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Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors

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Proteasomes perform the majority of proteolysis that occurs in the cytosol and nucleus of eukaryotic cells and, thereby, perform crucial roles in cellular regulation and homeostasis. Isolated proteasomes are inactive because substrates cannot access the proteolytic sites. PA28 and PA200 are activators that bind to proteasomes and stimulate the hydrolysis of peptides. Several protein inhibitors of the proteasome have also been identified, and the properties of these activators and inhibitors have been characterized biochemically. By contrast, their physiological roles – which have been reported to include production of antigenic peptides, proteasome assembly and DNA repair – are controversial. In this article, we briefly review the biochemical data and discuss the possible biological roles of PA28, PA200 and proteasome inhibitors.

Introduction

Proteasomes are large complexes that carry out crucial roles in many cellular pathways by degrading proteins in the cytosol and nucleus of eukaryotic cells to enforce quality control and to regulate many cellular processes [1]. The catalytic heart of these complexes, the 20S proteasome, has been highly conserved from yeast to humans, with simpler versions also found in some archaea and prokaryotes. The 20S proteasome is a barrel-shaped assembly of 28 protein subunits that possesses three distinct proteolytic active sites with different specificities (Figure 1). Together, the three active sites, present in the two central rings of β subunits, hydrolyze almost all peptide bonds, having trouble only with those bonds that follow glycine and proline. As revealed by structural studies performed by Huber and colleagues [2,3], the potentially catastrophic elimination of inappropriate substrates is prevented by sequestration of active sites within the hollow structure of the 20S proteasome. Substrates access the central catalytic chamber through axial ports in the end rings of α subunits [4], although in the absence of activators these channels are closed and proteasome activity is repressed (Figure 2).

Proteasomes are activated by protein complexes that bind to the end rings of α subunits (Figure 3). The best-known activator is PA700 [proteasome activator MW 700, also known as 19S or regulatory complex (RC)], which has been highly conserved from yeast to humans and binds to the 20S proteasome to form the 26S proteasome. PA700 is the only proteasome activator that is known to stimulate degradation of protein substrates, which it generally recognizes by a polyubiquitin modification and which it processes by an ATP-dependent mechanism. Thus, PA700 is thought to mediate most of the biological effects of the proteasome by facilitating substrate degradation. This biological role is well established, and PA700 and 26S proteasomes have been reviewed extensively elsewhere [5].

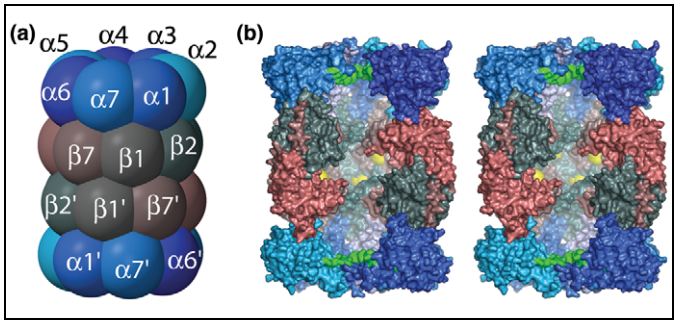

Figure 1. Architecture of the 20S proteasome. **(a)** Side view of the proteasome. Active sites are formed at the N-termini of β1, β2 and β5. The substrate preferences of these sites are: β1 – PGPH substrates; β2 – trypsin-like substrates; β5 – chymotrypsin-like substrates [3]. (Immunoproteasomes are the same as constitutive 20S proteasomes except that the constitutive catalytic subunits are replaced by inducible counterparts.) **(b)** Cutaway stereoview showing how the active sites (yellow) are sequestered within a central catalytic chamber. Substrates and products pass through an opening called the α-annulus (green) through the middle of the ring of α subunits [3].

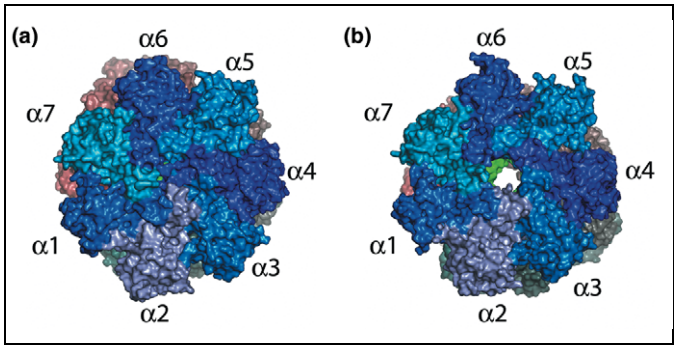

Figure 2. Proteasome entrance gate. **(a)** Top view of the proteasome in the closed conformation [3,64]. **(b)** Top view of the proteasome in the open conformation (as seen in complex with PA26) [9].

