Abstract

This study investigates the impact of ubiquitin mutants, including lethal variants UbEP42, UbL50P, and Ubl61T, on Candida albicans, revealing complex consequences on cell viability, surface integrity, and morphogenesis. Notably, ubiquitin mutations induce alterations in cell wall composition, resulting in increased β-glucan exposure and uneven chitin deposition compared to wild type and other mutants (UBS20F, UbA46S). Furthermore, these lethal mutants disrupt the G1 to S phase transition in the cell cycle, affecting cyclin-dependent kinase levels and cyclin expression patterns, thus impairing the protein degradation pathway. Interestingly, despite the expected reduction in Cdc28 levels conducive to hyphae formation, the mutants maintain the yeast form, accompanied by elevated expression of the bud-specific gene NRg1. The role of pH in yeast-to-hyphal transitions is highlighted, influencing the Fkh2 transcription factor associated with secretory aspartyl protease secretion. Under serum conditions promoting hyphal transformation, ubiquitin mutants suppress Fkh2 expression, correlating with low cyclin levels, hindering the activation of hyphal-specific genes like HGC1. Proteomic analysis reveals significant downregulation of cell wall- and oxidative stress-related proteins, including transcription factors RIM101, TEC1, and Fkh2, while compensatory upregulation occurs in proteins involved in cell wall integrity and stress response like EFG1 and Nrg1. Notably, proteins involved in sulfur metabolism pathways, such as MET3 and MET10, exhibit enhanced expression, suggesting a protective mechanism against ubiquitin dysregulation. Moreover, the study delves into the functional significance of the di-glycine motif in ubiquitin mutants, revealing its role in facilitating polyubiquitin chain assembly. Mutants lacking this motif show impaired ubiquitin polymerization and decreased chain linkages, affecting substrate recognition and degradation through the UPS pathway. Structural analysis elucidates distinct inter-residue geometries in mutants lacking the di-glycine motif, highlighting its importance in maintaining ubiquitin's regulatory functions. In conclusion, this research unveils intricate molecular mechanisms influenced by ubiquitin in C. albicans, impacting various cellular processes and shedding light on potential implications for fungal virulence and pathogenesis. Further proteomic and structural investigations are essential for a comprehensive understanding of ubiquitin-mediated regulation in fungal biology.