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Department of Biochemistry, Microbiology, and Molecular Biology

**GENOME-WIDE FUNCTIONAL ANALYSIS OF TAF1 AND MOT1,  
REGULATORS OF THE TATA BOX BINDING PROTEIN IN  
*SACCHAROMYCES CEREVISIAE***

A Thesis in

Biochemistry, Microbiology and Molecular Biology

by

Jordan Davis Welch Irvin

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The thesis of Jordan Davis Welch Irvin was reviewed and approved\* by the following:

B. Franklin Pugh  
Professor of Biochemistry and Molecular Biology  
Thesis Advisor  
Chair of Committee

Song Tan  
Associate Professor of Biochemistry and Molecular Biology

Richard J. Frisque  
Professor of Molecular Virology

Blake R. Peterson  
Associate Professor of Chemistry

Jerry L. Workman  
Investigator, The Stowers Institute for Medical Research  
Kansas City, MO  
Special Member

Robert A. Schlegel  
Professor of Biochemistry and Molecular Biology  
Head of the Department of Biochemistry, Microbiology and Molecular  
Biology

\*Signatures are on file in the Graduate School

## ABSTRACT

The TATA box binding protein (TBP) is a key protein in the process of gene expression, or transcription. The binding of TBP to DNA nucleates the formation of the pre-initiation complex of transcription factors at a gene. Numerous transcription factors regulate TBP both positively and negatively. TBP directs transcription from two multi-subunit complexes, SAGA and TFIID, that operate on separate classes of genes. The studies described here dissect the contributions that distinct domains of two transcription factors, TAF1 and MOT1, make on a genome-wide scale. TAF1 is an essential subunit of TFIID with several known activities, including binding TBP. MOT1 has been linked to the SAGA co-activator complex through genetic, biochemical, and genome-wide studies, and is known to remove TBP from DNA via ATP hydrolysis.

We find that all activities of TAF1 tested are required for proper expression of TFIID-dominated genes (~90% of the genome), and a domain of TAF1 plays a role in preventing TAF1 from associating with SAGA dominated genes. Deletion of functional domains of TAF1 contribute to protein instability, dominant toxicity, and can not support viability as the sole source of TAF1.

In contrast to the results from TAF1, several distinct patterns of MOT1-mediated regulation emerge. MOT1 appears to be a major regulator of transcription at a small subset of yeast genes. A genome-wide assessment indicates MOT1 acts primarily to activate TFIID-dominated (housekeeping) genes and repress SAGA-dominated (stress response) genes. The N-terminus of MOT1, which interacts with TBP is necessary for repression of stress response genes. A subset of genes depends on most or all of the

regions of MOT1 that were mutated for proper expression. Interestingly, the TBP-associated complex NC2, which makes positive and negative contributions to gene expression, appears to work in concert with MOT1 to regulate TBP. At genes where MOT1 is acting positively, NC2 also plays an activating role. Where MOT1 exerts repressive effects, NC2 also works to down-regulate expression.

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**ABBREVIATIONS**

$\alpha$ , anti  
 $\Delta$ , deletion  
aa, amino acid  
ATP, adenosine triphosphate  
ATP1, FHT MOT1  $\Delta$ 1403-1479. (ATP-ase activity region)  
bp, base pair  
C, carboxy  
ce, *Caenorhabditis elegans*  
CSM, complete synthetic media  
d, *Drosophila melanogaster*  
Da, dalton  
DAPI, 4',6-Diamidino-2-phenylindole  
dm, *Drosophila melanogaster*  
DT1, FHT-TAF1  $\Delta$ 10-88. (Deletion of TAND domain)  
DTT, dithiothreitol  
E., *Escherichia coli*  
FHT, Flu-6His-TEV  
Flu, influenza virus epitope (also known as HA)  
FOA, 5-fluoroorotic acid  
h, human  
HA, hemagglutinin  
HAT, histone acetyltransferase  
HFD, histone fold domain  
hs, *Homo sapiens* (human)  
HT4, 645-768. (Deletion of HAT region)  
II, RNA polymerase II associated factors  
K, kilo  
KDa, kilodalton  
min, minute  
MW, molecular weight  
N, amino  
NLS, nuclear localization signal  
NMR, nuclear magnetic resonance  
nt, nucleotide  
OD, optical density  
PAGE, polyacrylamide gel electrophoresis  
PB1, FHT-TAF1  $\Delta$ 912-992. (Deletion of promoter binding activity)  
PCR, polymerase chain reaction  
PEG, polyethylene glycol  
PIC, pre-initiation complex  
PMSF, phenylmethylsulphonylfluoride  
RT, room temperature (~22 °C)

S., *Saccharomyces*  
SAGA, Spt-Ada-Gcn5-acetyltransferase  
SDS, sodium dodecyl sulfate  
SLIK, SAGA like  
sp, *Schizosaccharomyces pombe*  
TAF, TBP-associated factor  
TAND, TAF1 N-terminal domain  
TBP, TATA-box binding protein  
TBP1, FHT MOT1  $\Delta$ 5-80. (Deletion of TBP interaction region #1)  
TBP2, FHT MOT1  $\Delta$ 307-432. (Deletion of TBP interaction region #2)  
TEV, Tobacco Etch Virus  
TF, transcription factor  
TF1; FHT-TAF1  $\Delta$ 208-303. (Deletion of Taf interaction region)  
TFIIA, transcription factor IIA  
TFIIB, transcription factor IIB  
TFIID, transcription factor IID  
ts, temperature sensitive  
UK1, FHT MOT1  $\Delta$ 1090-1259. (Unknown function)  
WT, wild-type  
WT1, FHT-TAF1 WT or FHT-MOT1 WT  
y, yeast (*Saccharomyces cerevisiae*)  
YPG, yeast extract/peptone/galactose  
YPR, yeast extract/peptone/raffinose

Note: The nomenclature used for the TBP associated factors (TAFs) established in:  
Tora, L. (2002) A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. *Genes & Development*, **16**, 673-675.

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## Chapter 1

### Introduction

#### 1.1 Overview of Gene Regulation

For a living cell to grow and survive, it must respond to the intracellular and extracellular environment. These responses must be coordinated in a timely and specific fashion to minimize unnecessary expenditure of cellular energy. One of the many processes that dynamically growing cells perform is gene expression. This is an ongoing process, as the cell responds to environmental changes, grows and develops, depletes nutrients, synthesizes proteins and enzymes, and many other functions. Genetic information stored in DNA is decoded by a series of protein complexes and converted into RNA through a process called transcription. Translation of the genetic information contained in RNA into a protein is the final step in gene expression. As a cell grows, develops, and responds to its environment, the need for certain proteins arises, and thus gene expression changes. For a single celled organism, such as the yeast *Saccharomyces cerevisiae*, expression of a set of genes may be triggered by a change in environmental temperature. A developing human cell may express a group of genes to differentiate into a particular type of cell.

Timely and efficient access to a cell's genetic material is intricate, complex, and highly regulated. These processes are regulated positively in order to increase expression

of a particular gene. Negative regulation can either prevent unnecessary expression (non-activating conditions) or dampen activated expression and return it to a basal level.

Negative regulation prevents the cell from synthesizing unnecessary proteins, depleting resources and substrates, and expending energy on non-productive adventures. Factors such as DNA accessibility, mRNA stability, rate of transcription, duration of transcription, and rate of translation all contribute to gene expression. The DNA of eukaryotic cells is compacted in the nucleus by wrapping the DNA around histone octamers to form nucleosomes. The nucleosome is the basic subunit of chromatin and contains 146 base pairs of DNA and two sets of histone H2A-H2B and H3-H4 pairs. Chromatin provides a physical barrier to DNA access and therefore prevents transcription. A number of chromatin modifying and remodeling enzymes are present in eukaryotic cells to overcome the obstruction that the nucleosomes present.

Transcription of RNA from DNA is catalyzed by RNA polymerases. Eukaryotes have three common RNA polymerases (pol I, II, III), each which synthesizes a particular class of RNA in a DNA-dependent manner. Additionally, a fourth class of polymerase (pol IV) was discovered in plants, but has not yet been identified in other eukaryotes (Onodera et al., 2005). Pol IV is non-essential and transcribes siRNA. Polymerase I transcribes rRNAs that help form the structure of the ribosome. While there are relatively few pol I genes, they are very heavily transcribed and account for greater than 90% of the RNA in a yeast cell. Polymerase III genes are distinguished by a distinct promoter structure. There are two conserved motifs, the A and B blocks, in all known pol III genes. A unique feature of the pol III promoter element is that it is internal to the open reading frame of the gene itself. Lastly, pol II transcribes the protein coding genes.

In yeast there are at least 6,000 known and predicted protein coding genes. Polymerase II transcripts are called mRNA since they carry the genetic message from DNA for conversion into protein. mRNA can vary widely in length and abundance, but as a whole compose ~2-3% of RNA in a living yeast cell.

## 1.2 Regulation of RNA Polymerase II Expression

Coordination of mRNA transcription by RNA polymerase II is highly regulated, involves hundreds of individual proteins, enzymes and several multi-subunit complexes. While a comprehensive review of all factors involved in transcriptional regulation is not possible, some of the important factors are reviewed below.

The RNA polymerase II complex transcribes all messenger RNA in yeast. Polymerase II is composed of 12 subunits (Rpb1-12), including the largest and catalytic subunit, Rbp1. The crystal structures of a 10 subunit “core” polymerase and the complete 12 subunit complex have been solved. While RNA polymerase is the enzyme responsible for transcription, a number of other proteins also play key functions in proper gene expression *in vivo*. Polymerase II requires general transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIIF, TFIIH) for transcription.

One key transcription factor is the TATA box binding protein, TBP, which is a subunit of TFIID. TBP is an essential polypeptide (~28 KDa in yeast) that binds eight nucleotides of DNA and initiates transcription. The TATA box is a conserved DNA motif (TATA(A/T)A(A/T)(A/G)) found in the promoters of ~20% of the yeast genes (Basehoar et al., 2004). TBP is required for expression from polymerase II (and

polymerase I, III) (Cormack and Struhl, 1992; Schultz et al., 1992), regardless of presence of a TATA box (Basehoar et al., 2004; Huisinga and Pugh, 2004; Kim and Iyer, 2004; Pugh and Tjian, 1991).

TBP contains a very highly conserved carboxy-terminal core domain of ~180 amino acids, and a divergent amino terminus (Hoffmann et al., 1990; Lee and Struhl, 2001). Crystallographic studies show the C-terminal core of TBP from several species to be saddle shaped and is the region of the protein that binds DNA (Chasman et al., 1993; Kim et al., 1993a; Kim et al., 1993b; Nikolov et al., 1992; Patikoglou et al., 1999). Biochemical analysis has shown that TBP binding to promoter DNA is a rate-limiting step for transcription initiation (Coleman and Pugh, 1997; Coleman et al., 1995). Due to its central role in gene expression, the binding of TBP to DNA is tightly regulated, both positively and negatively (Alexander et al., 2004; Chitikila et al., 2002; Coleman et al., 1995; Jackson-Fisher et al., 1999; Kou et al., 2003; Weideman et al., 1997).

At any particular gene, activators, repressors, co-activators, chromatin remodeling complexes, or chromatin modifying complexes may be present and contribute to gene expression. The requirements for many factors, such as activators and co-activators, are often gene specific. Many sequence specific repressors or *cis*-acting silencers are important for preventing improper gene expression. There are many examples of repressive effects on transcription such as the Ssn6/Tup1 complex; Sir2, Sir3, and Sir4 mediated repression; and Gal80 binding the transcriptional activator Gal4 and preventing expression (Carlson, 1998; Huh et al., 1999; Wyrick et al., 1999).

### 1.3 Chromatin Structure and Gene Expression

DNA is compacted and packaged into a hierarchical structure, chromatin, by binding to histone proteins. This binding and condensation provides a physical barrier to transcription factor access. Alterations in chromatin structure are necessary for transcription, DNA repair and replication. The basic subunit of chromatin is the nucleosome core particle, composed of 146 base pairs of DNA and eight histone proteins. Two copies of histone proteins H2A, H2B, H3 and H4 form an central octamer around which DNA is wound. Two copies of H2A-H2B and H3-H4 heterodimer pairs are formed via interactions in the histone fold domain of each protein. Additionally, linker DNA connects the nucleosomes, and this varies in length. A fifth histone particle, H1, helps stabilize the core particle, and in combination with the nucleosome core particle and linker DNA forms the nucleosome. Variant histones, such as the H3-like protein Cse4, involved in centromere structure and H2A.Z, often associated with active chromatin and localized to promoters, play specific roles but comprise a small percentage of the total cellular level of histones.

Common features of the major histones are highly basic composition, a central core domain with unstructured N and C termini, and strong evolutionary conservation. The histones are rich in lysine and arginine, have low numbers of aromatic amino acids, and lack tryptophan.

Due to their central role in formation of chromatin, histone proteins can be modified in a variety of ways. They are acetylated, methylated, O-phosphorylated on serines, poly-ADP ribosylated and ubiquitinated. Serine and threonine are the most

important sites for phosphorylation. In *S. cerevisiae*, histone H3 serine 10 phosphorylation has been shown to be important for transcription (reviewed in (Nowak and Corces, 2004)). Additionally, modification of histones by methylation can play an important role in transcription. H3 lysine four (K4) methylation has been shown as a marker for active genes, where H3 K9 is usually associated with inactive regions of chromatin (Hampsey and Reinberg, 2003; Ng et al., 2003). Ubiquitination of lysine 123 may play a role as a precursor to methylation (Ng et al., 2002b).

Other proteins can contribute to the formation of chromatin and present additional methods of transcriptional repression. Two examples are the Sir family of proteins, which are associated with heterochromatin and the Ssn6-Tup1 co-repressor complex. Sir proteins are found in high density at silent regions of the genome such as the telomeres. Sir3 preferentially associates with hypo-acetylated histone H4K16 tails. The Ssn6-Tup1 co-repressor complex is usually associated with silent chromatin. The complex does not bind directly to DNA, but Tup1 interacts with the N-terminal tails of histones H3 and H4 and regulates chromatin structure (reviewed in (Blander and Guarente, 2004)). Genetic evidence also indicates that Ssn6-Tup1 may interfere with transcription machinery and prevent transcription through more than one mechanism (reviewed in (Hampsey, 1998)).

#### **1.4 Chromatin Remodeling Complexes**

Chromatin remodeling enzymes use the energy of ATP hydrolysis to reposition nucleosomes. Remodeling can increase or decrease transcription factor accessibility to target DNA. The ATP dependent chromatin remodelers are divided into two main

families, the SWI/SNF family and the ISWI family. In yeast there are two members of the SWI/SNF family of remodelers, the Swi/Snf and the RSC complex. Yeast also contain two ISWI remodelers, Isw1 and Isw2. Protein homologues of ySwi/Snf include hSwi/Snf (Brg1) and dSwi/Snf (Brm). In higher eukaryotes NURF, ACF, CHRAC and RSF are all members of the ISWI family.

In yeast, Swi/Snf is a non-essential complex composed of at least 11 subunits with Swi2/Snf2 as the catalytic subunit (reviewed in (Becker and Horz, 2002; Eberhardter and Becker, 2004). RSC (Remodel the Structure of Chromatin) on the other hand, is essential, contains at least 15 subunits, and the catalytic subunit is Sth1. The RSC complex is involved in DNA repair and is required for expression of sporulation genes (Bungard et al., 2004; Ng et al., 2002a). ISWI is also a non-essential complex which has been implicated in promoting transcriptional elongation and separately, repressing initiation (Fazio and Tsukiyama, 2003; Goldmark et al., 2000; Imbalzano and Xiao, 2004).

It is possible that remodeling of chromatin takes place through nucleosome sliding, where the nucleosome is not detached from the DNA, but is repositioned in relative location. Movement of nucleosomes in *cis* appears to be the major method of action of remodelers, whereas relocation of a nucleosome in *trans* accounts for a small percentage of remodeled nucleosomes (reviewed in (John et al., 2000). By altering the location of the nucleosomes, these enzymes enhance the accessibility of DNA to the transcription machinery. In contrast, relocating the nucleosomes to the promoter and open reading frame can prevent factor access, and therefore transcription.

## 1.5 Histone Acetylation

Histone acetyltransferase enzymes have been highly studied in terms of their roles in transcription. These enzymes function by transferring an acetyl group (from acetyl-coenzyme A) to the  $\epsilon$ -amino position of lysines in the N-terminal tails of the histones. The prevailing thought on the mechanism of action for acetylation is that binding of a neutral acetyl group to a positively charged lysine residue reduces the ionic interactions between histone tails and the phosphate backbone of DNA (reviewed in (Belotserkovskaya et al., 2000)). Hyper-acetylation would create a local environment in which DNA is more easily accessible, and is often associated with active chromatin (Kurdistani et al., 2004; Robert et al., 2004; Xu et al., 2005). Conversely, hypo-acetylated regions of the genome, such as telomers and heterochromatin are generally transcriptionally inactive.

Yeast contain both multisubunit HAT complexes (SAGA (Gcn5p), ADA (Gcn5p), NuA3 (Sas3p), NuA4 (Esa1p), Elongator (Elp3p), TFIID (TAF1p)), and two individual HATs (Sas2 and Hpa2), (Belotserkovskaya et al., 2000; Mizzen et al., 1996). The activities of histone acetyltransferases (HATs) are countered by histone deacetylases (HDACs). While HATs increase acetylation, which is often linked to increases in transcription, HDACs generally have repressive functions.