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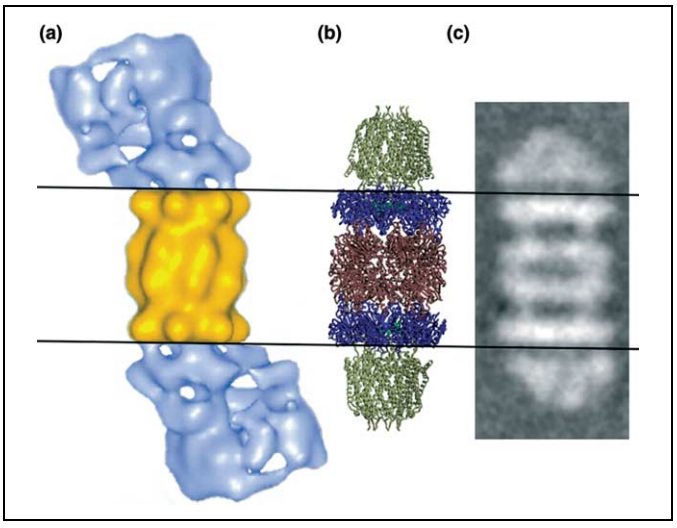

Figure 3. Proteasome–activator complexes. The horizontal lines delineate the ends of the 20S proteasome in these complexes. **(a)** Cryoelectron microscopy structure of the PA700–20S-proteasome complex (26S proteasome). Image adapted, with permission, from Ref. [5]. © (1999) Annual Reviews (www.annualreviews.org). **(b)** Crystal structure of the PA26–20S-proteasome complex [9]. **(c)** Averaged negative-stain electron micrograph of a bovine PA200–20S-proteasome complex. These images show the 20S proteasome bound by activators at both ends. Singly capped 20S-proteasome–activator complexes are also observed.

In contrast to PA700, two other evolutionarily conserved protein complexes, PA28 (also known as 11S or REG) [6] and PA200 [7], that have been shown to bind specifically to and activate 20S proteasomes against model peptide substrates do not recognize ubiquitinated proteins or use ATP. PA28 family members, which are found in higher eukaryotes but are, apparently, absent from yeasts, exist as homo- or heteromeric complexes of seven ~28-kDa subunits. PA200 is a single-chain protein of ~200 kDa, with homologs present in yeast, worms and humans. The biological roles of PA28 and PA200 are understood less well than those of PA700, although their biochemical activities and evolutionary conservation implies that they have important roles in cellular physiology, and several important functions have been proposed. In this article, we focus on the possible biological functions of PA28 and PA200. We also discuss possible roles of protein inhibitors of the proteasome that, like PA28 and PA200, have been characterized biochemically but have controversial biological functions.

Structural and biochemical properties of PA28 and PA200

The mechanism by which PA28 binds to and stimulates 20S proteasomes has been revealed, at least in part, by the crystal structure of a complex formed between the yeast 20S proteasome and PA26, the distant PA28 homolog from *Trypanosoma brucei* [8,9]. The structure shows that activator binding induces opening of the entrance and exit gate of the proteasome and that a central channel formed through the center of the activator aligns with the open entrance gate of the proteasome. The simplest interpretation of the structural data is that gate opening enables peptide substrates to diffuse through the central channel of the activator and into the proteasome interior. This could explain activation, although it is possible that peptide hydrolysis is further modulated by long-range conformational changes induced at the catalytic sites [10].

Overall, the biochemical properties of PA28 and PA200 are clear, although their biological roles are more controversial. One attractive possibility is that PA28 and PA200 normally function in mixed complexes in which one 20S proteasome is bound at one end by PA700 and at the opposite end by either PA28 or PA200 [7,11]. PA28 and PA200 might function as adaptors in these 'hybrid proteasomes' by, for example, recruiting proteasomes to specific intracellular locations. An adaptor function is consistent with the sequence analysis by Kajava *et al.* [12], who proposed that PA200 adopts a 'solenoid' structure – an architecture typically associated with protein–protein recognition. Possible biological roles for PA28 and PA200 are discussed in more detail later (Figure 4).

