and binding of the substrates themselves. So the α-subunits are able to change both the activity and specificity of the proteasome. The proteolytic centers found in the inner rings are encoded by three different β-subunits (β_1, β_2, and β_5). Thus, due to the symmetric arrangement of the different rings, the inner chamber contains 6 different proteolytic centers, protected inside the proteasome in the evolutionary higher form of the proteasome, but 14 in the ancestral one. The inside of the proteasome is subdivided into two "ante chambers" (between the α- and β-rings) and one single "main chamber," found between the two β-rings (see Fig. 2). The "main chamber" is also the location of the catalytic centers.

As the proteasome is today referred to as a proteolytic system, several regulators are binding to the core proteasome, modulating the proteasomal activity. Today, a set of several different proteasomal regulators are known, all binding to the α-subunits of the outer proteasomal rings. The 11S regulator particle, in most organisms termed "PA28" or "REG," is formed of three different subunits (PA28α, PA28β, and PA28γ arranged in several diverse combinations). In *Trypanosoma brucei*, this ATP-independent regulator is called "PA26." Another important regulator is the ATP-dependent "19S," also known as "PA700" regulator; its analogue in archaea is termed "PAN." Several other regulators are known, including the nuclear regulator "PA200," which is known in three different isoforms (PA200i, PA200ii, and PA200iii) and

TABLE I

HERE, THE MOLECULAR MASSES (AFTER POSTTRANSCRIPTIONAL PROCESSING, AS FOUND IN THE ASSEMBLED WHOLE 20S "CORE" PROTEASOME) OF THE PROTEASOMAL SUBUNITS FROM BOTH HUMAN AND YEAST PROTEASOME ARE LISTED, AS WELL AS THE SUBUNITS OF TWO PROTEASOMAL REGULATOR CAPS (11S AND 19S)

20S "core" proteasome

Systematic	*S. cerevisiae*	*Homo sapiens*	Mass [kDa]	Literature
α_1	C7/Prs2	HsPROS27/HsIota	27.5	42
α_2	Y7	HsC3	25.9	43,44
α_3	Y13	HsC9	29.5	45,46
α_4	Pre6	HsC6/XAPC7	27.9	47,48
α_5	Pup2	HsZeta	26.4	49–51
α_6	Pre5	HsC2/HsPROS30	30.2	52
α_7	C1/Prs1	HsC8	28.4	53–56
β_1	Pre3	HsDelta/Y	25.3 (21.9)	57–59
β_{1i}	–	Lmp2	23.2 (20.9)	60–63
β_2	Pup1	Z	20.0 (24.5)	64–66
β_{2i}	–	Mecl1	28.9 (23.8)	67,68
β_3	Pup3	HsC10-II	22.9	69,70
β_4	C11 / Pre1	HsC7-I	22.8	69,71
β_5	Pre2	X/MB1	N/A (22.4)	71
β_{5i}	–	Lmp7	30.4 (21.2)	67,72–74
β_6	C5/Prs3	HsC5	26.5 (23.3)	75,76
β_7	Pre4	HsN3/HsBPROS26	29.2 (24.4)	69,77,78

11S (PA28) activator cap

Systematic	Other names	Mass [kDa]	Literature
11S subunit α	REGα or PA28α	28.723	79,80
11S subunit β	REGβ or PA28β	27.348	20
11S subunit γ	REGγ or PA28γ	30.886	81–84

19S (PA700) regulator cap

Systematic	Other names	Mass [kDa]	Literature
ATPase-subunits			
Rpt1	S7 or p48, Mss1, Yta3, Cim5	48.633	85
Rpt2	S4 or p56Yhs4, Yta5, Mts2	49.184	86–88
Rpt3	S6b or p48, Tbp7, Yta2, Ynt1, MS73	47.336	89,90
Rpt4	S10b or p42, Sug2, Pcs1, Crl13, CADp44	44.173	91,92
Rpt5	S6a or p50, Tbp1, Yta1	49.118	93,94
Rpt6	S8 or p45, Trip1, Sug1, Cim3, Crl3, Tby1, Tbp10	45.653	95–97
Non-ATPase-subunits			
Rpn1	S2 or p97, Trap2, Nas1, Hrd2, Rpd1, Mts4	100.199	98
Rpn2	S1 or p112, Sen3	105.866	99,100
Rpn3	S3 or p58, Sun2	61.005	101

(*Continues*)

TABLE I (*Continued*)

19S (PA700) regulator cap			
Systematic	Other names	Mass [kDa]	Literature
Rpn4	Son1 or Ufd5	60.152	102–104
Rpn5	P55 or Nas5	52.904	105–107
Rpn6	S9 or p44.5	47.447	108–110
Rpn7	S10a or p44, HUMORF07	45.531	111–113
Rpn8	S12 or p40, Mov-34, Nas3	37.060	114
Rpn9	S11 or p40.5, Les1, Nas7	42.945	115–117
Rpn10	S5a or p54, ASF1, Sun1, Mcb1, Mbp1	40.736	118–120
Rpn11	S13 or Poh1, Mpr1, Par1	34.577	121–123
Rpn12	S14 or p31, Nin1, Mts3	30.004	98,124,125
Rpn15	DSS1 or SHFM1 (in human), SEM1 (in yeast)	8.146	126,127

The molecular masses in brackets represent the peptide mass before posttranscriptional processing. According to their position (α- or β-ring) in the mature proteasome, the systematic names of the subunits were defined. The "i" in the systematic names indicates an γ-interferon-"inducible" proteasomal subunit. The molecular masses of the proteasome are according to Coux *et al.*,[128] and the information about the regulator caps is according to Finley *et al.*[129] and Baumeister *et al.*.[130] The molecular weights of the single proteins were taken from the corresponding literature and checked using the site "www.wolframalpha.com." Molecular weights from "www.wolframalpha.com" were very similar to the weights taken from the literature, except for the subunits Rpt1 (57.199 kDa according to www.wolfram.com), Rpn4 (24.551 kDa), and Rpn12 (39.481 kDa).

contributes to spermatogenesis[131] and DNA repair.[132] However, only PA200i seems to bind to the proteasome, while the other two isoforms can be found in nuclear foci[133] without any proteasomal interaction.

Furthermore, PR39 and PI31, which work as cellular proteasomal inhibitors, are known. PR39 is a short peptide of only 39 amino acids, first extracted from porcine bone marrow, and functions as a noncompetitive inhibitor, both in yeast and mammals. Its mechanism of inhibition is unique: via binding to the α_7-subunit of the proteasome, an allosteric change of the whole proteasomal structure is induced that decreases its proteolytic activity and affects the binding to the 19S-regulator.[134] PI31, a mammalian protein first discovered by DeMartino,[135] competes with the α- and β-form of PA28 for binding of the proteasome.[136]

Several other proteins interact with the proteasomal system and are able to regulate the proteasomal proteolytic activities. The heat shock protein 90 (Hsp90) is another known cellular proteasomal regulator.[137–139] Furthermore, the proteasomal activity seems to be regulated by phosphorylation of its substrates or of components of the proteasomal system itself[53,95,140,141] and the nuclear proteasome by poly-ADP-ribose.[142–144]

As mentioned, the proteolytic activity is localized inside the proteasome in the main chamber. While only the subunits β_1, β_2, and β_5 show proteolytic activity, the others do not; furthermore, in some mammalian cells, the active

