The unique functions of tissue-specific proteasomes

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The 26S proteasome is the main protease in eukaryotes. Proteolysis occurs within the cylindrical 20S proteasome that is constitutively expressed in most tissues. However, three tissue-specific versions of the 20S proteasome have been discovered to date. The immunoproteasome is optimized to process antigens and it directs the differentiation of T helper (Th) cells. The thymoproteasome is selectively expressed in cortical epithelial cells of the thymus where it plays an essential role in the positive selection of T lymphocytes. Finally, the spermatoproteasome is found in the testes where it is required during spermatogenesis. Here, we outline how tissue-specific proteasomes adapt to functional needs in their respective tissues and how their selective inhibition may be used to interfere with autoimmune diseases and cancer.

The proteasome: evolution towards complexity and specialization

The proteasome is an ancient enzyme that has steadily evolved towards a higher complexity of subunits and regulators while preserving its cylindrical architecture constituted from four stacked rings. In the archebacterium *Thermoplasma acidophilum*, the 20S proteasome consists of two outer rings with seven copies of a single α-type subunit and two inner rings with seven copies of the same β-type subunit [1] (Figure 1). In the eubacterial actinomycete *Rhodococcus* sp., the 20S proteasome is constructed from two different α-type and two different β-type subunits [2], whereas in the eukaryotic yeast *Saccharomyces cerevisiae*, it consists of seven different α-type and seven different β-type subunits [3]. Proteolysis takes place in the inner chamber of the proteasome formed by the two β rings. Although each of the β subunits of the archebacterial and eubacterial proteasomes carry peptidolytically active centers, only three of the seven eukaryotic β-type subunits are catalytically active. It appears that the increase in complexity is accompanied with a reduction in the number of active β subunits; the driving force for which has remained elusive. This tendency goes along with a

specialization of substrate specificity of these three β subunits, which is measured via the hydrolysis of small fluorogenic peptides with certain amino acids in the P1 position. These define the chymotrypsin-like activity (cleavage after hydrophobic residues), the trypsin-like activity (cleavage after basic residues), and the caspase-like activity of the proteasome (cleavage after acidic residues). Although the archaeal and bacterial proteasomes only show chymotrypsin-like activity, the eukaryotic proteasomes show all three activities that are associated with the β1 (caspase-like activity), β2 (trypsin-like activity), and β5 (chymotrypsin-like activity) subunits. These β-type subunits of eukaryotes have been named according to their position in the β ring [3] (Figure 1).

Interestingly, for the normal housekeeping function of the proteasome in proteolysis of regulatory or misfolded proteins, this diversification is not necessary. Yeast mutants that only harbor the chymotrypsin-like activity remained viable [4]. Whether specialized proteasome subunits are needed to cope with an enhanced protein degradation load is currently debated (see below). However, when looking at the peptide products of the proteasome, the cleavage preferences of β1, β2, and β5 make a big difference. Proteasome products are used by the immune system to enable antigen recognition by T lymphocytes and proteasomal peptide products may even have as yet unknown signaling functions in spermatogenesis or other aspects of cell biology. The functional specialization of β1, β2, and β5 enables the modification of the cleavage preferences of the proteasome by replacing them with inducible or tissue-specific homologs. Next to the β1–β2–β5 containing 'constitutive' proteasome, three further proteasome subtypes with differing subunit composition have been found: the immunoproteasome, the thymoproteasome, and the spermatoproteasome (Figure 2). Recent insights, such as how their special subunit composition enables them to serve functions that the constitutive proteasome can only exert suboptimally, are discussed in this review.

The immunoproteasome in antigen processing

The immunoproteasome is a tissue-specific complex because it is continuously expressed in cells of the immune system such as T cells, B cells, monocytes, macrophages, dendritic cells, or medullary thymic epithelial cells [5]. However, it is also strongly inducible by the proinflammatory cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α in virtually all tissues except for the brain, where