## 1.6 The SAGA Co-Activator Complex

The SAGA (Spt, Ada, Gcn5, acetyltransferase) co-activator complex was first identified in 1997 after biochemical purification of Gcn5 from yeast cell extracts (Grant

et al., 1997). Gcn5 is the catalytic HAT subunit of this non-essential ~1.8 MDa complex of at least 22 subunits, and acetylates H3 K14, H4 K8 and K16 (Kuo et al., 1996; Roth and Allis, 1996; Zhang et al., 1998). There are four groups of proteins in the SAGA complex: the Ada group (Ada1, Ada2, Ada3, Ada4 = Gcn5, and Ada5 = Spt20), the Spt group (Spt3, Spt7, Spt8, Spt20), the TAFs 5, 6, 9, 10, 12 and Tra1, one of the largest proteins in yeast. Interestingly, SAGA does not contain TBP (Spt15), but Spt3, Spt8, and the TAFs have been shown through biochemical and genetic studies to interact with TBP. *spt7*, *spt20* and *ada1* mutants disrupt the SAGA complex completely (Sternier et al., 1999). Additionally, a C-terminal truncation of Spt7 causes a loss of Spt8. Complexes with the altered Spt7 and absent Spt8 are termed SLIK (SAGA like) and SALSA (SAGA Altered, Spt8 Absent) (Pray-Grant et al., 2002; Sternier et al., 2002; Wu and Winston, 2002).

Genome wide studies have shown that approximately 10% of the yeast genome's expression is highly dependent on the SAGA complex (Huisinga and Pugh, 2004; Lee et al., 2000). The genes governed by SAGA are highly inducible, many are involved in stress response, and are regulated by many factors (Huisinga and Pugh, 2004). Interestingly, the majority of promoters of SAGA dominated genes have a TATA box. (Basehoar et al., 2004; Huisinga and Pugh, 2004).

## 1.7 General Transcription Factors

General transcription factors were initially isolated as chromatographic fractions that could supplement purified mammalian polymerase II and reconstitute *in vitro* transcription from a DNA template (Matsui et al., 1980). TFIIA (Toa1p, Toa2p) helps stabilize TBP and TFIID on promoter DNA, and TFIIB (Sua7p) aids in positioning TBP at the transcriptional start site. TFIIF (Flp1p, Tfg1p, Tfg2p, TAF14p), a component of both the RNA polymerase II holoenzyme and the mediator complex, aids in polymerase recruitment. After TFIIF and polymerase are recruited, TFIIE (Tfa1, Tfa2) and TFIIH (Ssl1p, Met18p, Tfb3p, Tfb2p, Cll1p, Tfb4p, Tfb5p) bind and form the preinitiation complex. TFIIE modulates the function of Ssl1p, the kinase in TFIIH at phosphorylated serine 2 of the C-terminal domain (CTD) of polymerase. Phosphorylation of the CTD converts polymerase from the preinitiation (inactive) to elongation competent form.

### 1.7.1 TFIID

The general transcription factor TFIID contains the TATA box binding protein, TBP, and binding of TBP (TFIID) to DNA is the rate-limiting step in transcription (Coleman and Pugh, 1997; Coleman et al., 1995; Kim and Iyer, 2004). TFIID controls expression of greater than 90% of the genes in the yeast genome (Huisinga and Pugh, 2004). These genes are not regulated by large numbers of other factors, and are termed “housekeeping” genes (Huisinga and Pugh, 2004). The SAGA complex is involved in expression of the remaining 10% of the genome, which are stress response genes (Huisinga and Pugh, 2004). However, in the absence of SAGA, which is a non-essential

complex, TFIID can fully compensate and regulate transcription initiation of the entire yeast genome (Huisinga and Pugh, 2004).

Mass-spectrometry analysis of highly purified TFIID complexes indicates association of a number of minor components (Rsp5, Bul1, Ubp3, Bre5, Cka1 and Cka2) (Auty et al., 2004; Bai et al., 1997; Sanders et al., 2002a; Sanders and Weil, 2000).

Additionally, the precision of the mass spectrometry established the stoichiometry of the TFIID complex (Auty et al., 2004; Bai et al., 1997; Sanders et al., 2002a; Sanders and Weil, 2000). These results agree with previously published information on interactions of the TAFs, antibody staining/electron microscopy imaging, and the estimated size of the complex, which is calculated at 1.2 MDa by gel filtration chromatography or 0.7 MDa by ultracentrifugation (Andel et al., 1999; Sanders et al., 2002a; Sanders et al., 2002b).

Mass spectrometry data indicates that TAF1 and TAF5 are ubiquitinated, and TAF5 is methylated, but no TAFs were phosphorylated (Auty et al., 2004). TAF11 and TAF1 are frequently proteolytically processed. TAF1, missing up to 198 N-terminal amino acids, was often detected. Cleavage of the N-terminus of TAF1 could result in unbound TBP and TBP-free forms of TFIID as have been reported (Kuras et al., 2000; Mencia et al., 2002), since the major region of interaction for TBP with TFIID is on the N-terminus of TAF1 (Bai et al., 1997; Kou et al., 2003; Mencia and Struhl, 2001).

Several TAFs of the yTFIID complex have a histone fold domain (HFD). It has been proposed that, similar to the nucleosome, these domains mediate protein-protein interactions. Interactions like those seen between H2A and H2B, have been demonstrated for TAF 4/12 and 6/9 pairs (Brand et al., 1999; Leurent et al., 2002).

TFIID as a whole was proposed to mimic the structure of the nucleosome and facilitate wrapping of DNA (Oelgeschlager et al., 1996). Winding of DNA around the TFIID complex supports the idea of extended contacts with promoter DNA as seen in footprinting and crosslinking experiments (Auty et al., 2004) and could position the PIC in relation to the transcriptional start site (Hoffmann et al., 1996).

Genome-wide studies using heat inactivation of temperature sensitive mutants to all 13 essential TAFs demonstrate a wide range of transcriptional dependency. Several gene-specific effects are observed, indicating that TAFs have selective functions across the genome. Protein-DNA crosslinking and immunopurification reveals three classes of genes, based on their dependency of the TAFs. TAF-dependent genes have a high level of association of the majority of the TAFs, and their expression decreases upon thermal inactivation of temperature sensitive TAF alleles. A second class of genes is largely transcriptionally regulated in a TAF-independent manner. Lastly, some genes appear to require a subset of the TAFs for accurate transcription (Apone et al., 1998; Li et al., 2000; Mencia et al., 2002; Shen et al., 2003).

**Table 1-1**

Table 1-1: Yeast TAF Summary Table

yTAF	related to	yeast ORF (nt)	yeast size (AA)	yeast MW (KDa)	chrom	complexes	HFD	HFD partner	synthetic lethality	cell cycle arrest	% genome <sup>1</sup> req
TBP		720	240	27	V	IID/SAGA					
TAF1		3198	1066	121	VII	IID	no				~10
TAF2		3141	1047	161	III		no			G2/M	3
TAF3		1059	353	40	XVI	IID	yes				10
TAF4	H2A	1164	388	42	XIII	IID	yes	TAF12		--	11
TAF5		2394	798	89	II	IID/SAGA	no			G2/M	8
TAF6	H4	1548	516	58	VII	IID/SAGA	yes	TAF9			18
TAF7		1770	590	67	XIII	IID	no		<i>bdf1</i>		24
TAF8		1530	510	58	XIII	IID	yes				51
TAF9	Chs6, H3	471	157	17	XIII	IID/SAGA	yes	TAF6			~60
TAF10		618	206	23	IV	IID/SAGA	yes				~17
TAF11		1038	346	40	XIII	IID	yes				18
TAF12	H2B	1617	539	61	IV		yes	TAF4			9
TAF13		501	167	19	XIII		yes				19
TAF14	Sas5, Yaf9	732	244	27	XVI	Swi/Snf	no				
BDF1		2058	686	77	XII				<i>bdf2</i>		

Information on size in amino acids and molecular weight are for yeast TAFs only.  
nt = nucleotides; AA = amino acids; HFD = histone fold domain..

Molecular weight, when mature, in Kilo Daltons

\* TAF14 is also a member of the INO80, TFIIF and TFIIS complexes.

### 1.7.1.1 TFIID Subunit TAF1

One the TAFs unique to TFIID is TAF1 (h250, dm230, y130/145). TAF1 interacts directly with TBP and several of the TAFs. TAF1 is not a shared TAF; it is a member of TFIID but not SAGA. Biochemical analysis of higher homologues reveals a key structural role of TAF1 in TFIID integrity. Order-of-addition experiments suggest that TAF1 is required for further assembly of TFIID (Verrijzer et al., 1995). Mutations to TAF1 not only disrupt the complex, but lead to degradation of the subunits (Chen et al.,

<sup>1</sup> Data from Shen, W.C., Bhaumik, S.R., Causton, H.C., Simon, I., Zhu, X., Jennings, E.G., Wang, T.H., Young, R.A. and Green, M.R. (2003) Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly. *Embo J*, **22**, 3395-3402.

1994). These data from higher eukaryotes led to the notion that TAF1 was the “scaffold” upon which TFIID was assembled. In yeast, heat inactivation of temperature sensitive mutants of TAF1 leads to rapid protein degradation and a drastic drop in transcription (Walker et al., 1996). In yeast, three main protein-protein interaction regions are well defined. The N-terminus of TAF1 (TAND domain) interacts with TBP (Kokubo et al., 1998; Kotani et al., 1998; Liu et al., 1998). A deletion of the TAND domain is fatal at elevated temperatures in yeast, and displays synthetic toxicity with mutations in TBP or TAF12 (Kobayashi et al., 2003; Kobayashi et al., 2001; Kotani et al., 1998). Amino acids 208-303 are critical for association with the majority of the TAFs in TFIID (Bai et al., 1997; Mencia and Struhl, 2001). Additionally, TAF7 interacts with a broad region at the C-terminus of TAF1 (Singh et al., 2004).

*Drosophila* TAF250 increases the footprint of TBP to the TATA box (-30) of the *hsp70* promoter (Chalkley and Verrijzer, 1999; Verrijzer et al., 1995; Verrijzer et al., 1994). There is additional protection of the Inr region (~-5 to +20) after the addition of TAF250 and TAF150 (Verrijzer et al., 1995). Deletion analysis and chromatin immunoprecipitation reveals a small region of yTAF1 near the C-terminus is required for association with DNA (Mencia and Struhl, 2001). Yeast TAF1 increases the TBP footprint both proximal and distal to the TATA box (Auty et al., 2004).

All homologues of TAF1 have been shown to possess enzymatic HAT activity (Matangkasombut et al., 2000; Mizzen et al., 1996), but the *in vivo* relevance of this activity is unclear. Current work in the Pugh lab supports the notion that yeast TAF1 does not have a major biological HAT function (Durant and Pugh, unpublished).

While the TAF homologues are typically highly similar between species, TAF1 is not highly conserved (Moqtaderi et al., 1996; Tora, 2002). The yeast TAF1 (~120KDa) is considerably smaller than its counterparts in *Drosophila* (~230KDa) or humans (~250KDa). Only the higher homologues have amino and carboxy terminal kinase activities (O'Brien and Tjian, 1998; Sawa et al., 2004). TAF1 is also reported to contain an HMG box (Jantzen et al., 1990; Matangkasombut et al., 2004; Ruzzi et al., 1997). Homology comparisons between yeast and human TAF1 indicate that the smaller yTAF1 is missing the C-terminal bromodomain human TAF1. However, the yeast bromodomain protein Bdf1 is homologous to the C-terminus of human TAF1 (Matangkasombut et al., 2000). Together, yeast TAF1 and Bdf1 are functionally equivalent to hTAF1 (Jacobson et al., 2000; Matangkasombut et al., 2000).

### **1.8 Bromodomain Factor 1**

BDF1 (bromodomain factor) is a TFIID associated transcription factor that is involved sporulation, salt tolerance, cold stress tolerance, growth at 37 °C, resistance to caffeine, and growth on alternative carbon sources (de Jesus Ferreira et al., 2001; Hsu et al., 1995; Lygerou et al., 1994). BDF1 resembles the C-terminus of human TAF1, which contains a kinase domain, and similarly, BDF1 contains autophosphorylation activity (Matangkasombut et al., 2000). Genome-wide expression analysis indicates ~9% of the genome is regulated in part by BDF1 (Ladurner et al., 2003; Swanson et al., 2003). Genetic evidence (synthetic lethality) indicates BDF1 interacts with BDF2, histone H4 (HHF1, HHF2), histone H3 (HHT1, HHT2), the HAT complex Esa1, the chromatin

remodeling complex SWR-C, and the Spt20 subunit of SAGA (Kobor et al., 2004; Matangkasombut and Buratowski, 2003; Mizuguchi et al., 2004; Sawa et al., 2004).

### **1.9 The TATA Box Binding Protein (TBP)**

The TATA box binding protein (TBP) was identified through a genetic screen as a suppressor of a transposable element which inserted and disrupted genes (Simchen et al., 1984; Winston et al., 1987), and genetic experiments show that the gene encoding TBP, *SPT15*, is essential (Cavallini et al., 1989; Cavallini et al., 1988; Hahn et al., 1989; Hoey et al., 1990; Horikoshi et al., 1989; Kao et al., 1990; Schmidt et al., 1989). The sequence of the TBP protein is highly conserved evolutionarily, and diverse species have very similar TBP structures, showing functional importance (Hernandez, 1993; Patikoglou et al., 1999). Genetic studies show single amino acid substitutions in TBP that increase transcription. TBP binds to and activates transcription from TATA less promoters (Pugh and Tjian, 1991), and is involved in transcription of pol I, II, and III genes (Cormack and Struhl, 1992). *In vivo* crosslinking and chromatin immunoprecipitation studies demonstrate a clear correlation between levels of TBP bound to a promoter and level of transcription (Kuras and Struhl, 1999).

### **1.10 MOT1**

MOT1 (BUR3), or modifier of transcription, is an essential gene, encoding a very large yeast protein (~210 KDa) (Davis et al., 1992). MOT1 is homologous to human

TAF170/172 (BTAF1) (Chicca et al., 1998; Davis et al., 1992). MOT1 is a member of the Snf2/Swi2 helicase family of conserved ATP-ase DNA remodeling proteins, yet MOT1p does not display helicase activity (Adamkewicz et al., 2001). MOT1 uses the energy of ATP hydrolysis to remove TBP from DNA (Auble et al., 1994). MOT1 can accelerate TBP-DNA dissociation, even though TBP-DNA interactions are very stable (Muldrow et al., 1999; Prelich and Winston, 1993; Wade and Jaehning, 1996). While MOT1 can act to repress basal transcription (Andrau et al., 2002; Auble et al., 1994; Collart, 1996; Darst et al., 2003; Geisberg et al., 2002; Muldrow et al., 1999), MOT1 activates transcription, possibly through a mechanism where limiting quantities of TBP are recycled from non-active DNA templates (i.e. non-transcribed promoters or nonpromoter regions) to actively transcribed regions (Auble et al., 1994; Dasgupta et al., 2002).

It has also been hypothesized that MOT1 regulates TBP placement on DNA, possibly allowing for proper start site selection or asymmetric binding of TBP to symmetric TATA sequences (Darst et al., 2003)

MOT1 contains four conserved blocks (A, B, C, D) corresponding to HEAT repeats (Darst et al., 2003). The A block is essential for MOT1 activity (Adamkewicz et al., 2001; Auble et al., 1997). Located at the N-terminus of MOT1 is a large region (~800 residues) that binds TBP (Meisterernst and Roeder, 1991; Meisterernst et al., 1991). The central portion of MOT1 has no known function. The C-terminus contains the essential ATP-ase domain.

### 1.11 The Yeast NC2 Complex

NC2 (NC2 $\alpha$ /DRAP1 and NC2  $\beta$  /Dr1) is an essential hetero-dimer complex that was originally isolated as an inhibitor of basal polymerase II transcription from human cell extracts (Inostroza et al., 1992). Homologous proteins were identified in yeast that also demonstrated ability to bind TBP and prevent transcription (Bur6/NCB1 and YDR1/NCB2) (Kamada et al., 2001).

Structural determination of the NC2/TBP/DNA complex shows that NC2 interacts with DNA both upstream and downstream of TBP, and establishes how NC2 can stabilize TBP on DNA (Gilfillan et al., 2005; Klejman et al., 2004). Human NC2-TBP can bind to DNA in the absence of a canonical TATA box (Cang et al., 1999; Goppelt and Meisterernst, 1996; Goppelt et al., 1996; Kim et al., 1997; Yeung et al., 1997). NC2 negatively regulates transcription by binding to and stabilizing TBP on DNA in a manner that is competitive with positively acting transcription factors TFIIA and TFIIB (Bleichenbacher et al., 2003). However, the regions of TBP bound by human NC2 and TFIIA do not largely overlap (Coleman et al., 1999; Klejman et al., 2004; Weideman et al., 1997). The affinity for human NC2-TBP with DNA is weaker than that of TFIIA-TBP-DNA (Cang et al., 1999). A TBP point mutation (F182V) that was discovered through a genetic screen for mutations that would increase transcription from basal promoters, was found to be defective for NC2 interactions (Cang et al., 1999; Cang and Prelich, 2002; Creton et al., 2002; Geisberg et al., 2001; Lemaire et al., 2000; Willy et al., 2000).

