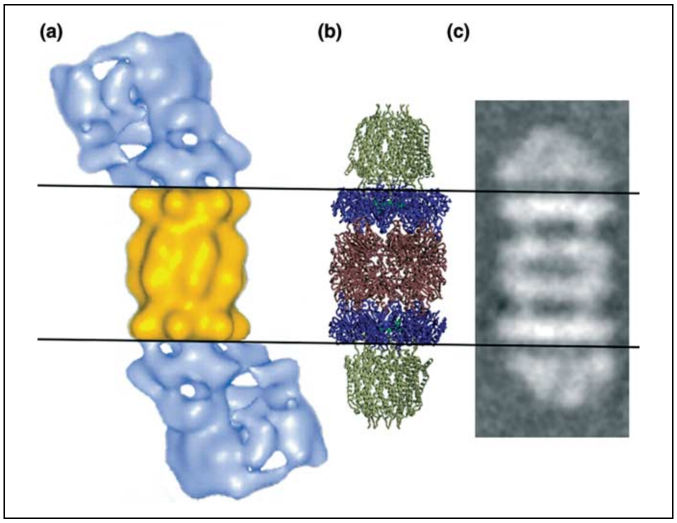

Figure 3. Proteasome–activator complexes. The horizontal lines delineate the ends of the 20S proteasome in these complexes. **(a)** Cryoelectron microscopy structure of the PA700–20S-proteasome complex (26S proteasome). Image adapted, with permission, from Ref. [5]. © (1999) Annual Reviews (www.annualreviews.org). **(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