Biological properties of PA28αβ and PA28γ

There are three PA28 homologs, called α, β and γ. The α and β subunits form a heteroheptamer, whereas γ forms a homoheptamer. PA28γ is found in worms, insects and higher animals, but not in yeast or plants [13]; PA28αβ is confined to jawed vertebrates. Sequence analyses indicate that duplication and divergence of the gene encoding PA28γ produced the gene encoding PA28α, which duplicated in turn to produce the gene encoding PA28β. PA28αβ appeared during evolution at roughly the same time as vertebrate cellular immunity. Although PA28α and β subunits are expressed in many organs, they are particularly abundant in immune tissues and are virtually absent from the brain. By contrast, the brain contains large amounts of PA28γ, compared with moderate levels in other organs. The intracellular distribution of PA28αβ and PA28γ also differs. PA28αβ is mainly cytoplasmic, whereas PA28γ is confined to the nucleus. Finally, PA28αβ is induced by interferon (IFN)γ and infection, whereas PA28γ is unaffected by IFNγ and can be markedly reduced during infection [14]. These general properties suggest that PA28αβ has a role in the immune system, although they are not particularly informative about the function of PA28γ.

PA28αβ and cellular immunity

Acquired immunity in vertebrates involves two distinct responses. Humoral responses are characterized by circulating antibodies that are directed against peptide epitopes generated mainly in endosomes and presented on MHC class II molecules [15]. Cellular responses are mediated by cytotoxic T lymphocytes (CTLs) that lyse infected cells after recognizing foreign peptides generated in the cytosol and transferred to the cell surface bound to class I molecules [16]. Proteasomes generate the vast majority of 8–11-residue peptides displayed on class I molecules. Proteasomal cleavage products are transported into the lumen of the endoplasmic reticulum, in which they bind to empty class I molecules that are then sent to the plasma membrane. By the late 1990s, it was generally accepted that PA28αβ contributes to class I presentation, based on the high levels of PA28αβ in immune tissues, IFNγ induction of PA28αβ and many components of the class I pathway, and direct production of some class I