While the negative role of the NC2 complex has been well established through biochemical, genetic, and structural studies, a less understood positive function of NC2 also exists (Lemaire et al., 2000; Prelich, 1997). NC2 is required for expression of some genes, including *GAL1*, *GAL10*, *HIS3* and *HIS4* (Cang and Prelich, 2002). Genome-wide studies indicate that NC2 $\alpha$  associates with active promoters in yeast (Creton et al., 2002; Lemaire et al., 2000). It appears that the alpha subunit (Bur6/NCB1) functions positively, especially in response to changing environmental conditions (Klejman et al., 2004). The NC2 $\alpha$  subunit binds to BTAF1 (human homologue of MOT1) and stimulates transcription while NC2 $\beta$  does not associate directly with BTAF1 and inhibits transcription (Shen et al., 2003).

The work presented here is a study of two factors involved in polymerase II transcription, TAF1 and MOT1, in the common laboratory yeast *Saccharomyces cerevisiae*. Both TAF1 and MOT1 interact with TBP and contribute to regulation of gene expression. The physiological relevance of the TATA binding protein's interactions with the N-terminus of TAF1 will be described in chapter 2. Genome-wide studies on functional domains of the TBP associated factor TAF1 are summarized in chapter 3. Chapter 4 focuses on an essential protein with both positive and negative roles in gene expression, MOT1. MOT1 is a Swi/Snf related helicase that can decrease expression of genes by removal of TBP from DNA in an ATP-dependent manner.

## 1.12 Bibliography

- Adamkewicz, J.I., Hansen, K.E., Prud'homme, W.A., Davis, J.L. and Thorner, J. (2001) High affinity interaction of yeast transcriptional regulator, Mot1, with TATA box-binding protein (TBP). *J Biol Chem*, **276**, 11883-11894.
- Alexander, D.E., Kaczorowski, D.J., Jackson-Fisher, A.J., Lowery, D.M., Zanton, S.J. and Pugh, B.F. (2004) Inhibition of TATA binding protein dimerization by RNA polymerase III transcription initiation factor Brf1. *J Biol Chem*, **279**, 32401-32406.
- Andel, F., 3rd, Ladurner, A.G., Inouye, C., Tjian, R. and Nogales, E. (1999) Three-dimensional structure of the human TFIID-IIA-IIB complex. *Science*, **286**, 2153-2156.
- Andrau, J.C., Van Oevelen, C.J., Van Teeffelen, H.A., Weil, P.A., Holstege, F.C. and Timmers, H.T. (2002) Mot1p is essential for TBP recruitment to selected promoters during in vivo gene activation. *Embo J*, **21**, 5173-5183.
- Apone, L.M., Virbasius, C.A., Holstege, F.C., Wang, J., Young, R.A. and Green, M.R. (1998) Broad, but not universal, transcriptional requirement for yTAFII17, a histone H3-like TAFII present in TFIID and SAGA. *Mol Cell*, **2**, 653-661.
- Auble, D.T., Hansen, K.E., Mueller, C.G., Lane, W.S., Thorner, J. and Hahn, S. (1994) Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes & Development*, **8**, 1920-1934.

- Auble, D.T., Wang, D., Post, K.W. and Hahn, S. (1997) Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP- driven enzyme that dissociates TATA-binding protein from DNA. *Mol Cell Biol*, **17**, 4842-4851.
- Auty, R., Steen, H., Myers, L.C., Persinger, J., Bartholomew, B., Gygi, S.P. and Buratowski, S. (2004) Purification of active TFIID from *Saccharomyces cerevisiae*: Extensive promoter contacts and co-activator function. *J Biol Chem*.
- Bai, Y., Perez, G.M., Beechem, J.M. and Weil, P.A. (1997) Structure-function analysis of TAF130: identification and characterization of a high-affinity TATA-binding protein interaction domain in the N terminus of yeast TAF(II)130. *Mol Cell Biol*, **17**, 3081-3093.
- Basehoar, A.D., Zanton, S.J. and Pugh, B.F. (2004) Identification and distinct regulation of yeast TATA box-containing genes. *Cell*, **116**, 699-709.
- Becker, P.B. and Horz, W. (2002) ATP-dependent nucleosome remodeling. *Annu Rev Biochem*, **71**, 247-273.
- Belotserkovskaya, R., Sterner, D.E., Deng, M., Sayre, M.H., Lieberman, P.M. and Berger, S.L. (2000) Inhibition of TATA-Binding Protein Function by SAGA Subunits Spt3 and Spt8 at Gcn4-Activated Promoters. *Mol Cell Biol*, **20**, 634-647.
- Blander, G. and Guarente, L. (2004) The Sir2 family of protein deacetylases. *Annu Rev Biochem*, **73**, 417-435.
- Bleichenbacher, M., Tan, S. and Richmond, T.J. (2003) Novel interactions between the components of human and yeast TFIIA/TBP/DNA complexes. *J Mol Biol*, **332**, 783-793.

- Brand, M., Leurent, C., Mallouh, V., Tora, L. and Schultz, P. (1999) Three-dimensional structures of the TAFII-containing complexes TFIID and TFTC. *Science*, **286**, 2151-2153.
- Bungard, D., Reed, M. and Winter, E. (2004) RSC1 and RSC2 are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*. *Eukaryot Cell*, **3**, 910-918.
- Cang, Y., Auble, D.T. and Prelich, G. (1999) A new regulatory domain on the TATA-binding protein. *Embo J*, **18**, 6662-6671.
- Cang, Y. and Prelich, G. (2002) Direct stimulation of transcription by negative cofactor 2 (NC2) through TATA-binding protein (TBP). *Proc Natl Acad Sci U S A*, **99**, 12727-12732.
- Carlson, M. (1998) Regulation of glucose utilization in yeast. *Curr Opin Genet Dev*, **8**, 560-564.
- Cavallini, B., Faus, I., Matthes, H., Chipoulet, J.M., Winsor, B., Egly, J.M. and Chambon, P. (1989) Cloning of the gene encoding the yeast protein BTF1Y, which can substitute for the human TATA box-binding factor. *Proc Natl Acad Sci U S A*, **86**, 9803-9807.
- Cavallini, B., Huet, J., Plassat, J.L., Sentenac, A., Egly, J.M. and Chambon, P. (1988) A yeast activity can substitute for the HeLa cell TATA box factor. *Nature (London)*, **334**, 77-80.
- Chalkley, G.E. and Verrijzer, C.P. (1999) DNA binding site selection by RNA polymerase II TAFs: a TAF(II)250-TAF(II)150 complex recognizes the initiator. *Embo J*, **18**, 4835-4845.

- Chasman, D.I., Flaherty, K.M., Sharp, P.A. and Kornberg, R.D. (1993) Crystal structure of yeast TATA-binding protein and model for interaction with DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8174-8178.
- Chen, J.L., Attardi, L.D., Verrijzer, C.P., Yokomori, K. and Tjian, R. (1994) Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell*, **79**, 93-105.
- Chicca, J.J., 2nd, Auble, D.T. and Pugh, B.F. (1998) Cloning and biochemical characterization of TAF-172, a human homolog of yeast Mot1. *Mol Cell Biol*, **18**, 1701-1710.
- Chitikila, C., Huisinga, K.L., Irvin, J.D., Basehoar, A.D. and Pugh, B.F. (2002) Interplay of TBP Inhibitors in Global Transcriptional Control. *Molecular Cell*.
- Coleman, R.A. and Pugh, B.F. (1997) Slow dimer dissociation of the TATA binding protein dictates the kinetics of DNA binding. *Proc Natl Acad Sci U S A*, **94**, 7221-7226.
- Coleman, R.A., Taggart, A.K., Benjamin, L.R. and Pugh, B.F. (1995) Dimerization of the TATA binding protein. *Journal of Biological Chemistry*, **270**, 13842-13849.
- Coleman, R.A., Taggart, A.K.P., Burma, S., Chicca II, J.J. and Pugh, B.F. (1999) TFIIA regulates TBP and TFIID dimers. *Molecular Cell*, **4**, 451-457.
- Collart, M.A. (1996) The NOT, SPT3, and MOT1 genes functionally interact to regulate transcription at core promoters. *Mol Cell Biol*, **16**, 6668-6676.

- Cormack, B.P. and Struhl, K. (1992) The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. *Cell*, **69**, 685-696.
- Creton, S., Svejstrup, J.Q. and Collart, M.A. (2002) The NC2 alpha and beta subunits play different roles in vivo. *Genes Dev*, **16**, 3265-3276.
- Darst, R.P., Dasgupta, A., Zhu, C., Hsu, J.Y., Vroom, A., Muldrow, T. and Auble, D.T. (2003) Mot1 regulates the DNA binding activity of free TATA-binding protein in an ATP-dependent manner. *J Biol Chem*, **278**, 13216-13226.
- Dasgupta, A., Darst, R.P., Martin, K.J., Afshari, C.A. and Auble, D.T. (2002) Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. *Proc Natl Acad Sci U S A*, **99**, 2666-2671.
- Davis, J.L., Kunisawa, R. and Thorner, J. (1992) A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Molecular & Cellular Biology*, **12**, 1879-1892.
- de Jesus Ferreira, M.C., Bao, X., Laize, V. and Hohmann, S. (2001) Transposon mutagenesis reveals novel loci affecting tolerance to salt stress and growth at low temperature. *Curr Genet*, **40**, 27-39.
- Eberharter, A. and Becker, P.B. (2004) ATP-dependent nucleosome remodelling: factors and functions. *J Cell Sci*, **117**, 3707-3711.
- Fazio, T.G. and Tsukiyama, T. (2003) Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. *Mol Cell*, **12**, 1333-1340.

- Geisberg, J.V., Holstege, F.C., Young, R.A. and Struhl, K. (2001) Yeast NC2 associates with the RNA polymerase II preinitiation complex and selectively affects transcription in vivo. *Mol Cell Biol*, **21**, 2736-2742.
- Geisberg, J.V., Moqtaderi, Z., Kuras, L. and Struhl, K. (2002) Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **22**, 8122-8134.
- Gilfillan, S., Stelzer, G., Piaia, E., Hofmann, M.G. and Meisterernst, M. (2005) Efficient binding of NC2.TATA-binding protein to DNA in the absence of TATA. *J Biol Chem*, **280**, 6222-6230.
- Goldmark, J.P., Fazio, T.G., Estep, P.W., Church, G.M. and Tsukiyama, T. (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell*, **103**, 423-433.
- Goppelt, A. and Meisterernst, M. (1996) Characterization of the basal inhibitor of class II transcription NC2 from *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **24**, 4450-4455.
- Goppelt, A., Stelzer, G., Lottspeich, F. and Meisterernst, M. (1996) A mechanism for repression of class II gene transcription through specific binding of NC2 to TBP-promoter complexes via heterodimeric histone fold domains. *EMBO Journal*, **15**, 3105-3116.
- Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate

- nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev*, **11**, 1640-1650.
- Hahn, S., Buratowski, S., Sharp, P.A. and Guarente, L. (1989) Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the SPT15 suppressor of Ty element insertions. *Cell*, **58**, 1173-1181.
- Hampsey, M. (1998) Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol Mol Biol Rev*, **62**, 465-503.
- Hampsey, M. and Reinberg, D. (2003) Tails of intrigue: phosphorylation of RNA polymerase II mediates histone methylation. *Cell*, **113**, 429-432.
- Hernandez, N. (1993) TBP, a universal eukaryotic transcription factor? *Genes & Development*, **7**, 1291-1308.
- Hoey, T., Dynlacht, B.D., Peterson, M.G., Pugh, B.F. and Tjian, R. (1990) Isolation and characterization of the Drosophila gene encoding the TATA box binding protein, TFIID. *Cell*, **61**, 1179-1186.
- Hoffmann, A., Chiang, C.M., Oelgeschlager, T., Xie, X.L., Burley, S.K., Nakatani, Y. and Roeder, R.G. (1996) A histone octamer-like structure within TFIID. *Nature*, **380**, 356-359.
- Hoffmann, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M. and Roeder, R.G. (1990) Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). *Nature*, **346**, 387-390.

- Horikoshi, M., Wang, C.K., Fujii, H., Cromlish, J.A., Weil, P.A. and Roeder, R.G. (1989) Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. *Nature (London)*, **341**, 299-303.
- Hsu, Y.S., Tang, F.M., Liu, W.L., Chuang, J.Y., Lai, M.Y. and Lin, Y.S. (1995) Transcriptional regulation by p53. Functional interactions among multiple regulatory domains. *Journal of Biological Chemistry*, **270**, 6966-6974.
- Huh, J.R., Park, J.M., Kim, M., Carlson, B.A., Hatfield, D.L. and Lee, B.J. (1999) Recruitment of TBP or TFIIB to a promoter proximal position leads to stimulation of RNA polymerase II transcription without activator proteins both in vivo and in vitro. *Biochem Biophys Res Commun*, **256**, 45-51.
- Huisinga, K.L. and Pugh, B.F. (2004) A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell*, **13**, 573-585.
- Imbalzano, A.N. and Xiao, H. (2004) Functional properties of ATP-dependent chromatin remodeling enzymes. *Adv Protein Chem*, **67**, 157-179.
- Inostroza, J.A., Mermelstein, F.H., Ha, I., Lane, W.S. and Reinberg, D. (1992) Dr1, a TATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. *Cell*, **70**, 477-489.
- Jackson-Fisher, A.J., Chitikila, C., Mitra, M. and Pugh, B.F. (1999) A role for TBP dimerization in preventing unregulated gene expression. *Mol. Cell*, **3**, 717-727.
- Jacobson, R.H., Ladurner, A.G., King, D.S. and Tjian, R. (2000) Structure and function of a human TAF(II)250 double bromodomain module [In Process Citation]. *Science*, **288**, 1422-1425.

- Jantzen, H.M., Admon, A., Bell, S.P. and Tjian, R. (1990) Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature*, **344**, 830-836.
- John, S., Howe, L., Tafrov, S.T., Grant, P.A., Sternglanz, R. and Workman, J.L. (2000) The Something About Silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. *Genes Dev*, **14**, 1196-1208.
- Kamada, K., Shu, F., Chen, H., Malik, S., Stelzer, G., Roeder, R.G., Meisterernst, M. and Burley, S.K. (2001) Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex. *Cell*, **106**, 71-81.
- Kao, C.C., Lieberman, P.M., Schmidt, M.C., Zhou, Q., Pei, R. and Berk, A.J. (1990) Cloning of a transcriptionally active human TATA binding factor. *Science*, **248**, 1646-1650.
- Kim, J. and Iyer, V.R. (2004) Global role of TATA box-binding protein recruitment to promoters in mediating gene expression profiles. *Mol Cell Biol*, **24**, 8104-8112.
- Kim, J.L., Nikolov, D.B. and Burley, S.K. (1993a) Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature*, **365**, 520-527.
- Kim, S., Na, J.G., Hampsey, M. and Reinberg, D. (1997) The Dr1/DRAP1 heterodimer is a global repressor of transcription in vivo. *Proc Natl Acad Sci U S A*, **94**, 820-825.
- Kim, Y., Geiger, J.H., Hahn, S. and Sigler, P.B. (1993b) Crystal structure of a yeast TBP/TATA-box complex. *Nature*, **365**, 512-520.
- Klejman, M.P., Pereira, L.A., van Zeeburg, H.J., Gilfillan, S., Meisterernst, M. and Timmers, H.T. (2004) NC2alpha interacts with BTAF1 and stimulates its ATP-

dependent association with TATA-binding protein. *Mol Cell Biol*, **24**, 10072-10082.

Kobayashi, A., Miyake, T., Kawaichi, M. and Kokubo, T. (2003) Mutations in the histone fold domain of the TAF12 gene show synthetic lethality with the TAF1 gene lacking the TAF N-terminal domain (TAND) by different mechanisms from those in the SPT15 gene encoding the TATA box-binding protein (TBP). *Nucleic Acids Res*, **31**, 1261-1274.

Kobayashi, A., Miyake, T., Ohyama, Y., Kawaichi, M. and Kokubo, T. (2001) Mutations in the TATA-binding protein, affecting transcriptional activation, show synthetic lethality with the TAF145 gene lacking the TAF N-terminal domain in *Saccharomyces cerevisiae*. *J Biol Chem*, **276**, 395-405.

Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., Madhani, H.D. and Rine, J. (2004) A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol*, **2**, E131.

Kokubo, T., Swanson, M.J., Nishikawa, J.I., Hinnebusch, A.G. and Nakatani, Y. (1998) The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA-binding protein. *Mol Cell Biol*, **18**, 1003-1012.

Kotani, T., Miyake, T., Tsukihashi, Y., Hinnebusch, A.G., Nakatani, Y., Kawaichi, M. and Kokubo, T. (1998) Identification of highly conserved amino-terminal segments of dTAFII230 and yTAFII145 that are functionally interchangeable for inhibiting TBP- DNA interactions in vitro and in promoting yeast cell growth in vivo. *J Biol Chem*, **273**, 32254-32264.

- Kou, H., Irvin, J.D., Huisinga, K.L., Mitra, M. and Pugh, B.F. (2003) Structural and functional analysis of mutations along the crystallographic dimer interface of the yeast TATA binding protein. *Mol Cell Biol*, **23**, 3186-3201.
- Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature*, **383**, 269-272.
- Kuras, L., Kosa, P., Mencia, M. and Struhl, K. (2000) TAF-Containing and TAF-independent forms of transcriptionally active TBP in vivo [In Process Citation]. *Science*, **288**, 1244-1248.
- Kuras, L. and Struhl, K. (1999) Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. *Nature*, **399**, 609-613.
- Kurdistani, S.K., Tavazoie, S. and Grunstein, M. (2004) Mapping global histone acetylation patterns to gene expression. *Cell*, **117**, 721-733.
- Ladurner, A.G., Inouye, C., Jain, R. and Tjian, R. (2003) Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Mol Cell*, **11**, 365-376.
- Lee, M. and Struhl, K. (2001) Multiple functions of the nonconserved N-terminal domain of yeast TATA-binding protein. *Genetics*, **158**, 87-93.
- Lee, T.I., Causton, H.C., Hostege, F.C., Shen, W.C., Hannett, N., Jennings, E.G., Winston, F., Green, M.R. and Young, R.A. (2000) Redundant roles for the TFIID and SAGA complexes in global transcription. *Nature*, **405**, 701-704.