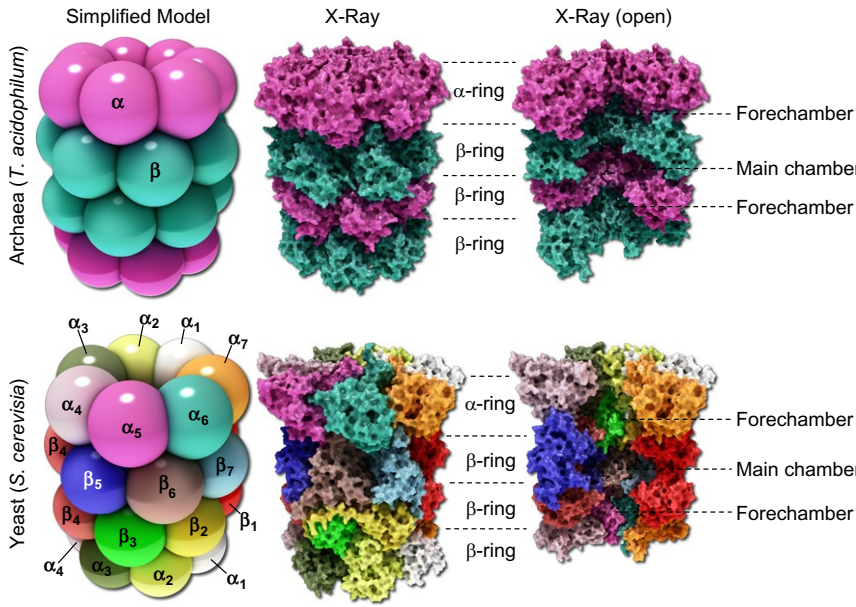

FIG. 2. Structures of the archaea and eukaryotic 20S proteasomes. Here, the structures of the archaea and the eukaryotic proteasomes are compared. The upper line of images shows a simple descriptive model (left), a highly detailed model calculated from X-ray structure analysis (middle), and the inside of the archaea 20S proteasome (from *Thermoplasma acidophilum*) after removal of several α- and β-subunits. The simple ball model shows the α-rings in pink and the β-rings in turquoise, while the more detailed one alternates those colors in order to accentuate the single rings. The right image shows the inner structure of the prokaryotic proteasome, subdivided into two fore chambers between the α- and β-rings, and the main chamber formed by the two β-rings. The bottom row of images shows the same for the eukaryotic 20S proteasome from *Saccharomyces cerevisiae*. This type of proteasome contains seven different α- and β-subunits, each arranged in two α- and β-rings. The single subunits are color coded in the same way in the simple ball model on the left and the more complex models from X-ray structure analysis (middle and right). The right image reveals the inner structure of the yeast 20S proteasome, showing the same subdivision into two fore and one main chamber, as found in the archaea proteasome too.

subunits can be replaced by their γ-interferon (IFN-γ)-inducible isoforms β_{1i}, β_{2i}, and β_{5i}. However, in fact, that is not a replacement but a *de novo* synthesis of new proteasomes. The presence of the IFN-γ-inducible isoforms of the proteasome results in a change of the fragment length of the product of oligopeptides. It is surmised that the inducible proteasomal forms play a significant role in the antigen presentation of the adaptive immune response. Interestingly, in the thymus, a specific third variation of the β_5-subunit was recently found by Hirano, the replacement of the β_5-subunit by the so-called β_{5t} one.[145] The β-subunits of the proteasome provide their own class of

proteases that show no evolutionary relation to other known proteases but a very high relationship to each other, suggesting a common ancestor.[36] According to the arrangement of the proteasomal subunits as found in the archaeic form from *T. acidophilum*, the different subunits have been divided into two classes, the α- and β-subunits.[146] Normally, Eubacteria contain no 20S proteasome. Nevertheless, in a subgroup of Eubacteria, the so-called actinobacteria, proteasomal genes and even proteasomes were found. In those actinobacteria, HslVU (also known as ClpQY), an ATP-dependent hexameric protease[147,148] that shows structural similarities to the β-subunits of the 20S proteasome, was identified. It is a dimer containing both the proteasome-related protease HsllV and HslU, an ATPase.[149]

The proteasome is not the only proteolytic system of the cell, but one of the most important ones. Among the others are the lysosomal system,[150,151] containing many different cathepsins,[152–154] and the calpains associated with the cytoskeleton. While the main task of the lysosomal system includes the degradation of intracellular organelles, the proteasomal system is the most important one regarding the recognition and degradation of (damaged) proteins. It is assumed that between 70% and 90%[155,156] of the misfolded, (oxidatively) damaged, or no longer needed proteins are degraded via the proteasomal pathway. While the pure degradation of dysfunctional or misfolded proteins occurs in an ATP-independent way, the regulatory degradation of functional proteins is ATP-dependent and, moreover, the proteins targeted for degradation have to be labeled with a chain of ubiquitin molecules (polyubiquitination).

In mammalian cells, the amount of proteasome can be up to 1% of the whole protein pool (in liver and kidney cells).[157] The proteasome can be found both in the cytosolic and in the nuclear compartment of a cell, and can also be bound to the endoplasmic reticulum (ER) as well as being in association with the cytoskeleton.[158] The mammalian form of the 20S proteasome was first discovered and isolated from human red blood cells by Harris in 1968 and termed "cylindrin"[159,160] following the shape of the large protein complex. Other scientists have termed it "macroxyproteinase,"[161–163] "hollow cylinder" protein,[164,165] "multicatalytic proteinase complex,"[166–170] or "prosome."[158,171] Today, the term "proteasome" is used the most often.

A. The Proteasomal α-Subunits

Both recognition and access of the substrate into the inner proteolytic chamber of the proteasome is regulated by the α-rings. After contact with a substrate, an (oxidatively) damaged/misfolded, and (partly) defolded protein, a conformational change of the α-rings is induced that virtually "unlocks" the gate they form to control and regulate substrate entrance to the inner chamber. That gate is formed by the N-terminal ends of the three subunits α_2, α_3, and α_4.[172] The N-terminal ends of those three subunits are pointing in the direction

of the proteasomal symmetric axis and block mechanically the entrance to the proteolytic centers formed by the two β-rings. Incubation of isolated proteasomes with low concentrations of sodium dodecylsulfate (SDS), an agent that induces a slight defolding of the proteasomal subunits, resulted in a significant increase of proteolytic activity, caused by an opening of the proteasomal gate that facilitates substrate entrance[128] (Fig. 3).

Similar effects could be induced by repeated freezing–thawing cycles as well as under conditions of low ionic strength. Those experimental results suggested an involvement of structural changes of the α-subunits that regulate proteasomal substrate entrance. A further possibility of substrate entrance modulation is the binding of a regulatory subunit (like 11S, 19S, or PA200) to the proteasomal α-rings. Such a binding can increase proteasomal activity up to 10-fold[173,174] and induce a change in substrate specificity, which is induced by the maximal opening of the substrate channel of the proteasome via a conformational rearrangement of the blocking N-terminal ends of some α-subunits.

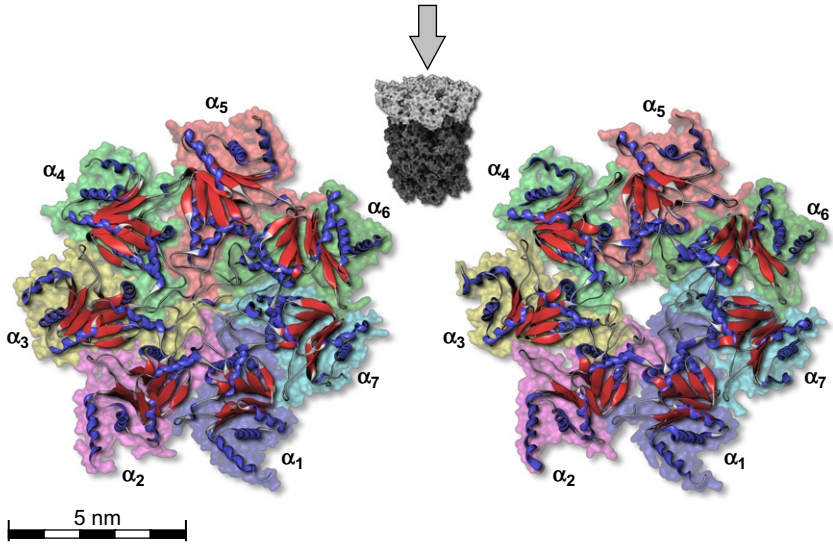

FIG. 3. Gating of the eukaryotic 20S proteasome. This image shows a structural rearrangement of the α-rings of the yeast 20S proteasome without and after activation. Activation can be induced by binding of a substrate protein, short oligopeptides, or regulating proteins (in this case, Blm10; see the text). After activation, the substrate accessibility of the proteasome increases, mainly due to an opening of the gate that is formed by the N-terminal structures of the α-subunits. Please note the massive reorganization of the N-terminus of α_3 (yellow). After activation, the α-rings open a channel of about 13 Å to the inner proteolytic centers. The small icon in the middle shows the point of view (arrow) from which the reader is looking at the depicted α-rings.

