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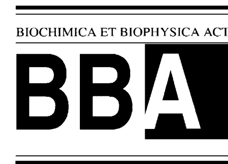

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Review

The proteasome and MHC class I antigen processing

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Abstract

By generating peptides from intracellular antigens, which are then presented to T cells, the ubiquitin/26S proteasome system plays a central role in the cellular immune response. Under the control of interferon-γ the proteolytic properties of the proteasome are adapted to the requirements of the immune system. Interferon-γ induces the formation of immunoproteasomes and the synthesis of the proteasome activator PA28. Both alter the proteolytic properties of the proteasome complex and enhance proteasomal function in antigen presentation. Thus, a combination of several of regulatory events tunes the proteasome system for maximal efficiency in the generation of MHC class I antigens.
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Keywords: Proteasome; Immune response; Antigen presentation; MHC-complex; Intereferon-γ; Interferon; Antigen processing

1. Introduction

The proteasome system is the central proteolytic system of the eukaryotic cell [1] and plays an important role in the generation of MHC-class I peptides. The major functions of the proteasome are the selective ATP-dependent degradation of cytosolic, nuclear and membrane bound proteins [2] as well as the regulation of such important cellular processes [3–5]. The enzyme complex responsible for the selectivity of intracellular protein degradation is the 26S proteasome that degrades poly-ubiquitinated substrates or in some cases even unmodified proteins in an ATP dependent manner. The 26S proteasome complex is formed by the 20S core particle harbouring the proteolytically active sites and two 19S regulatory particles that are responsible for substrate interaction.

2. The 20S proteasome

The 20S catalytic core, i.e., 20S proteasome, is a barrel-shaped particle composed four heptameric rings. The two outer rings consist of seven different but homologous α subunits (α1–α7) that provide the proteasome structure, control the access to the catalytic chamber and interact with regulatory factors and complexes [6]. The two inner rings are each composed of seven β subunits (β1–β7) three of which (i.e., β1-δ, β2-Z and β5-MB1) display catalytic activity. The central gate formed by the α-subunits is normally closed by the N-termini of the seven α-subunits that keep the proteasome in a proteolytically inactive state [6,7]. Thereby the N-terminus of the α3 subunit interacts with conserved residues on the other α-subunits [8]. Deletion of the N-terminus of the α3 subunit results in the opening of the gate and the facilitated access of substrates. The three active sites within one β-ring differ in their peptide hydrolyzing properties as was shown using short fluorogenic peptide substrates. However, the cleavage preferences of the different active sites are far from absolute since cleavage of a given peptide bond within a longer substrate seems to depend on the quality of neighbouring residues as well as on their interplay with residues which are more distant from the actual cleavage site [9,10]. Not neglecting certain preferences, cleavage site usage by the proteasome is promiscuous in that almost every amino acid residue can serve as a cleavage site, giving the enzyme complex a high degree of flexibility with regard to quality of the products that are generated [3].

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3. The 26S proteasome

The 26S proteasomes is formed through the ATP-dependent association of two 19S regulator complexes [11] with the two outer α-rings of the 20S core [12,13]. The 19S regulator is made up by approximately 18 subunits consisting of two functionally different entities [14]. The base is formed by six different ATPases of the AAA family (*A*TPases *a*ssociated with a variety of cellular *a*ctivities) and two additional non-ATPase subunits. The base binds to the outer α-rings and is responsible for the ATP-dependent opening of the central gate and hence the activation of the 20S core [15] as well as the reverse-chaperone-like unfolding of protein substrates [16,17]. The upper part of the 19S regulator is called the lid. The lid, which provides the specificity to proteolysis, is composed of at least 10 non-ATPase subunits and contains the binding sites for ubiquitinated substrates, non-ubiquitinated substrates and also enzymes for the breakdown and recycling of ubiquitin chains. Although the base is sufficient for unfolding a protein substrate and for opening the gate, the lid components are thought to play a role in the recognition of poly-ubiquitinated proteins and are essentially required for their degradation.

4. The properties of MHC class I antigens

The generation of peptide loaded MHC class I molecules mostly requires the proteolytic generation of peptides with a preferred length of 8–10 amino acids in the cytosol, the transport of peptides via TAP-proteins (transporter associated with antigen processing) into the endoplasmic reticulum [3,4]. There, peptides bind to MHC class I proteins followed by transport of the peptide loaded MHC molecules to the cell surface. Peptide binding to the MHC class I protein occurs through the peptide's side chains with pockets in the peptide binding groove of the MHC protein [18]. The specificity of this interaction is mediated by so-called anchor residues. One of these anchor residues is located at the carboxy-terminus of the peptide. These C-terminal anchors are enriched with hydrophobic or basic residues. To allow peptide binding, the anchor residue positions must be occupied by specific residues defined by the haplotype of the MHC class I molecules. In addition, several of the other residues can influence the stability of the peptide interaction through contacts with the peptide binding groove. The observation that the proteasome prefers cleavage behind hydrophobic or basic residues and the cytokine induced expression of some of its components suggested that the proteasome may be involved in antigen processing. It is now generally accepted that the proteasome system is indeed responsible for the generation of the majority of MHC class I ligands [4]. In some cases, other proteases such as TPPII, TOP or trim-peptidases will also contribute to the pool of antigenic peptides [19–22].

5. IFN-γ and the proteasome system

The first indication that proteasomes may play an important role in the generation of MHC class I presented peptides was the identification of the two IFN-γ inducible proteasome β subunits LMP2 (βi1)and LMP7 (βi5) that are encoded in the MHC class II region in direct neighbourhood to the TAP-genes (transporter associated with antigen presentation) [23–28]. A third subunit whose synthesis is also cytokine-dependent is MECL-1 (βi2) but not encoded within the MHC region [29]. All three interferon-γ inducible proteasome subunits of the β-type harbour active sites and replace the corresponding constitutive β-subunits upon de novo proteasome synthesis. In consequence, new 20S core complexes with altered proteolytic properties are generated.

Stimulation of cells with IFN-γ also induces the synthesis of the α and β subunits of the proteasome activator PA28 [30]. Interestingly, the expression profile of the two subunits largely correlates with that of the immuno-subunits [31]. In concert with the 19S regulator, these five IFN-γ inducible components of the proteasome system form the immunoproteasome, indicating its connection with the cellular immune response [32,33].