- Lemaire, M., Xie, J., Meisterernst, M. and Collart, M.A. (2000) The NC2 repressor is dispensable in yeast mutated for the Sin4p component of the holoenzyme and plays roles similar to Mot1p in vivo. *Mol Microbiol*, **36**, 163-173.
- Leurent, C., Sanders, S., Ruhlmann, C., Mallouh, V., Weil, P.A., Kirschner, D.B., Tora, L. and Schultz, P. (2002) Mapping histone fold TAFs within yeast TFIID. *Embo J*, **21**, 3424-3433.
- Li, X.Y., Bhaumik, S.R. and Green, M.R. (2000) Distinct classes of yeast promoters revealed by differential TAF recruitment [In Process Citation]. *Science*, **288**, 1242-1244.
- Liu, D., Ishima, R., Tong, K.I., Bagby, S., Kokubo, T., Muhandiram, D.R., Kay, L.E., Nakatani, Y. and Ikura, M. (1998) Solution structure of a TBP-TAF(II)230 complex: protein mimicry of the minor groove surface of the TATA box unwound by TBP. *Cell*, **94**, 573-583.
- Lygerou, Z., Conesa, C., Lesage, P., Swanson, R.N., Ruet, A., Carlson, M., Sentenac, A. and Seraphin, B. (1994) The yeast BDF1 gene encodes a transcription factor involved in the expression of a broad class of genes including snRNAs. *Nucleic Acids Res*, **22**, 5332-5340.
- Matangkasombut, O., Auty, R. and Buratowski, S. (2004) Structure and function of the TFIID complex. *Adv Protein Chem*, **67**, 67-92.
- Matangkasombut, O., Buratowski, R.M., Swilling, N.W. and Buratowski, S. (2000) Bromodomain factor 1 corresponds to a missing piece of yeast TFIID [In Process Citation]. *Genes Dev*, **14**, 951-962.

- Matangkasombut, O. and Buratowski, S. (2003) Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. *Mol Cell*, **11**, 353-363.
- Matsui, T., Segall, J., Weil, P.A. and Roeder, R.G. (1980) Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. *J Biol Chem*, **255**, 11992-11996.
- Meisterernst, M. and Roeder, R.G. (1991) Family of proteins that interact with TFIID and regulate promoter activity. *Cell*, **67**, 557-567.
- Meisterernst, M., Roy, A.L., Lieu, H.M. and Roeder, R.G. (1991) Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. *Cell*, **66**, 981-993.
- Mencia, M., Moqtaderi, Z., Geisberg, J.V., Kuras, L. and Struhl, K. (2002) Activator-specific recruitment of TFIID and regulation of ribosomal protein genes in yeast. *Mol Cell*, **9**, 823-833.
- Mencia, M. and Struhl, K. (2001) Region of yeast TAF 130 required for TFIID to associate with promoters. *Mol Cell Biol*, **21**, 1145-1154.
- Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S. and Wu, C. (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science*, **303**, 343-348.
- Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y. and Allis, C.D. (1996) The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. *Cell*, **87**, 1261-1270.

- Moqtaderi, Z., Yale, J.D., Struhl, K. and Buratowski, S. (1996) Yeast homologues of higher eukaryotic TFIID subunits. *Proc Natl Acad Sci U S A*, **93**, 14654-14658.
- Muldrow, T.A., Campbell, A.M., Weil, P.A. and Auble, D.T. (1999) MOT1 can activate basal transcription in vitro by regulating the distribution of TATA binding protein between promoter and nonpromoter sites. *Mol Cell Biol*, **19**, 2835-2845.
- Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2002a) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. *Genes Dev*, **16**, 806-819.
- Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell*, **11**, 709-719.
- Ng, H.H., Xu, R.M., Zhang, Y. and Struhl, K. (2002b) Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J Biol Chem*, **277**, 34655-34657.
- Nikolov, D.B., Hu, S.H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N.H., Roeder, R.G. and Burley, S.K. (1992) Crystal structure of TFIID TATA-box binding protein. *Nature*, **360**, 40-46.
- Nowak, S.J. and Corces, V.G. (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet*, **20**, 214-220.
- O'Brien, T. and Tjian, R. (1998) Functional analysis of the human TAFII250 N-terminal kinase domain. *Mol Cell*, **1**, 905-911.

- Oelgeschlager, T., Chiang, C.M. and Roeder, R.G. (1996) Topology and reorganization of a human TFIID-promoter complex. *Nature*, **382**, 735-738.
- Onodera, Y., Haag, J.R., Ream, T., Nunes, P.C., Pontes, O. and Pikaard, C.S. (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell*, **120**, 613-622.
- Patikoglou, G.A., Kim, J.L., Sun, L., Yang, S.H., Kodadek, T. and Burley, S.K. (1999) TATA element recognition by the TATA box-binding protein has been conserved throughout evolution. *Genes Dev*, **13**, 3217-3230.
- Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R., 3rd and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol Cell Biol*, **22**, 8774-8786.
- Prelich, G. (1997) *Saccharomyces cerevisiae* BUR6 encodes a DRAP1/NC2alpha homolog that has both positive and negative roles in transcription in vivo. *Mol Cell Biol*, **17**, 2057-2065.
- Prelich, G. and Winston, F. (1993) Mutations That Suppress the Deletion of an Upstream Activating Sequence in Yeast - Involvement of a Protein Kinase and Histone-H3 in Repressing Transcription In vivo. *Genetics*, **135**, 665-676.
- Pugh, B.F. and Tjian, R. (1991) Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes & Development*, **5**, 1935-1945.
- Robert, F., Pokholok, D.K., Hannett, N.M., Rinaldi, N.J., Chandy, M., Rolfe, A., Workman, J.L., Gifford, D.K. and Young, R.A. (2004) Global position and recruitment of HATs and HDACs in the yeast genome. *Mol Cell*, **16**, 199-209.

- Roth, S.Y. and Allis, C.D. (1996) Histone acetylation and chromatin assembly: a single escort, multiple dances? *Cell*, **87**, 5-8.
- Ruzzi, M., Marconi, A., Saliola, M., Fabiani, L., Montebove, F. and Frontali, L. (1997) The sequence of a 8 kb segment on the right arm of yeast chromosome VII identifies four new open reading frames and the genes for yTAFII145. *Yeast*, **13**, 365-368.
- Sanders, S.L., Garbett, K.A. and Weil, P.A. (2002a) Molecular characterization of *Saccharomyces cerevisiae* TFIID. *Mol Cell Biol*, **22**, 6000-6013.
- Sanders, S.L., Jennings, J., Canutescu, A., Link, A.J. and Weil, P.A. (2002b) Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. *Mol Cell Biol*, **22**, 4723-4738.
- Sanders, S.L. and Weil, P.A. (2000) Identification of two novel TAF subunits of the yeast *saccharomyces cerevisiae* TFIID complex [In Process Citation]. *J Biol Chem*, **275**, 13895-13900.
- Sawa, C., Nedeá, E., Krogan, N., Wada, T., Handa, H., Greenblatt, J. and Buratowski, S. (2004) Bromodomain factor 1 (Bdf1) is phosphorylated by protein kinase CK2. *Mol Cell Biol*, **24**, 4734-4742.
- Schmidt, M.C., Kao, C.C., Pei, R. and Berk, A.J. (1989) Yeast TATA-box transcription factor gene. *Proc Natl Acad Sci U S A*, **86**, 7785-7789.
- Schultz, M.C., Reeder, R.H. and Hahn, S. (1992) Variants of the TATA-binding protein can distinguish subsets of RNA polymerase I, II, and III promoters. *Cell*, **69**, 697-702.

- Shen, W.C., Bhaumik, S.R., Causton, H.C., Simon, I., Zhu, X., Jennings, E.G., Wang, T.H., Young, R.A. and Green, M.R. (2003) Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly. *Embo J*, **22**, 3395-3402.
- Simchen, G., Winston, F., Styles, C.A. and Fink, G.R. (1984) Ty-mediated gene expression of the LYS2 and HIS4 genes of *Saccharomyces cerevisiae* is controlled by the same SPT genes. *Proc Natl Acad Sci U S A*, **81**, 2431-2434.
- Singh, M.V., Bland, C.E. and Weil, P.A. (2004) Molecular and genetic characterization of a Taf1p domain essential for yeast TFIID assembly. *Mol Cell Biol*, **24**, 4929-4942.
- Sterner, D.E., Belotserkovskaya, R. and Berger, S.L. (2002) SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. *Proc Natl Acad Sci U S A*, **99**, 11622-11627.
- Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L. and Berger, S.L. (1999) Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA- binding protein interaction. *Mol Cell Biol*, **19**, 86-98.
- Swanson, M.J., Qiu, H., Sumibcay, L., Krueger, A., Kim, S.J., Natarajan, K., Yoon, S. and Hinnebusch, A.G. (2003) A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. *Mol Cell Biol*, **23**, 2800-2820.

- Tora, L. (2002) A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. *Genes & Development*, **16**, 673-675.
- Verrijzer, C.P., Chen, J.L., Yokomori, K. and Tjian, R. (1995) Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. *Cell*, **81**, 1115-1125.
- Verrijzer, C.P., Yokomori, K., Chen, J.L. and Tjian, R. (1994) Drosophila TAF<sub>II</sub>150: similarity to yeast gene TSM-1 and specific binding to core promoter DNA. *Science*, **264**, 933-941.
- Wade, P.A. and Jaehning, J.A. (1996) Transcriptional corepression in vitro: a Mot1p-associated form of TATA-binding protein is required for repression by Leu3p. *Molecular & Cellular Biology*, **16**, 1641-1648.
- Walker, S.S., Reese, J.C., Apone, L.M. and Green, M.R. (1996) Transcription activation in cells lacking TAFIIS. *Nature*, **383**, 185-188.
- Weideman, C.A., Netter, R.C., Benjamin, L.R., McAllister, J.J., Schmiedekamp, L.A., Coleman, R.A. and Pugh, B.F. (1997) Dynamic interplay of TFIIA, TBP and TATA DNA. *J Mol Biol*, **271**, 61-75.
- Willy, P.J., Kobayashi, R. and Kadonaga, J.T. (2000) A basal transcription factor that activates or represses transcription. *Science*, **290**, 982-985.
- Winston, F., Dollard, C., Malone, E.A., Clare, J., Kapakos, J.G., Farabaugh, P. and Minehart, P.L. (1987) Three genes are required for trans-activation of Ty transcription in yeast. *Genetics*, **115**, 649-656.
- Wu, P.Y. and Winston, F. (2002) Analysis of Spt7 function in the *Saccharomyces cerevisiae* SAGA coactivator complex. *Mol Cell Biol*, **22**, 5367-5379.

- Wyrick, J.J., Holstege, F.C., Jennings, E.G., Causton, H.C., Shore, D., Grunstein, M., Lander, E.S. and Young, R.A. (1999) Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature*, **402**, 418-421.
- Xu, F., Zhang, K. and Grunstein, M. (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell*, **121**, 375-385.
- Yeung, K., Kim, S. and Reinberg, D. (1997) Functional dissection of a human Dr1-DRAP1 repressor complex. *Mol Cell Biol*, **17**, 36-45.
- Zhang, W., Bone, J.R., Edmondson, D.G., Turner, B.M. and Roth, S.Y. (1998) Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. *Embo J*, **17**, 3155-3167.

## Chapter 2

### Interaction of the Amino-Terminal Domain of yTAF1 and TBP

#### 2.1 Introduction

The general transcription complex TFIID is composed of 14 TAFs and TBP and regulates transcription of ~90% of the yeast genome (Huisinga and Pugh, 2004). Typically, the occupancy of TBP bound to the promoter of a gene upstream correlates with the level of transcription of that gene (Geisberg et al., 2002; Kuras et al., 2000). However genome-wide studies indicate this correlation is not universal (Zanton and Pugh, 2004). Enhancement of TBP binding through the action of activators and co-activator complexes increases the expression levels of reporter genes and those in their natural environment. TBP binds directly to DNA, and TAF1 increases the region bound both upstream and downstream of TBP. TBP/TFIID binding to DNA is stimulated by other factors such as TFIIA and TFIIB.

Studies on human TAF1 show a region at the N-terminus that interacts with TBP and prevents TBP-TATA association (Takada et al., 1992). Subsequent studies demonstrate that TAF1 homologues in flies and yeast also contain the TBP binding region (Kokubo et al., 1993; Kokubo et al., 1998; Kokubo et al., 1994; Kotani et al., 1998; Nishikawa et al., 1997). This region was termed the TAND or TAF1 N-terminal domain. Binding of TAND to TBP is competitive with TBP-DNA and TBP-TFIIA association, which both promote transcription (Kokubo et al., 1998). While the other

TAFs in TFIID are TBP-associated factors, their interaction is mainly mediated through TAF1; although few TAFs interact weakly with TBP (Leurent et al., 2002; Leurent et al., 2004; Yatherajam et al., 2003). The TAND domain therefore links TBP, TAF1 and TFIID.

The residues in yeast TBP that mediate the interaction with TAF1 and how TBP-TAF1 interactions regulate transcription were unknown. Previous studies in the Pugh lab isolated mutant derivatives of TBP that increase transcription *in vivo*. We sought to address if these mutants had impaired interactions with the repressive TAND domain. Answers to these questions would lead to a greater understanding of the molecular interactions inside of TFIID and the physiological importance of these interactions for living cells.

## **2.2 Identification of TAF1 N-Terminal Domain (TAND) and Association with TBP**

The TAND (TAF1 N-terminal domain) was formally identified as a region (residues 18-77) of *Drosophila melanogaster* (dm) TAF1 (formerly TAF230) that could interact with TBP (Kokubo et al., 1994). This binding of TAND to TBP is antagonistic to TBP-TATA interaction. A second, highly conserved region that increased affinity of TAND for TBP was later identified and named dTANDII (aa 118-143) (Nishikawa et al., 1997). Database searching indicates that TANDII is conserved between flies and yeast. However, in yeast, TAF1 is considerably smaller than *Drosophila*, and yeast TAND (I and II) only comprises amino acids 10-76 (Kokubo et al., 1998; Kotani et al., 1998). While smaller than the *Drosophila* counterpart, y TAND I and II could stably bind TBP.

Recently a third, novel region of yeast TAF1 (residues 82-139) was also found to bind TBP (Takahata et al., 2003). Other biochemical studies such as fluorescence anisotropy, co-immunoprecipitation and NMR spectroscopy, further demonstrate the existence of TAF1-TBP interactions (Bai et al., 1997; Banik et al., 2001; Kobayashi et al., 2001; Kokubo et al., 1998; Kokubo et al., 1994; Kotani et al., 2000; Kotani et al., 1998; Liu et al., 1998; Mencia and Struhl, 2001; Takahata et al., 2003).

Genetic evidence indicates a link between the TAND domain and TBP. The general transcription factor TFIIA helps increase the rate and stabilize the binding of TBP (TFIID) to DNA (Coleman et al., 1999b; Weideman et al., 1997). TAND-TBP binding is competitive with TFIIA-TBP binding *in vitro*, indicating a negative regulation of TBP (Kokubo et al., 1998). The TAND domain is dispensable for yeast growth at 25 °C, but at 37 °C it becomes essential (Kotani et al., 1998). Overexpression of the subunits of TFIIA (Toa1, Toa2) or TBP could suppress the temperature sensitive phenotype in  $\Delta$ TAND strains. The genetic evidence indicates that TFIIA positively regulates TBP association with DNA and that the TAND domain operates negatively.

The ability of TAND to operate on TBP in a negative manner independent of TAF1 is demonstrated by fusing the TAND domain to TAF5 or TAF11 in a  $\Delta$ TAND temperature sensitive strain and monitoring growth at the permissive temperature (Takahata et al., 2004). Transcription of TAF-dependent genes and growth at 37 °C were not disrupted in strains deleted for the TAND domain and having TAND expressed on the C-terminus of TAF1. Fusion of the TAND domain to the N-terminus of TAF11, or either the N or C-terminus of TAF5 restores transcription, showing that TAND is important for transcription and that TAND can operate autonomously (Takahata et al.,

2004). Placing the TAND domain at the C-terminus of TAF11 can not recapitulate the WT transcription levels or growth, due to compromised TAF11-TFIID interactions.

Chimeric TAND proteins constructed from yeast and *Drosophila* TAF1 are functionally interchangeable *in vitro*, but yeast and *Drosophila* TAND domains can not be swapped *in vivo* (Kotani et al., 1998). The discrepancy between *in vitro* and *in vivo* results may be explained by the relative locations and size differences between the yeast and *Drosophila* TAND I and TAND II domains. The *Drosophila* TAND is larger than yeast, and the two dTAND domains are spaced further apart than their corresponding yeast domains. However, even if a spacer region is included in the chimeras, the dTAND does not support viability in yeast (Kotani et al., 1998).

### **2.3 TAND Homology**

Sequence alignment of the homologous yeast and *Drosophila* TAF1 N-terminal regions illustrates several key differences between the two proteins (Figure 2-2). The N-termini of the yeast and *Drosophila* TAF1 homologues are poorly conserved (11% identity) (Mal et al., 2004). However, the TAND domains are highly similar and contain a high representation of negatively charged amino acids (Kotani et al., 1998; Mal et al., 2004). Yeast TAND I and II (residues 10-71) is 36% negatively charged and dTAND I (11-77) contains 22% acidic amino acids. The acidic nature of the TAND I domain and the high degree of positively charged residues in TBP contributes to the ionic interaction between TAND and TBP (see below and Figure 2-2).

