

**Functional characterization of dosage-dependent lethal mutation of ubiquitin in *Saccharomyces cerevisiae***

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**Abstract**

Ubiquitin is a eukaryotic protein with 96% sequence conservation from yeast to human. Ubiquitin plays a central role in protein homeostasis and regulation of protein function. We have reported on the generation of variants of ubiquitin by in vitro evolution in *Saccharomyces cerevisiae* to advance our understanding of the role of the invariant amino acid residues of ubiquitin in relation to its function. One of the mutants generated, namely UbEP42, was a dosage-dependent lethal form of the ubiquitin gene, causing lethality to UBI4-deficient cells but not to ubiquitin wild-type cells. In the present study we investigated the functional reasons for the observed lethality. Expression of UbEP42 in a UBI4-deleted stress-sensitive strain resulted in an increased generation time due to a delayed S phase caused by decreased levels of Cdc28 protein kinase. Cells expressing UbEP42 displayed heightened sensitivity towards heat stress and exposure to cycloheximide. Furthermore, its expression had a negative effect on the degradation of substrates of the ubiquitin fusion degradation pathway. However, UbEP42 is incorporated into polyubiquitin chains. Collectively, our results establish that the effects seen with the mutant ubiquitin protein UbEP42 are not due to malfunction at the stage of polyubiquitination.

**Keywords:** functions of ubiquitin; in vitro evolution of ubiquitin; mutations in ubiquitin; structure of ubiquitin; ubiquitin