6. Immuno-subunits and MHC class I antigen processing

As noted above, under conditions of IFN-γ induction the entire catalytic machinery of the proteasome complex is replaced. In fact, cells with antigen presenting functions as found in the thymus, spleen and lymph nodes express the IFN-γ inducible subunits constitutively [31,34,35]. Furthermore, also other stimuli like TNFα and IFNβ as well as stimulation of dendritic cells can influence the expression levels of the three immuno-subunits [36–38]. First experimental attempts to demonstrate a connection between the proteasome system and MHC class I antigen processing showed that purified constitutive (standard) as well as immunoproteasomes are able generate a correct 9mer epitope derived from the IE pp89 protein of the murine cytomegalovirus [39]. In addition, Michalek et al. [40] presented evidence that defects in the ubiquitinylation pathway disturbed antigen processing of chicken albumin. The availability of membrane-permeable proteasome inhibitors finally allowed to demonstrate a direct correlation between cellular proteasome activity and the production of antigenic peptides that bind to MHC class I molecules [1,41].

In vitro digestion of polypeptide substrates encompassing antigenic peptides by purified 20S proteasomes and studies of antigen presentation with intact cells which were transfected with N-terminally and C-terminally extended antigenic peptides showed that proteasomes liberate the correct C-terminus of an antigenic peptide [39,42–44]. Cleavage at the N-terminus of an epitope is, however, less precise. In

many cases N-terminally extended epitope precursor peptides are generated that are trimmed to the correct size by either ER-resident or cytosolic trim-peptidases [45–51].

To elucidate the role of IFN-γ inducible subunits, purified immunoproteasomes were tested with respect to their peptide hydrolysing activities [35,39,52–56]. These studies showed a clear reduction of the caspase like activity upon incorporation of the immuno-subunits and due to the replacement of β1 by β1i. However, inconsistent changes in the activities displayed by the other two β-subunits, i.e., β5/β5i chymotrypsin-like and β2/β2i trypsin-like, were observed. Furthermore, detailed analysis of cleavage products generated from larger polypeptides and protein substrates by both standard and immunoproteasomes demonstrated that activities of the three different catalytic centers towards longer substrates are less well defined [9,57]. In other words, while cleavages after basic residues in fluorogenic peptide substrates (lysine/arginine) are exclusively mediated by the β2/β2i pair of catalytic subunits [58], cleavages after the same residues within polypeptide substrates appear to be performed by different active site subunits. In addition, also the analysis of mutant cell lines with defective LMP2 and LMP7 expression or expression of mutant, inactive LMP2 did not reveal any consequences on antigen presentation [59–62]. On the other hand, it was found that LMP7 expression was essential for the processing of an influenza virus-derived CTL-epitope [63].

Transfection experiments with cDNA encoding β1i, β2i and β5i in connection with extensive analyses of CTL epitope generation demonstrated that the presence of immuno-subunits enhances the presentation of a major subset of virus-derived antigen peptides [64–67] although this effect does not apply to all viral epitopes.

Despite this accumulating information on the role of the immuno-subunits in MHC class I antigen presentation, the mechanisms that are involved are not fully understood. In vitro digestion experiments with purified 20S proteasomes and synthetic polypeptides encompassing the antigenic peptides and natural flanking sequences as substrate confirmed in almost all cases the observations made in intact cellular systems [64,65]. Analysis of peptide fragments generated in vitro showed that the presence of immuno-subunits altered the cleavage site preference of proteasomes. This resulted in a more frequent usage of specific cleavage sites with the consequence that the relative abundance of certain peptides within the generated peptide pool is changed. Whether, or to which degree, the presence of immuno-subunits also changes the quality of cleavage sites used is at the moment difficult to access.

Interestingly, experiments in which HeLa cells were infected with vaccinia virus expressing the hepatitis B virus (HBV) core antigen showed that the $HBVcAg_{141-151}$ was only efficiently liberated after IFN-γ stimulation. Furthermore, although the concerted presence of all three immuno-subunits seemed to be required an inactive β5i subunit, it also supported epitope generation. These experiments demonstrated that the altered cleavage profile found to be associated with immuno-subunit incorporation was due not only to cleavage specificity but most likely also to structural changes imposed on the proteasome complex by the incorporation of the immuno-subunits [65]. The idea that the primary effect of immuno-subunit incorporation is the alteration of proteasome structure is also supported by the changed chromatographic properties of immunoproteasomes that is caused by changes in their surface charge [68].

7. The proteasome system and T cells

Under non-inflammatory conditions many cell types lack expression of immuno-subunits and express PA28 differentially and only at low levels [69]. This suggests that a continuous presence of these molecules in all tissues is either not required or desired. In fact, neither the incorporation of immuno-subunits nor the up-regulated expression of PA28 in transfectant cells seems to influence the antigen processing independent functions of the proteasome system. Therefore, one possible function of immuno-subunits and PA28 may lie in the development of the CD8+ cell repertoire. In particular, the observation that in some cases immuno-subunits may influence antigen processing of foreign and self-antigens differently appears indicative for this assumption [70]. The expression of immuno-subunits in dendritic cells within the thymus may result in a negative selection of T cells reactive against peptides derived from self antigens, leaving only those T cells that react against peptides generated by constitutive proteasomes as potential targets for autoimmune CTL.

Interestingly, Morel et al. [70] reported that immunoproteasomes are unable to produce several self antigen-derived CTL epitopes including an important CTL epitope derived from Melan-A. These data have been the first example of CTL epitopes that are only generated by constitutive proteasomes and appear to be destroyed by immunoproteasomes. At the same time, it must also be stated that even stimulated dendritic cells always contain a mixed population of proteasomes comprising constitutive as well as immunoproteasomes [31]. Sun et al. [71] recently also reported on a tyrosinase-related protein 2 (TRP2)-derived tumor epitope whose surface presentation was down-regulated in response to IFN-γ in Melanoma 18a cells. However, in that case it could be shown that this was not due to the presence of immunoproteasomes.

8. The role of PA28

The proteasome activator PA28 is a heptameric 180–200-kDa complex composed of α and β subunits [72,73]. PA28 attaches in an ATP-independent way to the outer α-rings of the 20S proteasome and in vitro strongly stimulates the

hydrolysis of short fluorogenic peptide substrates. The turnover rate of cellular native proteins is, however, not to be affected. Detailed kinetic analysis showed that PA28 activates the 20S proteasome without affecting the active sites by either facilitating substrate entry or product exit [74].

A structural explanation for the observed kinetic effects of PA28 binding was obtained when the crystal structure of a complex between the trypanosome 11S regulator PA26 [75] and the yeast 20S proteasome was obtained [76].

The PA26/20S core complex crystal structure reveals that PA26 induces conformational changes via its C-termini in the N-terminal parts of the α-subunits with the effect that the central gates in the two outer α-rings of the 20S core complex are opened [77]. Based on this and on the observation that PA28 facilitates product exit, it was proposed that such an open conformation might allow the release of slightly longer peptides and, in consequence, might support the presentation of MHC class I antigen presentation.