The "activation" of the proteasome seems to be mediated via the binding of the α-rings to hydrophobic amino acids (normally buried inside the correctly folded/native form of a globular and water-soluble proteins) that are exposed by (oxidatively) damaged or unfolded/misfolded proteins; that binding induces the conformational change that results in an opening of the proteasomal gate.[175]

The proteasomal gate shows a maximal diameter of about 13 Å (archaea proteasome) in its maximal opened state: this is sufficient to enable the entrance of a defolded substrate protein, usually represented by a single chain of amino acids. The individual N-terminal ends of the single gating subunits reveal unique three-dimensional structures that are essential for the gating of the proteasome. Some of the involved structures are highly conserved in eukaryotic cells. The so-called YDR-motif (Tyr8-Asp9-Arg10) can be found in every single α-subunit, as well as in archaea and eukaryotic cells, and may be working as a joint, bending the gating structures in order to modulate proteasomal activities.[172] The most important part seems to be played by the α_3 subunit: $\alpha_3\Delta N$ mutant of yeast, which miss the last nine amino acids (GSRRYDSRT) that are found in the wild type, and show a permanently increased proteasomal activity that cannot be modulated/increased any further by SDS-exposure of the proteasome.[172] However, the characteristics of substrate binding or degradation are not or significantly less affected. In contrast, the $\alpha_7\Delta N$ mutant did not reveal any significant increase in proteolytic activity, while the $\alpha_3\alpha_7\Delta N$ mutant induced significantly more activity in casein degradation than either of the single deletions.[176] So, especially the YDR motive of the α_3-subunits seems to be essential for stabilization of the gate, involving allosteric effects that affect also the subunits α_2 and α_4.

Interestingly, in the archaea proteasome (from *T. acidophilum*), where the α-rings are built of seven identical subunits, some oligopeptides revealed the ability to induce a conformational change in the N-termini of the α-subunits resulting in an opening of the gate. In this conformational change, the last 13 amino acids are involved as well as a slight turn (about 4°) of every single subunit.

The same gate-opening reconfiguration can be induced by the attachment of PAN in its ATP-bound confirmation, as cryo-electron microscopic experiments have revealed.[177] The oligopeptides mentioned above (seven or more amino acid residues) are the different C-terminal sequences of PAN subunits[178] that are able to bind the gaps between the single α-subunits of the proteasome. Those residues are termed the HbXY motifs. After binding of PAN or an oligopeptide, the gate of the archaea proteasome "opens" by a structural rearrangement. The diameter of the "closed" gate is usually about 9 Å and thus notably smaller than the channel directing to the inner proteolytic chamber of the proteasome with a diameter of about 23 Å. As mentioned earlier, after "activation" the diameter of the open gate increases to about 13–20 Å. A similar

mechanism was revealed in studies of the PA26-binding: the gate structures were opened by the C-termini of PA26 and a so-called activation loop that induces both a movement of the N-terminal structures of the α-rings and a faint turning of every α-subunit.[177]

Usually, the 20S proteasome is found in the cell in its "inactive" state but can be "activated" by regulators, unfolded proteins, or proteasomal substrates. Furthermore, it has to be discriminated between the proteasomal peptidase and protease activities: while "peptidase activities" represent the degradation of small oligopeptides and are almost independent of the gate status, "protease activities" stand for the degradation of a whole unfolded protein and are considerably dependent on the gate status. This suggests that the gating α-subunits have only little interaction with small peptide fragments but a key function in the degradation of whole protein substrates.

B. The Proteasomal β-Subunits

In contrast to the gating/regulating function of the proteasomal α-units, the main task of the β-units is the proteolytic process itself.

The ancient archaea proteasome contains seven identical β-subunits in one ring and thus seven proteolytic centers, too. In contrast, further developed yeast and the mammalian proteasomes contain only three different centers per β-subunit ring, localized on the subunits β_1, β_2, and β_5. In 2002, Unno *et al.* proposed a novel N-terminal nucleophile hydrolase activity,[40,41] formed by the Thr8 residue of subunit β_7, after the analysis of bovine proteasomes' X-ray crystal structure. The proteolytic centers of the active subunits are found in the inner chamber of the 20S proteasome complex. Figure 4 shows the catalytic centers of the archaea proteasome. Each of the three known proteolytic subunits shows a different preference for substrate binding:

- β_1 Shows a peptidyl–glutamyl–peptide hydrolysing activity (caspase-like activity, cleaving after *acidic* amino acids, thus also termed as "post-glutamyl-peptide hydrolytic" activity).[179]
- β_2 Shows a trypsin-like activity, cleaving after *basic* amino acids.
- β5 Shows a chymotrypsin-like activity and cleaves after *neutral* amino acids.[180]

In all three active subunits, the active center is formed by the N-terminus (Thr1) of the corresponding proteasomal β-subunits (Fig. 5). Noteworthy, the proteases that show similar specificities like trypsin[181] or chymotrypsin[182] are serine-proteases. Typical products of proteasomal degradation are oligopeptides with lengths between 2 and 35 amino acids.[183] That distribution shows three different maxima: 2–3, 8–10, and 20–30 amino acids, while the average length is about 8–12 residues.[86]

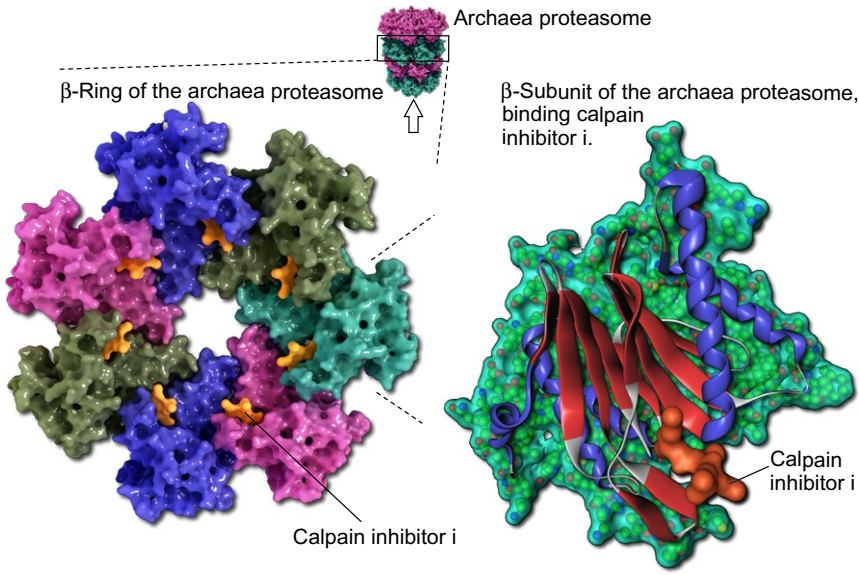

FIG. 4. Active centers of the archaea 20S proteasome. Here, the β-ring of the archaea proteasome from *Archaeoglobus fulgidus* is shown. The small icon in the upper left corner shows the angle of view (arrow) and the part of the proteasome the reader is looking at, seeing the side of the β-ring facing the proteolytic main chamber of the proteasome. The single identical β-subunits of the displayed archaea β-ring are color coded, and the small orange molecule bound to every single one is the calpain inhibitor i. The right image shows a single β-subunit in complex with calpain inhibitor i (orange).

