Chapter 3

Status of cyclins in the various stages of cell cycle in *C. albicans* expressing ubiquitin mutations.

3.1. Introduction:

In earlier studies, to understand the relationship between the conserved primary structure of ubiquitin and the multiple cellular functions in which it serves as a posttranslational modifier, mutants of ubiquitin were generated and examined in *Saccharomyces cerevisiae* (Prabha et al., 2010, Mishra et al., 2011). Studies on the effects of ubiquitin mutations like UbEP42 and the four mutations UbS20F, UbA46S, UbL50P, and UbI61T derived from it led to several interesting observations, in *S. cerevisiae*. Interestingly, the mutations UbEP42, UbL50P, and UbI61T negatively influenced the growth and various other functions in *S. cerevisiae* (Doshi et al., 2014, Doshi et al.,2017). Cyclin levels under ubiquitin mutations studied in *S. cerevisiae* (Doshi et al., 2017) have been employed for preliminary study in *C. albicans* strain to gain insights into cell cycle dynamics and morphogenesis in *C. albicans*, especially concerning the transition from yeast to hyphal form, an essential step in pathogenesis at the molecular level with compatible vector in fast-growing Strain BWP17.

C. albicans the cyclin B level is modulated by the mitotic transcription factor Fkh2 (Berman et al., 2006). This factor is analogous to the Phd, Sok2, and StuA transcription factors (Mattos et al., 2020), which influence the activity of secretory aspartyl proteases in *Aspergillus* species. In this context, it would be interesting to see what would be the effect of ubiquitin mutations on cell cyclins level in *C.* albicans and how their level is associated in yeast and hyphal conditions in relation to ubiquitin mutations.

3.2 Material and methods

3.2.1. Strains and media

The auxotrophic *C. albicans* strain, BWP17 has been used in the present study (Greig et al., 2015). BWP17 (ura3/ura3 his1Δ/his1Δ arg4Δ/arg4Δ) derivative of CAI4, was a gift from Prof. Yue Wang from IMCB Singapore. It is an avirulent strain of *C. albicans* (Hussain et al.,2021. BWP17 was grown in YPD (1% w/v yeast extract, 2 % w/v peptone and 2 % w/v dextrose) supplemented with or without 50 μg/ml uridine for selection as previously described (Chapa et al., 2005).

3.2.2. Analysis of the expression levels of cyclins

Quantitative real-time PCR was carried out for comparative analysis of the expression of cyclins and virulence-associated transcription factors. Total RNA of the BWP17 transformants of the wild type and the mutant forms were isolated using TRIzol reagent (Takara, Japan). Poly-A tailing of small RNAs was performed using E. coli Poly-A Polymerase (New England Biolabs, UK) at 37 °C for 30 min. cDNA was synthesized using a cDNA prime script synthesis kit (Takara, Japan). One-way analysis of variance (ANOVA) followed by Bonferroni's test were used for intergroup variation analysis. Statistical significance was established at $p < 0.05$.

3.3 Results:

3.3.1. Status of cyclins in the various stages of cell cycle in *C. albicans* expressing ubiquitin mutations

It was reported earlier that decline in Cdc28 level leads to formation of hyphal stage in *C. albicans* (Wójcik-Mieszawska et al., 2023). We have observed earlier that dosage dependent lethal mutations of ubiquitin hamper yeast to hyphal transition. However, the effect of ubiquitin mutations on the levels of Cdc28 and cyclins has not been investigated. During the growth phase of yeast, cell shape closely correlates with the progression of the cell cycle due to the synchronized function of Cdc28 and cyclin complexes in *C. albicans*. In the G1 phase of cell cycle in *C. albicans*, the G1 cyclins Ccn1 and Cln3 are produced. When these cyclins bind to Cdc28, they facilitate apical growth and bud stretching (Hossain et al., 2021, Chapa et al., 2005). As the cell cycle moves forward, the G1 cyclins are broken down and are succeeded by the G2 cyclins, Clb2 and Clb4. These cyclins then interact with Cdc28, inhibiting polarized growth and promoting bud growth (Besen et al., 2005). This harmonized transition between bud stretching results in the creation of fully distinct, oval-shaped mother cell and daughter buds. Consequently. any imbalance in the synthesis or breakdown of cyclins can lead to significant alterations in cell shape (Berman et al., 2006). The mRNA levels of Cdc28 and cyclins regulating different stages of cell cycle were monitored in cells expressing the wild type and the mutant forms of ubiquitin. It was observed that G1 cyclins Ccn1 and Cln3 were not degraded in UbEP42, UbL50P and UbI61T, instead they were upregulated and not replaced by the G2 cyclins. The G2 cyclins Clb2 and Clb4 bind to Cdc28 and suppress polarized growth. In our study, we found that the transcript

of G2 cyclin Clb2 levels were upregulated and the expression of hyphal-specific gene Hgc1 was down regulated **(Figure 3.1)**. The G_0/G_1 arrest observed with the three mutants could be due to impairment in the ubiquitin-mediated degradation pathway because previously it was observed that UbEP42, UbL50P and UbI61T prevented the formation of polyubiquitin chain Lys63 and Lys48 linkages (Dantuslia et al., 2023).

