Abstract

Posttranslational modification with the **s**mall **u**biquitin-related **mo**difier SUMO is an important regulatory mechanism implicated in many cellular processes including several of biomedical relevance. Sumoylation is a reversible process. In the yeast *Saccharomyces cerevisiae*, the balance between SUMO conjugation and deconjugation is important for cellular homeostasis. For most substrates, the regulation of their SUMO modification and its effect on the function are still unknown. To gain insight into the complex control of sumoylation, a combination of yeast two-hybrid screening and selection of spontaneous suppressors of the temperature-sensitive phenotype of a SUMO isopeptidase mutant was undertaken.

Budding yeast contains two desumoylating enzymes Ulp1 and Ulp2. In contrast to Ulp1, Ulp2 is not essential for cell viability. However, *ulp2*Δ mutant cells are temperature-sensitive and hypersensitive to the microtubule destabilizing drug thiabendazole and to the DNA damaging agent methyl methanesulfonate (MMS). Due to the deconjugation defect, this mutant accumulates sumoylated proteins some of them with a high molecular weight SUMO chains. In the screen for spontaneous suppressors of the temperature-sensitive growth phenotype of *ulp2*Δ, mutant alleles for *ULP1* (*sul8*, *sul9*), *UBA2* (sul25-2), *AOS1* (sul22-2) and a null mutation in *NUP84* (*sul23-1*) were isolated. All of them were mutations decreasing SUMO conjugation, which supported the assumption that the precise regulation of SUMO conjugation and deconjugation is important for normal growth of the cells.

Six SUMO interacting proteins (SIPs) were identified in the yeast two-hybrid screen. A consensus site called SUMO interacting motif (SIM) was deduced by sequence analysis of the minimal SIPs clones. Two of the SIPs Uls1 and Uls2 turned out to be RING-finger-type ubiquitin ligases that mediate the proteolytic downregulation of sumoylated proteins. Genetic and biochemical evidence indicated that the dynamic function of SUMO substrates involves the inactivation of their sumoylated forms by polysumoylation and/or ubiquitin-dependent degradation. Inhibition of both mechanisms leads to severe phenotypic defects. Ubiquitin-dependent proteolytic control of SUMO conjugates appears to be conserved from yeast to humans. Treatment of HeLa cells with proteasome inhibitors resulted in an accumulation of SUMO2/3 conjugates whereas SUMO1 conjugate levels were less affected. Ubiquitin-dependent proteolytic control thus provides a novel functional distinction between SUMO isoforms in human cell.