Besides Thr1, the amino residues Asp17 and Lys33 turned out to be essential for the functionality of all three active centers. At the same time, the residues Ser129/166/169, which are localized surrounding Thr1, seem to be important for stabilization of the three-dimensional structure of each active center (Fig. 6). Possible allosteric effects between the single active centers are still discussed: Some experimental outcome suggests such interactions,[184] but most of the proteasomal inhibitors bind to the β_5-subunit, resulting only in a significant reduction of the associated specificity (chymotrypsin like), but no change in the activities of the other two proteolytic subunits. In contradiction to these experimental results is the "bite and chew" model from Kisselev[185] that proposes indeed allosteric interactions between the active subunits; according to this model, substrates for the β_5-subunit like Suc-LLVY-MNA or Suc-FLF-MNA significantly increased the activity of β_1 (caspase-like). In the same way, inhibiting substrates for one activity indirectly had an effect on the other two activities. Thus, Kisselev concluded a cyclic mechanism in the degradation of a protein substrate. The chymotrypsin-like activity of β_5 initiates degradation of an amino acid chain and

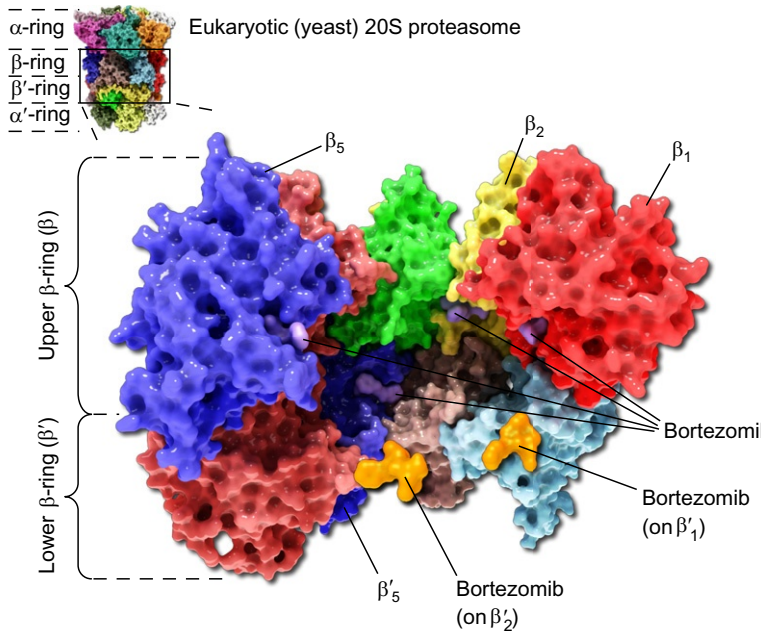

FIG. 5. Active centers of the eukaryotic 20S proteasome.

triggers further cleavage by the β_1-subunit (showing peptidyl–glutamyl–peptide hydrolysing activity); during β_1-mediated substrate cleavage ("chewing"), β_5 activity is inhibited. If no further cleavage by β_1 is possible, then β_5 is "reactivated," starting the cycle over and over again. According to Kisselev's model, allosteric interactions between the active subunits are essential for substrate degradation.[185] However, knockout mutants of yeast revealed that active proteasomal subunits are important for cellular survival and functionality (cell division) and showed that the proteolytic capacities of the single subunits vary in their importance: $\beta_5 >> \beta_2 > \beta_1$. This results in the fact that double knockout mutants β_1/β_5 and β_1/β_2 are still viable, while β_2/β_5 are not.[186,187]

C. Intracellular Assembly of the 20S Proteasome

The first step in proteasome assembly is the association of the α-ring. In order to prevent any unspecific oligomerization of the α-subunits, this process is guided by different chaperones. These chaperones are called proteasome assembly chaperones (PAC). Until now four different forms of these chaperones are known: PAC 1–4 (in humans)[188–190] and their equivalents from yeast,

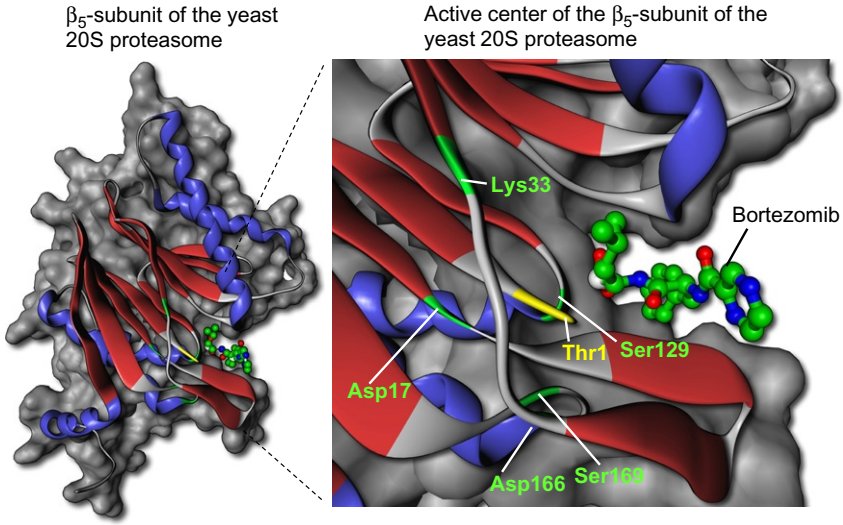

FIG. 6. Amino acids in the active center of the eukaryotic 20S proteasome. The small icon in the upper left corner shows the part of the whole 20S proteasome (from *Saccharomyces cerevisiae*) that is shown in the enlarged image: the two β-rings. Some of the subunits are removed in order to expose the main chamber with its six proteolytic centers overall. The upper β-ring still contains β_1 (red), β_2 (yellow), and β_5 (blue), and the lower ring only β_5' (blue). Every single of those active subunits binds a bortezomib molecule (purple) to its active center. The two bortezomib molecules that are bound to the removed active subunits of the lower β-ring (β_1' and β_2') are shown in orange. Here, the β_5-subunit of a 20S proteasome (from *S. cerevisiae*) is shown. The left part of the image shows the whole structure, and the right part the active center and the most important amino acids that are involved. The center binds a single molecule of bortezomib.

termed proteasome biogenesis-associated protein (Pba) 1–4.[191,192] However, such a terminology is not uniquely used and so sometimes in yeast, Pba1 is called POC1, Pba2 POC2 or PAC2, Pba3 PAC3 or Dmp2[188] or POC3, and finally Pba4 Dmp1 or POC4. These chaperones form two different heterodimers to become active: the first one is PAC1–PAC2 in humans and Pba1–Pba2 (POC1–POC2) in yeast; the second one is PAC3–PAC4 in humans and Pba3–Pba4 (Dmp1–Dmp2) in yeast.[189] PAC1–PAC2 are involved in the assembly of the α-ring. The PAC1–PAC2 heterodimer first binds to the subunits α_5 and α_7, followed by stepwise incorporation of α_6 and α_1 (both bind on the α_7 side), then followed by α_2 (binds to α_1), α_3 (binds α_2), and α_4 (binds both to α_3 and α_5), driven by mutual interactions.[193] This assembly is supplemented by the PAC3–PAC4 heterodimer, attaching to the α_2-subunit.

After this, the PAC3–PAC4 heterodimer (in yeast Pba3–Pba4/Dmp1–Dmp2) provides incorporation of the single β-subunits one after the other. In a first step, PAC3–PAC4 binds and incorporates first β_2 and then β_3. In further

steps, the subunits β_4, β_5, β_6, β_1, and β_7[145] are incorporated. At this stage, the active β-subunits (β_1, β_2, and β_5) are still in their inactive form (proβ_1, proβ_2, and proβ_5), due to a later removed prosequence. β_6 and β_7 are not proteolytic active, but nevertheless contain a prosequence. The PAC3–PAC4 heterodimer detaches from the complex after β_3 is incorporated. This ends up with the formation of a so-called half proteasome built with one α- and one β-ring. The corresponding intermediates of a complete α-ring binding different β-subunits including the so-called half-mer (a complete α-ring ring with all β-subunits incorporated except β_7) have already been identified, suggesting the sequential characteristic of that assembly.[194] Another identified intermediate is the so-called 13S (built with a complete α-ring and the subunits β2, β3, and β4).[195] Until now, the exact order of β-subunit incorporation in yeast is still unclear. In yeast, Ump1 binds after β_2, β_3, and β_4 are already recruited in the complete α-ring, while in humans it binds the α-ring together with β_2.[145]

So, two of those "half proteasomes" finally assemble at the holoproteasome, the functional 20S "core" particle. In yeast, assembly of the holoproteasome is mediated by the proteasome maturation factor Ump1[196,197] (also called "proteassemblin"[193,198] or "POMP",[199–201] for proteasome maturation protein). The human form of Ump1 is termed "hUmp1." One Ump1 binds to every single "half proteasome." After the last β-subunit (β_7) is incorporated into the "half-mer," the dimerization of two "half mers" is induced. That whole complex (also termed "16S intermediate") now contains a complete α- and β-ring, as well as one Ump1 and the PAC1–PAC2 heterodimer. In this process, the extended C-terminus of β_7 from one "half proteasome" interacts between the β_1- and β_2-subunit of the opposite β-ring from the other "half proteasome." Incorporation of β_7 seems to be a rate-limiting step in this process, since overexpression of β_7 massively increases 16S dimerization.[202] Assembly of the holoproteasome is followed by an autocatalytic "activation" of the proteolytic β-subunits: Their N-terminal ends are degraded (setting free the N-terminal Thr1 on β_1, β_2, and β_3), followed by degradation of both Ump1 molecules inside the proteolytic chamber as the first substrate of the functional 20S holoproteasome. After this, the two attached PAC1–PAC2 heterodimers are degraded, too.