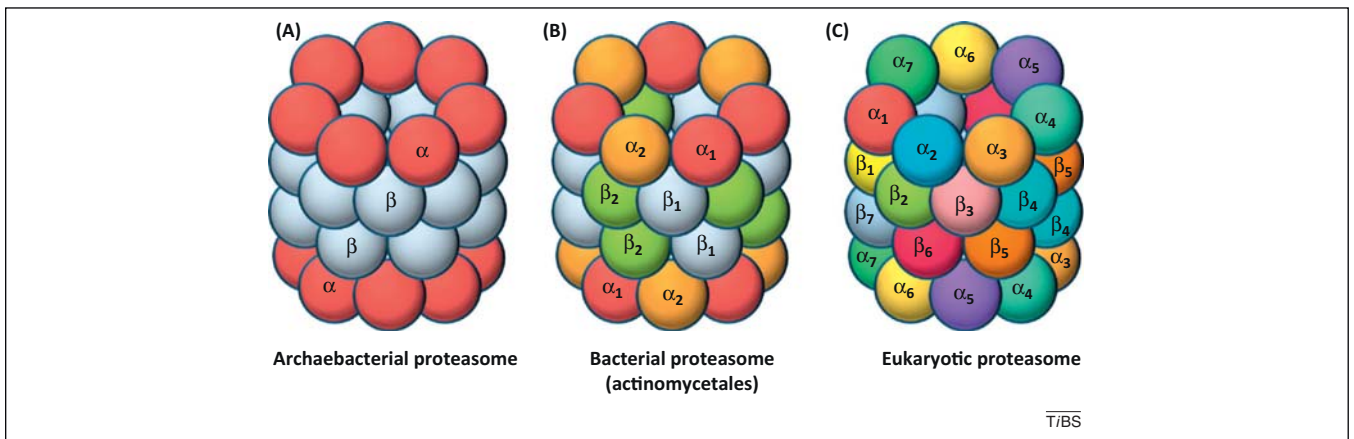**Figure 1**. The proteasome in three kingdoms of life. 20S proteasome models in the barrel-shaped $\alpha_{1-7}-_{1-7}\beta_{1-7}\alpha_{1-7}$ pattern. **(A)** Proteasome from archaebacterium *Thermoplasma acidophilum*. The archaebacterial proteasome is composed of two outer and two inner rings; each of which contains seven identical α subunits (in red) or β subunit (gray), respectively. **(B)** Among bacteria, proteasomes have been found in eubacterial actinomycetales. The proteasome from *Rhodococcus erythropolis* is built of two different α (red and orange) and two different β subunits (gray and green), which are most likely randomly arranged in the respective α and β rings. **(C)** Proteasome from the eukaryote *Saccharomyces cerevisiae*. Seven different α and seven different β subunits have their defined positions in the eukaryotic 20S proteasome.

immunoproteasome expression is largely confined to microglia and invading leukocytes [6]. Remarkably, immunoproteasomes replace up to 90% of constitutive proteasomes in tissues in the course of viral, bacterial, or fungal infections [5–7], which raises the question why the immune system induces such an extensive change in proteasome composition. Interestingly, this argues that immunoproteasomes can take over the housekeeping functions of constitutive proteasomes for several days without harming the inflamed tissues.

In the immunoproteasome all three active site bearing subunits of the constitutive 20S proteasome (β1, β2, and β5) are replaced by homologous, cytokine-inducible subunits named β1i [low molecular mass polypeptide (LMP)2], β2i [(multicatalytic endopeptidase complex-like-1 (MECL-1)], and β5i (LMP7), respectively. Two subunits of the immunoproteasome, β1i and β5i, were discovered during genomic sequencing of the major histocompatibility complex (MHC) gene locus over two decades ago, whereas the β2i subunit was discovered later because it is not encoded in the MHC locus [8]. Many members of the MHC class I and II antigen presentation processing pathways are encoded within the MHC locus, which had focused immunoproteasome research on their role in antigen processing [9].

Proteasome activity is required for MHC class I but not class II restricted antigen presentation. This was shown with broad spectrum proteasome inhibitors that interfered with MHC class I release from the endoplasmic reticulum (ER) and class I cell surface expression [10–12], whereas the classical MHC class II pathway was barely affected by them. The proteasome generates peptide fragments from protein antigens that bind into the peptide-binding groove of class I molecules. They can be generated in their final length by proteasomes either in the cytoplasm or in the nucleus. They can also be generated as N-terminally extended precursors that are subsequently trimmed either in the cytoplasm or after transport into the lumen of the ER where they meet partially folded MHC class I/β2-microglobulin complexes [8]. Only if a given peptide binds tightly into the peptide ligand cleft of class I molecules does the trimeric complex finally fold and get released from the ER

to the cell surface. There, the antigen receptor of cytotoxic T lymphocytes can recognize peptide plus MHC and kill the cell that presents their cognate antigens. The criteria for tight peptide–class I binding are (i) a length of 8–9 amino acids and (ii) the presence of anchor residues at the C terminus of the peptide and somewhere else within the peptide sequence. The C-terminal anchor residues in humans can be of basic or hydrophobic nature, whereas mouse class I molecules only accept peptides with hydrophobic C termini. These rather tight acceptance criteria render it difficult to cleave out a sufficient number of peptide ligands to mount an immune response especially against small viruses that encode only a few proteins. It is certainly one of the contributions of the immunoproteasome to a successful cellular immune response against viruses or intracellular bacteria that its altered cleavage priorities enhance the versatility of antigen processing and the chance to generate a pivotal antigenic peptide [8].