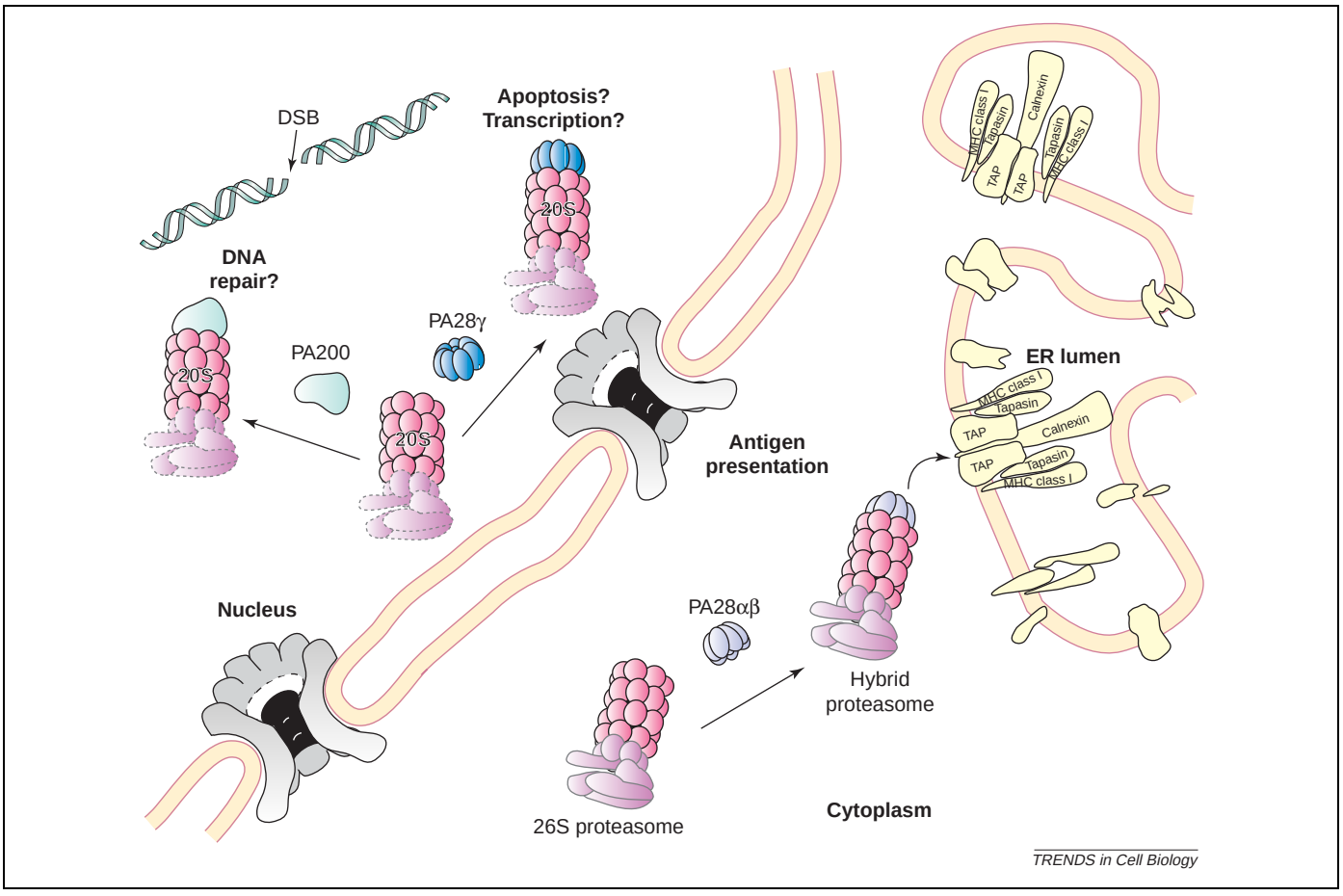

Figure 4. Possible biological functions of PA200 and PA28 homologs. PA200 and PA28γ are nuclear and are thought to be involved in DNA repair, and transcription or apoptosis, respectively. In the nucleus, the 19S regulatory complex (PA700) has a broken outline because it is not clear whether PA200 and PA28γ associate with the 26S proteasome or only with the 20S enzyme. PA28αβ is shown in the cytoplasm forming a hybrid proteasome that functions in class I antigen presentation. These properties of PA28αβ are reasonably well established.

epitopes by PA28αβ–proteasome complexes (for review, see Ref. [17]).

Several recent articles reinforce the connection between PA28αβ and cellular immunity. Two groups have reported that mice lacking PA28αβ exhibit impaired class I presentation, although the severity of the immune defects and the proposed reasons for them differ greatly between the two studies. Preckel *et al.* [18] disrupted the *PA28β* gene and observed that the knockout mice, which did not express either PA28β or PA28α, exhibited a general impairment in CTL responses and greatly reduced immunoproteasome levels. They concluded that PA28 is necessary for immunoproteasome assembly and that defects in class I presentation resulted from the lack of immunoproteasomes. In mice, *PA28α* and *PA28β* genes are within six kilobase pairs of each other, which enabled Murata *et al.* to disrupt both of them in a single step [19]. In contrast to Preckel *et al.*, they observed efficient immunoproteasome assembly and normal cellular immunity following influenza virus infection. The $PA28\alpha\beta^{-/-}$ mice were, however, unable to process a specific epitope from the melanoma antigen TRP-2. Murata *et al.* concluded that PA28αβ is not required for immunoproteasome assembly or class I antigen presentation in general but that it is necessary for the presentation of certain epitopes.

Overexpression of PA28αβ in fibroblasts supports the idea that PA28αβ enhances antigen processing independently from any effect on immunoproteasome synthesis [20]. Furthermore, studies of dendritic cells, the professional antigen-presenting cells of the body, showed that immunoproteasome subunits are upregulated early in the maturation process, whereas PA28αβ subunits and many other components of the class I pathway are synthesized later [21]. Thus, it seems unlikely that PA28αβ is involved in immunoproteasome assembly.

Experiments by Yamano *et al.* [22] have addressed how PA28αβ might function in the class I pathway. By examining the presentation of a class I epitope from ovalbumin (OVA) in $PA28\alpha\beta^{+/+}$ and $PA28\alpha\beta^{-/-}$ cells, they found that both heat-shock protein (hsp)90 and PA28αβ enhanced OVA epitope production. The hsp90 inhibitor geldanamycin completely suppressed epitope presentation in $PA28\alpha\beta^{-/-}$ cells, but only partially in $PA28\alpha\beta^{+/+}$ cells. Moreover, when PA28αβ levels were increased in $PA28\alpha\beta^{+/+}$ cells by IFNγ treatment, geldanamycin had no effect on OVA epitope presentation. Because hsp90 is essential for class I presentation when PA28αβ is absent and because it is not needed when PA28αβ levels are elevated, Yamana *et al.* proposed that hsp90 and PA28αβ serve as parallel chaperones that transfer peptides from the proteasome to the class I

peptide-loading complex in the endoplasmic reticulum membrane [22].