Another protein involved in 16S dimerization is Hsc73: one is bound to each 16S, and detaches after assembly of both 16S. Since intracellular immunolocalization revealed a stable binding of Ump1 to the outer membrane of the ER,[199] it is supposed that the complete α-ring is bound to Ump1 and is not released before all of the β-subunits are recruited. As much as 75–88% of the proteasome maturation intermediates were colocalized with the ER membrane, while formation of the holoproteasome seems to take place in the cytosol of the cell.[203]

Interestingly, in contrast, assembly of the archaea proteasome (from *T. acidophilum*), which only contains a single type of both α- and β-subunits, is independent of any chaperone proteins: a coexpression of both subunits in

Escherichia coli resulted in the formation of functional archaea 20S proteasomes.[36,146] This auto-assembly is enabled by characteristic loops (L-loops) of the α-subunits that can assemble with other α-subunits. The β-subunits have no such structure and, thus, their assembly depends of a complete α-ring that functions as an assembly "platform."[204]

D. Modeling of the 20S Proteasomal Proteolysis

In order to describe 20S proteasomal (with and without regulators) degradation, several mathematical models have been developed. For the prediction of antigens presented by the immune system, a model describing cleavage sites and fragment length of a given oligopeptide may be useful. For this purpose, two main strategies exist: one concentrates on the predictions of the fragments generated during proteolytic degradation of a substrate, though the results may become inaccurate if the produced fragments overlap[205]; the other strategy is focused on the prediction of potential cleavage sites, returning the probability of the occurrence of a specific fragment.[206] In this model, the produced fragments are normally determined by a potential cleavage site between two amino acids with respect to one or two residues neighboring that locus on each side. In contrast, other models try to simulate the process of degradation from a mechanistic point of view, considering kinetic rates in order to calculate the velocity of proteolytic degradation for a given substrate protein. The computed results have to be proven in an empiric way, while the models are based on "learning sets" that result from the fragments occurring after degradation of a known protein.

III. Regulation of the 20S Proteasome

In order to prevent uncontrolled and unregulated proteolytic degradation in a cell, the proteasomal degradation has to be carefully regulated. Therefore, during evolution, a set of regulators has developed that are able to control both recognition and degradation of proteasomal substrates. The regulated entrance into the proteolytic inner chamber of the 20S core proteasome is realized via the gating α-rings that may be "activated" by binding to the exposed hydrophobic stretches of oxidatively damaged proteins. Usually, these hydrophobic structures are buried inside the soluble proteins, but after (oxidative) modification, these structures may be exposed, whereas native and correctly folded proteins have to be targeted (via polyubiquitination) for proteasomal degradation. The most important regulator for the recognition of ubiquitin-labeled proteins is the so-called 19S regulator complex that cooperates with another cellular machinery, the ubiquitination system.

A. The 19S Regulator

The 19S regulator, also known as "PA700" or "proteasome activator 700 kDa"[207–210] is built up of two main structures: a ring-shaped base that binds to the α-rings of the 20S "core" proteasome and a lid that recognizes and binds polyubiquitinated proteins, thus regulating substrate entrance to the 20S proteasome.

The base ring contains at least 10 different subunits (Rpt1–Rpt6, Rpn1, Rpn2, Rpn10, and Rpn13). The lid contains nine subunits (Rpn3, Rpn5–Rpn9, Rpn11, Rpn12, and Rpn15) that are also termed "DSS1" or "SHFM1" in humans, and "SEM1" in yeast. The Rpt-subunits show an ATPase activity, while the Rpn-subunits do not. Furthermore, Rpn11 in the lid contains a Zn^{2+}-dependent proteolytic center that is able to catalyze the proteolytic degradation of polyubiquitin chains that label native substrate proteins for degradation; after this, the ubiquitin molecules are released for reuse of the polyubiquitination machinery.

The Rpt2 (in humans also called S4 or p56, and in yeast YTA5 or mts2), Rpt3 (human form termed as S6, Tbp7, or P48, and the yeast form as YTA2), and Rpt5 (S60 or Tbp1 in humans, and YTA1 in yeast) subunits of the base ring play a role in gate opening of the attached α-subunits of the 20S proteasome,[211] while Rpn10 (S5a or Mbp1 in humans, and SUN1, MCB1, or pus1 in yeast) and Rpn13 (ADRM1 in humans, and DAQ1 in yeast) function as polyubiquitin receptors.[211] The main role of the small protein ubiquitin is the labeling of native proteins for proteasomal degradation, in order to regulate their intracellular amount of life span.

One 19S regulator might attach to each of the α-rings of the 20S "core" proteasome, forming a large particle of about 2 MDa,[212] termed the "26S proteasome." In a mechanism that is known from other regulators of the proteasome, the 19S particle makes substrate access to the "core" particle easier by "opening" the gating α-rings. It has been shown in yeast that the Rpt2-ATPase of the base ring is involved in this process.[86] Until now, no data from X-ray crystallography of the 19S regulator are available[211] and only some of the interactions of the single subunits are known[213]; thus, in Fig. 7, only a hypothetical structure of the 19S regulator cap can be displayed.

B. The Immunoproteasome

A special inducible form of the 20S proteasome is called the immunoproteasome (i20S). The i20S proteasome can be induced by IFN-γ, and on the site of the subunits β_1, β_2, and β_5 their inducible equivalents (β_{1i}, β_{2i}, and β_{5i}) are located.[140,214–219] To achieve this, proteasomes have to be synthetized de novo.[220,221] Further inducers are both tumor necrosis factor alpha (TNF-α)[222,223] and lipopolysaccharides.[224] In general, the three inducible β-subunits

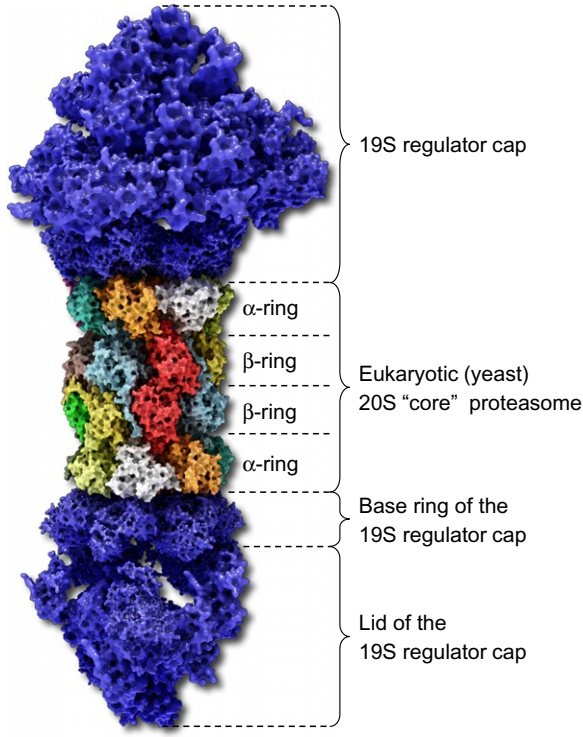

FIG. 7. A model of the structure of the eukaryotic 26S proteasome. This image shows a model of a eukaryotic 20S "core" proteasome (from *Saccharomyces cerevisiae*) bound to two 19S regulator caps. Since there are no data from X-ray structure analysis for the 19S regulator, this is just a model showing the very basic shape of 19S that is divided into a "base"-ring containing six subunits binding the α-ring of the 20S "core" proteasome and a "lid" containing nine subunits, responsible for recognition, binding, and unfolding (in an ATP-dependent way) of polyubiquitinated substrates, feeding them into the 20S proteasome for terminal degradation. Overall, the 19S regulator cap contains some 18 different subunits (see Table I), 10 for the whole "base" structure and nine for the "lid."