The data obtained from X-ray structure analysis are supported by mutational and structural analysis of yeast proteasomes. A deletion of the N-terminal nine amino acids of the α3 subunits or a mutation within the N-terminus of the α3 subunits results in the complete opening of the central gate controlled by the N-termini of the α-subunits and the full activation of the otherwise latent or inactive proteasome [78,79]. Based on these studies, the so-called “gating model” finds widespread acceptance at the moment.

One important aspect of the current gating model is that opening of the gate by regulatory molecules, i.e., PA28, 19S regulator, does not induce structural changes on the 20S proteasome with the effect that the basic proteolytic activity within the β-rings changed. In contrast, the measured proteolytic activity using short fluorogenic peptide substrates correlates directly with the width of the gate [80,74]. Based on mutational analysis of PA28γ/Ki, a sequence homologue of PA28αβ□ the group around Rechsteiner suggested that binding of the mutated PA28γ/Ki to the α-rings results in a specific allosteric activation of certain active sites [81]. Recent data obtained from tissue culture experiments support Rechsteiner’s conclusion that PA28 induces structural changes on the proteasome [71,82]. From these data it was inferred that the binding of PA28αβ, in vivo and in vitro, may effect substrate channelling and the accessibility of active sites for a given natural substrate, rather then affecting the characteristics of the active sites themselves.

Since proteasome-dependent cellular protein breakdown in general requires ATP and the presence of the 19S regulator, the importance of PA28/20S complexes in vivo is still unclear. Recent investigations showed that PA28 in connection with the 20S core and the 19S regulator may form the so-called hybrid proteasomes [33,80,83,84]. Interestingly, these PA28–20S–19S hybrid proteasomes exhibit a proteolytic activity against fluorogenic peptide substrates, which is almost identical to that of the 19S–20S–19S proteasome. Thus, one can conclude that at least one of the functions of the two different types of proteasome regulator is the opening of the central gates in the two outer α-rings of the 20S core complex, whereby both PA28 and the 19S regulator appear to open the gate to its maximal width.

Stimulation of cells with IFN-γ not only induces the expression of immuno-subunits but also that of PA28αβ. Although PA28, unlike the immuno-subunits, is found in almost any cell type even in the absence of cytokine stimulation, enhanced constitutive levels are observed in cells with specialized antigen presenting function [85]. The investigation of viral antigens showed that PA28 enhances the presentation of some viral epitopes without increasing overall protein turnover or the turnover of viral protein substrates [67,86] while the presentation of other virus-derived epitopes was not effected. Furthermore, this enhanced peptide presentation was independent of the presence of immuno-subunits in the 20S proteasome [64,74] excluding the possibility that PA28 might exert its function by increasing immunoproteasome formation as was proposed recently [86].

Analysis of the effects of PA28 on proteasomal cleavage site usage revealed that PA28 does not induce new cleavage specificities. Furthermore, there is no evidence that PA28 has a major impact on substrate turnover rates. Instead, PA28 enhances the frequency at which specific cleavage sites are used, resulting in the immediate liberation of the peptides residing in between two of these preferred cleavage sites [71,87,88].

The so far most striking example for PA28 dependency of an epitope was recently obtained when the processing and presentation of two CTL epitopes derived from a melanoma differentiation antigen, i.e., TRP2, was analysed. Detailed biochemical and molecular experiments showed that the inability of melanoma 18a cells to present the $TRP2_{360–368}$ epitope correlated with a strongly impaired expression of PA28 in these cells, which also was not restored by IFN-γ treatment. Epitope presentation and the ability to activate $TRP2_{360–368}$ epitope-specific CTLs by melanoma 18a cells were, however, fully restored by transient transfection with cDNAs encoding the PA28α and PA28β subunits. These experiments demonstrated for the first time that PA28 expression can alter the immunological phenotype of a cell.

How then does PA28 exert its effect on antigen processing and presentation? Based on the structural data and the observation that PA28 facilitates product exit, it was proposed that an open conformation of the proteasome might allow the release of slightly longer peptides [71]. In this scenario, these peptides would be rescued from further degradation, which in turn may support the generation of certain CTL epitopes and in particular those that are produced from N-terminally extended precursor peptides.

In fact, it has been shown that even epitopes which are generated by the proteasome with correct size for MHC class I binding are often also produced with short N-terminal extension, requiring trimming by aminopeptidases such TOP or bleomycin hydrolase (BH) [88,89]. In

consequence, if PA28 indeed supports the generation of longer peptides, the result would not only be the generation of a new quality of peptides but also the amount of peptide available would be increased.

However, since both the 19S regulator complex as well as PA28 induce the complete opening of the gate, it is obvious that this gating hypothesis, as attractive as it appears, does not fully explain the effects on substrate cleavage alone. Furthermore, if an increase in peptide length as a result of PA28 action would be of general benefit for MHC class I-dependent antigen presentation, one would expect to find an increased MHC class I surface expression upon PA28 induction. However, in none of the studies performed so far such an effect has been observed. Therefore, an alternative hypothesis, which does not in principle exclude the above discussed gating model, would be that PA28, by binding to the outer α-ring, not only opens the gate at the site opposite to the 19S regulator binding sites but also induces subtle structural changes which will allow better access to the actives sites of those epitope-containing domains (for example by gaining higher affinity to the substrate binding grooves), which otherwise possess only a strongly reduced affinity.

9. The PI31 protein

PI31 is a protein of approximately 31 kDa that presumably functions as homodimer. In vitro PI31 inhibits 20S-mediated cleavage of short fluorogenic substrates and of polypeptides and competes with the binding of PA28 to 20S proteasomes [90,91]. This suggests that PI31 binds the α-rings of the 20S proteasome and thereby obstructs the access to the catalytic cavity. Nevertheless, transfection of PI31 into intact cells recently showed that, rather than inhibiting proteasome function, PI31 acts as a selective modulator of the proteasome-mediated steps in MHC-class I antigen processing. Overexpression of PI31 had no impact on proteasome-mediated proteolysis but interfered selectively with the maturation of immunoproteasome. The in vivo function of PI31 may therefore differ from the previously proposed function as a general inhibitor of proteasome activity [92].

10. The proteasome and immune escape

As the proteasome is the central generating source for antigenic peptides, it is not surprising that viruses and also tumor cells have evolved strategies to interfere with proteasome function. In a number of tumor types, such as colon carcinoma, small cell lung carcinoma, pancreatic carcinoma, hepatocellular carcinoma, renal cell carcinoma and melanoma, a down-regulation of immuno-subunit expression was observed [93–95]. Although the absence of PA28α and -β seems to be less frequently effected there, complete absence has been reported for several melanoma cell lines [96,71]. In some cases the expression of immuno-subunits and/or PA28αβ expression appeared coordinately down-regulated while in other instances only single subunits were affected. In most cases treatment with IFN-γ is able to restore the expression of the proteasome components but there are also exceptions [71].

