Abstract

Posttranslational modification is a prominent tool of the cell to regulate a protein's function. SUMO belongs to the ubiquitin like proteins (Ubls) and shares similarities in structure and conjugating mechanism with ubiquitin. A hallmark of Ubl conjugation is the successive activity of activating (E1), conjugating (E2) and ligating (E3) enzymes which lead to the modification of the target protein. Ubl modification is a reversible process which is catalyzed by specific de-conjugating enzymes.

Modification with SUMO is a very dynamic and transient process. The modification state of a protein is controlled by adjustment of the conjugating or de-conjugating activity of the corresponding enzymes. One of the two reported de-conjugating enzymes in *Saccharomyces cerevisiae*, Ulp2, is assumed to be mainly responsible for the removal of SUMO from its substrates. In this study we demonstrate that Ulp2 undergoes cell cycle regulated phosphorylation, which peaks during G2/M. Phosphorylation is not an essential prerequisite for cell growth under normal conditions but rather a fine tuning mechanism which adjusts Ulp2 function under certain stress conditions. One such example is treatment with lethal concentrations of the DNA damaging drug MMS, which leads to a drastic decrease in Ulp2 levels, that is more prominent for phosphorylated Ulp2.

Another function of Ulp2 is associated with proteasomal degradation. *ulp2Δ* cells stabilize a proteasomal substrate which is rapidly degraded in wildtype cells. This stabilization is due to the accumulation of SUMO conjugates in *ulp2Δ* as the suppressor mutation *uba2-ts* reverts this effect and shows a similar turnover of the test substrate as the wildtype strain. Gel filtration analysis revealed that Ulp2 is associated with high molecular weight complexes, possibly interacting with the 19S cap of the 26S proteasome.

An additional finding of this work was the *in vitro* ubiquitin-ligase activity of the SUMO binding complex Hex3/Slx8. Both proteins are characterized by SUMO interacting motifs (SIM) and RING finger domains. We also demonstrate that this heterodimeric complex interacts specifically with high molecular weight SUMO conjugates (HMW-SC) and that SUMO conjugates are subjected to proteolytic turnover.

Taken together these results indicate that Hex3 and Slx8 target sumoylated proteins for ubiquitylation and subsequent proteasomal degradation, suggesting a new degradation pathway involving SUMO-dependent ubiquitylation. This is in line with the assumption that Ulp2 is interacting with the 19S cap of the 26S proteasome to remove SUMO from the target protein and release it to the pool of free SUMO in the cell, additionally preventing blocking of the proteasome by accumulation of SUMO conjugates.