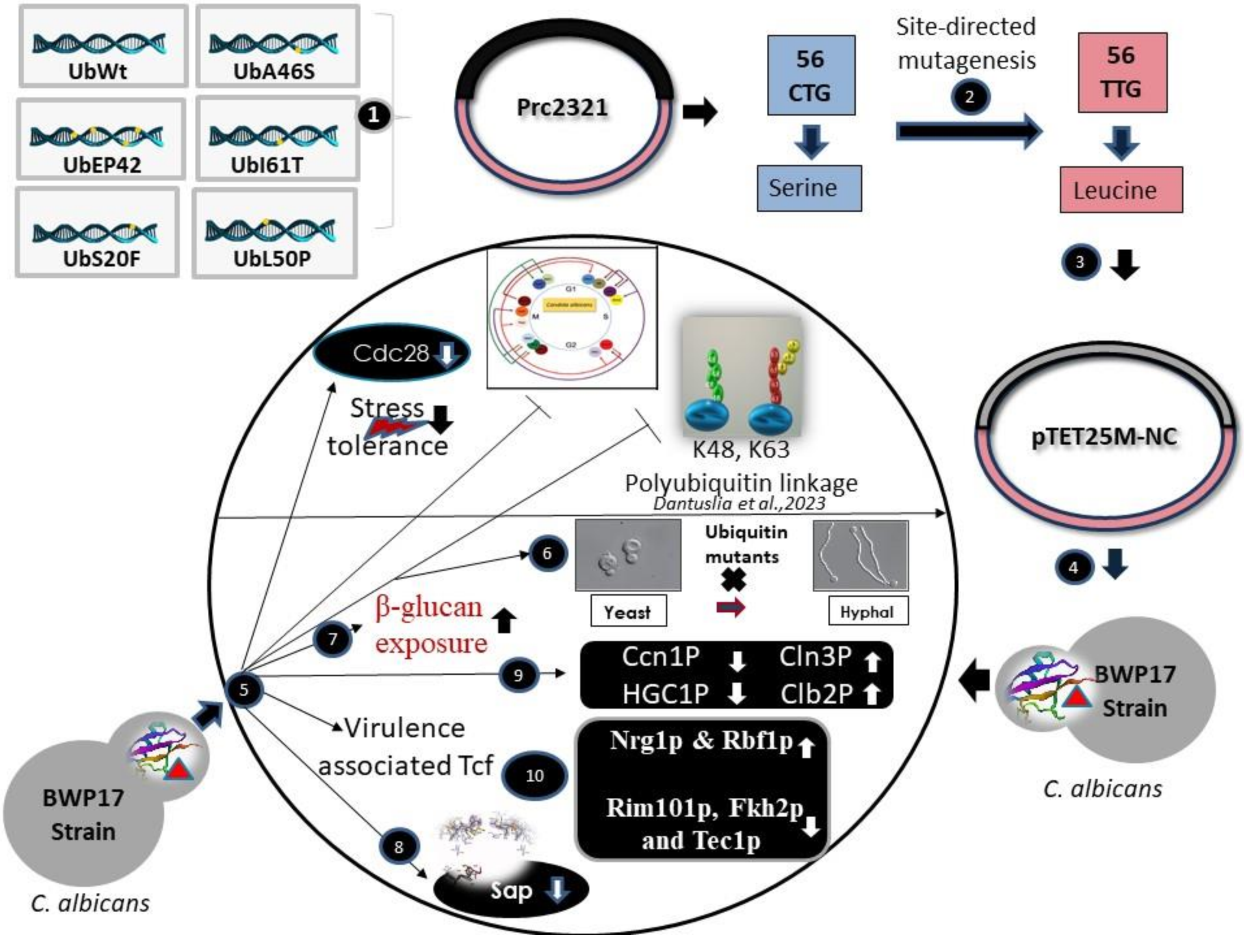
Summary

This study investigates the impact of ubiquitin mutants, namely lethal Ubiquitin mutants UbEP42, UbL50P, and UbI61T, on *Candida albicans*, elucidating a complex array of consequences affecting cell viability, surface integrity, and morphogenesis. Remarkably, ubiquitin mutations alter the cell wall composition, resulting unmasking of higher β-glucan exposure towards cell surface and uneven chitin deposition and aggregates formation compared to wild type and other mutants (UBS20F, UbA46S). The lethal ubiquitin mutants disrupt the G1 phase to S phase transition in the cell cycle, characterized by alterations in cyclin-dependent kinase (Cdk) levels, elevated expression of G1 cyclins (Ccn1 and Cln3), and reduced expression of G2 cyclins (Clb2 and Clb4), attributed to the impairment of the protein degradation pathway. Interestingly, despite the typical hyphae-inducing reduction in Cdc28 levels, the mutants sustain the yeast form, accompanied by elevated expression of the bud-specific gene NRg1. The study highlights the significance of pH in yeast-to-hyphal transitions, influencing the Fkh2 transcription factor associated with secretory aspartyl protease secretion. Under serum conditions, where hyphal transformation is common, ubiquitin mutants suppress Fkh2 expression, correlating with low cyclin levels that impact Fkh2 phosphorylation, hindering the activation of hyphal-specific genes like HGC1. In conclusion, this research unveils intricate molecular mechanisms influenced by ubiquitin in C. albicans, impacting various cellular processes. Further investigations, particularly proteomics analysis, are imperative to unravel the complete molecular details and understand the potential implications for fungal virulence and pathogenesis.



Further proteomics analysis profile in *Candida albicans* under ubiquitin mutants, shedding light on the molecular mechanisms underlying their phenotypic outcomes. There were 61 downregulated proteins and 12 upregulated proteins with >twofold difference identified from the *Candida albicans* under ubiquitin mutants’ condition UbEP42, UbL50P, and UbI61T. Mutant expression causes downregulated several cell wall- and oxidative stress-related Proteins and pH response transcription factor RIM101, Transcription activator TEC1, Secretory aspartyl protease associated Fork-head transcription factor Fkh2 and Transcription factor RBF1; Transcriptional activator that binds to the RPG box and telomeres and involved in the regulation of the transition between yeast and filamentous forms and plays a role