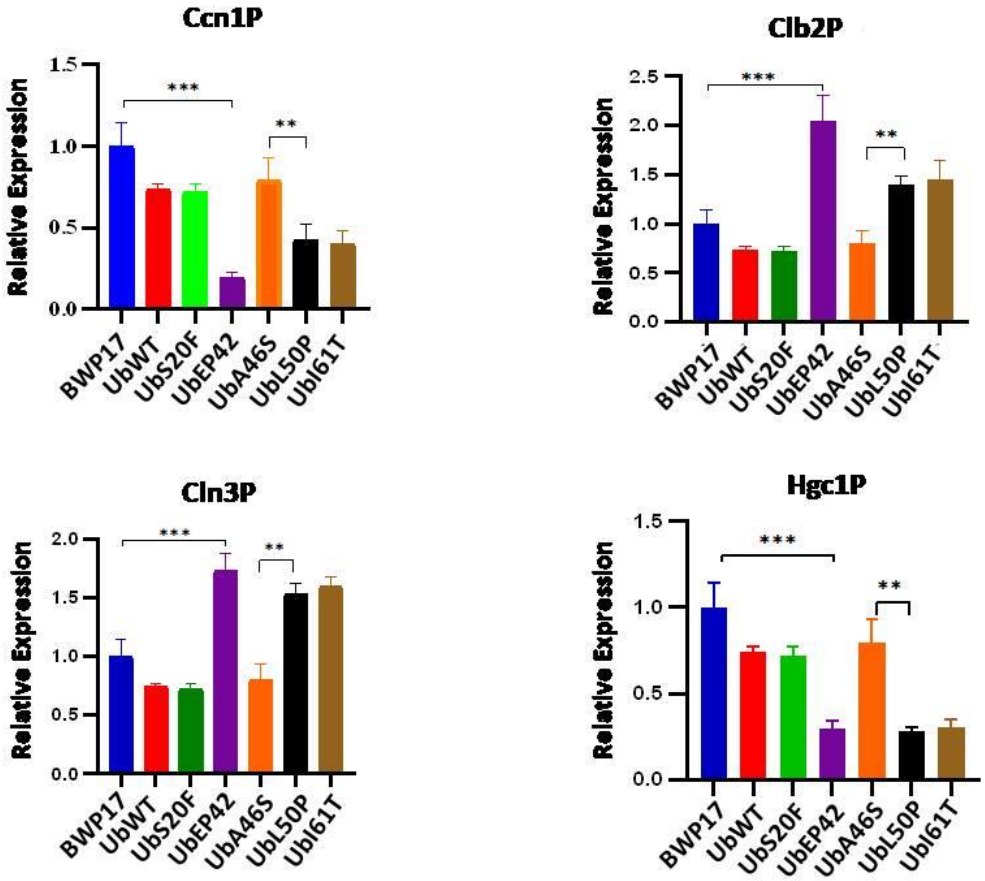

Figure 3.1 Effect of ubiquitin mutant on cyclins level in *Candida albicans*. N=3 All values are presented as Mean+ SEM *P<0.05; **P<0.01 and*P<0.001 as compared to control.**

3.4 Discussion

The present study explains at the molecular level the earlier findings of decreased viability, failure of morphogenesis, cell cycle arrest, in *C. albicans*, under the influence of the ubiquitin mutations UbEP42, UbL50P and UbI61T affected morphogenesis from yeast to hyphal

form(Chapter 2) by decreasing the levels of Nrg1transcription factor (Chapter 4). These lethal mutants hamper G1 to S transition during cell cycle because of: (i) alteration in the levels of Cdc28, (ii) upregulation of G1 cyclins Ccn1 and Cln3 and (iii) decreased expression of the G2 cyclins Clb2 and Clb4, which bind to Cdk (Cdc28) leading to suppression of polarized growth. These effects could be due to the impairment of the protein degradation pathway caused by ubiquitin mutants UbEP42, UbL50P and UbI61T. Usually, a reduction in Cdc28 levels leads to formation of hyphae. However, in the case of the mutants UbEP42, UbL50P, and UbI61T, yeast to hyphal transition did not occur (chapter 2). Interestingly, these mutants show elevated expression of the bud-specific gene Nrg1(Chapter 4), which could be seen as the possible reason for continuing in yeast form leading to G1 cyclins increasing level regulate Nrg1 expression positively. A low level of HGC1 possible assumption is that ubiquitin mutants suppress the expression of Fkh2 in *C. albicans* grown in serum conditions (Chapter 4). Further, it was reported in the literature that low levels of cyclins lead to alteration in the phosphorylation of Fkh2, preventing it from activating the expression of hyphal-specific genes such as HGC1. It was observed in the present study that ubiquitin mutations led to decreased expression of HGC1. Further research is needed to fully understand the molecular details of these effects by carrying out proteomics analysis to understand the metabolic pathways and their potential implications for fungal virulence and pathogenesis. Further, it is possible to identify and earmark possible targets for developing new strategies to treat the infections caused by C. albicans.