There are two other possible functions of PA28αβ in the class I pathway. Products of proteasomal degradation range from 3–4 to >30 amino acids in length [23]. Most, however, are 6–8 residues long, which is too short for optimal binding to class I molecules. Whitby *et al.* [8] proposed that, by opening a wide aqueous channel through the α ring of the proteasome, PA28αβ increases the length of peptides that exit the enzyme and that are, therefore, available for binding to class I molecules. Hence, class I epitope presentation would be more efficient. Although this is an attractive idea, Cascio *et al.* [24] reported that PA28αβ did not increase the length of products released from 26S proteasomes. PA28αβ-dependent presentation of the TRP-2 epitope [19] illustrates a second way in which the proteasome activator could augment class I presentation – PA28αβ might simply alter proteasomal cleavage sites within a polypeptide, thereby generating unique epitopes. Because the three potential functions of PA28αβ, namely specifying, lengthening or channeling proteasomal cleavage products, are not mutually exclusive, all could serve to enhance the production of class I epitopes (Figure 5).

Non-immune functions of PA28αβ

Several physiological or pathological conditions that seem to be unrelated to the immune response can affect cellular levels of PA28αβ. Chronic stimulation of rabbit skeletal muscle produces a threefold increase in the number of 20S-proteasome subunits and an impressive 70-fold increase in levels of PA28αβ [25]. Serum withdrawal, amino acid starvation or crowding of human skin fibroblasts produces a more modest twofold increase in levels of PA28αβ [26], as does aging of human keratinocytes [27]. Although PA28β is virtually absent from the brain, the α subunit is present, which suggests that PA28α alone functions in nervous tissue. Because adult neurons do not express MHC class I molecules, PA28α function in the brain is unlikely to be immunological. In cases in which levels of PA28αβ increase in the absence of an immune response, the proteasome activator presumably generates more hybrid 26S proteasomes, thereby increasing proteolytic efficiency [11]. Alternatively, PA28αβ might promote protein repair because it has been reported to assist in the hsp90-mediated refolding of denatured luciferase [28].

Potential functions of PA28γ

Two groups have generated and characterized mice lacking PA28γ. Murata *et al.* observed that PA28γ-deficient mice were normal at birth but grew more slowly and were ~10% smaller than wild-type mice at maturity [29]. *PA28γ*$^{-/-}$ embryonic fibroblasts were larger and displayed lower saturation density and a higher proportion of G_1 cells, suggesting that PA28γ functions in cell-cycle progression. More recently, Barton *et al.* found that *PA28γ*$^{-/-}$ mice did not clear pulmonary fungal infections as efficiently as wild-type mice did, which suggests an immune role for PA28γ [30]. They confirmed the smaller body size of *PA28γ*$^{-/-}$ mice and the increased proportion of embryonic fibroblasts in G_1 cells. In addition, they observed that apoptosis was increased almost threefold in *PA28γ*$^{-/-}$ embryonic fibroblasts. An effect of PA28γ on cell division was seen in *Drosophila* cells in which RNA interference (RNAi)-mediated depletion of the proteasome activator also resulted in a higher proportion of G_1 cells [31]. Moreover, a promoter element found in *Drosophila* genes that are involved in DNA replication lies just upstream of the *Drosophila PA28γ* gene [31]. These findings echo an earlier study showing that PA28γ expression correlates with cell proliferation [32]. Thus, results from mice and flies are consistent with a role for PA28γ in cell-cycle traverse, apoptosis or both.