The recently reported high-resolution X-ray crystallographic structures of the mouse constitutive proteasome and immunoproteasome allow a side by side comparison of the three different substrate-binding pockets that define the cleavage priorities of the two complexes [13]. The most striking difference is that between β1 and β1i. Although the former accommodates peptides with an acidic P1 residue (caspase-like activity), the latter binds peptides with a hydrophobic P1 residue. For unknown reasons, the hundreds of allelic variants of mouse and human MHC class I molecules do not accept peptide ligands with acidic C-terminal anchor residues. The proteasome is the main determiner of the C terminus of antigenic peptides, therefore, β1 products are lost for antigen presentation. The replacement of β1 with β1i avoids this loss, generating useful class I ligands with hydrophobic C termini, therefore enough ligands become available to sustain the approximately tenfold upregulation of class I cell surface expression after stimulation with IFN-γ. This advantage most likely explains, at least in part, a phenotype of immunoproteasome-deficient mice: they show a 50% reduced MHC class I surface expression of lymphocytes and dendritic cells [14–16]. Also the replacement of β5 by β5i
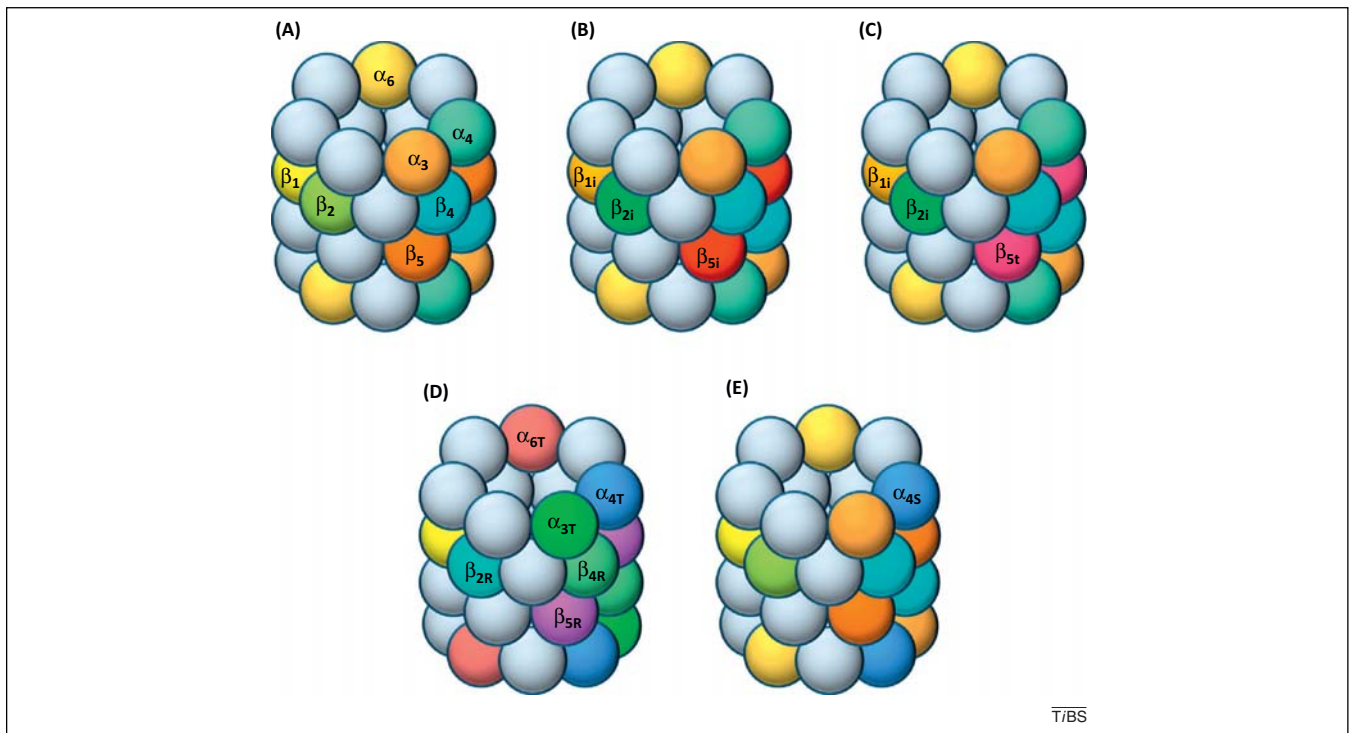
Figure 2. Eukaryotic 20S proteasome subtypes. **(A)** Constitutive proteasome; subunits that differ in tissue specific 20S proteasomes are highlighted in different colors. **(B)** Vertebrates possessing an adaptive immune system can additionally express the inducible proteolytic subunits β1i [low molecular mass polypeptide (LMP)2], β2i (multicatalytic endopeptidase complex-like-1; MECL-1), and β5i (LMP7) that are incorporated into immunoproteasomes. **(C)** The thymoproteasome expressed in cortical thymic epithelial cells (cTECs) contains the proteolytic subunits β1i, β2i, and the thymus-specific subunit β5t. **(D)** Spermatoproteasomes from *Drosophila melanogaster* are composed of several alternative subunits. Testis-specific subunits are: α3T, α4T1/2, α6T, β2R1/2, β4R1/2, and β5R1/2. **(E)** Mammalian spermatoproteasomes characteristically incorporate the testis-specific α4s subunit.

likely contributes to this phenomenon because the S1 pocket of β5i better accommodates larger hydrophobic amino acid side chains, which are the preferred C-terminal anchor residues for many class I molecules. Moreover, β5i has a more hydrophilic surrounding for the active site threonine and forms a specific hydrogen bond that stabilizes the tetrahedral transition state during catalysis, which could together kinetically favour the peptidolytic activity of β5i [13]. By contrast, the replacement of β2 with β2i is enigmatic, because both substrate pockets are virtually identical and they show the same trypsin-like activity [17,18]. Nevertheless, β2i knockout mice have clear phenotypes (see below), which may have a structural rather than a catalytic basis [19,20].