Direct functional evidence for viral interference with the proteasome system is only just beginning to emerge. In this context, it is important to realize that many viruses are dependent on proteasome activity for their survival. For instance, proteasomes process the HIV gag polyprotein into different gag proteins and that interference with this process impairs maturation of HIV particles [97]. Human papilloma virus (HPV) affects the proteasome system by various mechanisms. For example, the HPV16 E7 protein binds one of the six ATPase subunits of the base thereby enhancing proteasome activity and inducing increased turnover of the Rb retinoblastoma tumor suppressor protein [98]. In addition, HPV16 E6 targets the p53 tumor suppressor protein for proteasome-dependent degradation, by recruiting the ubiquitin ligase E6-AP [99], thus contributing to oncogenic transformation of HPV-infected cells.

Evidence for direct interaction with proteasome subunits has been obtained for the human T-cell leukemia virus typ1 (HTLV-1) Tax protein [100], HBV pX protein [101–103], HIV Nef [104] and Tat [105], and adenovirus (Ad5) E1A. For the Tax protein it was shown that it binds the N3 and C9 subunits and also activates the transcription factor NF-κB. Both the HIV Tat and HBV pX protein bind to subunits of the α-ring (α4) and interfere with the binding of PA28. Interestingly, most recently it could be shown that the binding of HIV Tat to the α-rings also affects the capacity of PA28 to enhance antigen presentation [106].

In addition, both the Adenovirus type 12 E1A and HPV type 18 E7 proteins repress the β1i and/or β5i promoter and, consequently, subunit expression [107–109]. A completely different strategy for proteasome inhibition is introduced by the Epstein–Bar virus nuclear antigen-1 (EBNA1). This protein contains an internal glycine/alanine repeat that hinders the proteasome-dependent degradation of this proteins and the generation of EBNA1-derived CTL epitopes [110,111].

11. Computer models

To simplify CTL epitope identification strategies, computer prediction programs that predict cleavage site usage within proteins have been established. So far, two prediction program-based different parameters have been published [112–114]. One program was trained using 20S cleavage data of the enolase-1 protein; the other program is based on polypeptide cleavage data generated in a large number of studies. Both programs predict proteasomal cleavages with high fidelity whereby the latter program not only identifies

proteasome cleavage sites but also predicts the probability with which specific fragments are generated. Indeed, the application of this program in combination with a program that predicts MHC class I binding affinity led to the identification of eight CTL epitopes derived from the entire deduced proteome of *Chlamydia trachomatis* that are presented by HLA-B27 molecules in patients suffering from rheumatoid arthritis [115].

In summary, these computer-assisted approaches enable a rapid and easy identification of CTL Epitopes that can be included in the aimed CTL inducing vaccine and will help the selection of Epitope flanking spacers that improve proteasome-mediated epitope processing. The existing proteasome cleavage programs are currently tested for general applicability whereby the availability of new cleavage data obtained from in vitro digestion of designed constructs with purified proteasomes, in turn, will allow the designers to improve their programs.

References

[1] K.L. Rock, C. Gramm, L. Rothstein, K. Clark, R. Stein, L. Dick, D. Hwang, A.L. Goldberg, Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules, Cell 78 (1994) 761–771.

[2] R.K. Plemper, D.H. Wolf, Retrograde transport translocation: reverse protein transport and its end in the proteasome, Trends Biochem. Sci. 24 (1999) 266–270.

[3] P.-M. Kloetzel, Antigen processing by the proteasome, Nat. Rev., Mol. Cell Biol. 2 (2001) 179–187.

[4] L.K. Rock, I.A. York, T. Saric, A.L. Goldberg, Protein degradation and the generation of MHC class I-presented peptides, Adv. Immunol. 80 (2002) 1–59.

[5] M.H. Glickman, D.M. Rubin, O. Coux, I. Wefes, G. Pfeifer, Z. Cjeka, W. Baumeister, V.A. Fried, D. Finley, A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3, Cell 94 (1998) 615–623.

[6] M. Groll, L. Ditzel, J. Löwe, D. Stock, M. Bochtler, H.D. Bartunik, R. Huber, Structure of 20S proteasome from yeast at 2.4 A resolution, Nature 386 (1997) 463–471.

[7] M. Groll, M. Bakorke, A. Kohler, L. Moroder, D.M. Rubin, R. Huber, M. Glickman, D. Finley, A gated channel into the proteasome core particle, Nat. Struct. Biol. 7 (2000) 1062–1067.

[8] A. Köhler, P. Cascio, D.S. Legget, K.M. Woo, A.L. Goldberg, D. Finley, The axial channel of the proteasome core particle is gated by the rpt2 ATPase and controls both substrate entry and product release, Mol. Cell 7 (2001) 1143–1152.

[9] A.K. Nussbaum, T.P. Dick, W. Keilholz, M. Schirle, S. Stevanovic, K. Dietz, W. Heinemeyer, M. Groll, D.H. Wolf, R. Huber, H.G. Rammensee, H. Schild, Cleavage motifs of the yeast proteasome βeta subunits deduced from digests of enolase 1, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12504–12509.

[10] N. Shimbara, H. Nakajama, N. Tanahasi, K. Ogawa, S. Niwa, A. Uenakla, E. Nakayama, K. Tanaka, K. Double, Cleavage production of the CTL epitope by proteasomes and PA28: role of the flanking region, Genes Cells 2 (1997) 785–800.

[11] K. Ferrell, C.R.M. Wilkinson, W. Dubiel, C. Gordon, Regulatory subunit interactions of the 26S proteasome, a complex problem, Trends Biochem. Sci. 25 (2000) 83–88.

[12] J.M. Peters, W.W. Franke, J.A. Kleinschmidt, Distinct 19S and 20S subcomplexes of the 26S proteasome and their distribution in the nucleus and the cytoplasm, J. Biol. Chem. 269 (1999) 7709–7718.

[13] D. Voges, P. Zwickl, W. Baumeister, The 26S proteasome: a molecular machine designed for controlled proteolysis, Annu. Rev. Biochem. 68 (1999) 1015–1068.

[14] M.H. Glickman, D.M. Rubin, H. Fu, C.N. Larsen, O. Coux, I. Wefes, G. Pfeifer, Z. Cjeka, R. Vierstra, W. Baumeister, V. Fried, D. Finley, Functional analysis of the proteasome regulatory particle, Mol. Biol. Rep. 26 (1999) 21–28.

[15] M.H. Glickman, A. Ciechanover, The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction, Physiol. Rev. 82 373–428.

[16] B. Braun, M. Glickman, R. Kraft, B. Dahlmann, P.-M. Kloetzel, D. Finley, M. Schmidt, The base of the proteasome regulatory particle exhibits ATP-dependent chaperone-like activity, Nat. Cell Biol. 1 (1999) 221–226.

