CONCLUSIONS & FUTURE PERSPECTIVES
VI. Conclusions and Perspectives

TAF sequences are conserved over evolution and they are essential transcriptional adapter proteins in yeast. Studies in yeast have revealed that TAFs are multifunctional proteins that function as a complex with 14 TAFs and TBP in TFIID. Five of the TAFs are also present in the SAGA histone acetylase complex. Yeast genome sequence data has shown that each TAF is encoded by a single gene, and no alternative forms of TAFs or TBP have been found. In contrast, higher eukaryotes including Drosophila and mouse encode TBP and TBP-like proteins and multiple isoforms of certain TAFs, which function in a tissue-specific manner.

We first examined the *C. albicans* genome for the presence of sequence orthologs of *S. cerevisiae* TAF proteins. For twelve out of the thirteen yTAF proteins we identified a single hit. However, for TAF12, we identified two proteins in *C. albicans* with high amino acid sequence similarity. Secondary structure prediction showed that histone H2B-like HFD was present in the two sequences. We therefore named the two proteins TAF12a (orf19.470) and TAF12b (orf19.6820). Phylogenetic analysis showed that TAF12a is evolutionarily closer to the canonical yTAF12 and its orthologs are present in all *Candida* clade organisms. The TAF12b protein sequence, however, was found in six fungal genomes *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *L. elongisporus* and *P. stipitis*, all belonging to the CTG group. Thus, although isoforms of several TAFs have been reported in higher eukaryotes, this is the first instance of the occurrence of TAF orthologs in unicellular eukaryotes. It would be interesting to further dissect the evolutionary origin and divergence of the two TAF12 isoforms and their functional relevance in the context of *C. albicans* and other closely related fungal organisms.

To investigate if the *C. albicans* TAF12-like sequences could complement the lethality of *S. cerevisiae taf12Δ* mutant, we expressed the two genes under the yeast
GAL1 promoter. Neither of the two constructs rescued the \( ytaf12\Delta \) lethality. Comparison of the two CaTAF12 sequences revealed that although the amino acid sequences of the C-terminal HF domains are highly conserved, the amino terminal regions of the TAF12a and TAF12b protein sequences are different. Moreover, TAF12a sequence has two CTG codons and TAF12b has one CTG codon outside their HF domains, but the importance of the encoded amino acid residue at these positions is not understood. Because CTG codon, which is translated as a serine in \( C. \) albicans (Santos et al., 1993), would instead encode a leucine, when the TAF12a and TAF12b proteins are expressed in \( S. \) cerevisiae and possibly affect their folding or function. Therefore we expressed the conserved HFD alone from the \( C. \) albicans TAF12-like sequences under the control of \( yTAF12 \) promoter and tested complementation. The HFD of TAF12a but not TAF12b was able to rescue the \( ytaf12\Delta \) lethal phenotype. These results taken together with the bioinformatic analysis suggest that CaTAF12a could be the functional ortholog of TAF12 in \( C. \) albicans.

We carried out genetic analyses in \( C. \) albicans to examine the TAF12a and TAF12b functions. We attempted to construct null mutants of \( TAF12a \) and \( TAF12b \) genes using several approaches. While \( taf12a\Delta \) mutants could be obtained, no \( taf12b\Delta \) null mutant could be isolated indicative of being essential for growth of \( C. \) albicans. This is in contrast to our assessment of TAF12a and TAF12b orthology to \( yTAF12 \) inferred from bioinformatics and yeast complementation studies. Therefore TAF12a and TAF12b functions were examined in \( C. \) albicans using promoter regulatable alleles of the two genes and the consequences of the promoter shut-off studied. The depletion of TAF12b upon expression from a regulatable promoter resulted in a severe growth defect and led to altered cellular morphologies, primarily composed of pseudohyphae. In addition, \( \sim 10\% \) of the cell population showed germ-
tube like short evaginations, previously shown to be indicative of a cell cycle defect (Berman, 2006; Cote et al., 2009; Sellam et al., 2010). Several TAFs including TAF12 in *S. cerevisiae* have previously been shown to play a role in cell cycle. Although TAF12b expression itself is not periodically regulated (Cote et al., 2009), our genome wide expression data suggests that indeed expression of several cell cycle related genes were down-regulated between two and four-fold upon depletion of TAF12b. It would therefore be interesting to study how TAF12b might be required for expression of cell cycle regulated genes and how this translates to a role for TAF12b in proper cell cycle progression in *C. albicans*.

On the other hand, TAF12a conditional as well as null mutant strains were viable, although they also exhibited slow growth and altered cellular and colony morphologies. Closer examination of the characteristic cellular morphology, comprising of chains of cells in which the daughter cells appear to remain attached to the mother cell, suggest a possible defect in cell separation. Such cell separation defects have been previously described for a few *C. albicans* genes, two of which (*NDT80* and *SUN41*) (Firon et al., 2007; Sellam et al., 2010) were also found to be downregulated in the TAF12a depleted strain in our microarray data. We also found that loss of TAF12a resulted in sensitivity to oxidative stress agents H₂O₂, menadione and cadmium.

Biochemical analyses involving coimmunoprecipitation experiments revealed that both TAF12a and TAF12b associated with TBP, albeit to different extents. However, their associations with the TFIID subunit TAF11 and the SAGA subunit ADA2 were found to be mutually exclusive. TAF12b associated with TAF11 but not with ADA2, suggesting that TAF12b is a likely constituent of TFIID complex and regulates the expression of a large number of genes. Therefore, depletion of TAF12b might compromise the function of the entire complex, resulting in a decrease in global
transcription which in turn might be responsible for the severe growth defect observed in our TAF12b regulatable expression strains. We observed an upregulation of TBP mRNA level in response to TAF12b depletion in our microarray data, and was validated by real time PCR analysis. It would be interesting to examine the significance of the TBP upregulation in TAF12b depleted cells.

TAF12a, on the other hand, was found to associate only with ADA2, shown previously to be a part of the SAGA complex as well as the small ADA complex, but not with the TFIID complex. Interestingly, the *C. albicans* ada2Δ mutant is also sensitive to oxidative stress and the ADA2 protein has previously been shown to be recruited to promoters of oxidative stress response genes and regulate their expression (Sellam et al., 2010). The SAGA complex is known to be involved in regulation of stress responsive genes in *S. cerevisiae* (Huisinga and Pugh, 2004). It is therefore likely that TAF12a, along with ADA2 is involved in the regulation of genes in response to stress conditions as a part of the SAGA complex. Future experiments would be directed to investigate how TAF12a and TAF12b associate with the two transcription regulatory complexes and how they differentially regulate gene expression. However, our current data does not rule out a function for TAF12a and TAF12b outside the TFIID and SAGA complexes, and would also be addressed by biochemical purification of TAF12a and TAF12b containing complexes from *C. albicans* and identification of associated proteins by mass spectrometry.