# Abstract

The ubiquitin proteasome system is the major pathway for selective intracellular protein degradation. At the center of the degradation mechanism stands the 26S proteasome, composed of the 20S proteasome (core particle) and the 19S proteasome (regulatory particle). A close inspection of the crystal structure of the yeast 20S proteasome revealed that a prominent connection between the two β-rings is mediated by the subunit β7/Pre4. Its C-terminal extension intercalates between the β1/Pre3 and β2/Pup1 subunits on the opposite ring.

This work demonstrates that deletion of the 19 amino acid residues from the β7/Pre4 C terminus leads to an accumulation of half-proteasome precursor complexes, denoting its function in stabilizing the newly formed dimer. Furthermore, it is required for the maturation and activity of the post-acidic catalytic site mediated by β1/Pre3.

Purification of the half-proteasome complex lead to the observation that β7/Pre4 is the only subunit not present in these complexes and that its overexpression leads to a faster assembly of precursor complexes into 20S core particles. It is also shown that the regulation of *PRE4* expression by Rpn4 differs from that of other proteasomal subunits. Moreover, Blm10, a 246 kDa protein assumed to be the homolog of the PA200 activator in yeast was also detected in half proteasomes. While a *blm10Δ* mutant did not display severe effects on the proteasome assembly and maturation, apparently because the 19S regulator is capable of replacing Blm10, the *blm10Δ pre4*ΔC19 double mutant resulted in striking assembly and maturation defects.

Taken together, these data demonstrate that incorporation of β7/Pre4 is a rate-limiting step in the dimerization of half proteasomes and, together with Blm10, it stabilizes the nascent 20S proteasomal structure and promotes its maturation.