are homologs of the constitutive ones and are indicated by an additional "i" (i20S); other names found in the literature for those inducible proteasomal subunits are low-molecular-weight protein 2 (LMP2, for β_{1i}), multicatalytic endopeptidase-like-complex-1 (MELC1, for β_{2i}), and LMP7 (for for β_{5i}).[225–227]

At the same time, the so-called 11S regulator (also known as PA28, PA26, or REG) is induced by the same cellular signal cascades. The major function of the immunoproteasome is the production of a specific short oligopeptide product pattern that can be presented by the major histocompatibility complex class I (MHC-I) on the cell's surface in immune response. Typical products of

the immunoproteasome are short protein fragments, made up of about 8–10 amino acids that are optimized for MHC-I-presentation. Since i20S induction mainly depends on the amount of cytokines that are released in the tissue, it is suggested that immunoproteasomes mainly release new self-determinants that prevent autoimmune response in the surrounding uninfected cells.[228] On the other hand, Yewdell proposed in 2005[228] that the main functions of i20S are not the generation of MHC-I-presented antigens and that further research in this field is needed.

INF-γ induces both the proteasome maturation factor Ump1 and the inducible forms of the proteolytic proteasomal subunits; paradoxically, the mRNA of Ump1 is increased, while the amount of free Ump1 decreases (as found in HeLa cells) and the half-life of that protein is reduced from 82 to only 21 min.[229] It turns out that this is caused by a massively increased proteasome formation that enhanced degradation of Ump1, which is the first proteasomal substrate (see above) after assembly of the holoproteasome. Considering this, the turnover of Ump1 can be used as an indicator for the formation rate of functional proteasomes. The formation of the immunoproteasome is turned into a highly dynamic process due to two main factors. The first one is the fact that the processed β_{5i} has a higher affinity to Ump1 than the propeptide proβ_{5i}, which generates a higher rate of i20S formation compared to the one of c20S (this fact suggests two different binding sites).[229] The second factor is the much shorter half-life of i20S (about 27 h) compared to c20S (about 8–12 days).[230–232] The interplay of these two factors allows both a very quick expression and a fast removal of i20S. Seven days of continuous stimulation can completely replace c20S by i20S,[21] while after a shorter stimulus the ratio of c20S/i20S decreases.

However, after expression of the inducible subunits, the de novo assembled proteasomes do not always contain all six inducible catalytic subunits; many forms are found that only show between 1 and 5 of the inducible β_i-subunits, while the others are the constitutive ones. Whether there is a special need or function for such intermediate proteasomes is still unclear. Nevertheless, both β_{5i} and Ump1 seem to be essential for the formation of i20S: cells that do not express β_{5i} or Ump1 are not able to form i20S, even after IFN-γ treatment.

In Ump1-knockdown cells, proteasomal-mediated proteolysis decreases rapidly to 60% after 24 h and to 40% after 48 h,[229] while the overall amount of cellular proteasomes is reduced significantly.[233] In the same way, an overexpression of Ump1 increases the proteasomal-mediated cellular proteolysis.[200] The number of β_i-subunits increases constantly over time in muscle tissue of aged rats compared to young ones (a three- to sixfold increase was found). The same results were detected for neurons, astrocytes, and endothelial cells in the hippocampus of elderly humans (about 70 years of age) compared to a younger control group (about 42 years of age).[234] Considering this,

the inducible β-subunits seem to accumulate over time in cells and tissues that normally only contain the "housekeeping" form of the proteasome (c20S) and especially in cells prone to postmitotic aging, as neurons and muscle cells.

C. The Thymus-Specific Proteasome (Thymoproteasome)

Another specific proteasomal subunit is the so-called β_{5t}, which was first discovered in mice, exclusively in cortical thymic epithelial cells (cTECs).[235] β_{5t} plays an important role in the positive selection of thymocytes.[236] Accordingly, the term "thymoproteasome" was suggested for the "β_{1i}, β_{2i}, and β_{5t}-configuration" of the 20S proteasome. It turns out that the thymoproteasome is responsible for the generation of short antigenic oligopeptides presented on the cell surface, resulting in the positive selection of $CD8^+$ T-cells. The self-peptide production is dependent on the thymoproteasome and is essential for the development of an immune-competent repertoire of $CD8^+$ T-cells.[237]

Genomic analyses of the gene coding β_{5t} (PSMB11) was performed by Sutoh *et al.*.[238] It turned out that teleost fish have two functional copies of PSMB11 (PSMB11a and PSMB11b), while chickens, turkeys, and zebra fish lost PSMB11, expressing neither thymo- nor immunoproteasomes. In mammals, reptiles, amphibians, and teleost fishes, PSMB11 is located close to PSMB5, which codes β_5 of the constitutive 20S proteasome. These results suggest that PSMB11 may originate from the older PSMB5 by tandem duplication. β_{5t} shows a close relation to both β_5 and β_{5i} and was found to be incorporated in about 20% of the thymic proteasomes.

In proteasomes containing β_{5t}, the inducible subunits β_{1i} and β_{2i} are preferentially incorporated compared to the constitutive ones β_1 and β_2. Antigens that are presented by the MHC-I[239–241] complexes show hydrophobic C-termini that function as an anchor in MHC-I binding[242] and that result from the characteristics of β_{5t}-mediated cleavage. In contrast to β_5 and β_{5i}, the proteolytic center of β_{5t} contains hydrophobic amino residues that reduce the chymotrypsin-like proteasomal activity by 60–70%, without any effect on the other two activities.[235] The maximal velocity of proteolysis, as well as the Michaelis constant, is lower in β_{5t} compared to both β_5 and β_{5i}. The result is a significantly decreased amount of oligopeptides released with a hydrophobic C-terminus that are preferably incorporated in the binding grove of MHC-I. Thus, β_{5t} seems to reduce the amount of MHC-I-presentable antigens.

The result is both a lowered production and presentation of MHC-I-bound oligopeptides and thus a decreased interaction of cTECs with the αβ-T-cell antigen receptor, which causes a higher probability of positive selection of those cells.[235,236]

β_{5i}-deficient mouse models revealed an imperfect development of $CD8^+$ T-cells and a resulting decrease of those cells by about 80%,[243] suggesting that β_{5i} may enhance the selection of $CD8^+$ T-cells. However, the amount of antigen-loaded MHC-I molecules presented on the surface of β_{5t}-deficient cells did not change. The lysosomal cathepsin S is a necessary factor in antigen presentation in most cells, but in contrast, this task is performed by cathepsin L in thymus cortical epithelial cells. After deletion of cathepsin L in those cells, the selection of $CD4^+$ was reduced without any influence on the amount of MHC-II. The presentation of different antigenic oligopeptides by MHC-I (in $CD8^+$ cells mediated by the proteasome) and MHC-II (in $CD4^+$ cells mediated by the lysosomal system/cathepsin L) decides the positive or negative selection of mature T-cells. T-cells showing a high affinity to self-antigens are sorted out, otherwise causing autoimmune reactions. In contrast, T-cells with a low affinity to MHC molecules will maturate, while mediocre affinity usually triggers positive selection.