[17] E. Strickland, K. Hakala, P.J. Thomas, G.N. DeMartino, Recognition of misfolded proteins by PA 700, the regulatory subcomplex of the 26S proteasome, J. Biol. Chem. 275 (2000) 5565–5572.

[18] D.R. Madden, The three-dimensional structure of peptide–MHC complexes, Annu. Rev. Immunol. 13 (1995) 587–622.

[19] U. Seifert, C. Maranon, A. Shmueli, J.F. Desoutter, L. Wesoloski, K. Janek, P. Henklein, S. Diescher, M. Andrieu, H. De La Salle, T. Weinschenk, H. Schild, D. Laderach, A. Galy, G. Haas, P.-M. Kloetzel, Y. Reiss, A. Hosmalin, An essential role for tripeptidyl peptidase in the generation of an MHC class I epitope, Nat. Immunol. (2003) 375–379.

[20] T. Serwold, S. Gaw, N. Shastri, ER aminopeptidases generate a unique pool of peptides for MHC class molecules, Nat. Immunol. 2 (2001) 644–651.

[21] F. Levy, L. Burri, S. Morel, A.L. Peitrequin, N. Levy, A. Bachi, U. Hellman, B.J. Van den Eynde, C. Servis, The final N-terminal trimming of a subaminoterminal proline-containing HLA class I-restricted antigenic peptide in the cytosol is mediated by two peptidases, J. Immunol. 169 (2002) 461–471.

[22] L. Saveanu, D. Fruci, P. van Endert, Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases, Mol. Immunol. 39 (2002) 203–214.

[23] R. Glynne, S. Powis, S. Beck, A. Kelly, L.A. Kerr, J. Trowsdale, A proteasome related gene between the two abc transporter loci in the calls II region of the human MHC, Nature 353 (1991) 357–360.

[24] M.G. Brown, J. Driscoll, J.J. Monaco, Structural and serological similarity of MHC linked LMP and proteasome (multicatalytic proteinase complex), Nature 353 (1991) 355–358.

[25] A. Kelly, S.H. Powis, R. Glynne, J. Trowsdale, Second proteasome-related gene in the human MHC class II region, Nature 353 (1991) 667–668.

[26] V. Ortiz-Narvarete, A. Seelig, M. Gernold, S. Frentzel, P.-M. Kloetzel, G.J. Hämmerling, Subunit of the 20S proteasome (multicatalytic proteinase) encoded by the major histocompatibility complex, Nature 353 (1991) 662–672.

[27] C.K. Martinez, J.J. Monaco, Homology of proteasome subunits to a major histocompatibilty linked LMP2 gene, Nature 353 (1991) 664–665.

[28] Y. Yang, K. Fruh, J. Chambers, J.B. Waters, L. Wu, T. Spies, P.A. Peterson, Major histocompatibility complex (MHC)-encoded HAM2 is necessary for antigenic peptide loading onto class I MHC molecules, J. Biol. Chem. 267 (1992) 11669–11672.

[29] M. Groettrup, S. Standera, R. Stohwasser, P.-M. Kloetzel, The subunits MECL-1 and LMP2 are mutually required for incorporation into the 20S proteasome, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8970–8975.

[30] D. Nandi, H. Jiang, J.J. Monaco, Identification of MECL-1 (LMP-10) as the third IFN-gamma-inducible proteasome subunit, J. Immunol. 156 (1996) 2361–2364.

[31] A. Macagno, M. Gillet, F. Sallust, A. Lanzavecchia, F.O. Nestle, M. Groettrup, Dendritic cells up-regulate immunoproteasomes and the proteasome regulator PA28 during maturation, Eur. J. Immunol. 29 (1999) 4037–4042.

[32] M. Aki, N. Shimbara, M. Takashina, K. Akiyama, S. Kagawa, T. Tamura, N. Tanahashi, T. Yoshimura, K. Tanaka, A. Ichihara, Interferon-gamma induces different subunit organizations and functional diversity of proteasomes, J. Biochem. 115 (1994) 257–269.

[33] K.B. Hendil, S. Khan, K. Tanaka, Simultaneous binding of PA28 and PA700 activators to 20S proteasomes, Biochem. J. 332 (1998) 749–754.

[34] R. Stohwasser, S. Standera, I. Peters, P.-M. Kloetzel, M. Groettrup, Molecular cloning of the mouse proteasome subunits MC14 and MECL-1: reciprocally regulated tissue expression of interferon-gamma-modulated proteasome subunits, Eur. J. Immunol. 27 (1997) 1182–1187.

[35] A.M. Eleuteri, R.A. Kohanski, C. Cardozo, C. Orlowski, Bovine spleen multicatalytic proteinase complex (proteasome): replacement of X, Y, and Z subunits by LMP7; LMP2 and MECL1 and changes in properties and specificity, J. Biol. Chem. 272 (1997) 11824–11831.

[36] M. Jamaluddin, S. Wang, R.P. Garofalo, T. Elliott, A. Casola, S. Baron, A.R. Brasier, IFN-beta mediates coordinate expression of antigen-processing genes in RSV-infected pulmonary epithelial cells, Am. J. Physiol., Lung Cell. Mol. Physiol. 280 (2001) 248–257.

[37] A. Loukissa, C. Cardozo, C. Altschuller-Felberg, J.E. Nelson, Control of LMP7 expression in human endothelial cells by cytokines regulating cellular and humoral immunity, Cytokine 12 (2000) 1326–1330.

[38] U. Kuckelkorn, T. Ruppert, B. Strehl, P.R. Jungblut, U. Zimny-Arndt, S. Lamer, I. Prinz, I. Drung, P.-M. Kloetzel, S.H. Kaufmann, U. Steinhoff, Link between organ-specific antigen processing by 20S proteasomes and CD8(+) T cell-mediated autoimmunity, J. Exp. Med. 195 (2002) 983–990.

[39] B. Boes, H. Hengel, T. Ruppert, G. Multhaup, U. Koszinowski, P.-M. Kloetzel, IFN-□ stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes, J. Exp. Med. 179 (1994) 901–909.

[40] M.T. Michalek, E. Grant, C. Gramm, A.L. Goldberg, K.L. Rock, A role for the ubiquitin-dependent proteolytic pathway in MHC class I restricted antigen presentation, Nature 363 (1993) 552–554.

[41] C.V. Harding, Class I MHC presentation of exogenous antigens, J. Clin. Immunol. 16 (1996) 90–96.

[42] T. Dick, T. Ruppert, M. Groettrup, P.-M. Kloetzel, L. Kuehn, U. Koszinowski, S. Stevanovic, H.J. Schild, H.G. Rammensee, Coordinated dual cleavages induced by the proteasome regulator PA28 lead to dominant MHC ligands, Cell 86 (1996) 253–262.