Two-hybrid screens have identified several proteins that interact with PA28γ. Interestingly, they all display a relationship with apoptosis. PA28γ was recovered in a screen using the mitogen-activated protein (MAP) kinase kinase MEKK3 as bait [33]. Expression of MEKK3 in Cos cells increased PA28γ levels, and *in vitro* studies indicated that PA28γ is phosphorylated by MEKK3 [33]. MEKK3 is an upstream activator of c-Jun N-terminal kinases (JNKs), which have long been implicated in apoptosis [34]. Screens in which PA28γ was used as bait have identified four proteins involved in apoptosis. S. Wilk (personal communication) recovered FLASH, a protein implicated in Fas-mediated activation of caspase 8 [35]. X. Gao (personal communication) recovered Daxx, RanBPM and PIAS1 in two-hybrid screens of human brain or HeLa cells. All three of these proteins affect apoptosis. For example, Daxx was identified initially as an activator of JNK and apoptosis [36]. Although there is controversy as to whether Daxx is pro- or anti-apoptotic, there is general consensus that Daxx influences cell death [37]. RanBPM interacts with the neurotrophin receptor p75NTR, which is a member of the tumor necrosis factor receptor family that facilitates apoptosis through the JNK pathway [38]. PIAS1 is reported to have pro-apoptotic activity that is also mediated through the JNK pathway [39]. Thus, three proteins implicated in JNK-mediated apoptosis and the pro-apoptotic protein FLASH have been found to interact with PA28γ. These findings, coupled with the observation that *PA28γ*$^{-/-}$ fibroblasts exhibit increased levels of apoptosis, suggest that PA28γ is an anti-apoptotic factor. The proposed anti-apoptotic properties of PA28γ could explain its high concentration in the adult brain because neurons are the last cells that an organism would want to self destruct. An anti-apoptotic function could also explain the high levels of PA28γ in malignant thyroid cells [40].

How might PA28γ suppress apoptosis? There is increasing evidence that the ubiquitin–proteasome system has a direct role in transcription [41]. Perhaps PA28γ recruits the 26S proteasome to specific promoters – a possibility suggested by the recent report that Daxx and FLASH modulate transcription mediated by the mineralocorticoid and glucocorticoid receptors [42]. Alternatively, PA28γ could promote the proteolysis of specific pro-apoptotic components – a possibility suggested by the report that PA28γ speeds degradation of hepatitis C virus core protein [43]. Or perhaps PA28γ simply binds to pro-apoptotic factors and prevents their action. It is neither

