ABSTRACT

The conserved ubiquitin/proteasome system is the major intracellular machinery for selective protein degradation in all eukaryotes. Many essential cellular functions are regulated by this pathway and inhibition of the proteasome is lethal. The ubiquitin-conjugates degrading 26S proteasome is a large ca. 2 MDa complex of more than 30 different subunits. It is assembled from precursor complexes in a highly coordinated mechanism.

Key steps in the assembly pathway are proteasome maturation (i.e. processing of inactive β subunit precursors), dimerization of two half-proteasomes, and degradation of the maturation factor Ump1.

In this work, we tested various hypotheses regarding proteasome maturation in *Saccharomyces cerevisiae* by using biochemical and genetic approaches. In vivo analyses of different mutants with defective 19S particles revealed a strong stimulatory effect of the 19S regulator on proteasome maturation.

Further characterization of these mutants with defective 19S particles demonstrated that the 19S base subunit Rpn2 plays an important role in the stability of 26S proteasomes.

Nas6, a protein previously described either as bona fide 19S regulator subunit or as a proteasome interacting protein was found in purified 19S complexes. Differential elution of matrix-bound 19S complexes indicated that Nas6 may belong to the lid subcomplex of the 19S regulator as a bona fide subunit.

Furthermore, we established an in vitro system based on a 'whole extract' approach that, for the first time, enabled us to recapitulate the maturation process in more detail and under controlled conditions. These in vitro experiments indicated a new function for the molecular chaperone Hsp90 and the presence of an ATP hydrolysis-dependent mechanism during proteasome maturation.

The experimental in vitro settings established in this study greatly enhance the toolbox to decipher further details of proteasome maturation in the future.

Based on the results of this work, we propose a modified model for proteasome maturation that extends the current view by adding an additional layer of complexity. In this layer, Hsp90, ATP, and the 19S cap are involved in the formation of stable and mature 20S core particles.