Figure 2-1

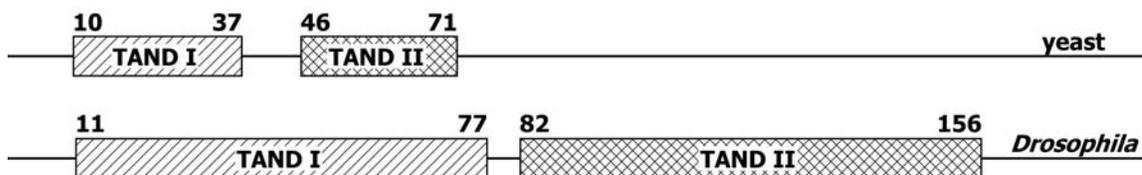


Figure 2-1: Schematic of yeast and Drosophila TAND I and II domains.

Figure 2-2

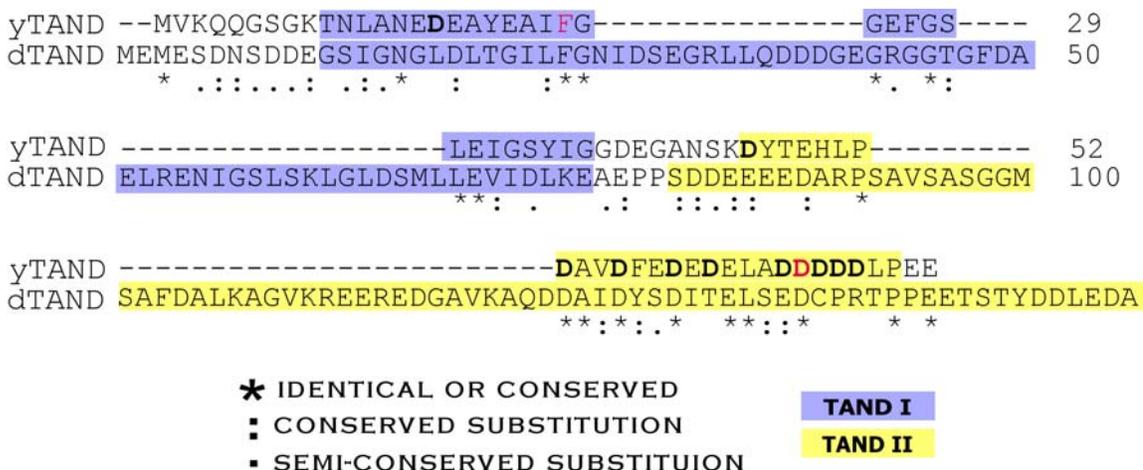


Figure 2-2: Sequence homology of the TAND domain.

Alignment of yeast (upper row) and Drosophila (bottom row) TAND regions. Acidic residues in yeast TAND are in bold. Yeast residues F23 and D66 are colored red. Blue and yellow boxes bracket the defined TAND I and TAND II regions, respectively. Alignment performed with ClustalW (Thompson et al., 1994).

## 2.4 Structure of TBP

The crystal structure of the evolutionarily conserved 179 C-terminal amino acids (“core”) of TBP was solved by separate groups in 1993 and 1996, demonstrating that

TBP crystallized as a homodimer (Chasman et al., 1993; DeDecker et al., 1996; Nikolov et al., 1992). The DNA binding surface of TBP, as determined by crystallography (see Figure 2-5) (Kim et al., 1993a; Kim et al., 1993b; Nikolov et al., 1996), is occluded by the arrangement of the two TBP monomers. TBP resembles a saddle, with a concave region (the DNA binding surface) and two stirrups formed in part by strands of an anti-parallel B-sheet (Nikolov et al., 1992). The C-terminal core domain of TBP is almost perfectly symmetrical with a pseudodyad running through the concave surface (see Figure 2-3). Several independent crystal structures of TBP from different species in different complexes show remarkable structural homogeneity, indicating a precise structure-function relationship (Geiger et al., 1996; Kim et al., 1993b; Nikolov et al., 1995; Patikoglou et al., 1999; Tan et al., 1996). The TBP protein is very basic (15%), which might aid interaction with acidic transcription factors such as activators or the TAND domain.

Previous studies in the Pugh laboratory focused on the *in vitro* and *in vivo* biochemical properties of TBP and interaction with other transcription factors. From these earlier studies several important conclusions were made. First, both human and yeast TBP self associate with a slow disassociation rate (Coleman and Pugh, 1997; Jackson-Fisher et al., 1999a; Taggart and Pugh, 1996; Weideman et al., 1997). This activity was demonstrated with a variety of *in vitro* experiments such as pulldown assays, gel filtration, TATA binding (filter binding assays), and ultracentrifugation. *In vivo* crosslinking and *B-galactosidase* reporter gene assays support these conclusions (Coleman et al., 1995; Coleman et al., 1999a; Daugherty et al., 1999; Daugherty et al., 2000; Jackson-Fisher et al., 1999a; Jackson-Fisher et al., 1999b; Taggart and Pugh,

1996). TBP dimer dissociation is the rate limiting step for transcription initiation (Coleman and Pugh, 1997). TFIIA stabilizes TBP on DNA and promotes the dissociation of TBP dimers (Coleman et al., 1999a; Weideman et al., 1997).

To further characterize TBP biochemical properties, amino acids located within 4 angstroms of other residues along the dimerization interface (see Figure 2-3) were targeted for individual site-directed mutagenesis (Jackson-Fisher et al., 1999b; Kou et al., 2003). To test the physiological relevance of TBP dimerization, engineered mutations in TBP were created, based on the crystallographic structure, to potentially disrupt TBP self-association. A class of TBP mutants displaying decreased dimerization and elevated basal (non-activated) transcription are termed TBP<sup>EB</sup> mutants (Chitikila et al., 2002; Jackson-Fisher et al., 1999b). Using *B-galactosidase* activity assay and the *lacZ* reporter gene, a direct relationship between TBP dimer instability and increased transcriptional rate is observed, (Jackson-Fisher et al., 1999b). The most unstable TBP mutants are degraded rapidly and have the most powerful phenotypic effects, such as inability to support viability and toxicity (Jackson-Fisher et al., 1999b; Kou et al., 2003). However, since TBP binds a number of transcription factors, defects in these interactions could explain the increase in transcription displayed in the TBP<sup>EB</sup> mutants. Biochemical and genetic studies performed by several other research groups indicates TBP interacts with several factors, NC2, MOT1, Spt3 (SAGA), activators, and the N-terminus of TAF1 (TFIID) (reviewed in (Burley and Roeder, 1996; Struhl, 1995)).

Figure 2-3

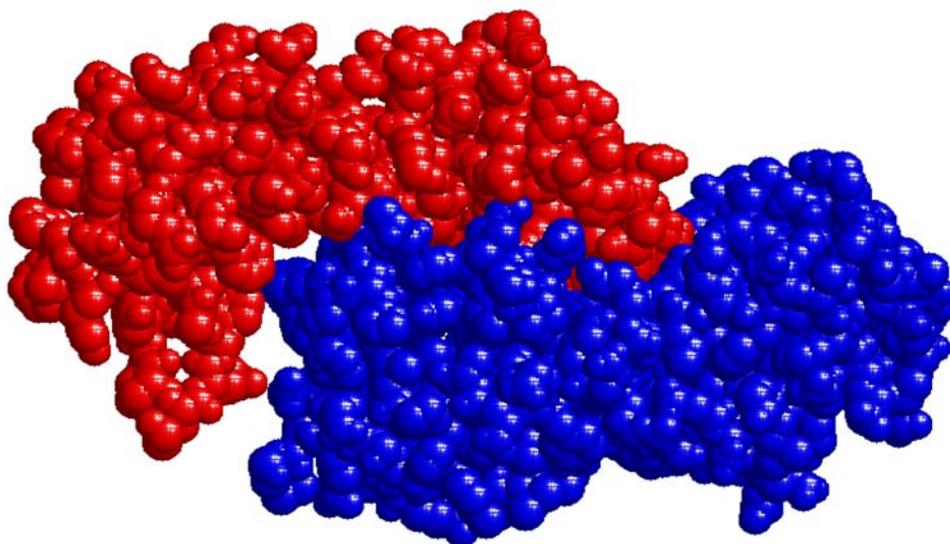


Figure 2-3: Crystal structure of yeast TBP.

Conserved 179 Carboxyl-terminal amino acids of yeast TBP (core) crystallizes as a dimer (Chasman et al., 1993). Spacefill model showing one TBP monomer in red and a second monomer in blue.

Figure 2.4



Figure 2.4: Ribbon structure of yTBP.

179 C-terminal core residues from TBP crystal structure (Chasman et al., 1993) displayed as ribbons. Alpha helices H1, H1', H2 and H2' are indicated. Strands (from B-sheet) S2 and S2' form the N-terminal and C-terminal stirrups, respectively. Pseudodyad symmetry such that the left and right sides of TBP are nearly symmetrical.

Figure 2-5

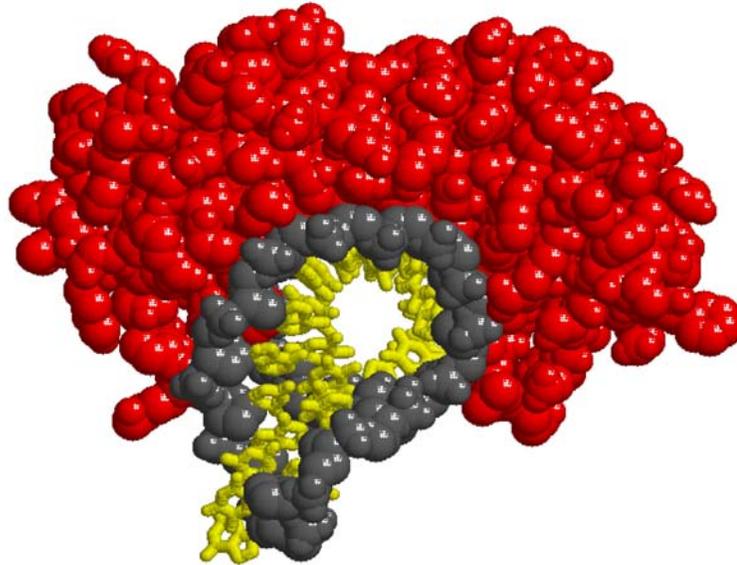


Figure 2-5: Crystal structure of yTBP-TATA DNA complex.

Crystal structure of yTBP-TATA DNA complex (Kim et al., 1993b). Spacefilling model of yTBP shown in red. Spacefilling representation of DNA backbone shown in black and nucleotides shown in yellow wireframe.

## 2.5 TAND Structure Mimics the TATA Box

The nuclear magnetic resonance (NMR) structure of *Drosophila* TAND (residues 11-77) complexed with conserved core of yeast TBP (residues 49-240) was solved (Liu et al., 1998). Comparison of the TAND-TBP solution structure to the TBP-TATA DNA crystal structure illustrates that TAND adopts a very similar configuration to the partially unwound DNA in the TATA box upon TBP binding (Liu et al., 1998). In the TBP-TATA crystal structure, the eight nucleotides of the minor groove of the DNA double helix are in contact with TBP. TBP binding to the DNA induces nearly a 90-degree bend and partially unwinds the DNA (Chasman et al., 1993; DeDecker et al., 1996; Flatters et

al., 1997; Kim and Burley, 1994; Kim et al., 1993a; Kim et al., 1993b; Nikolov et al., 1996; Nikolov et al., 1995; Tan et al., 1996). While the interaction between the TBP and TAND is largely hydrophobic, electrostatic interactions are important for positioning the TBP and TAND molecules (Liu et al., 1998). The basic TBP residues (R98, R105, K120, K211, K218) that make electrostatic interactions with the DNA backbone are the same residues involved in electrostatic contacts with TAND. Importantly, the TAND domain adopts a curved structure similar to the slightly unwound conformation displayed in TATA DNA upon TBP binding (Kim et al., 1993a; Kim et al., 1993b; Liu et al., 1998). The eight base region of TATA DNA are mimicked by TAND residues D29, E31, E51, E70, D73.

Mutagenesis of several TAND residues results in a decreased ability to bind TBP, specifically at *Drosophila* residue F25 (yeast F23) and yeast D66 (see below) (Kokubo et al., 1998; Kokubo et al., 1994; Nishikawa et al., 1997). F25 is in the central portion of the TBP-TAND NMR structure that makes obvious contacts with TBP. The homologous residue of yeast D66 is D138 in *Drosophila*, which is outside of the peptide used in the structural determination.

Before the NMR solution structure was solved, it was thought that the separable domains of yTAND (I, II, and later, III) interacted with the concave and convex surfaces of yTBP respectively (Kotani et al., 1998). TAND I was thought to bind to the N-terminal half of yTBP and stirrup #1. In the NMR, structure the largely unstructured loop between dTAND I and II protrudes outside of the concave surface of yTBP. After formation of the loop, the backbone returns to the underside of TBP (Figure 2-6). This structure only shows TAND I, and does not indicate where TAND II would bind to TBP

(i.e. it does not show the TAND backbone leading towards the convex surface of TBP, as has been proposed for TAND binding).

Figure 2-6

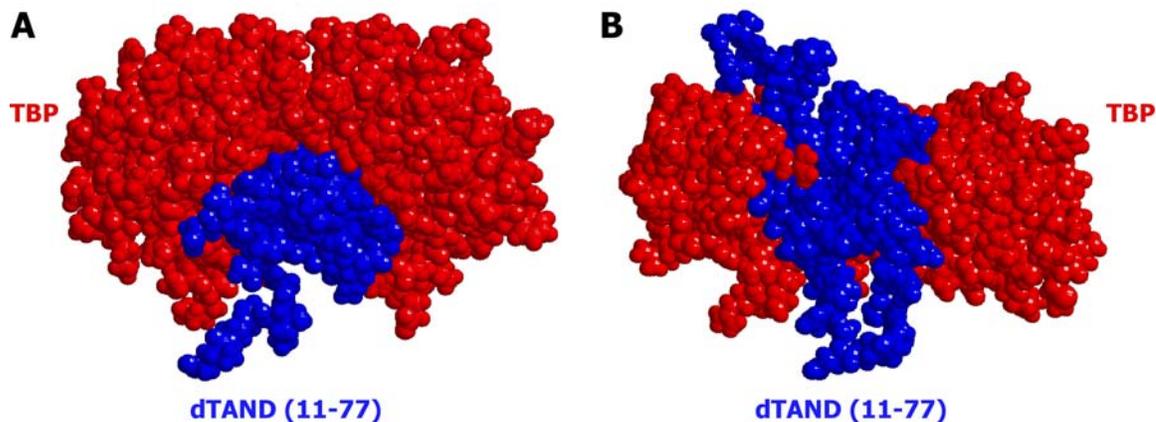


Figure 2-6: Solution structure of dTAND and yTBP.

A. Spacefilling model of the nuclear magnetic resonance (NMR) solution structure of *Drosophila* TAND (residues 11-77, blue) and yTBP (residues 49-240, red). B. Model was rotated  $\sim 90$  degrees from A; view is looking into concave surface of TBP.

## 2.6 Mutagenesis of TBP and effects on TAND binding

A mutation to the concave surface of TBP (L114K) destabilizes the binding of TBP with the acidic viral activator, VP16 (Nishikawa et al., 1997). Nearby residues, E93, F99, I103, F116, and V122 were found to be critical for TAND-TBP interaction in *Drosophila* (Nishikawa et al., 1997). It is postulated that the TAND domain prevents binding of activators to TBP through competition with the concave surface. This mechanism of competitive binding would dampen transcription by limiting TBP-activator and TBP-DNA access. A model for this interaction involves two distinct steps. First, the TAND domain binds TBP and prevents spurious TBP-DNA interactions. When acidic activators are present, they compete for the binding by the TAND domain. The activators bring TBP/TFIID to promoter DNA via interactions with a DNA binding domain. This is

called a two-step handoff, since TBP was passed from TAND to an activator and then to DNA. The biological relevance of this model had not been tested in an *in vivo* situation.

Previous work in the Pugh laboratory on TBP identifies six mutations that destabilize TBP dimers *in vitro*, increase transcription from a basal promoter, and lead to increased TBP degradation. These mutations can not support viability on their own, and their expression is toxic to yeast cells (Jackson-Fisher et al., 1999b). TBP dimerization plays an important biological role in regulation of TBP. Mutations that destabilize TBP dimers increase transcription rates (Jackson-Fisher et al., 1999b). TBP dimerization prevents TBP-DNA access and is in competition with several other TBP interactions, such as TFIIA (Jackson-Fisher et al., 1999b). It is possible that loss of other interactions (i.e. NC2 or MOT1), and not dimerization is responsible for the increased transcription, toxicity and protein instability of the TBP mutants.

To address the potential that the TBP mutants increase transcription due to loss of interaction with the repressive TAND domain, I directly measured the strength of TAND-TBP interactions *in vitro* via GST-pulldown assays. Yeast strains with a deletion of the TAND domain ( $\Delta$ TAND) or a TBP mutant defective for NC2 interactions (F182V) (Cang et al., 1999; Geisberg et al., 2001; Kamada et al., 2001; Lee et al., 1998; Prelich, 1997) were used to measure genome-wide expression by Carmelata Chitikila and Kathryn Huisinga. Haiping Kou performed the TBP toxicity, TBP-TATA affinity, and *in vivo* transcription experiments. The results of these studies were published previously in two journals and are partially reprinted here, with permission.

