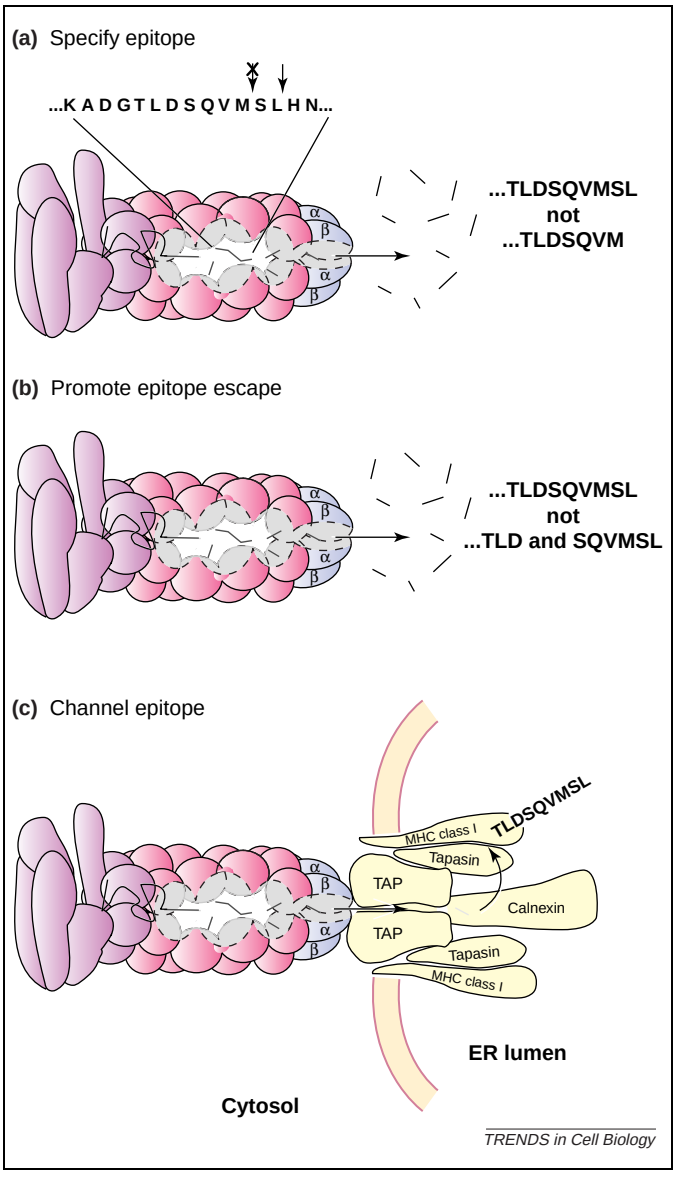


**Figure 5.** Three ways in which PA28αβ could promote presentation of a class I epitope. **(a)** The proteasome activator could bias peptide-bond cleavage so that the C-terminal residue of the epitope is leucine, not methionine. **(b)** By opening a channel through the proteasome α ring, PA28αβ could 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