Compared to the relatively minor consequence of immunoproteasome deficiency on bulk MHC class I peptide loading and surface expression, the loss of β1i, β2i, or β5i can have enormous positive and negative consequences for single epitopes. Some epitopes are immunoproteasome dependent in their generation [21–24], whereas others – including important T cell epitopes of tumor antigens – are destroyed by immunoproteasomes [25,26]. A special case, where the expression of a catalytically inactive β1i subunit could rescue an immunoproteasome-dependent T cell epitope, was recently explained: the inactive β1i takes the place of β1, which would have destroyed the epitope with its caspase-like activity [27]. Previously, the impact of immunoproteasomes on the peptide repertoire presented by MHC class I molecules has generally been underestimated [28]. The proteomic analysis of peptides eluted from class I molecules

of splenocytes from mice either proficient or deficient for all three immunoproteasome subunits revealed that about one-third of peptides were only presented by wild type cells, one-third were presented only by β1i$^{-/-}$β2i$^{-/-}$β5i$^{-/-}$ cells, and another third were presented by splenocytes from both mouse strains [16]. This result implies that more T cell epitopes must be affected by immunoproteasome induction in inflamed tissues than previously expected.

Initial reports of the biological effects of immunoproteasome deficiency were disappointing. Lymphocytic choriomeningitis virus was cleared normally in immunoproteasome-deficient mice [15], whereas there was a defect reported to clear *Listeria monocytogenes* from the liver but not the spleen of β5i$^{-/-}$ mice [29]. However, a recent study revealed that β5i-deficient mice succumb to an infection with *Toxoplasma gondii* that wild type mice can survive [30]. Therefore, it is warranted to test further pathogens in immunoproteasome-deficient mice including the recently generated triple knockout mice. If more pathogens that rely on immunoproteasomes for their clearance can be identified, and if immunoproteasomes fulfill the proteolytic housekeeping functions as efficiently as constitutive proteasomes, one may wonder why immunoproteasomes are not constitutively expressed in all tissues.

Several years ago we proposed the hypothesis that the induction of immunoproteasome expression in inflamed tissues would focus the effector phase of the cytotoxic T cell response on immunoproteasome-dependent epitopes that would not be presented in uninflamed tissues. This could prevent accidentally activated self-reactive bystander

T cells in inflamed tissues from finding the same self-epitopes elsewhere in the body [31]. We predicted that immunoproteasomes would thus contribute to protection from autoimmune diseases. Recently, Zaiss *et al.* reported evidence supporting this hypothesis. They showed that β2i$^{-/-}$β5i$^{-/-}$ mice, when irradiated and reconstituted with bone marrow from the same strain, developed insulin-dependent diabetes mellitus that was caused by cytotoxic T lymphocytes recognizing β-islet antigens [32]. Moreover, it was shown that certain allelic variants of β5i or β1i were associated with human type 1 diabetes, and other human autoimmune or autoinflammatory diseases [33–36]. Taken together, it appears that on the one hand the immunoproteasome enhances and diversifies the set of class I ligands and on the other hand it may contribute to the avoidance of autoimmunity.

The immunoproteasome in Th cell differentiation and the pathogenesis of autoimmunity

A new unexpected function of the immunoproteasome, which is unrelated to its role in antigen processing, has recently been discovered. When T cells lacking β2i, β5i or, to a lesser extent, β1i were transferred into virus-infected recipient mice, they failed to survive in contrast to immunoproteasome-proficient T cells [19,23]. This was true for both Th cells and cytotoxic T cells, therefore, this phenomenon could not be related to MHC class I antigen processing. The notion that T cells need the immunoproteasome to survive in an inflammatory environment sparked the idea that a selective inhibition of β5i may interrupt the pathogenetic function of T cells in chronic inflammatory diseases like autoimmune diseases. Remarkably, the treatment of mice with the β5i selective inhibitor ONX 0914 (formerly called PR-957) was able to prevent the progression of rheumatoid arthritis in two preclinical mouse models and the induction of T cell-mediated type 1 diabetes [37]. Moreover, the inhibition or the deletion of β5i or β2i abrogated the dextran-sulfate-sodium-mediated induction of colitis [20,38,39], and β5i inhibition prevented the development of lupus-erythematosus-like disease in lupus-prone mice [40]. These findings may open a new approach for the treatment of proinflammatory autoimmune diseases using immunoproteasome selective inhibitors. In addition, they raise the interesting question whether the immunoproteasome is mechanistically involved in these diseases. A first step in support of this idea may be the observation that the inhibition or deletion of β5i partially suppressed the differentiation of the proinflammatory Th1- and Th17-type cells, while the development of anti-inflammatory regulatory T (Treg) cells was suppressed [41]. Both Th1 and Th17 cells are involved in the development of autoimmune diseases, whereas Treg cells suppress them. Mechanistically, silencing β5i suppresses phosphorylation of the transcription factors signal transducer and activator of transcription (STAT)1 and STAT3, which drive the differentiation of naïve T helper cells to Th1 and Th17 cells, respectively [41,42]. How exactly β5i enables STAT1 and STAT3 phosphorylation remains to be determined. An intriguing hypothesis is that β5i processes a factor that promotes Th1 and Th17 differentiation differently than β5. In general, the proteasome is able to generate factors from precursor proteins by limited proteolysis as has been shown for the nuclear factor (NF)-κB precursor protein p105 [43] and the plasma membrane resident transcription factor precursor Spt23/MGA2 [44]. In fact, it has been reported that processing of p105 to p50 is specifically performed by the immunoproteasome [45], but this concept could not be confirmed by others [46,47] and remains a matter of experimentation and debate.