“Interplay of TBP inhibitors in global transcriptional control.” Carmelata Chitikila, Kathryn L. Huisinga, Jordan D. Irvin, Andrew D. Basehoar and B. Franklin Pugh. *Mol Cell*. 2002 Oct;10(4):871-82.

“Structural and functional analysis of mutations along the crystallographic dimer interface of the yeast TATA binding protein.” Haiping Kou, Jordan D. Irvin, Kathryn L. Huisinga, Madhusmitra Mitra, and B. Franklin Pugh. *Mol Cell Biol*. 2003 May;23(9):3186-201.

## 2.7 Results

The important question of the physiological relevance of TBP-TAND interaction and its role in transcriptional repression is critical to our understanding of TAF1's role in regulation of TBP. A caveat to any biochemical study on mutant proteins is they are still conformationally functional. Two separate indications of the functionality of these TBP mutants are shown in Table 2-1. First, these TBP mutants still are able to bind to TATA-DNA as measured in mobility shift experiments. A range of affinity for TATA DNA is expected based on the mutation made and the TBP-DNA crystal structure (Chitikila et al., 2002; Jackson-Fisher et al., 1999b; Kou et al., 2003). Secondly, many of these TBP mutants increase transcription from a reporter gene, indicating that, *in vivo*, these proteins are still active. Many of the TBP mutants are expressed at a low steady-state level *in vivo*, and therefore have very high specific activity, indicating they are functional *in vivo*.

To test the interaction of the TBP mutants with the yTAND domain, glutathione S-transferase (GST) fusion proteins of yTAF1 (10-88) were created. Two yeast residues

(F23, D66) were previously shown to be important for TAND interactions (Kokubo et al., 1998), and a plasmid encoding a double point mutation in the TAND domain (F23K, D66K) was generously provided by T. Kokubo (Yokohama City University, Yokohama, Japan).

A schematic of the proteins (and mutations) in the GST pulldowns is shown in Figure 2-7. GST-yTAND (WT, F23K, or F23K/D66K) was incubated with WT or 28 TBP single amino acid substitution mutants (mutation denoted with “x”). The majority of the TBP mutations are in the concave surface, but K133E is on the convex surface, where TANDII was proposed to interact. These mutants were bound to three GST-yTAND (residues 10-88) derivatives: WT, D66K, and F23K/D66K, which destabilizes the interaction (Kokubo et al., 1998) and served as a negative control. GST-TAND fusion proteins and interacting TBP were purified using glutathione-agarose (GTA) resin, which binds GST. Figure 2-8 shows GST-pulldowns of yTAND (10-88) and yTBP, and results of the interaction between the TBP mutants and WT TAND are quantitated in Figure 2-9.

We find that WT yTBP associates with WT yTAND, as expected. The D66K mutation in the TANDII domain weakens the interaction, supporting the notion that TANDII makes contacts that strengthen the binding to TBP. The double mutant F23K/D66K greatly destabilizes the binding to WT TBP (Figure 2-8).

Replacement of the original side chains with bulky, charged residues impaired the ability of TBP and TAND to associate. Since the interaction between the acidic TAND domain and the basic TBP concave surface is primarily ionic (Irvin, ; Kokubo et al., 1998) it was expected that mutation of TBP residues to glutamic acid (E) would disrupt interaction with the acidic TAND domain. Likewise, replacement of TAND residues

with the positively charged lysine (i.e. F23K and D66K) was expected to weaken interaction with TBP.

Figure 2-7

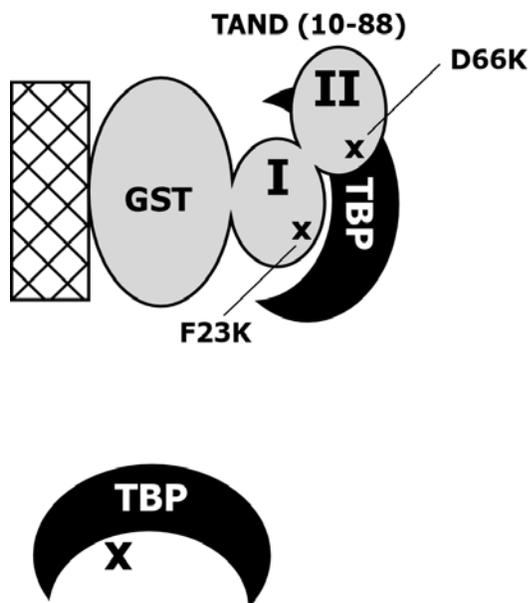


Figure 2-7: Schematic of GST pulldowns.

GST was fused in-frame to the  $\gamma$ TAND (residues 10-88, domains (I, II)). The mutation F23K is located in TAND I, and D66K is in TAND II (mutations denoted by "x"). Mutations to TBP are mainly in the concave surface. GST-TAND fusion proteins and associated TBP were purified using GTA resin (hatched).

Figure 2-8

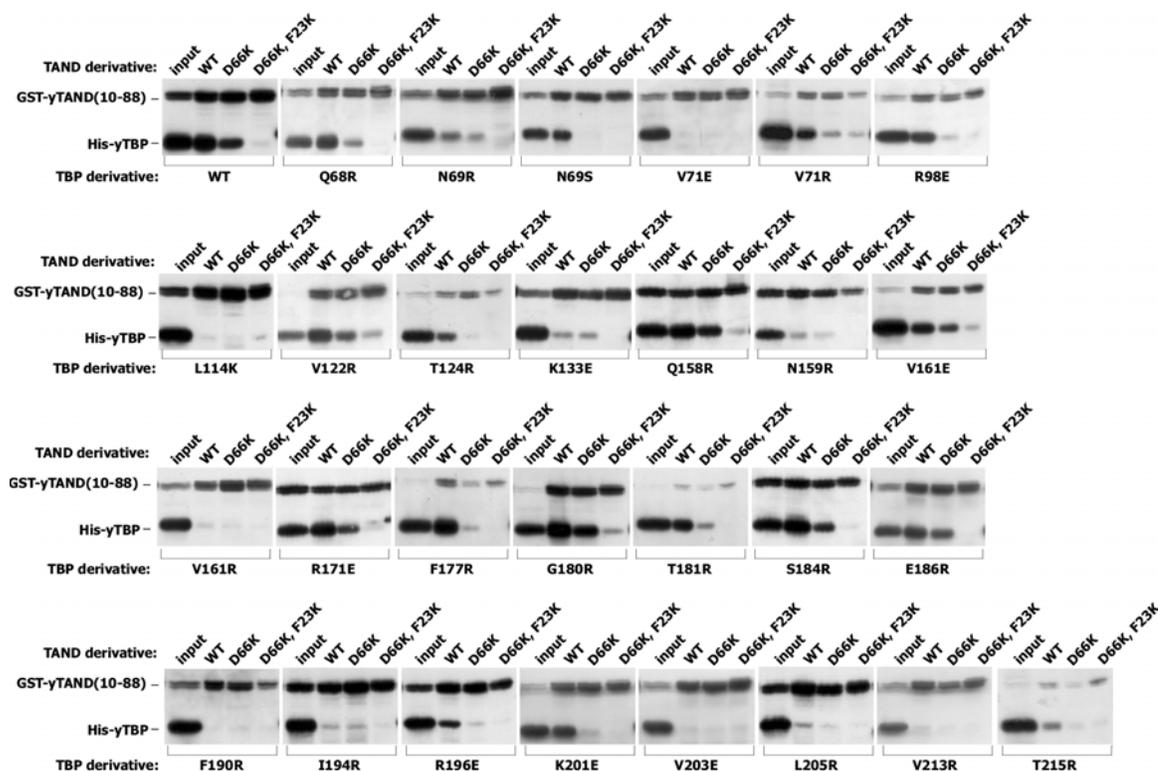


Figure 2-8: Western blots of GST pulldown of yTAND (10-88) and TBP mutants.

Three derivatives of the TAND domain were used, WT, the single mutant in TAND II (D66K), or the double mutant (F23K, D66K), and are labeled across the top. Mutant TBPs are listed across the bottom. Five percent (5%) of the reaction was loaded in the first lane of each panel (“input”), and 50% of the pulldown loaded in subsequent lanes. Proteins were boiled in protein sample buffer and run on 10% SDS-PAGE, transferred to nitrocellulose and immunoblotted with anti-TBP (rabbit), anti-GST-HRP, and anti-rabbit-HRP IgG (goat) antibodies. The upper band shows the ~35 KDa GST-TAND construct and the lower band the ~28 KDa his-yTBP, as indicated on the left.

The results of the *in vitro* pulldown results are presented in Figure 2-8, which is arranged by TBP residue. After performing *in vitro* pulldowns and quantifying the immunoblots, we classified these results into three major groups: residues with near WT binding (>75%), those with a moderate level of binding (~25-75%), and those with

severely disrupted interactions (<25%). The quantitation of the pulldown is color coded blue, yellow, and red, respectively, graphed in Figure **2-9** and plotted on the crystal structure of TBP in Figure **2-10**.

Two separate mutations to V161 (V161E, V161R) reduced the binding to TAND (WT). However, not all replacements at a particular residue affect the interactions similarly. Particular TBP substitutions displayed near WT level of binding (e.g. N69S, V71R), while others at these same residues (N69R, V71E) weakened the binding to <20% of the WT binding.

The TBP mutation L114K disrupts the interaction between *Drosophila* TAND and yTBP (Nishikawa et al., 1997) and L114K does not interact with yTAND in our system. L114K is the most N-terminal residue in the concave surface that abolishes interaction with the TAND domain, indicating a potential limit of interaction. Other than K133E, which is proposed to interact with yTAF1 (Kokubo et al., 1998; Kotani et al., 1998), the residues outside of the concave saddle have less of an impact on the pulldown (see residues in blue, Figure **2-10**).

A number of the hydrophobic TBP residues that interact with dTAND as determined by NMR structure, are replaced here (V122R, T124R, V161R, I194R, L205R, V213R, T215R, F190R). These residues cluster to the C-terminal side of the concave surface. These mutations add a bulky, positively charged arginine, which could potentially disrupt the hydrophobic interaction reported between dTAND(11-77) and yTBP (Liu et al., 1998). As displayed in Figure **2-9** and Figure **2-10**, these mutations display the weakest interactions, and all cluster to the underside of yTBP. These results are partially in agreement with the published statement that F190K, I194K, V205K, and

V213K show no detectable binding to dTAF230 (2-81) (Nishikawa et al., 1997), indicating that yeast and *Drosophila* TAND occupy a similar space under TBP.

Figure 2-9

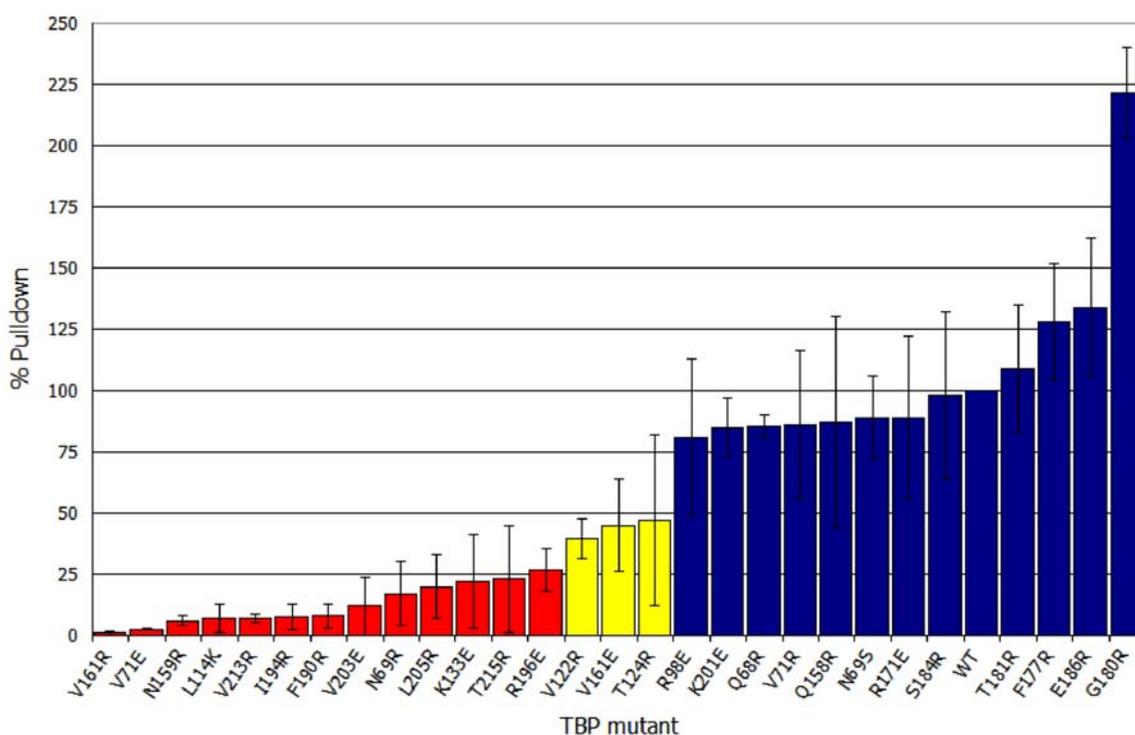


Figure 2-9: Quantitation of interaction between yTBP and WT yTAND.

Immunoblots were scanned into Densitometer (Molecular Dynamics) and quantitated. WT TBP pulldown was set to 100%. TBP mutants with pulldown < ~25% were colored red. Pulldown between 25-75% of WT were colored yellow. Pulldown >75% of WT were colored in blue.

Figure 2-10

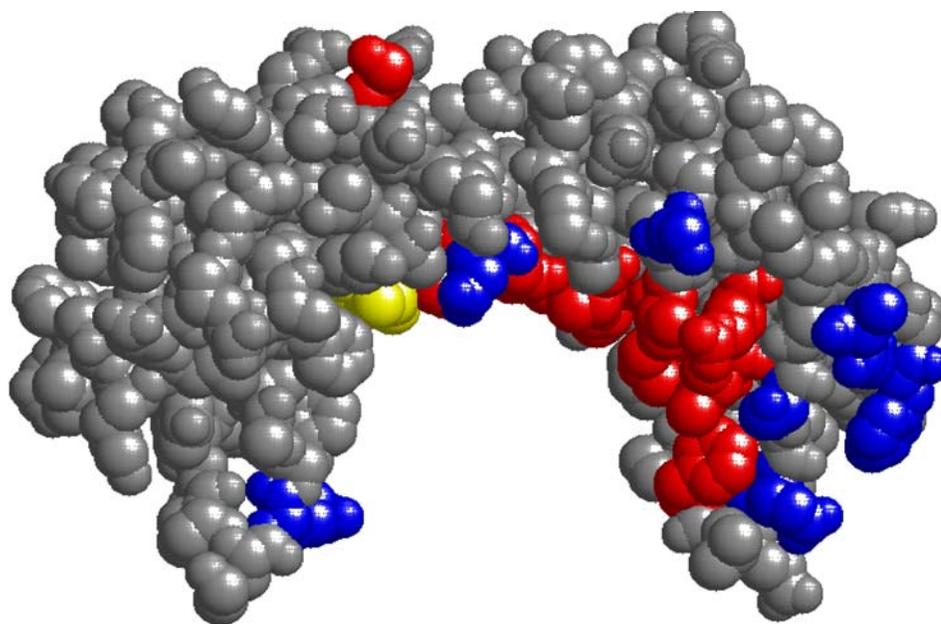


Figure 2-10: TBP residues that interact with yTAND domain.

Yeast TBP residues that are critical for interaction (<25% of WT pulldown) with GST-yTAND (10-88) (WT) are colored in red. Pulldown values between 25-75% of WT (3 residues) are shown in yellow. Residues with ~ WT or greater levels of binding are shown in blue.

Table 2-1

Table 2-1: Properties of TBP mutants.

TBP mutant	Dimer Stability	TBP-TAND Stability	TBP-TATA Stability	$\beta$ -Gal
WT	100	100	100	1
Q68R	58	90	100	5
N69R	9	20	20	190
V71R	18	90	5	81
R98E	23	80	50	1
L114K	41	10	5	7
V122R	23	40	10	44
T124R	25	50	10	31
Q158R	37	90	100	8
N159R	14	5	10	120
V161R	8	5	5	150
R171E	34	90	100	1
F177R	83	130	100	1
G180R	66	220	100	4
T181R	61	110	100	1
S184R	58	100	100	3
E186R	66	130	100	1
F190R	6	5	5	3
I194R	10	5	10	2
R196E	4	30	5	4
K201E	23	90	10	3
V203E	10	10	10	6
L205R	5	20	10	3
V213R	5	10	10	110
T215R	9	20	20	23

WT values of TBP dimer, TBP-TATA and TBP-TAND stability are 100%. Relative fold  $\beta$ -galactosidase activity, where WT = 1.

## 2.8 Discussion

At the time these studies were completed, the results were the most detailed assessment of *in vitro* TBP-TAND interactions and *in vivo* implications of TAND regulation of TBP. The conclusion that TAND makes contacts throughout the underside

of TBP was unexpected. While the NMR structure of dTAND shows potential contact points, the smaller yTAND domain tested (aa 10-88) was not predicted to fill the TBP concave pocket. Potentially, yTAND does not protrude outward from TBP to the extent that dTAND does (Figure 2-6). Alternatively, if yTAND does not adopt a largely folded shape (is mainly linear) yTAND could “trace” the underside of yTBP.