D. The 11S Regulator

The 11S regulator of the proteasome, also called "PA28," "REG," or "PA26" (in *T. brucei*), has different structures. Three different subunits of the 11S activator are known: PA28α, PA28β, and PA28γ. There are hexameric or heptameric structures described and, in addition to that, under defined conditions, various homo- or heteropolymerization products of the individual subunits are formed. Results indicated first that the 11S regulator has an $\alpha_3\beta_3$ structure, where both subunits were arranged alternatively.[244,245] However, later, an $\alpha_3\beta_4$ complex was detected. This particle interestingly contains a β–β dimer, but no α-subunit dimer.[246] There seem to exist several 11S forms in cells, as $PA28\alpha_3\beta_3$, $PA28\alpha_4\beta_3$, $PA28\alpha_3\beta_4$ (in each case with alternating arrangement of the α- and β-subunits), and $PA28\gamma_7$.[247] If PA28α and PA28β subunits are mixed in vitro in a ratio of α to β of 1.2, both $PA\alpha_3\beta_4$ and $PA\alpha_4\beta_3$ can be detected.[246] The PA28α-heptamer is instable, but can be formed in vitro, whereas the PA28β-heptamer cannot. However, PA28γ forms a stable heptamer.[248,249]

The base diameter of the 11S regulator is some 90 Å and the complex is about 60 Å in height; with a central cavity 20–30 Å wide. In general, the various 11S regulators are able to bind to the outer α-rings of the proteasome and change substrate degradation properties. However, like the degradation by the 20S proteasome, the degradation of substrates by the 11S–20S complex is ATP-independent (Fig. 8), suggesting that only unfolded proteins are substrates.[247] 11S binding increases the β_2-catalyzed cleavage about 10-fold and the β_1- and β_5-catalyzed cleavages by about 50-fold.[250,251] The PA28γ-isoform activates only β_2.[248] Binding of PA28 changes the conformation of the proteasome, thereby making it more efficient in proteolytic activity.[252]

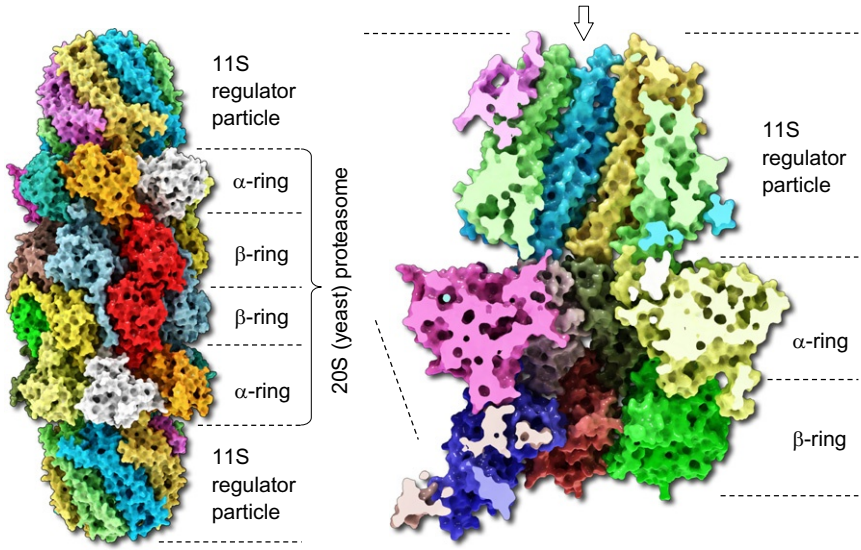

FIG. 8. The immunoproteasome and the 11S regulator. Here, the structure of the so-called immunoproteasome, a eukaryotic 20S "core" proteasome capped with two 11S regulators, is shown on the left side of the image. The right part shows a cross-section of the 11S regulator cap and an α- and β-ring of the 20S proteasome (a half proteasome). Please note that the gate of the "core" proteasome is "opened" and a channel through the regulator cap (arrow above the structure) enables substrate access to the main chamber of 20S and the proteolytic centers within.

Interestingly, PA28α and PA28β are located only in the cytosol, but all three PA28 isoforms can be found in the nucleus.[253,254] The PA28γ isoform activates only β_2.[248] The PA28α,β regulators seem to be involved in the generation of oligopeptides in the immune response (see also immunoproteasome). So, PA28β knockout animals have a reduced immune function.[255] Interestingly, PA28γ-knockout animals show malfunctions in cell cycle regulation and apoptosis,[255,256] due to the role of PA28γ_7 in the degradation of nuclear lysine-free proteins.[257,258]

E. The Hybrid Proteasome (PA28–20S–PA700)

The hybrid proteasome, thus termed by Tanahashi *et al.*,[259] contains both a PA700 (19S) and a PA28 (11S) regulator cap. Each of the regulators is bound to either end of the 20S proteasome. Tanahashi *et al.* determined the relative amounts of the different possible hybrid proteasomes (see Fig. 9). The 11S regulator is present in its hexameric form $(\alpha\beta)_3$ and the two different heptameric versions $(\alpha\beta)_3\alpha$ and $(\alpha\beta)_3\beta$ in hybrid proteasomes, whereas the

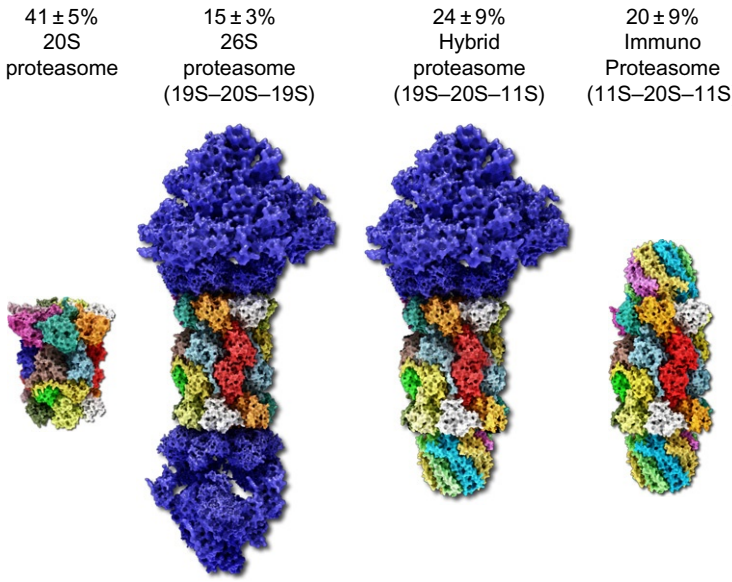

FIG. 9. The relative amount of proteasome types as found in the cytosol of HeLa cells. Here, the relative amount of proteasomal types are shown, starting with the uncapped 20S (on the left, "20S proteasome") and the amounts of the different fractions like 26S proteasome (19S–20S–19S, second from left), hybrid proteasome (19S–20S–11S, second from right), and the immunoproteasome (11S–20S–11S, on the right).

heptameric PA28γ (γ_7) is not. Via immunoprecipitation it is possible to isolate the hybrid proteasomal forms that can be induced in cells in an IFN-γ-mediated way.[260] ATP is needed for attachment of PA28 to the 20S core proteasome, as well as in the ATP-dependent protein degradation of the PA28–20S–PA700 complex.[259] Considering the ATP-dependence of the regulator "core" particle attachment, the formation of PA28–20S–PA700 is very similar to that of PA700–20S–PA700 (the 26S proteasome).

Though the exact cellular function of those hybrid proteasomes is still unknown, it might be possible that the proteolytic specificities of the core proteasome bound to 19S change by binding of an additional PA28 regulator,[260] causing a different set of oligopeptide products produced during proteolytic degradation of a substrate. The proteolytic activity of 26S was found to be higher than that shown by the hybrid forms. However, a cooperation of immunoproteasome and hybrid forms of the proteasome in antigen processing might be possible, since both proteasomal forms can be induced via IFN-γ. So, it was suggested that most of the proteolysis required for MHC-I antigen presentation is performed via the 26S proteasome or the so-called hybrid

proteasomes (PA28–20S–19S). Since the PA28 activator cap is not able to mediate the degradation of natively folded proteins, it appears that the substrate protein has to be unfolded first in an ATP-dependent way that is mediated by the 19S-regulator cap.