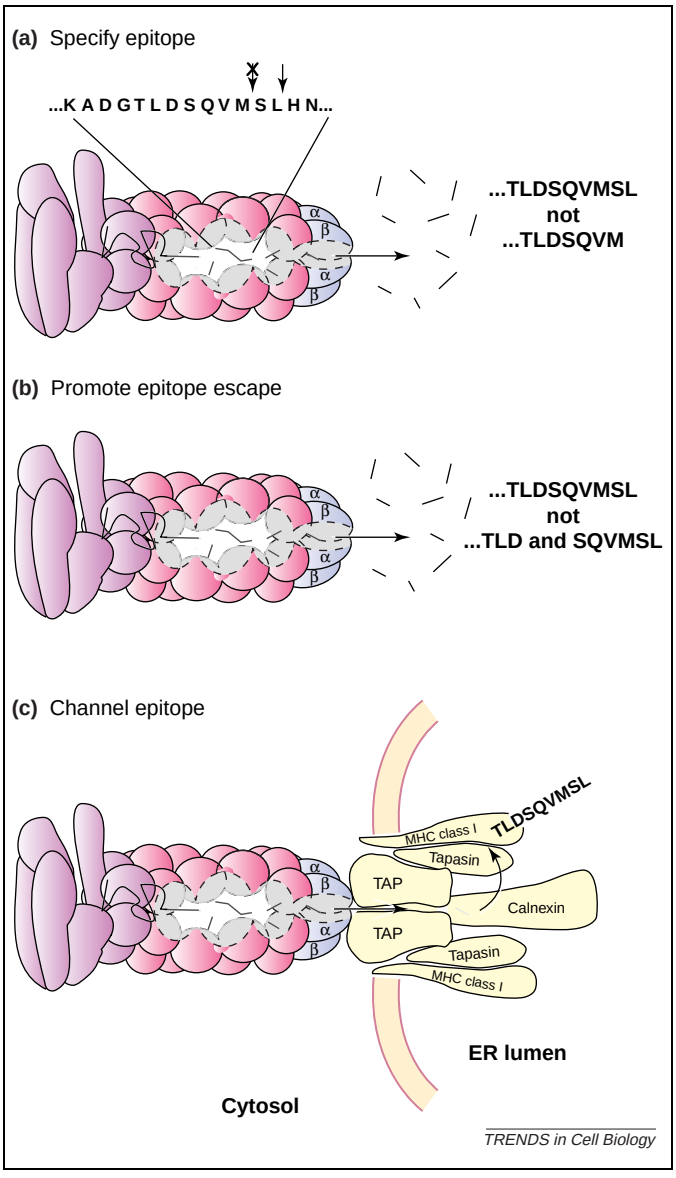

Figure 5. Three ways in which PA28αβ could promote presentation of a class I epitope. **(a)** The proteasome activator could bias peptide-bond cleavage so that the C-terminal residue of the epitope is leucine, not methionine. **(b)** By opening a channel through the proteasome α ring, PA28αβ could facilitate peptide diffusion from the central chamber before further cleavage destroys the epitope. **(c)** PA28αβ might directly couple the hybrid proteasome to the peptide-loading complex, thereby channeling the epitope to empty class I molecules. In the figure, these examples are illustrated with TLDSQMSL, which is a PA28αβ-dependent epitope from the melanoma protein TRP-2.

clear how PA28γ affects apoptosis nor whether it affects apoptosis directly.

Biological properties of PA200

PA200 is the most recent proteasome activator to be discovered [7]. The original description of this proteasome activator proposed it to be involved in DNA repair. A variety of evidence was presented in favor of this hypothesis. Mammalian PA200 is homologous to the yeast protein Blm3p, mutation of which was reported to confer sensitivity to the DNA-damaging agent bleomycin. Blm3p was also found to interact with Sir4p, a yeast protein that relocates to DNA double-strand breaks. In mice, both *PA200* mRNA and protein are abundant in testes, where double-strand breaks occur at high frequency during meiotic recombination. Finally, PA200 is present within the nucleus and, as with several DNA-repair components in mammalian cells, forms foci after γ-irradiation. Several recent articles, however, cast doubt over the DNA-repair hypothesis. Deletion of *BLM3* (now known as *BLM10*) does not result in bleomycin sensitivity [44] but it does increase the rate of proteasome assembly in yeast [45]. (Surprisingly, Fehlker *et al.* [45] also report that blm3p does not stimulate proteasome activity *in vitro*.) Moreover, PA200 is upregulated at least threefold in four different models of muscle wasting [46]. Because there is no obvious relationship between increased turnover of skeletal muscle proteins and DNA repair, it is difficult to rationalize increased PA200 levels during muscle wasting. Although, at present, there is no doubt that PA200 binds to the ends of the 20S proteasome, the physiological significance of this association is not clear.

Protein inhibitors of the 20S proteasome

The small-molecule proteasome inhibitor Velcade® has proved to be remarkably effective against refractory multiple myeloma and has sparked considerable clinical interest in proteasomes [47]. Several cellular and viral proteins have also been found to inhibit 20S proteasome activity *in vitro*. They will be discussed briefly because several antagonize activation by PA28αβ and they might affect proteasome function *in vivo*.

We have already noted that hsp90 might shepherd proteasomal cleavage products to the class I peptide-loading complex [22]. *In vitro*, this abundant chaperone inhibits hydrolysis of fluorogenic peptides by the 20S proteasome approximately twofold [48]. Interestingly, constitutive 20S proteasomes, but not immunoproteasomes, are inhibited by hsp90, and inhibition is abrogated by low levels of PA28αβ [49]. Failure of hsp90 to inhibit immunoproteasomes could be physiologically relevant, considering the proposal by Yamano *et al.* [22] that it shuttles peptides in the class I pathway. However, all organs except the brain express PA28αβ, so hsp90 inhibition of the 20S proteasome might not be important *in vivo*.

PI31 is a proline-rich 30K protein that inhibits proteasomal degradation of fluorogenic peptides and unstructured proteins *in vitro* [50]. PI31 homologs are present in plants and higher eukaryotes, but not in yeast, and *PI31* mRNA is expressed in many organs. Immunofluorescence localizes PI31 to the endoplasmic reticulum of mouse fibroblasts [51]. Enzyme assays have shown that PI31 inhibits the chymotrypsin (CT) and post-glutamyl-peptidyl-hydrolyzing (PGPH) active sites of the proteasome more than it inhibits the trypsin (T)-site [52], which is a pattern of inhibition similar to that seen with another proline-rich inhibitor: PR39. PI31 is a competitive inhibitor of PA28αβ activation, with an affinity for the 20S proteasome that is ~50-fold higher than that of PA28αβ [52]. Yet, surprisingly, inhibition of PA28αβ–proteasome complexes or 26S proteasomes has not been observed to be greater than 50%, even at extremely high levels of PI31 [50]. Overexpression of PI31 in cultured mammalian cells has been reported to impair immunoproteasome formation

and class I antigen presentation, rather than inhibit proteasome function [51]. However, the presence of PI31 in plants and lower animals that lack immunoproteasomes or class I responses suggests that PI31 serves other purposes in nonvertebrate organisms.