[43] M. Eggers, T. Ruppert, B. Boes-Fabian, P.-M. Kloetzel, U. Koszinowski, The cleavage preference of the proteasome governs the yield of antigenic peptides, J. Exp. Med. 182 (1995) 1865–7180.

[44] G. Niedermann, R. Grimm, E. Geier, M. Maurer, C. Realini, C. Gartmann, J. Soll, S. Omura, M.C. Rechsteiner, W. Baumeister, K. Eichmann, Potential immunocompetence of proteolytic fragments produced by proteasomes before evolution of the vertebrate immune system, J. Exp. Med. 186 (1997) 209–220.

[45] F. Ossendorp, M. Eggers, A. Neisig, T. Ruppert, M. Groettrup, A. Sijts, E. Mengede, P.-M. Kloetzel, J. Neefjes, U. Koszinowski, C. Melief, A single residue exchange within a viral CTL epitope alters proteasome-mediated degradation resulting in lack of antigen presentation, Immunity 5 (1996) 115–124.

[46] A. Craiu, T. Akopian, A.L. Goldberg, K.L. Rock, Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10850–10855.

[47] C. Knuehl, P. Spee, T. Ruppert, U. Kuckelkorn, P. Henklein, J. Neefjes, P.-M. Kloetzel, The murine cytomegalovirus pp89 immunodominant H-2Ld epitope is generated and translocated into the endoplasmic reticulum as an 11-mer precursor peptide, J. Immunol. 167 (2001) 1515–1521.

[48] J. Beninga, K.L. Rock, A.L. Goldberg, Interferon-gamma can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase, J. Biol. Chem. 273 (1998) 18734–18744.

[49] T. Serwold, F. Gonzalez, J. Kim, R. Jacob, N. Shastri, ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum, Nature 419 (2002) 480–483.

[50] L. Stoltze, M. Schirle, G. Schwarz, C. Schroter, M.W. Thompson, L. Hersh, H. Kalbacher, S. Stevanovich, H.G. Rammensee, H.J. Schild, Two new proteases in the MHC class I processing pathway, Nat. Immunol. 1 (2000) 413–418.

[51] T. Saric, S.H. Chang, A. Hattori, I.A. York, S. Markant, K.L. Rock, M. Tsujimoto, A.L. Goldberg, ERAP1, an interferon-□-induced aminopeptidase in the endoplasmic reticulum, that trims precursors to MHC class I-presented peptides, Nat. Immunol. 3 (2002) 1169–1176.

[52] J. Driscoll, M.G. Brown, D. Finley, J.J. Monaco, MHC-linked *LMP* gene products specifically after peptidase activities of the proteasome, Nature 365 (1993) 262–264.

[53] U. Kuckelkorn, S. Frentzel, R. Kraft, S. Kostka, M. Groettrup, P.-M. Kloetzel, Incorporation of major histocompatibilty complex encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-γ, Eur. J. Immunol. 25 (1995) 2605–2611.

[54] V. Ustrell, G. Pratt, M. Rechsteiner, Effects of interferon gamma and major histocompatibilty complex encoded subunits on peptidase activities of human multicatalytic proteases, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 584–588.

[55] M. Gacynska, K.L. Rock, A.L. Goldberg, Gamma interferon and expression of MHC genes regulate peptide hydrolysis, Nature 363 (1993) 262–264.

[56] M. Groettrup, A. Soza, U. Kuckelkorn, P.-M. Kloetzel, Peptide antigen production by the proteasome: complexity provides efficiency, Immunol. Today 17 (1996) 429–435.

[57] G. Schmidtke, M. Eggers, T. Ruppert, M. Groettrup, U. Koszinowski, P.-M. Kloetzel, Inactivation of a defined active site in the mouse 20S proteasome complexes enhances MHC class I presentation of a murine cytomegalovirus protein, J. Exp. Med. 187 (1998) 1641–1646.

[58] U. Salzmann, S. Kral, B. Braun, S. Standera, M. Schmidt, P.-M. Kloetzel, A. Sijts, Mutational analysis of subunit i beta2 (MECL-1) demonstrates conservation of cleavage specificity between yeast and mammalian proteasomes, FEBS Lett. 54 (1999) 11–15.

[59] F. Momburg, V. Ortiz-Navarrete, J. Neefjes, E. Goulmy, Y. van de Wal, H. Spits, S.J. Powis, G.W. Butcher, J.C. Howard, P. Walden, et al., Proteasome subunits encoded by the major histocompatibility complex are not essential for antigen presentation, Nature 360 (1992) 174–177.

[60] D. Arnold, J. Driscoll, M. Androlewicz, E. Hughes, P. Cresswell, T. Spies, Nature 360 (1993) 171–174.

[61] J. Yewdell, C. Lapham, I. Bacik, T. Spies, J. Bennink, MHC-encoded proteasome subunits LMP2 and LMP7 are not required for efficient antigen presentation, J. Immunol. 152 (1994) 1163–1170.

[62] G. Schmidtke, M. Eggers, T. Ruppert, M. Groettrup, U. Koszinowski, P.-M. Kloetzel, Inactivation of a defined active site in the mouse 20S proteasome complexes enhances MHC class I presentation of a murine cytomegalovirus protein, J. Exp. Med. 187 (1998) 1641–1646.

[63] V. Cerundolo, A. Kelly, T. Elliott, J. Trowsdale, A. Townsend, Genes encoded in the major histocompatibility complex affecting the generation of peptides for TAP transport, Eur. J. Immunol. 25 (1995) 554–562.

[64] K. Schwarz, M. Eggers, A. Soza, U.H. Koszinowski, P.-M. Kloetzel, M. Groettrup, The proteasome regulator PA28 αβ can enhance