A special role of the immunoproteasome in the accelerated degradation of polyubiquitin conjugates?

Still another special function of the immunoproteasome is the enhanced degradation of polyubiquitin conjugates, which was recently proposed by Seifert *et al.* [48]. It was reported that after stimulation of fibroblasts or HeLa cells with IFN-γ polyubiquitin conjugates accumulated for 8 h and declined thereafter. The decline of ubiquitin conjugates correlated with the induction of immunoproteasomes in these cells, which led to the hypothesis that immunoproteasomes might be needed to handle the excess of ubiquitin conjugates in IFN-γ-stimulated cells. Such a transient rise and decline in polyubiquitin conjugates was not observed by Seifert *et al.* in IFN-γ-stimulated mouse embryonal fibroblasts (MEFs) from β5i-deficient mice, supporting this concept. Instead, an accumulation of ubiquitin-containing protein aggregates was observed in β5i-deficient MEFs as well as in the brain of β5i$^{-/-}$ mice suffering from experimental autoimmune encephalomyelitis (EAE); a model for multiple sclerosis. In apparent agreement with the latter result, higher EAE disease scores were recorded for β5i$^{-/-}$ as compared to wild type mice. The notion that the 26S immunoproteasome would degrade polyubiquitylated proteins faster than the constitutive 26S proteasome was then confirmed by monitoring the degradation of polyubiquitylated mucin by the two proteases *in vitro* [48].

That IFN-γ stimulation might induce a protease that would more efficiently degrade the misfolded proteins that it also induces is an appealing notion. However, none of the aforementioned results by Seifert *et al.* [48] that indicated a higher capacity of the immunoproteasome to degrade polyubiquitylated proteins could be confirmed in the same or very similar experiments performed by Nathan *et al.* [49]. Instead, it was found that the immunoproteasome and the constitutive proteasome do not differ in their ability to bind and to degrade polyubiquitylated proteins. To identify details in experimental procedures that may account for the strikingly different outcomes of the experiments in these two studies is beyond the scope of this review. However, it should be mentioned that no difference in the amounts of polyubiquitylated proteins in splenocytes from wild type mice and mice triply deficient for β1i, β2i, and β5i could be detected by Rock and colleagues [16]. Furthermore, there is no evidence that immunoproteasomes associate preferentially with any of the known proteasome regulators including the 19S regulator, which polyubiquitylated proteins need to dock onto in order to be unfolded under ATP hydrolysis to enter the 20S proteasome or 20S immunoproteasome [50]. This is consistent with the recently elucidated high resolution crystal structures of mouse constitutive- and immunoproteasomes,

which structurally do not differ in their α-endplates where all known regulators bind [13]. Therefore, it is difficult to envision how immunoproteasomes would degrade polyubiquitylated proteins faster than constitutive proteasomes do. Taken together, structural considerations and compelling experimental data [16,49] argue that immunoproteasomes do not degrade ubiquitin conjugates faster than constitutive proteasomes.

The role of the thymoproteasome in selecting developing T lymphocytes

Sometimes data mining can be more rewarding than gold mining. When Shigeo Murata, Keiji Tanaka, and colleagues were searching a genome data base for proteasome-related genes they found an open reading frame with homology to β5 and β5i. This gene was exclusively expressed in the thymus, which led to its designation β5t (t for thymus) [51]. A more detailed analysis of subpopulations of cells in the thymus revealed that β5t was exclusively expressed in epithelial cells of the thymic cortex. The thymus is a primary lymphoid organ where immature T cells gain their antigen receptor specificity and where those T cells that bind to the MHC molecules encoded by the individual (self-MHC) are positively selected to survive and mature [52]. This positive selection occurs in the cortex of the thymus and is mediated by the highly specialized cortical thymic epithelial cells (cTECs). By contrast, T cells that recognize self-MHC and a self-peptide have the potential to cause autoimmunity, thus they need to be selected against and induced to die by apoptosis. This process of negative selection is mediated by medullary thymic epithelial cells (mTECs) and dendritic cells, which both express about equivalent levels of constitutive and immunoproteasomes [53,54]. To determine the function of the new proteasome subunit β5t, Murata *et al.* generated a β5t-deficient mouse that turned out to have a dramatic phenotype. Although the selection of mature CD4$^+$ T helper cells was normal, the selection of mature CD8$^+$ cytotoxic T cells was reduced by 80%, leading to a similarly strong reduction of CD8$^+$ T cells in the spleen [51]. Consequently, β5t$^{-/-}$ mice succumbed to an influenza virus infection that wild type mice survived [55].