Originally isolated as an antibacterial peptide secreted by macrophages, PR39 is a proline- and arginine-rich 39-residue peptide that readily crosses cell membranes [53]. The peptide was linked with the ubiquitin–proteasome system when a two-hybrid screen identified the 20S-proteasome subunit α7 as being an interacting protein [54]. PR39 is reported to stimulate angiogenesis and suppress inflammation, apparently by inhibiting proteasome-mediated degradation of hypoxia-inducible factor 1α [55] and IκBα [54], respectively. Mechanistic studies *in vitro* showed that PR39 is a noncompetitive reversible inhibitor of 20S proteasomes, 26S proteasomes [56] and PA28αβ–proteasome complexes (M. Gaczynska, personal communication). Like PI31, PR39 inhibits the CT and PGPH sites of the proteasome, while sparing T-site activity. Atomic-force microscopic images of 20S and 26S proteasomes are markedly altered in the presence of PR39, leading to the proposal that the peptide prevents both enzymes from switching between open and closed conformations [56].

Tat is an 86-residue HIV protein that functions as a transcriptional activator. Large amounts of Tat are secreted from HIV-infected cells, and exogenous Tat can enter cells directly through the plasma membrane. After Tat was shown to interact with 26S-proteasome subunits [57], Seeger *et al.* tested whether Tat affects proteasome activity *in vitro* [58], finding that it inhibits 20S peptidase activity and interferes with PA28αβ activation of the 20S proteasome. A subsequent study reported that Tat reduces presentation of a PA28αβ-stimulated class I epitope; the authors also presented a model for Tat interaction with the 20S proteasome [59]. The most recent study identified α4 and α7 proteasome subunits as being Tat-interacting proteins [60]. Hepatitis B virus is a causative agent of chronic and acute hepatitis. Hepatitis B virus X protein (HBx), a viral phosphoprotein that transactivates viral and cellular promoters, is rapidly degraded by the ubiquitin–proteasome system [61]. Two-hybrid screens identified the 20S-proteasome subunit α7 as being an HBx interactor [62]. Expression of HBx in HepG2 cells produced modest inhibition of the proteasome [61], and a peptide comprising residues 116–138 of HBx inhibited activation of the proteasome by PA28αβ *in vitro* [63].

Physiological significance of macromolecular proteasome inhibitors

Three of the inhibitors that compete with PA28αβ for binding to the proteasome – PR39, Tat and HBx – were identified as two-hybrid interactors with proteasome α subunits. In fact, all three bind to the α7 subunit, which has a long, highly charged C-terminal extension. This raises the possibility that the observed two-hybrid and *in vitro* interactions are predominantly ionic and relatively nonspecific. Because activation by PA28αβ requires its binding to the entire upper surface of the proteasome α ring, one would imagine that any protein bound to an α subunit would sterically block PA28αβ binding. Thus, competition by PR39, Tat or HBx could be an *in vitro* artifact. However, one or more of the inhibitors might affect the ubiquitin–proteasome system under normal physiological or pathological conditions. Whether proteasome inhibition by these proteins is artifactual or physiological is, at present, an open question.

Concluding remarks

The understanding of the regulation of proteasome activity is mixed. On the one hand, it is clear that PA700, as part of the 26S proteasome, mediates degradation of polyubiquitinated substrate proteins and that this activity has a major impact on a broad range of biological processes. On the other hand, whereas the biochemical basis for stimulation of proteasome activity by PA28 is largely well characterized, the biological role of PA28 is incompletely understood and the relevance of the induced peptidase activity is obscure. In the case of PA200, there is a lack of both structural information and a clearly defined biological role. Nevertheless, we believe that the evolutionary conservation of PA28 and PA200 as proteasome-binding components reflects important biological functions that are probably mediated in the context of hybrid proteasomes by targeting the degradative potential of PA700–20S–PA28 and PA700–20S–PA200 complexes. The various proteasome inhibitors discussed might represent a newly appreciated level of proteasome regulation, although it will be important to establish the biological relevance of their effects. Regulation of proteasome activity is of profound importance for cellular function, and understanding the biochemical and biological functions of associating proteins will be a priority for future research efforts.

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