The biological significance of the broad occupancy of TAND in the concave region of TBP is that yTAND(10-88) could prevent other proteins (activators, NC2, MOT1, TFIIA, TFIIB, TBP) and DNA from making necessary *in vivo* relationships with TBP. Preventing access to the concave surface of TBP for factors that increase transcription (DNA, TFIIB, TFIIA) or repress transcription (TBP, NC2, MOT1) by binding broadly to a common surface would be a mechanism where the TAND domain could exert both positive and negative effects.

Genomic expression patterns from  $\Delta$ TAND mutants show the TAND domain has minor contributions both positively and negatively on transcription (Chitikila et al., 2002). Additionally, TBP mutants display widespread effects in a  $\Delta$ TAND strain, indicating that loss of interactions with the TAND domain is not solely responsible for changes in gene expression (Chitikila et al., 2002). Synthetic toxicity of  $\Delta$ TAND with TBP mutants and the expression studies indicate that TAND does play a minor inhibitory role, but the effects are largely masked by other inhibitors of transcription (Chitikila et al., 2002; Kou et al., 2003).

A few of the replacement mutations caused an increase in binding of TBP to the TAND domain, (S184R, T181R, F177R, E186R, G180R). The increased TBP-TAND affinity *in vitro* could be due to a slight conformational change induced in TBP by the

addition of arginine at each of these positions. Additionally, since arginine is a positively charged amino acid and the TAND domain is largely acidic, the increase could be due to strengthened physiological contacts. Since these residues lie outside of the region of TBP proposed to interact with TAND from NMR studies Figure **2-10**, and display modest decreases in TBP dimerization Table **2-1**, it is possible these mutations promote TBP dimer dissociation without contributing negatively to TBP-TAND interaction. In this manner, these TBP mutants would increase the pool of TBP monomers for binding to the TAND domain.

After our studies had been completed (Chitikila et al., 2002; Kou et al., 2003), a NMR structure of the yeast TAND domain was solved (Mal et al., 2004). Since structural information is not available, a depiction of the yTAND could not be generated. However, shown in Table **2-1** are nine residues where mutations cause a wide range of TBP-TAND interactions. The positions of these residues shift in NMR spectrum after binding to TAND. Six of the nine residues (N69, V71, L114, N159, F190, L205), when mutated, display a decrease in binding. The correlation between the residues which decrease in binding in our mutants and show interaction in NMR indicates our results provide high resolution of the interaction between TBP and TAND.

There are several possibilities why other mutated residues (i.e. Q68R, Q158R) did not decrease the pulldown but did shift the NMR signature. The amino acid substitution might not be sterically positioned to disrupt the TBP-TAND interaction or the change in amino acid might not cause disrupt an interaction, where a different substitution (see V71E and V71R in Table **2-1**) at the same position could. We also used a larger portion of yTAF1 (10-88) than was used in NMR studies (residues 10-73), which could stabilize

weak disruptions. Lastly, the conditions used in each study were quite different for technical reasons. There were mutated residues that affect the  $\gamma$ TBP- $\gamma$ TAND pulldown (i.e. V161R, N69R), but the WT residue shows no change after binding in the NMR studies. The reasons for this are unclear, but are probably due to the amino acid substitution repositioning of nearby side chains creating a local disruption, which affects the pulldown.

Table 2-2

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Table 2-2: GST-TAND pulldown value (%) of TBP residues shifted in NMR studies<sup>2</sup>.

Q68R	85
N69R	17
N69S	89
V71E	3
V71R	86
L114K	7
V122R	40
Q158R	87
N159R	6
F190R	8
L205R	20
WT	100

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## 2.9 Materials and Methods

### 2.9.1 Subcloning of F23K and F23K/D66K Mutations

A plasmid containing the F23K/D66K double mutation in the TAND domain and a plasmid containing the WT TAND domain (pYN1) were gifts of T. Kokubo (Yokohama City University, Japan). Oligonucleotides containing restriction sites were synthesized (Gibco) to PCR amplify the region for amino acids 10-88. For the upstream primer, a silent amino acid change (changing codon GCA to GCG to preserve an alanine

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<sup>2</sup> Mal, T.K., Masutomi, Y., Zheng, L., Nakata, Y., Ohta, H., Nakatani, Y., Kokubo, T. and Ikura, M. (2004) Structural and functional characterization on the interaction of yeast TFIID subunit TAF1 with TATA-binding protein. *J Mol Biol*, **339**, 681-693.

at residue 18 in TAND domain) was necessary to knock out an existing NdeI site. PCR conditions used 1X Gene Choice reaction buffer, 0.2 mM dNTPs, 0.1 uM primers, 0.025 U gene choice polymerase, 0.0002 U Pfu and 30 cycles of 1 minute at 45 °C, 1.5 minutes at 72 °C, 1 minute at 95 °C and ~10ng of plasmid DNA as a template for the reaction. PCR products were subcloned in frame into NdeI (5') and EcoRI (3') sites of digested, phosphatased, and gel purified pGEX-yTBP(181C) plasmid to produce GST fusions of TAND WT, F23K, or F23K/D66K. pGEX-yTBP(181C) was derived from pGEX-GST-yTBP, which was constructed from pGEX-1λT. Subcloning was confirmed with restriction digestion and DNA sequencing (performed at the Penn State University Nucleic Acid Facility).

### **2.9.1 GST-TAND purification**

*E. coli* (DE3) cells were grown in YT media (1% tryptone, 1% yeast extract, 0.5% NaCl) containing 0.8% glucose and 400 ug/mL ampicillin to optical density (OD<sub>595</sub>) of ~0.8 at 37 °C. Isopropylthio-β-D-galactoside (IPTG) was added to 0.1 mg/mL and cells were cultured at 30 °C for 2 hrs. Cells were harvested by centrifugation (Sorvall GS3 at 5,000 rpm for 5 min, 4 °C), and resuspend in 15 mL lysis buffer (25 mM HEPES, 200 mM potassium chloride, 12.5 mM magnesium chloride, 10% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, and 1% v/v protease inhibitor cocktail (Sigma)). Cells were lysed by addition of 0.67 mg/mL lysozyme (Sigma), 0.067% IGEPAL CA-630 (Sigma), and 100 mM potassium chloride before two sessions of sonication (30 seconds each) with a Branson Sonifier 450 set to maximum. Cellular extracts were centrifuged at

15,000 rpm (Sorval RC5C, SS34) for 15 minutes at 4 °C. Soluble GST proteins were purified over a glutathione agarose (GTA) affinity column. GTA (Sigma) was rehydrated in ddH<sub>2</sub>O and resuspended as 50:50 slurry in H1 buffer (20 mM HEPES, 20% glycerol, 2 mM magnesium chloride, 1 M potassium chloride, 10 mM imidazole, 0.1 mM phenylmethylsulfonyl fluoride, 1mM dithiothreitol ). Cell lysate was applied to a 0.75 mL column, washed with 10 mL H1 buffer, and eluted with 2.25 mL of glutathione elution buffer (0.1 M reduced glutathione, 50 mM Tris, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, pH 8.0), and samples were dialyzed into H.35 (20 mM HEPES, 20% glycerol, 2 mM magnesium chloride, 350 mM potassium chloride, 10 mM imidazole, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol) overnight, centrifuged for 5 minutes 15,000 rpm, 4 °C, divided into aliquots and stored -80 °C.

### **2.9.1 Purification of 6His-TBP mutants**

*E. coli* BL21 cells containing pET16b-yTBP mutants were grown in 500 mL YT with 0.4% glucose and 200 ug/mL ampicillin at 37 °C to OD<sub>595</sub> of 0.7 and cooled to 30 °C. 20 ug/mL IPTG was added and the culture was induced for 60 minutes. Cells were harvested at 6,000 rpm for 6 minutes at 4 °C in a Sorvall GS3 rotor. Cells were resuspended in 20 mL of lysis buffer (25 mM Hepes, 200 mM potassium chloride, 12.5 mM magnesium chloride, 10% glycerol, 0.25 mM phenylmethylsulfonyl fluoride). Lysozyme was added to 0.4 mg/mL and potassium chloride increased to 2 M before two sonication sessions (30 seconds each) with a Branson Sonifier 450 set to maximum. Lysate was spun at 15,000 rpm for 30 minutes at 4 °C in Sorval SS-34 rotor. Soluble

portion was applied to 300  $\mu$ L bed volume of Talon (Clontech) metal affinity resin in H.0 (20 mM HEPES, 20% glycerol, 2 mM magnesium chloride, 10 mM imidazole, 0.1 mM phenylmethylsulfonyl fluoride) for 90 minutes at 4 °C. The resin was washed with H1 buffer, H.35 buffer + 20 mM imidazole and 6His-TBP mutants were eluted with 600  $\mu$ L of I.6 buffer (20% glycerol, 2 mM magnesium chloride, 20 mM Hepes, 0.35 M potassium chloride, 600 mM imidazole, 0.1 mM phenylmethylsulfonyl fluoride, pH 7.8) before dialysis into TSB<sup>+</sup> (20 mM Tris acetate, 2 mM magnesium chloride, 20% glycerol, 0.1 mM EDTA, 200 mM potassium glutamate, 0.04% NP40) overnight. To determine concentration and purity of TBP, dialyzed proteins were centrifuged at 14,000 rpm for 5 minutes at 4 °C and the soluble fraction was quantitated by Bradford assay (Bio-Rad) and 10% SDS-PAGE gels were silver stained.

### **2.9.1 GST Pulldowns**

840 ng of his-yTBP (300 nM) and 1050 ng (300 nM) of GST-TAND were mixed in 100  $\mu$ L 150 mM binding buffer (0.15 M potassium chloride, 20 mM Tris-Cl pH 8.0, 12.5 mM magnesium chloride, 10% glycerol, 1 mM dithiothreitol, 50  $\mu$ g/mL bovine serum albumin pH 8.0) in silanized eppendorf tubes. Reactions were incubated on ice for 30 minutes. 5  $\mu$ L (5% of initial volume; ~42 ng TPB) was removed as “input”. 15  $\mu$ L of GTA:binding buffer (50:50 slurry) was added, and incubated on rotating wheel at 4 °C for 30 minutes. Samples were microfuged at 3000 rpm for 30 seconds at room temperature. Supernate was removed and beads were washed with 500  $\mu$ L of ice cold 150 mM potassium chloride binding buffer three times. Beads were resuspended in 15

uL of 2X Lamelli protein sample buffer (PSB) and boiled at 100 °C for 2 minutes. SF9 extract (insect cells) was added to 50 % of the reaction (to improve electrophoretic transfer). Samples were boiled at 100 °C for 4 minutes before loading onto 10% acrylamide denaturing gels (SDS-PAGE). Proteins were transferred to nitrocellulose at constant 1.5 Amps for 60 minutes. Nitrocellulose was incubated in 10 mL of non-specific proteins solution (blocker) consisting of 5% dry milk/TBST for 30 minutes at room temperature. 2.5 uL of  $\alpha$ -yTBP serum (Rockford) was incubated for at least one hour. Blot was washed four times with 10 mL of TBST for four minutes. 2ul of anti-rabbit IgG HRP secondary antibody (Amersham) and 3ul of anti GST-HRP conjugated antibody (Amersham) were added in 10 mL of 5% dry milk/TSBT and incubated for one hour at room temperature. Blot was washed with 10 mL of TBST four times for four minutes each. HPR conjugated antibodies were detected with ECL kit (Amersham) and hyperfilm (Amersham).

### 2.9.1 Molecular Structures

All images were generated with Rasmol (Sayle and Milner-White, 1995). Crystal structure coordinates (see below for ID numbers) were downloaded from Brookhaven protein data bank (PDB; <http://www.rcsb.org/>) (Berman et al., 2000; Chasman et al., 1993). **yTBP:** (PDB ID# 1TBP, (Chasman et al., 1993)). **yTBP-DNA:** (PDB ID# 1YTB (Kim et al., 1993a; Kim et al., 1993b)). **dTAND:** (PDB ID# 1TBA, (Liu et al., 1998)). **yTAND:** After our studies were completed, a NMR structure for yTBP and

yTAND (residues 10-73) was determined (Mal et al., 2004). However, to date, structural information is not publicly available.

## 2.10 Bibliography

- Bai, Y., Perez, G.M., Beechem, J.M. and Weil, P.A. (1997) Structure-function analysis of TAF130: identification and characterization of a high-affinity TATA-binding protein interaction domain in the N terminus of yeast TAF(II)130. *Mol Cell Biol*, **17**, 3081-3093.
- Banik, U., Beechem, J.M., Klebanow, E., Schroeder, S. and Weil, P.A. (2001) Fluorescence-based analyses of the effects of full-length recombinant TAF130p on the interaction of TATA box-binding protein with TATA box DNA. *J Biol Chem*, **276**, 49100-49109.
- Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E. (2000) The Protein Data Bank. *Nucleic Acids Res*, **28**, 235-242.
- Burley, S.K. and Roeder, R.G. (1996) Biochemistry and structural biology of transcription factor IID (TFIID). *Annu Rev Biochem*, **65**, 769-799.
- Cang, Y., Auble, D.T. and Prelich, G. (1999) A new regulatory domain on the TATA-binding protein. *Embo J*, **18**, 6662-6671.
- Chasman, D.I., Flaherty, K.M., Sharp, P.A. and Kornberg, R.D. (1993) Crystal structure of yeast TATA-binding protein and model for interaction with DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8174-8178.

- Chitikila, C., Huisinga, K.L., Irvin, J.D., Basehoar, A.D. and Pugh, B.F. (2002) Interplay of TBP Inhibitors in Global Transcriptional Control. *Molecular Cell*.
- Coleman, R.A. and Pugh, B.F. (1997) Slow dimer dissociation of the TATA binding protein dictates the kinetics of DNA binding. *Proc Natl Acad Sci U S A*, **94**, 7221-7226.
- Coleman, R.A., Taggart, A.K., Benjamin, L.R. and Pugh, B.F. (1995) Dimerization of the TATA binding protein. *Journal of Biological Chemistry*, **270**, 13842-13849.
- Coleman, R.A., Taggart, A.K., Burma, S., Chicca, J.J., 2nd and Pugh, B.F. (1999a) TFIIA regulates TBP and TFIID dimers. *Mol Cell*, **4**, 451-457.
- Coleman, R.A., Taggart, A.K.P., Burma, S., Chicca II, J.J. and Pugh, B.F. (1999b) TFIIA regulates TBP and TFIID dimers. *Molecular Cell*, **4**, 451-457.
- Daugherty, M.A., Brenowitz, M. and Fried, M.G. (1999) The TATA-binding protein from *Saccharomyces cerevisiae* oligomerizes in solution at micromolar concentrations to form tetramers and octamers. *J Mol Biol*, **285**, 1389-1399.
- Daugherty, M.A., Brenowitz, M. and Fried, M.G. (2000) Participation of the amino-terminal domain in the self-association of the full-length yeast TATA binding protein. *Biochemistry*, **39**, 4869-4880.
- DeDecker, B.S., O'Brien, R., Fleming, P.J., Geiger, J.H., Jackson, S.P. and Sigler, P.B. (1996) The crystal structure of a hyperthermophilic archaeal TATA-box binding protein. *J Mol Biol*, **264**, 1072-1084.
- Flatters, D., Young, M., Beveridge, D.L. and Lavery, R. (1997) Conformational properties of the TATA-box binding sequence of DNA. *J Biomol Struct Dyn*, **14**, 757-765.

- Geiger, J.H., Hahn, S., Lee, S. and Sigler, P.B. (1996) Crystal structure of the yeast TFIIA/TBP/DNA complex. *Science*, **272**, 830-836.
- Geisberg, J.V., Holstege, F.C., Young, R.A. and Struhl, K. (2001) Yeast NC2 associates with the RNA polymerase II preinitiation complex and selectively affects transcription *in vivo*. *Mol Cell Biol*, **21**, 2736-2742.
- Geisberg, J.V., Moqtaderi, Z., Kuras, L. and Struhl, K. (2002) Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **22**, 8122-8134.
- Huisinga, K.L. and Pugh, B.F. (2004) A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell*, **13**, 573-585.
- Irvin, J.D., Pugh, B.F. Data not shown.
- Jackson-Fisher, A.J., Burma, S., Portnoy, M., Schneeweis, L., Coleman, R.A., Mitra, M., Chitikila, C. and Pugh, B.F. (1999a) Dimer dissociation and thermosensitivity kinetics of the *Saccharomyces cerevisiae* and human TATA binding proteins. *Biochemistry*, **38**, 11340-11348.
- Jackson-Fisher, A.J., Chitikila, C., Mitra, M. and Pugh, B.F. (1999b) A role for TBP dimerization in preventing unregulated gene expression. *Mol. Cell*, **3**, 717-727.
- Kamada, K., Shu, F., Chen, H., Malik, S., Stelzer, G., Roeder, R.G., Meisterernst, M. and Burley, S.K. (2001) Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex. *Cell*, **106**, 71-81.
- Kim, J.L. and Burley, S.K. (1994) 1.9 Å resolution refined structure of TBP recognizing the minor groove of TATAAAAG. *Nature Structural Biology*, **1**, 638-653.