The strong phenotype of β5t$^{-/-}$ mice raised the question of why the β5 or β5i subunits cannot compensate for the loss of β5t. A problem with proteasome assembly could be excluded because with β5t deficiency the so-called thymoproteasome of mouse cTECs uses the β5i active site subunit in its place [55]. Insight into the unique function of β5t in thymic positive selection was derived when comparing the peptidolytic activities of β5t- and β5-containing proteasomes. The β5t$^+$ proteasomes had a much lower chymotrypsin-like activity than β5$^+$ proteasomes, whereas their caspase-like and trypsin-like activities were not affected by β5t incorporation into the proteasome [51]. This change in activity profile can be explained when looking at the amino acids that line the substrate binding pocket of β5t. The S1 pockets of β5 and β5i accommodate larger hydrophobic amino acids, whereas four replacements of hydrophobic amino acids in the S1 pocket of β5 and β5i by the hydrophilic amino acids serine or threonine in β5t predict a cleavage preference for β5t after hydrophilic or less

hydrophobic amino acids. The resulting peptide products of β5t$^+$ proteasomes are expected to be weak ligands of mouse MHC class I molecules. Murata *et al.* proposed that such weak β5t-dependent class I ligands, which have a high off rate, might be a prerequisite for a successful positive selection of T cells in accordance with the affinity model of thymic selection. The affinity model states that low-affinity binding of the T cell receptor (TCR) to MHC/peptide complexes will lead to positive selection, whereas high-affinity TCR binding will lead to negative selection [56].

The structural, enzymatic and functional data on β5t all are remarkably consistent. There is only one experimental aspect that is difficult to reconcile with a large body of data. It has been shown that MHC class I ligands with weak C-terminal anchor residues are poor stabilizers of class I cell surface expression [57]. Nevertheless, a detailed analysis of surface expression of three different class I molecules on cTECs from wild type and β5t$^{-/-}$ mice revealed that the lack of β5t did not affect class I surface expression [55]. Perhaps cTECs have developed mechanisms that allow the stabilization even of weak peptide/class I/β2-microglobulin complexes on the cell surface. However, this and other hypotheses will be difficult to address experimentally because few cTECs can be isolated from a murine thymus.

