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.

Acknowledgements

We thank A. Förster and C. Gorbea for assistance with the figures, and A. Steven and J. Ortega for providing the image in Figure 1c.

References

1 Ciechanover, A. *et al.* (2000) Ubiquitin-mediated proteolysis: biological regulation via destruction. *Bioessays* 22, 442–451
2 Löwe, J. *et al.* (1995) Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 Å resolution. *Science* 268, 533–539
3 Groll, M. *et al.* (1997) Structure of 20S proteasome from yeast at 2.4Å resolution. *Nature* 386, 463–471
4 Groll, M. *et al.* (2000) A gated channel into the proteasome core particle. *Nat. Struct. Biol.* 7, 1062–1067
5 Voges, D. *et al.* (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu. Rev. Biochem.* 68, 1015–1068
6 Hill, C.P. *et al.* (2002) The 11S regulators of 20S proteasome activity. *Curr. Top. Microbiol. Immunol.* 268, 73–89
7 Ustrell, V. *et al.* (2002) PA200, a nuclear proteasome activator involved in DNA repair. *EMBO J.* 21, 3516–3525
8 Whitby, F.G. *et al.* (2000) Structural basis for the activation of 20S proteasomes by 11S regulators. *Nature* 408, 115–120
9 Forster, A. *et al.* (2003) The pore of activated 20S proteasomes has an ordered 7-fold symmetric conformation. *EMBO J.* 22, 4356–4364
10 Li, J. *et al.* (2001) Lysine 188 substitutions convert the pattern of proteasome activation by REGγ to that of REGs α and β. *EMBO J.* 20, 3359–3369
11 Tanahashi, N. *et al.* (2000) Hybrid proteasomes. Induction by interferon-γ and contribution to ATP-dependent proteolysis. *J. Biol. Chem.* 275, 14336–14345
12 Kajava, A.V. *et al.* (2004) New HEAT-like repeat motifs in proteins regulating proteasome structure and function. *J. Struct. Biol.* 146, 425–430