antigen presentation without affecting 20S proteasome subunit composition, Eur. J. Immunol. 165 (2000) 768–778.
[65] A. Sijts, T. Ruppert, B. Rehermann, M. Schmidt, U. Koszinowski, P.-M. Kloetzel, Structuralfeatures of immunoproteasomes determine the efficient generation of a viral CTL epitope, J. Exp. Med. 191 (2000) 503–513.
[66] A. Sijts, S. Standera, N. Beekmann, P.A. van Veelen, F.A Ossendorp, C.J. Melief, P.-M. Kloetzel, MHC class I antigen processing of an Adenovirus CTL epitope is linked to the levels of immunoproteasomes in infected cells, J. Immunol. 164 (2000) 450–456.
[67] T. van Hall, A. Sijts, M. Camps, R. Offringa, C. Melief, P.-M. Kloetzel, F. Ossendorp, Differential influence on CTL epitope presentation by controlled expression of either proteasome immuno-subunits or PA28, J. Exp. Med. 192 (2000) 483–492.
[68] B. Dahlmann, T. Ruppert, L. Kuehn, S. Merforth, P.-M. Kloetzel, Different proteasome subtypes in a single tissue exhibit different enzymatic properties, J. Mol. Biol. 303 (2000) 643–653.
[69] A. Soza, C. Knuehl, M. Groettrup, P. Henklein, K. Tanaka, P.-M. Kloetzel, Expression and subcellular localization of mouse 20S proteasome activator complex PA28, FEBS Lett. 413 (1997) 27–34.
[70] S. Morel, F. Levy, O. Burleit-Schiltz, F. Brasseur, M. Probst-Kepper, A.L. Peitrequin, B. Monsarrat, R. Van Velthoven, J.C. Certtini, T. Boon, J.E. Gairin, B.J. Van den Eynde, Processing of some antigens by standard proteasome but not by the immunoproteasome result in poor presentation by dendritic cells, Immunity 12 (2000) 101–117.
[71] Y. Sun, A. Sijts, M. Song, K. Janek, A.K. Nussbaum, S. Kral, M. Schirle, S. Stevanovic, A. Paschen, H.J. Schild, P.-M. Kloetzel, A. Schadendorf, Expression of the proteasome activator PA28 rescues the presentation of a cytotoxic T lymphocyte epitope on melanoma cells, Cancer Res. 62 (2002) 2875–2882.
[72] W. Dubiel, G. Pratt, K. Ferrell, M. Rechsteiner, Purification of an11S regulator of the multicatalytic proteinase, J. Biol. Chem. 267 (1992) 22369–22377.
[73] C.P. Ma, C.A. Slaugther, G.N. DeMartino, Identification, purification and characterisation of a protein activator (PA28) of the 20S proteasome (macropain), J. Biol. Chem. 267 (1992) 10115–10123.
[74] R. Stohwasser, U. Salzmann, J. Giesebrecht, P.-M. Kloetzel, H.G. Holzhütter, Kinetic evidences for facilitation of peptide channelling by the proteasome activator PA28, Eur. J. Biochem. 276 (2000) 6221–6229.
[75] F.G. Whitby, E.I. Master, L. Kramer, J.R. Knowlton, Y. Yao, C.C. Wang, C.P. Hill, Structural basis for the activation of 20S proteasomes by 11S regulators, Nature 408 (2000) 115–120.
[76] J.R. Knowlton, J.R. Johnston, F.G. Whitby, C. Realini, Z. Zhang, M. Rechsteiner, C.P. Hill, Structure of the proteasome activator REGalpha (PA28alpha), Nature 390 (1997) 639–643.
[77] C.P. Hill, E.I. Masters, F.G. Whitby, The 11S regulators of 20S proteasome activity, Curr. Top. Microbiol. Immunol. 268 (2002) 73–89.
[78] M. Groll, M. Bakorke, A. Kohler, L. Moroder, D.M. Rubin, R. Huber, M. Glickman, D. Finley, A gated channel into the proteasome core particle, Nat. Struct. Biol. 7 (2000) 1062–1067.
[79] A. Köhler, P. Cascio, D.S. Legget, K.M. Woo, A.L. Goldberg, D. Finley, The axial channel of the proteasome core particle is gated by the rpt2 ATPase and controls both substrate entry and product release, Mol. Cell 7 (2001) 1143–1152.
[80] F. Kopp, B. Dahlmann, L. Kuehn, Reconstitution of hybrid proteasomes from purified PA700-20 S complexes and PA28alpha-beta activator: ultrastructure and peptidase activities, J. Mol. Biol. 313 (2001) 465–471.
[81] J. Li, X. Gao, J. Ortega, T. Nazif, L. Joss, M. Bogyo, A.C. Steven, M. Rechsteiner, Lysine 188 substitutions convert the pattern of proteasome activation by REGgamma to that of REGs alpha and beta, EMBO J. 20 (2001) 3359–3369.
[82] A. Sijts, Y. Sun, K. Janek, A. Paschen, D. Schadendorf, P.-M. Kloetzel, The role of proteasome activator PA28 in antigen processing, Mol. Immunol. 39 (2002) 165–172.
[83] A.F. Kisselev, D. Kaganovich, A.L. Goldberg, Binding of hydrophobic peptides to several non-catalytic sites promotes peptide hydrolysis by all active sites of 20 S proteasomes: evidence for peptide-induced channel opening in the alpha-rings, J. Biol. Chem. 277 (2002) 22260–22270.
[84] N. Tanahashi, Y. Murakami, Y. Minami, N. Shimbara, K.B. Hendil, K. Tanaka, Hybrid proteasomes: induction by interferon-□ and contribution to ATP-dependent proteolysis, J. Biol. Chem. 275 (2000) 14336–14345.
[85] M. Groettrup, M. Soza, M. Eggers, L. Kuehn, T.P. Dickal, H.J. Schild, H.G. Rammensee, U.H. Koszinowski, P.-M. Kloetzel, A role for the proteasome regulator PA28 α in antigen presentation, Nature 381 (1996) 166–168.
[86] T. Preckel, W.P. Fung-Leung, Z. Cai, A. Vitiello, L. Salter-Cid, O. Winqvist, T.G. Wolf, E.M. Von Herrath, A. Angulo, P. Ghazal, J.D. Lee, A.M. Fourie, Y. Wu, J. Pang, K. Ngo, P.A. Peterson, K. Früh, Y. Yang, Impaired immunoproteasome assembly and immune response in PA28-/-mice, Science 286 (1999) 2162–2165.
[87] U. Kuckelkorn, E. Ferreira, I. Drung, U. Liewer, P.-M. Kloetzel, M. Theobald, The effect of the interferon-γ inducible processing machinery on the generation of a naturally tumor-associated human cytotoxic lymphocyte epitope within a wild type and mutant p53 sequence context, Eur. J. Immunol. 32 (2002) 1368–1375.
[88] L. Stoltze, M. Schirle, G. Schwarz, C. Schroter, M.W. Thompson, L.B. Hersh, H. Kalbacher, S. Stevanovic, H.G. Rammensee, H. Schild, Two new proteases in the MHC class I processing pathway, Nat. Immunol. 1 (2000) 413–418.
[89] E. Geier, G. Pfeifer, M. Wilm, M. Lucchiari-Hartz, W. Baumeister, K. Eichmann, G. Niedermann, A giant protease with potential to substitute for some functions of the proteasome, Science 283 (1999) 978–981.
[90] D.M. Zaiss, S. Standera, H. Holzhutter, P.-M. Kloetzel, A.J. Sijts, The proteasome inhibitor PI31 competes with PA28 for binding to 20S proteasomes, FEBS Lett. 457 (1999) 333–338.
[91] S.L. McCutchen-Maloney, K. Matsuda, N. Shimbara, D.D. Binns, K. Tanaka, C.A. Slaughter, G.N. DeMartino, cDNA cloning, expression, and functional characterization of PI31, a proline-rich inhibitor of the proteasome, J. Biol. Chem. 275 (2000) 18557–18565.
[92] D. Zaiss, S. Standera, P.-M. Kloetzel, A. Sijts, PI31 is a modulator of proteasome formation and antigen processing, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 14344–14349.
[93] K. Delp, F. Momburg, C. Hilmes, C. Huber, B. Seliger, Functional deficiencies of components of the MHC class I antigen pathway in human tumors of epithelial origin, Bone Marrow Transplant. 25(Suppl. 2) (2000) S88–S95.
[94] N.P. Restifo, F. Esquivel, Y. Kawakami, J.W. Yewdell, J.J. Mule, S.A. Rosenberg, J.R. Bennink, Identification of human cancers deficient in antigen processing, J. Exp. Med. 177 (1993) 265–272.
[95] B. Seliger, A. Hohne, A. Knuth, H. Bernhard, T. Meyer, R. Tampe, F. Momburg, C. Huber, Analysis of the major histocompatibility complex class I antigen presentation machinery in normal and malignant renal cells: evidence for deficiencies associated with transformation and progression, Cancer Res. 56 (1996) 1756–1760.
[96] B. Seliger, U. Wollscheid, F. Momburg, T. Blankenstein, C. Huber, Characterization of the major histocompatibility complex class I deficiencies in B16 melanoma cells, Cancer Res. 61 (2001) 1095–1099.
[97] U. Schubert, L.C. Antón, J. Gibbs, C.C. Norbury, J.W. Yewdell, S.F. Bennink, Rapid degradation of a large fraction of newly synthesised proteins by proteasomes, Nature 404 (2000) 770–774.
[98] E. Berezutskaya, S. Bagchi, The human papillomavirus E7 oncoprotein functionally interacts with the S4 subunit of the 26 S proteasome, J. Biol. Chem. 272 (1997) 30135–30140.
[99] M. Scheffner, J.M. Hulbregtse, R.D. Vierstra, P.M. Howley, The HPV-16 E6 and E6–AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53, Cell 75 (1993) 495–505.

