Abstract

Selective ubiquitin (Ub)-mediated proteolysis is the dominating mechanism in the degradation of cytosolic and nuclear proteins in eukaryotic cells. Substrate proteins are recognized by enzymes of the Ub-system, and poly-Ub-chains are attached to internal lysine residues. These poly-Ub-chains mediate interaction with the 26S-proteasome, a ~2MDa complex that unfolds and degrades the substrate proteins. Expression of proteasome subunit genes is regulated by an autoregulatory mechanism in dependence of proteasome activity. In this work it is shown that the transcriptional activator Rpn4 controls basal as well as activity-dependent expression of proteasome subunit genes and of the polyubiquitin gene. Deletion of *RPN4* resulted in a reduction of proteasome activity by 50% and led to severe growth defects in combination with mutants interfering with proteasome activity or assembly. Analysis of Rpn4 stability in different proteasome deficient mutants revealed that Rpn4 itself is a substrate of the proteasome which directly links Rpn4 abundance to the activity of the proteasome. This enables the cell to correlate proteasome activity and Rpn4 dependent de novo synthesis of proteasome complexes. Rpn4 is an unusual substrate of the proteasome that is degraded by ubiquitin-dependent and -independent mechanisms.

A selection based screen for mutants stabilizing Rpn4 led to the isolation of *dor2* and *dor3* (degradation of Rpn4). The *dor2* mutation seems to affect the *FHL1* gene that encodes a transcription factor involved in ribosomal RNA processing. The identity of the gene affected by *dor3* is currently unknown, but it could be shown that the mutation results in an impaired ubiquitin-dependent degradation of Rpn4.

In addition to regulating proteasome subunit gene expression, Rpn4 is also part of a regulatory network controlling cellular responses to a variety of stresses including DNA-damage. In the present work the effects of two substances that induce such stresses were analyzed. It could be shown that addition of the DNA-damaging drug methyl methanesulfonate (MMS) leads to an increase in *RPN4* gene expression while addition of caffeine induces a more rapid turnover of Rpn4 protein. The deletion of Rpn4 resulted in a strong sensitivity against MMS and caffeine. Overexpression of the *YAP1* or *SSZ1* gene suppressed this hypersensitivity. Both genes are known to act in cellular responses to various stresses. Analysis of the mechanisms underlying the regulation of Rpn4 could reveal interesting insights into the interplay between the ubiquitin/proteasome-system and cellular responses to a variety of different stresses.