in virulence. Whereas, mutants induced action was compensated by markedly upregulation of many other proteins involved in cell wall integrity and stress response like EFG1, and Nrg1. In Proteome analysis we found that MET3 and MET10 have better interactive scores which are involved in the sulfur metabolism pathway This pathway enhances antibiotic and heat stress tolerance in *C. albicans*, suggesting a protective mechanism to counteract the deleterious effects of ubiquitin dysregulation on fungal viability and survival observed in our study. Moreover, considerable expression changes were identified in biological processes including the Glucan catabolic process, Cellular aldehyde, amino acid glucan metabolic process, and metabolism affected including Butanoate, Tyrosine, Glyoxylate, dicarboxylate, beta-alanine metabolism Alanine, aspartate, and glutamate metabolism. Signature proteins essential for modulating cell wall integrity, stress response, and metabolic activities may account for the antifungal resistance of C. albicans biofilms.

Deletion of di-glycine at c-terminal ubiquitin mutants study shed light on, how our ubiquitin mutants are involved in functional polyubiquitination. Our results revealed a notable absence of polyubiquitin chains in ubiquitin mutants lacking the di-glycine motif, indicating having importance in impaired ubiquitin polymerization. This impairment suggests that the di-glycine motif in our ubiquitin mutants plays a crucial role in facilitating polyubiquitin chain assembly for dosage dependent lethality in C. albicans. Mutants lacking the di-glycine motif exhibited decreased K48 and K63 chain linkages upon overexpression of UbEP42GG-, suggesting in the absence of substrate recognition motifs its monomer or polymer forms degradation through UPS pathway. Structural bioinformatics analysis using the trRosetta server further elucidated the molecular basis of di-glycine deletion in ubiquitin mutants. The predicted 3D structures revealed distinct inter-residue geometries, potentially affecting protein-protein interactions and substrate binding. These structural alterations may underlie the observed functional differences in polyubiquitination profiles and chain linkages, highlighting the importance of the di-glycine motif in maintaining ubiquitin's regulatory functions. Moreover, the absence of significant differences in secondary structure elements between ubiquitin mutants with and without the di-glycine motif suggests that the functional disparities arise from alterations in inter-residue interactions rather than overall structural changes.