[100] R. Rousset, C. Desbois, F. Bantignies, P. Jalinot, Effects of the NF-kB1/105 processing of the interaction between HTLV-1 trans-activator Tax and the proteasome, Nature 381 (1996) 328–331.
[101] M. Fischer, L. Runkel, H. Schaller, HBx protein of hepatitis B virus interacts with the C-terminal portion of a novel human proteasome α-subunit, Virus Genes 10 (1995) 99–102.
[102] J. Huang, J. Kwong, E.C. Sun, T.J. Liasng, Proteasome complex as a potential cellular target for hepatitis B virus X protein, J. Virol. 70 (1996) 5582–5591.
[103] R. Stohwasser, H.G. Holzhütter, U. Lehmann, P. Henklein, P.-M. Kloetzel, Hepatitis B virus HBx peptide 116–138 and proteasome activator PA28 compete for binding to the proteasome α4/MC6 subunit, Biol. Chem. 384 (2002) 39–49.
[104] A.S. Turnell, R.J.A. Grand, C. Gorbea, X. Zhang Wang, J.S. Mymryk, P.H. Gallimore, Regulation of 26S proteasome by adenovirus, EMBO J. 19 (2000) 4759–4773.
[105] M. Seeger, K. Ferrell, R. Frank, W. Dubiel, HIV-tat inhibits the 20S proteasome and its 11S regulator-mediated activation, J. Biol. Chem. 272 (1997) 8145–8148.
[106] X. Huang, U. Seifert, U. Salzmann, P. Henklein, R. Preissner, W. Henke, A. Sijts, P.-M. Kloetzel, W. Dubiel, The RTP site shared by the HIV-1 TAT protein and the 11 regulator subunit a is crucial for their effects on proteasome function including antigen processing, J. Mol. Biol. 323 (2002) 771–782.
[107] J.A. Proffitt, G.E. Blair, The MHC-encoded TAP1/LMP2 bidirectional promoter is down-regulated in highly oncogenic adenovirus type 12 transformed cells, FEBS Lett. 400 (1997) 141–144.
[108] N.T. Georgopoulos, J.L. Proffitt, G.E. Blair, Transcriptional regulation of the major histocompatibility complex (MHC) class I heavy chain, TAP1 and LMP2 genes by the human papillomavirus (HPV) type 6b, 16 and 18 E7 oncoproteins, Oncogene 19 (2000) 4930–4935.
[109] A. Rotem-Yehudar, M. Groettrup, A. Soza, P.-M. Kloetzel, R. Ehrlich, LMP-associated proteolytic activities and TAP-dependent peptide transport for class I MHC molecules are suppressed in cell lines transformed by the highly oncogenic adenovirus 12, J. Exp. Med. 183 (1996) 499–514.
[110] J. Levitskaya, M. Coram, V. Levitsky, S. Imreh, P.M. Steigerwald-Mullen, G. Klein, M.G. Kurilla, M.G. Masucci, Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1, Nature 375 (1995) 685–688.
[111] J. Levitskaya, A. Sharipo, A. Leonchiks, A. Ciechanover, M.G. Masucci, Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly–Ala repeat domain of the Epstein–Barr virus nuclear antigen 1, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 12616–12621.
[112] J.H. Kessler, N.J. Beekman, S.A. Bres Vloemans, P. Verdijk, P.A. Van Veelen, A.M. Kloosterman-Joosten, D.C. Vissers, G.J. ten Bosch, M.G. Kester, A. Sijts, J.W. Drijfhout, F. Ossendorp, R. Offringa, C.J. Melief, Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen by proteasome-mediated digestion analysis, J. Exp. Med. 193 (2001) 73–88.
[113] H.-G. Holzhütter, P.-M. Kloetzel, A kinetic model of vertebrate 20S proteasome accounting for the generation of major proteolytic fragments from oligomeric peptide substrates, Biophys. J. 79 (2000) 1196–1205.
[114] C. Kuttler, A.K. Nussbaum, T.P. Dich, H.G. Rammensee, H. Schild, K.P. Hadeler, An algorithm for the prediction of proteasome cleavage, J. Mol. Biol. 298 (2001) 417–429.
[115] W. Kuon, H.G. Holzhutter, H. Appel, M. Grolms, S. Knollnberger, A. Traeder, P. Henklein, E. Weiss, A. Thiel, R. Lauster, P. Bowness, A. Radbruch, P.-M. Kloetzel, J. Sieper, Identification of HLA-B27-restricted peptides from *Chlamydia trachomatis* proteome with possible relevance to HLA-B27-associated diseases, J. Immunol. 167 (2001) 4738–4746.