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