Whether the short oligopeptide products that are released after 19S–20S-mediated proteolytic degradation are further processed by another proteasome, perhaps containing a PA28 regulator protein, is still unclear. According to the "molecular coupling hypothesis", a hybrid proteasome is attached to a TAP1–TAP2 complex (TAP, transporter associated with antigen processing) protein channel in the ER membrane via the PA28 regulator cap. So, a polyubiquitinated and natively folded antigenic protein is recognized and unfolded by the 19S regulator cap and then guided into the core proteasome (in this special case, an immunoproteasome), where it is degraded. Fragment lengths as well as the fragment characteristics are influenced by the PA28 proteasomal regulator cap that delivers antigenic oligopeptide fragments directly into the TAP1–TAP2 protein complex. The advantage of such a direct transport is the protection of the fragments from cytosolic proteases. Further processing of the antigenic fragments (like N-terminal trimming) is done via ER-resident proteases as endoplasmic reticulum aminopeptidase associated with antigen processing and endoplasmic reticulum aminopeptidase 1 and 2. This proposed mechanism was inspired by the identification of the highly conserved "KEKE"-motifs at the distal side of PA28 that are not involved in binding or activation of the 20S "core" proteasome. KEKE motifs may be involved in protein–protein interactions and have been found in four subunits of the 20S proteasome and five subunits of the 19S proteasomal regulator cap. Furthermore, they have been found in both HSP90 and calnexin, two other proteins that play a role in epitope loading of MHC-I proteins. Another hypothesis gaining from those results suggests that heat shock proteins may be involved in immune response.[261,262] However, this idea has been disproven in 2006 using the SIINFEKL epitope of ovalbumin: experiments revealed that there is no promotion of its MHC-I presentation.[263]

F. The PA200 Regulator Protein

The PA200 proteasomal activator cap is exclusively found in the nucleus of mammalian cells. The yeast homolog (from *Saccharomyces cerevisiae*), known as Blm10, shows a sequence homology of about 20%[264] to the mammalian form. First investigations of the PA200 structure and its binding to the 20S core proteasome were done using electron microscopy. Three-dimensional reconstructions of the gained data showed a slightly asymmetric dome structure (100 Å in diameter, as the 20S proteasomal α-ring, and about 60 Å in high) with an inner cavity that sits on one or both the α-rings like a cap[265] (Fig. 10). Differently from the other proteasomal regulators, PA200 is a monomeric

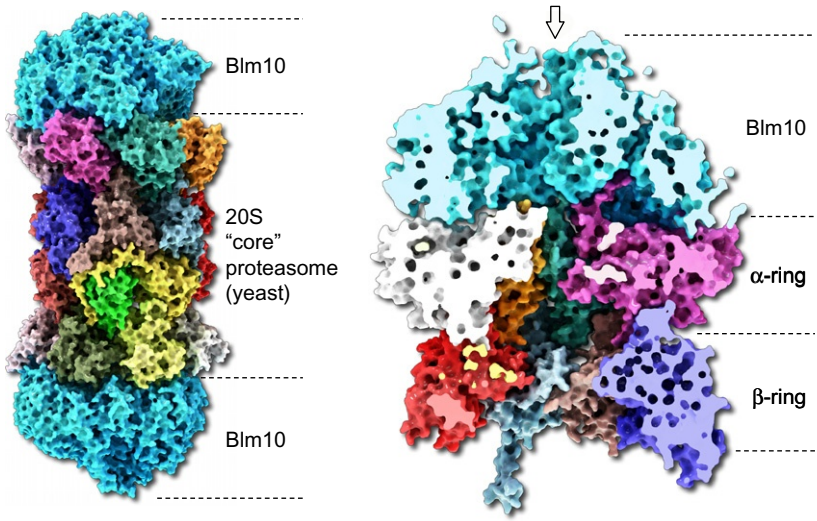

FIG. 10. The Blm10 regulator cap. The left part of the image shows a 20S "core" proteasome (from *Saccharomyces cerevisiae*) with two Blm10 regulator caps (the light blue dome-shaped structure on both ends of 20S) attached. The right part of the image shows a more detailed cross-section of a single Blm10 cap and one α- and β-ring of the corresponding 20S proteasome. The cross-section shows both an activated α-ring opening a channel to the inside of the proteasome and a channel in the Blm10 regulator cap (arrow on the right image) that enables substrate access, significantly increasing substrate turnover.

structure of about 200 kDa. PA200 binding to the 20S core proteasome is mediated by a structure that contains several HEAT-repeats and that contacts almost every α-subunit, except α_7. The yeast form (Blm10) binds to every single α-subunit.[264] Normally, the entrance to the inner proteolytic chamber is regulated by the N-termini of the α-subunits. This suggests that the PA200 regulator changes its structure in a way that enables enhanced substrate access to the "core" particle. This rearrangement turns out to be the main mechanism of activation by reconstructions from electron microscopic data.[265]

In the same way as the 11S regulator, PA200 increases the degradation of small protein fragments and releases fluorescent degradation products, but is not able to process a natively folded protein. A ratio of 20S/PA200–20S/PA200–20S–PA200 of 50:40:10 was measured using electron microscopic investigation of isolated proteasomes and PA200–proteasome complexes from bovine testes.[265] About the function of PA200, the current knowledge is very limited. Interestingly, experiments of gene deletion or overexpression did not induce significant change in the phenotype.[264] Only a role of PA200 in DNA repair after exposure to ionizing radiation or oxidizing agents seems to be ensured.[132]

So, in response to ionizing radiation, PA200 is expressed and accumulates in its hybrid form on chromatin.[133] After PA200 knockdown, cells showed genomic instabilities and reduced survival. The genome-stabilizing functions of PA200 seem to be a result of its ability to enhance the proteasomal β_1-mediated peptidyl—glutamyl-like proteolysis.[266] However, deletion of Blm10 in the yeast A364a-strain did not result in any effect on growth or viability after treatment of the cells with DNA-damaging agents like bleomycin or phleomycin.[264] Furthermore, no increased susceptibility of A364a to UV- or γ-irradiation, methyl methane sulfonate, camptothecin, or hydroxyurea could be detected. However, overexpression of Blm10 resulted in a reduced growth, but this can be an effect of increased binding to the 20S proteasome, thus detracting activity from other cellular functions of that protease. The presence of PA200 in the nucleus enables the formation of another proteasome complex: in yeast, Blm10 is able to form PA200–20S–19S.[132,267] After HeLa treatment with ionizing irradiation, immunoprecipitation revealed a coprecipitation of PA200 in complex with 20S–19S, even if the amounts of 20S and 19S were not increased. Thus, irradiation seems to induce the formation of the PA200–20S–19S complex in a DNA-damage response-mediated way.[266] Twenty-four hours after irradiation, the PA200–20S–19S complex showed an accumulation on chromatin. The trypsin-like (mediated by the β_2-subunit of the proteasome) activity associated with the chromatin showed a sixfold increase, and the peptidyl–glutamyl-like one (mediated by the β_1-subunit) up to a 19-fold increase, accompanied by a five- to eightfold increased amount of 20S on the chromatin.[268] That accumulation seems to be independent of ATM (a PI3-like kinase),[269,270] which starts the signal cascade after irradiation-mediated stress via triggering of the tumor suppressor p53.[271,272] One important function of PA200 might be an enhancement of the β_1 activity of the proteasome that is essential for the cellular survival after exposure to ionizing irradiation.[266]

IV. Conclusion

As shown in this chapter, the proteasomal system is complex and far from being well understood. We referred in this chapter only to the principal structures of the proteasome, not describing the interaction with the ubiquitination machinery, or other proteasomal regulators; it becomes clear that one can hardly imagine any aspect of cellular life not related to the function of the ubiquitin–proteasome system. The importance of this system is further underlined by the fact that it appeared early in evolution, as in Archae bacteria, and evolved to a more complex structure with new regulators and specialized subunits. The evolution toward function and organ-specific isoforms underlines this chapter.

In the further chapters of this book, the functions of the proteasome will be described in more detail, and the role of the proteasome in a selection of diseases and cellular stress situations will be highlighted, always on the basis of the principal structural features of the proteasome described above.

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