The ubiquitin/proteasome system (UPS) is the primary machinery for selective protein degradation of short-lived and abnormal proteins in eukaryotic cells. The 26S proteasome, which is the central component of the UPS, is composed of a 20S core particle (CP) (provides its proteolytic activity) and one or two 19S regulatory particles (mediate substrate recognition, unfolding, and translocation into the CP). The regulation of proteasome abundance is critical for cellular adaptation, and both decreased and increased proteasome function can cause diseases. At the level of transcription, yeast proteasome abundance is mainly regulated by the transcription activator Rpn4. Post-translationally, mature 26S proteasome levels are regulated by its assembly and its vacuolar degradation (proteaphagy). The present work provides new insights into all these processes and could therefore lead to new proteasome-related therapeutic approaches.

The present study provides the first, systematic analysis of the differential expression of proteasome subunits under normal and stress conditions. Interestingly, despite the stoichiometric presence in mature 20S proteasomes, the cellular levels of α-subunits were strikingly higher than the levels of β-subunits. Additionally, β-subunits were shown to be induced more strongly by Rpn4 than α-subunits, with β7, the last subunit to be incorporated into the dimerizing 15S complexes, showing the strongest dependency on Rpn4. Notably, upon overexpression of the rate-limiting β7-subunit, higher relative amounts of mature 20S CP were formed in both wild-type and *rpn4*Δ cells. We hypothesize that the observed system of differential subunit induction allows the cell to rapidly mobilize mature 20S proteasomes from pre-formed immature precursor complexes under stress conditions.

Until today, the eukaryotic conserved and chaperone-assisted step-wise process of 20S CP formation is poorly understood. Currently, there are two assembly models: the α-ring model, in which 20S CP assembly is thought to be initiated by the formation of rings of seven distinct α-subunits, and a new model, developed in our laboratory, in which two complementary precursor complexes, termed complex I and complex II, with distinct subsets of α- and β-subunits, constitute early assembly intermediates for the formation of the 15S precursor complex. Interestingly, the present study provides evidence for both assembly pathways. On the one hand, an α-ring assembly intermediate was purified from a mutant yeast strain overexpressing the chaperone proteins Pba1-Pba2 and Pba3-Pba4. On the other hand, complex I was detected by pulse-chase analysis as assembly intermediate in a yeast strain lacking Pba1-Pba2. Additionally, in an *in vitro* assembly assay, complex I (purified from yeast) and complex II (purified from *E.coli*) were demonstrated to be capable of 13S precursor complex formation. Therefore, the present study suggests a co-existence of both the α-ring and the complex I + complex II assembly pathway in yeast cells.

Surprisingly, until 2015, little attention has been given to possible mechanisms for the removal of misassembled or unrequired 26S proteasomes from the cell. As the process of proteaphagy has only

recently been discovered, little is known about its exact mechanism. The results of the present study confirmed the vacuolar degradation of the yeast 26S proteasome by showing that the 20S CP and, to a lower extent, also the 19S RP are capable of Atg8 binding. Additionally it was demonstrated that mainly free 20S CP and 19S RP, rather than intact 26S proteasomes bind to Atg8, indicating a regulatory mechanism, in which only defective, partly disassembled proteasomes are degraded, while intact 26S proteasomes escape vacuolar degradation. Free proteasome subunits, in contrast, were shown to be mainly degraded by the 26S proteasome itself.