International Journal of Biological Macromolecules, 2017 Jun;99:128-140.

ISSN: 1879-0003

Structural changes induced by L50P and I61T single mutations of ubiquitin affect cell cycle progression while impairing its regulatory and degradative functions in Saccharomyces cerevisiae

Ankita Doshi[1], Mrinal sharma[2] and C. Ratna Prabha [3]

[1] Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, 390002, India.
[2] Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, 390002, India

Abstract

Posttranslational conjugation of ubiquitin to proteins either regulates their function directly or concentration through ubiquitination dependent degradation. High degree of conservation of ubiquitin's sequence implies structural and functional importance of the conserved residues. Ubiquitin gene of Saccharomyces cerevisiae was evolved in vitro by us to study the significance of conserved residues. Present study investigates the structural changes in the protein resulting from the single mutations UbS20F, UbA46S, UbL50P, UbI61T and their functional consequences in the SUB60 strain of S. cerevisiae. Expression of UbL50P and UbI61T decreased Cdc28 protein kinase, enhanced Fus3 levels, caused dosage dependent lethality and at sublethal level produced drastic effects on stress tolerance, protein sorting, protein degradation by ubiquitin fusion degradation pathway and by lysosomes. UbS20F and UbA46S produced insignificant effects over the cells. All four mutations of ubiquitin were incorporated into polyubiquitin. However, polyubiquitination with K63 linkage decreased significantly in cells expressing UbL50P and UbI61T. Structural studies on UbL50P and UbI61T revealed distorted structure with greatly reduced α-helical and elevated β-sheet contents, while UbS20F and UbA46S show mild structural alterations. Our results on functional efficacy of ubiquitin in relation to structural integrity may be useful for designing inhibitors to investigate and modulate eukaryotic cellular dynamics.

Keywords: Ubiquitin functions; Ubiquitin structure; Yeast ubiquitin mutants