The spermatoproteasome in the generation of spermatids

The most extensive tissue-specific alteration of proteasome subunit composition has been reported for the testes and in particular for spermatids [58]. The differentiation from spermatogonial stem cells to mature sperms is a highly regulated cellular process that includes mitosis, meiosis, and morphological changes, which are steps that might all be dependent on proteasomal function. The existence of testis-specific proteasome subunits was first described for *Drosophila melanogaster* in the mid-1990s. Belote and coworkers reported two alternative α4-type subunits (α4T1 and α4T2) encoded by paralogous genes that are exclusively expressed in the male germline [59]. Subsequent studies revealed that proteasome subunit gene duplications are widespread in *D. melanogaster*: to date, 12 of the 33 26S proteasome subunits have been identified to exist in two or even three different isoforms. Although the conventional form of each subunit can be detected in all tissues and during all developmental stages examined, all additional isoforms are specifically expressed in the testes (reviewed in [58]). Some alternative subunits were studied in more detail using reporter transgenes. These investigations showed an expression pattern limited to male germ line cells during the mid to late stages of spermatogenesis, whereas only the conventional proteasome subunits are expressed in the early gonial stages [59–61]. In elongated spermatids proteasomal 'speckles' containing alternative subunits could be observed in close proximity to the individualization complex (IC) [60] (Figure 3). The cytoskeletal membranous IC harbors a cluster of actin cones and mediates sperm individualization by moving down the spermatid bundle, thus expelling cytoplasm and organelles and simultaneously packing each spermatid in its own plasma membrane. The functional consequence of the proteasomal subcellular localization at the IC has not yet been clarified
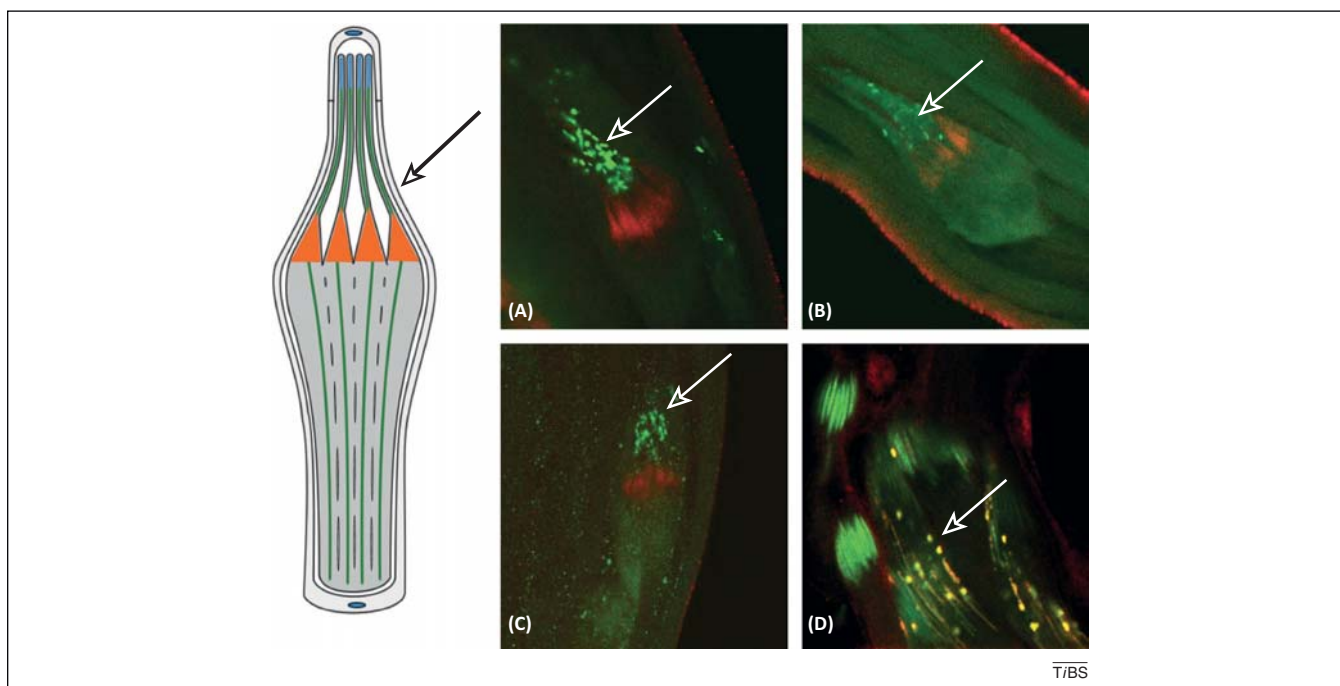
Figure 3. Images of testis-specific proteasome subunits α3T and α6T in the individualization complex (IC) of spermatids in *Drosophila melanogaster*. During the individualization of spermatids the syncytial spermatid bundle is resolved into separate sperm cells. On the left a cartoon of the IC is depicted; it is composed of actin cones (orange) with the cystic bulge, which is formed as the IC moves down the spermatid bundle. Sperm nuclei are at the top (blue). **(A)** Speckles of α3T-GFP (green) trailing the actin cones (red). **(B)** Speckles of α6T-GFP (green) trailing the actin cones (red). **(C)** Immunostaining of wild type testis with anti-proteasome antibodies (green). **(D)** Immunostaining of α6T-GFP in the testis of an α6T-GFP transgenic fly with anti-proteasome antibody showing the colocalization (yellow) of α6T-GFP and proteasome signals. Reproduced, with permission, from [58].

but, interestingly, knockout of the alternative subunit α6T in *D. melanogaster* resulted in male sterility based on a defective sperm individualization, which was accompanied by disrupted actin cone coordination in ICs [60].

Recent work detected the alternative α4-type proteasome subunit, α4s/PSMA8, in the mammalian testes. Its expression is restricted to spermatids and mature sperm, which correspond to the late stage expression of alternative subunits in *D. melanogaster* [62,63]. As the proteasomal outer α ring lacks catalytic activity, the function of alternative α subunits is less clear. One possibility could be a preferred interaction with certain regulatory particles. Besides α4s, mammalian testis proteasomes were reported to contain immunoproteasome subunits [63,64]. However, β1i, β2i, and β5i showed partly dissimilar cell type and differentiation stage expression patterns. Clearly, lower amounts of immunoproteasomes could be detected in testes compared to spleen [63].

In addition to the special subunit composition of the 20S complex, the associated regulatory particles are also testis specific. The proteasome regulator PA200 is expressed in different mammalian tissues but it is particularly abundant in testis [65], where PA200-capped proteasomes are present as single or double-capped complexes or in the form of hybrid proteasomes that carry both PA200 and the 19S regulator, each binding on opposing α-endplates of the 20S barrel [63]. The 200-kDa PA200 is a monomeric non-ATPase proteasome activator that attaches to the outer α rings of the 20S complex and enhances the proteasomal degradation of small peptides but not ubiquitylated protein substrates *in vitro* [65]. PA200 knockout mice are viable and show no obvious developmental abnormalities. However, PA200-deficient

males exhibit a marked reduction of fertility due to defective spermatogenesis [66]. A mechanistic explanation was recently suggested when Qian *et al*. showed that PA200 promotes the acetylation-dependent degradation of core histones during somatic DNA damage responses and spermatogenesis [63]. Histone acetylation weakens the histone–DNA interaction and leads to an open and transcriptionally active chromatin structure. During spermatogenesis, histone acetylation is followed by removal of histones from chromatin and their replacement by transition proteins and subsequently protamines, which allow denser DNA packing [67]. An interesting question, which has not been addressed yet, is whether the testis-specific α4s/PSMA8 subunit supports the docking of PA200 more than the conventional α4 subunit does. Taken together, PA200-dependent (and perhaps α4s/PSMA8 dependent) proteasomal degradation of acetylated histones might represent an important mechanism during spermatogenesis that enables transcription arrest, spermatid differentiation, and chromatin condensation.

