Abstract

Transcription initiation is a multistep process and involves ordered recruitment of basal transcription factors, coregulators and RNA polymerase II, which constitute the preinitiation complex (PIC) assembly at the core promoter sequences. Transcriptional activators play a central role to incorporate regulatory signals to the PIC assembly. Transcriptional activator proteins have been shown to interact with basal factors, coregulators such as mediator subunits, the TAF subunits in TFIID. TBP-associated factors, TAFs, 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 in the SAGA histone acetylase complex. The S. cerevisiae 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, mouse and humans encode TBP, TBP-like proteins and multiple isoforms of certain TAFs, all shown to function in a tissue-specific manner.

We were interested in the structure-function relationship of TAF proteins and therefore we carried out comparative analysis of orthologous sequences from 22 fungal genome sequences belonging to the subphylum Saccharomycotina. Surprisingly, we identified two TAF12-like proteins, named isoform TAF12a and isoform TAF12b. All other TAFs and TBP were found to be single proteins. Phylogenetic analysis of the 22 Saccharomycotina species examined showed that yeast TAF12/TAF12a-like sequences were present in all, but the TAF12b-like sequence was restricted to only six of the CTG group fungi including Candida albicans, five of which were diploid. However the evolutionary origin of the two TAF12 isoforms TAF12a and TAF12b is unclear.
We envisaged the following key questions:

Are both TAF12a and TAF12b bona fide TAFs and serve TAF12-like function? What is the functional relevance of the two TAF12-like proteins in \textit{C. albicans}? Do the two TAF12 proteins perform redundant functions or do they have specialized roles? What is the organization of TAF12-containing regulatory complexes?

We carried out functional complementation analysis in \textit{S. cerevisiae} and found that the histone-fold domain of TAF12a but not of TAF12b was able to rescue the \textit{ytaf12A} lethal phenotype. These results suggest that CaTAF12a might be the functional ortholog of TAF12. However, neither of the full length proteins complemented the \textit{ytaf12A} deletion. It is possible that, although the HF domains are very highly conserved, the function of the whole proteins in \textit{C. albicans} have diverged.

To carry out genetic analysis in \textit{C. albicans}, we attempted to delete \textit{TAF12a} and \textit{TAF12b} genes. We successfully obtained \textit{taf12a} null mutants but were unable to obtain viable \textit{taf12b} null mutants indicating that \textit{TAF12b} is essential for \textit{C. albicans} growth. Therefore we used strains bearing \textit{TAF12a} and \textit{TAF12b} genes under \textit{MAL2} promoter to study the effects of depletion of these proteins in \textit{C. albicans} biology. Western blot analysis showed that when the \textit{MAL2} promoter-regulated TAF12a and TAF12b were cultured for 6h in glucose, there were little or no detectable levels of the two proteins, indicating that the \textit{MAL2} promoter-regulated alleles could be effectively used for genetic analysis of TAF12 proteins in \textit{C. albicans}.

The TAF12a and TAF12b depleted cells gave different cellular, colony and stress response phenotypes. Both strains yielded a mixture of different cellular morphologies including normal yeast-form, as well as chain of cells comprised of mother and daughter cells joined together at the septa that could be broadly classified
as pseudohyphal-type morphology. About 10% of the TAF12b depleted cells were enlarged with rounded mother cells bearing small tubular projections similar to germ tubes that had incomplete septa between mother and daughter cells. A single nucleus was found in the evaginated daughter cells but no nucleus was found within the mother cell. This phenotype has been described as a cell cycle arrest phenotype. Thus it appears that TAF12b depletion induces defects in cell cycle progression, and further experiments would shed light on the role of TAF12b in cell cycle progression.

The TAF12a depleted cells and taf12aΔ cells exhibited distinct long, branched chain of cells of varying lengths. The TAF12a depleted cells were severely impaired for pseudohyphae formation and agar invasion in pseudohyphae-inducing medium. However, the TAF12a depleted cells retained the ability to form true hyphae although filamentation was less profuse compared to the wild-type control strain. The taf12aΔ mutant also formed wrinkled colonies like the wild-type strain but was defective in forming filamentous projections at the colony boundaries. Moreover, both TAF12a depleted strain and the taf12aΔ strain, were found to be impaired for growth under oxidative stress imposed by H2O2, menadione and cadmium. The TAF12a and TAF12b depleted cells and taf12aΔ strains were examined for their virulence potential in mice model of candidiasis. The onset of virulence was delayed with high statistical significance in mice injected with PMAL2-TAF12b strain, while the taf12aΔ strain was completely avirulent. Thus both TAF12a and TAF12b seem to be required for full virulence potential of C. albicans in mouse model of Candidiasis.

Our microarray study revealed that the expression of over 3% and 4% of the genes analyzed are regulated ≥4-fold in a TAF12a and TAF12b dependent manner respectively. The data showed little or no overlap in the highly regulated TAF12a and TAF12b dependent genes, but sizable overlap in the genes regulated between 2-
and 4-fold. It would be important to examine more datasets and carry out time-course of mRNA expression analysis to establish the full scope of the transcriptome regulated by TAF12a and TAF12b proteins.

To examine how TAF12 like proteins might regulate transcription in vivo, we examined the associations of the *C. albicans* TAF12 isoforms with TFIID and SAGA, previously shown to contain two molecules of TAF12 each. Coimmunoprecipitation experiments were conducted using cell extracts from strains expressing TAP tagged TBP and HA3-tagged TAF12a or TAF12b. Both TAF12a- and TAF12b-HA3 coimmunoprecipitated with TBP in a TAP-pulldown, but only TAF12b-HA3 efficiently immunoprecipitated TBP-TAP in the reciprocal pulldown with α-HA antibody. These results indicated that TAF12a and TAF12b proteins interact with TBP in vivo, and the stability and/or affinity of TBP interaction with TAF12b is stronger than that with TAF12a. Coimmunoprecipitation experiments conducted using the TFIID subunit TAF11, and the SAGA subunit ADA2 showed differential association of the two TAF12 isoforms. Remarkably, while TAF12a associated with ADA2/SAGA, TAF12b associated with TAF11/TFIID. Our data suggest that the two *C. albicans* TAF12 isoforms are functionally diverged and likely carry out specialized roles in transcriptional regulation.

In summary, our integrated study comprising bioinformatics, genetics and biochemistry led to the discovery of two TAF12 isoforms in *C. albicans* and five other fungal lineages out of the 22 examined. We conclude that TAF12a and TAF12b are specialized forms of TAF12 in *C. albicans* and indicate that they differentially regulate transcription by selectively associating with TFIID and SAGA complexes.