Concluding remarks and future perspectives

The existence of tissue-specific proteasomes or proteasomes that are only expressed during stimulation with cytokines pose many unanswered questions. Are there selective substrates of tissue-specific proteasomes? Do tissue-selective proteasomes process precursor proteins in a different manner as compared to constitutive proteasomes? Do tissue-selective proteasomes associate differently with the increasing number of proteasome regulators? Apart from these fundamental biological questions tissue-specific proteasomes offer interesting new
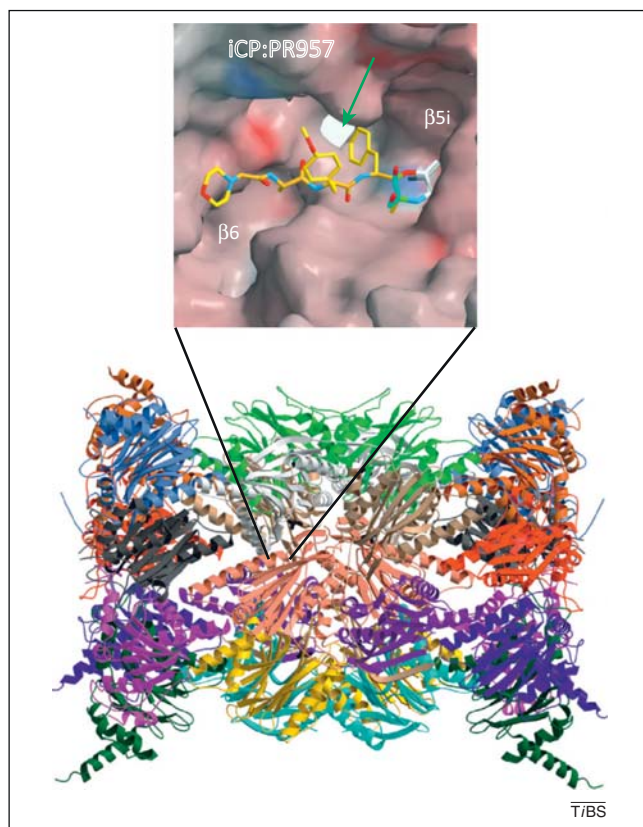
Figure 4. Structure of the mouse immunoproteasome with the β5i (low molecular mass polypeptide 7; LMP7) active site bound by the β5i inhibitor ONX 0914 (formerly called PR-957). Surface colors in the Conolly surface representation of β5i indicate positive and negative electrostatic potentials contoured from 50 kT/e (intense blue) to −50 kT/e (intense red). In order to illustrate conformational changes induced by the inhibitor, the amino acids 46–50 were removed. Thr1 of the β5i subunit is colored in white and the covalently bound inhibitor in yellow. The green arrow indicates where the phenyl side chain of the inhibitor would clash with the constitutive β5 subunit, whereas in the immunoproteasome subunit β5i, a reorientation of the CH3-S- group of Met45 avoids such a steric clash, thus explaining the immunoproteasome selectivity of ONX 0914. Adapted, with permission, from [13,72].

perspectives for their selective pharmacological inhibition while leaving the activity of constitutive proteasomes intact. Broad-spectrum proteasome inhibitors like bortezomib (Velcade) or carfilzomib (Kyprolis) are currently used to treat multiple myeloma but they need to be used close to the maximally tolerated dose to be effective. The dose-limiting side effects stem from the inhibition of the housekeeping functions of the constitutive proteasome needed in virtually all tissues [68]. Subunit-specific proteasome inhibitors, in contrast, may target diseases in a tissue specific manner. The β5i inhibitor ONX 0914 is a prominent example because it can suppress autoimmune diseases at doses far below the maximal tolerated dose in mice [37] (Figure 4). Other immunoproteasome-specific inhibitors such as UK-101 and IPSI, which both inhibit the β1i subunit, have been developed with the aim of improving cancer therapy [69,70]. Although cell-permeable inhibitors of the trypsin-like activity of the proteasome and immunoproteasome have been developed successfully, they do not discriminate between β2 and β2i, probably because their substrate-binding pocket is similar [71]. It will be interesting to follow the further development of subunit-selective inhibitors and to test their therapeutic potential in preclinical models and in humans. Apart from autoimmune diseases and cancer the

in vivo manipulation of antigen presentation and T cell selection in the thymus might offer unanticipated clinical opportunities. Testis-specific proteasome subunits or regulators, by contrast, may serve as targets for the development of male contraceptives.

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