- Kim, J.L., Nikolov, D.B. and Burley, S.K. (1993a) Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature*, **365**, 520-527.
- Kim, Y., Geiger, J.H., Hahn, S. and Sigler, P.B. (1993b) Crystal structure of a yeast TBP/TATA-box complex. *Nature*, **365**, 512-520.
- Kobayashi, A., Miyake, T., Ohyama, Y., Kawaichi, M. and Kokubo, T. (2001) Mutations in the TATA-binding protein, affecting transcriptional activation, show synthetic lethality with the TAF145 gene lacking the TAF N-terminal domain in *Saccharomyces cerevisiae*. *J Biol Chem*, **276**, 395-405.
- Kokubo, T., Gong, D.W., Yamashita, S., Horikoshi, M., Roeder, R.G. and Nakatani, Y. (1993) *Drosophila* 230-kD TFIID subunit, a functional homolog of the human cell cycle gene product, negatively regulates DNA binding of the TATA box-binding subunit of TFIID. *Genes & Development*, **7**, 1033-1046.
- Kokubo, T., Swanson, M.J., Nishikawa, J.I., Hinnebusch, A.G. and Nakatani, Y. (1998) The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA-binding protein. *Mol Cell Biol*, **18**, 1003-1012.
- Kokubo, T., Yamashita, S., Horikoshi, M., Roeder, R.G. and Nakatani, Y. (1994) Interaction between the N-terminal domain of the 230-kDa subunit and the TATA box-binding subunit of TFIID negatively regulates TATA-box binding. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 3520-3524.
- Kotani, T., Banno, K., Ikura, M., Hinnebusch, A.G., Nakatani, Y., Kawaichi, M. and Kokubo, T. (2000) A role of transcriptional activators as antirepressors for the

autoinhibitory activity of TATA box binding of transcription factor IID. *Proc Natl Acad Sci U S A*, **97**, 7178-7183.

Kotani, T., Miyake, T., Tsukihashi, Y., Hinnebusch, A.G., Nakatani, Y., Kawaichi, M. and Kokubo, T. (1998) Identification of highly conserved amino-terminal segments of dTAFII230 and yTAFII145 that are functionally interchangeable for inhibiting TBP- DNA interactions *in vitro* and in promoting yeast cell growth *in vivo*. *J Biol Chem*, **273**, 32254-32264.

Kou, H., Irvin, J.D., Huisinga, K.L., Mitra, M. and Pugh, B.F. (2003) Structural and functional analysis of mutations along the crystallographic dimer interface of the yeast TATA binding protein. *Mol Cell Biol*, **23**, 3186-3201.

Kuras, L., Kosa, P., Mencia, M. and Struhl, K. (2000) TAF-Containing and TAF-independent forms of transcriptionally active TBP *in vivo* [In Process Citation]. *Science*, **288**, 1244-1248.

Lee, T.I., Wyrick, J.J., Koh, S.S., Jennings, E.G., Gadbois, E.L. and Young, R.A. (1998) Interplay of positive and negative regulators in transcription initiation by RNA polymerase II holoenzyme. *Mol Cell Biol*, **18**, 4455-4462.

Leurent, C., Sanders, S., Ruhlmann, C., Mallouh, V., Weil, P.A., Kirschner, D.B., Tora, L. and Schultz, P. (2002) Mapping histone fold TAFs within yeast TFIID. *Embo J*, **21**, 3424-3433.

Leurent, C., Sanders, S.L., Demeny, M.A., Garbett, K.A., Ruhlmann, C., Weil, P.A., Tora, L. and Schultz, P. (2004) Mapping key functional sites within yeast TFIID. *Embo J*, **23**, 719-727.

- Liu, D., Ishima, R., Tong, K.I., Bagby, S., Kokubo, T., Muhandiram, D.R., Kay, L.E., Nakatani, Y. and Ikura, M. (1998) Solution structure of a TBP-TAF(II)230 complex: protein mimicry of the minor groove surface of the TATA box unwound by TBP. *Cell*, **94**, 573-583.
- Mal, T.K., Masutomi, Y., Zheng, L., Nakata, Y., Ohta, H., Nakatani, Y., Kokubo, T. and Ikura, M. (2004) Structural and functional characterization on the interaction of yeast TFIID subunit TAF1 with TATA-binding protein. *J Mol Biol*, **339**, 681-693.
- Mencia, M. and Struhl, K. (2001) Region of yeast TAF 130 required for TFIID to associate with promoters. *Mol Cell Biol*, **21**, 1145-1154.
- Nikolov, D.B., Chen, H., Halay, E.D., Hoffman, A., Roeder, R.G. and Burley, S.K. (1996) Crystal structure of a human TATA box-binding protein/TATA element complex. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 4862-4867.
- Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G. and Burley, S.K. (1995) Crystal structure of a TFIIB-TBP-TATA-element ternary complex. *Nature*, **377**, 119-128.
- Nikolov, D.B., Hu, S.H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N.H., Roeder, R.G. and Burley, S.K. (1992) Crystal structure of TFIID TATA-box binding protein. *Nature*, **360**, 40-46.
- Nishikawa, J., Kokubo, T., Horikoshi, M., Roeder, R.G. and Nakatani, Y. (1997) Drosophila TAF(II)230 and the transcriptional activator VP16 bind competitively to the TATA box-binding domain of the TATA box-binding protein. *Proc Natl Acad Sci U S A*, **94**, 85-90.

- Patikoglou, G.A., Kim, J.L., Sun, L., Yang, S.H., Kodadek, T. and Burley, S.K. (1999) TATA element recognition by the TATA box-binding protein has been conserved throughout evolution. *Genes Dev*, **13**, 3217-3230.
- Prelich, G. (1997) *Saccharomyces cerevisiae* BUR6 encodes a DRAP1/NC2alpha homolog that has both positive and negative roles in transcription *in vivo*. *Mol Cell Biol*, **17**, 2057-2065.
- Sayle, R.A. and Milner-White, E.J. (1995) RASMOL: biomolecular graphics for all. *Trends Biochem Sci*, **20**, 374.
- Struhl, K. (1995) Yeast transcriptional regulatory mechanisms. *Annu Rev Genet*, **29**, 651-674.
- Taggart, A.K. and Pugh, B.F. (1996) Dimerization of TFIID when not bound to DNA. *Science*, **272**, 1331-1333.
- Takada, R., Nakatani, Y., Hoffmann, A., Kokubo, T., Hasegawa, S., Roeder, R.G. and Horikoshi, M. (1992) Identification of human TFIID components and direct interaction between a 250-kDa polypeptide and the TATA box-binding protein (TFIID tau). *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 11809-11813.
- Takahata, S., Kasahara, K., Kawaichi, M. and Kokubo, T. (2004) Autonomous function of the amino-terminal inhibitory domain of TAF1 in transcriptional regulation. *Mol Cell Biol*, **24**, 3089-3099.
- Takahata, S., Ryu, H., Ohtsuki, K., Kasahara, K., Kawaichi, M. and Kokubo, T. (2003) Identification of a novel TATA element-binding protein binding region at the N

- terminus of the *Saccharomyces cerevisiae* TAF1 protein. *J Biol Chem*, **278**, 45888-45902.
- Tan, S., Hunziker, Y., Sargent, D.F. and Richmond, T.J. (1996) Crystal structure of a yeast TFIIA/TBP/DNA complex. *Nature*, **381**, 127-151.
- Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, **22**, 4673-4680.
- Weideman, C.A., Netter, R.C., Benjamin, L.R., McAllister, J.J., Schmiedekamp, L.A., Coleman, R.A. and Pugh, B.F. (1997) Dynamic interplay of TFIIA, TBP and TATA DNA. *J Mol Biol*, **271**, 61-75.
- Yatherajam, G., Zhang, L., Kraemer, S.M. and Stargell, L.A. (2003) Protein-protein interaction map for yeast TFIID. *Nucleic Acids Res*, **31**, 1252-1260.
- Zanton, S.J. and Pugh, B.F. (2004) Changes in genomewide occupancy of core transcriptional regulators during heat stress. *Proc Natl Acad Sci U S A*, **101**, 16843-16848.

## Chapter 3

### Genome-Wide Roles of TAF1 Functional Domains on Transcription

#### 3.1 Abstract

TFIID plays a central role in regulating the expression of most eukaryotic genes. Of the 14 TAF subunits that compose TFIID, TAF1 is one of the largest and most functionally diverse. Yeast TAF1 reportedly possesses at least four distinct activities including a histone acetyltransferase, and TBP, TAF, and promoter binding. Establishing the importance of each region in gene expression through deletion analysis has been hampered by the cellular requirement of TAF1 for viability. To circumvent this limitation we introduced galactose-inducible deletion derivatives of previously defined functional regions of TAF1 into a temperature sensitive (*taf1<sup>ts2</sup>*) yeast strain. After galactose-induction and temperature inactivation of the temperature-sensitive allele, we examined the properties and phenotypes of the mutants, including their impact on genome-wide transcription. Virtually all TAF1-dependent genes, which comprise ~90% of the yeast genome, displayed a strong dependency upon all regions of TAF1 that were tested. This might reflect the need for each region of TAF1 to stabilize TAF1 against degradation or that all TAF1-dependent genes require the many activities of TAF1. Paradoxically, deletion of the region of TAF1 that is important for promoter binding interfered with the expression of many genes that are normally TFIID-

independent/SAGA-dominated, suggesting that this region normally prevents TAF1 (TFIID) from interfering with the expression of this class of genes.

### **3.2 Introduction**

DNA binding sequence-specific activators regulate eukaryotic genes at many stages including the recruitment of chromatin remodeling factors that increase the accessibility of promoters to the transcription machinery. Activators also assist in the loading of the general transcription factors and RNA polymerase II at promoters to form a pre-initiation complex that is capable of transcribing the gene. The transcription machinery assembles at promoters via two major pathways in yeast; one that involves TFIID and the other involving a compositionally related complex called SAGA (Green 2000; Naar, Lemon et al. 2001). TFIID is composed of the TATA binding protein (TBP) and 14 TBP-associated factors (TAFs), of which all but one are essential for cell viability. Several of these TAFs, along with TBP, are also found in SAGA (Grant, Schieltz et al. 1998). Although TBP is widely regarded as being responsible for delivering TFIID to promoters through interactions of TBP with the TATA box, TFIID largely functions at TATA-less promoters (Basehoar, Zanton et al. 2004; Huisinga and Pugh 2004; Zanton and Pugh 2004). TATA-containing promoters tend to be TFIID-independent, and instead prefer to load TBP via the SAGA assembly pathway (Huisinga and Pugh 2004). The vast majority of all yeast genes (80-90%) are regulated through a TFIID/TATA-less arrangement, whereas a minority depend primarily on a SAGA/TATA arrangement. Strikingly, the latter class largely include stress-induced genes. Thus, transcription

complex assembly via the SAGA pathway might provide a greater level of inducibility that is characteristic of stress-induced responses (Gasch, Spellman et al. 2000; Huisinga and Pugh 2004; Zanton and Pugh 2004). Under normal growth conditions, SAGA is not essential for cell viability (Grant, Schieltz et al. 1998; Lee, Causton et al. 2000). In the absence of SAGA, expression of virtually the entire measurable yeast genome becomes TFIID-dependent. Thus, TFIID may be capable of setting up transcription complexes at all pol II-transcribed genes.

TAFs perform a variety of functions including interactions with transcriptional activators, other general transcription factors, and promoter DNA (Kokubo, Swanson et al. 1998; Durso, Fisher et al. 2001; Kirchner, Sanders et al. 2001; Mencia and Struhl 2001; Kirchner, vom Baur et al. 2002; Shen, Bhaumik et al. 2003). Genome-wide studies using temperature-sensitive alleles of various TAFs indicate that some TAFs may be selective in the genes they activate (Holstege, Jennings et al. 1998; Lee, Causton et al. 2000; Shen, Bhaumik et al. 2003). This suggests that distinct parts of TFIID might play important promoter-specific roles. Similarly, a variety of temperature-sensitive alleles located throughout TAF10 reveal potential promoter-selective roles for distinct regions of a single TAF (Kirchner, vom Baur et al. 2002). Conceivably, different parts of TFIID may be tailored for very promoter-specific roles.

TAF1 is considered to be a 'hallmark' of TFIID in that it resides only in TFIID and not in SAGA, and it may serve as a scaffold upon which TBP and TAFs assemble, although other TAFs might also play a scaffolding role (Chen, Attardi et al. 1994; Bai, Perez et al. 1997; Mencia and Struhl 2001; Singh, Bland et al. 2004). When the studies reported here were initiated, TAF1 had been systematically dissected into four functional

domains: an N-terminal TBP-binding domain termed TAND, a TAF-TAF interaction domain, a histone acetyltransferase domain, and a promoter recognition domain (Mizzen, Yang et al. 1996; Kokubo, Swanson et al. 1998; Kotani, Banno et al. 2000; Mencia and Struhl 2001; Sanders, Garbett et al. 2002; Singh, Bland et al. 2004). More recently, a fifth domain that interacts with TAF7 has been identified (Singh, Bland et al. 2004). Collectively, the potentially gene-specific roles of TAFs and the potential modularity of TAF1 and other TAFs lead us to consider whether the various functional domains of TAF1 play gene-specific roles *in vivo*. Since TFIID contributes to the expression of nearly the entire yeast genome, a greater understanding of the yeast gene regulatory network might be achieved by assessing the contribution of each of TFIID's activities on genome-wide transcription.

Any investigation into the genome-wide function of TAF1 or any other essential factor is hampered by the fact that deleterious mutations block cell growth. Creation of temperature-sensitive alleles have been a powerful and productive means of dissecting essential function regions. However, this approach can be biased and restrictive in that any mutation must knock out an essential function at the nonpermissive temperature, but render the protein functional at the permissive temperature. Since a large fraction of yeast genes are not essential for cell growth, this strategy could miss TAF1 mutations that are specifically defective in many nonessential genes. As an alternative strategy, we employed a systematic targeted approach by disrupting known functional domains of TAF1. To minimize potential indirect effects caused by constitutive expression of the TAF1 mutants, we chose to express each mutant under an inducible promoter. Since TAF1 is essential, this approach necessitated the use of a functional copy of TAF1 to

promote cell growth. However, when assaying for mutant function it was desirable to eliminate the functional TAF1 copy so that it would not obscure or suppress the deletion construct. This was achieved by using a temperature-sensitive allele of *TAF1*, *taf1<sup>ts2</sup>*, which supports viability at non-permissive temperatures (Walker, Shen et al. 1997; Huisinga and Pugh 2004).

Using this approach, I first assayed for the ability of an induced version of wild type or mutant TAF1 to functionally complement the growth defect of a temperature-sensitive TAF1 allele. Next, I characterized the expression, stability, and subcellular localization of the TAF1 mutants in order to better assess its potential to impact gene expression. Lastly, genome-wide expression studies were performed to evaluate the contribution of each functional domain to transcription.

### **3.3 Results and Discussion**

#### **3.3.1 Mutant Design**

Four functional regions of TAF1 were chosen based on previous studies and homology. Figure 3-1 shows a protein alignment of TAF1 homologues. The TAND domain, TAF interaction region, HAT function, and promoter binding region. A schematic representation of the TAF1 open reading frame (ORF) and relative location of four targeted functional regions are illustrated in Figure 3-2 A. Since *TAF1* is an essential gene it cannot be constitutively replaced by *taf1* mutants that knock out essential functions. However, transient replacement can be achieved by expressing TAF mutants

under the control of an inducible promoter in a strain harboring a second allele of *TAF1* that is temperature sensitive. To achieve this, a chromosomal *TAF1* allele was placed under control of the *GALI* promoter in the diploid wild type strain BY4743 (Figure 3-2 B). Deletion of each functional domain was achieved by homologous recombination using a PCR amplified cassette containing a *kanMX* gene flanked by *loxP* Cre recombination sites. The cassette also contained coding sequences that allowed the deleted region to be replaced by an FHT epitope tag, containing a triple HA tag, a 6x polyhistidine tag, and a TEV protease cleavage site. After selection for recombinants on G418 plates, and subsequent excision of *kanMX* with the Cre recombinase, the deleted region contained the FHT tag and a single *loxP* site, both of which maintain an open reading frame through the deleted region. The location of each mutation (Figure 3-2 C) was verified by PCR and by immunoblot analysis for the presence of an appropriate size band that reacted with HA antibodies and derived from galactose-treated but not glucose-treated cells (Figure 3-2 D). Most mutants, except “TF1”, were expressed at levels that were comparable to endogenous TAF1 (Figure 3-2 D left panel). High levels of expression of this mutant from the endogenous *TAF1* promoter has been noted previously (Mencia and Struhl 2001).

Figure 3-1

```

dm -----MEMESDNSDDEGSIGNG-LDLTGILFGNIDSEGRLLODDDGE 42
hs MGPGCDLLLRATAATIATAAIMSDTSDSDSAGGGPFLAGFLGNINGAGQLE----- 53
ce -----
sc -----
sp -----

dm RGGTGFDAELRENIGSLSKLGLDSMLLEVIDLKEAEPSPDDEEEDARPSAVSASEGMSA 102
hs -GESVLDDECKKHLGALGLGSLITELTANEELTGTG----- 92
ce -----
sc -----
sp -----

dm FDALKAGVKREDGAVKAQDDAIDYSDITELSEDCPRTPEETSTYDDLEDAIP-ASKVEA 161
hs -----ALVNDEGWVRSTEDAVDYSDINEVAEESRRYQQTMGSLQPLCHSDYDEDDYDA 146
ce -----MNNTHHTNGYRTEKPKNEEDLEDPYANLSDFYKNHPAARNEACSSA 47
sc -----MVKQQGSGKTNLANEDEAYEAFI FGGEFGSLEIGSYIGGDEGANSKDYTEH 50
sp -----MSFDGLIVENENTKSGYNDGN 21
      : . . .

dm KLTKDDKELMPPP---SAPMRSSGSG----GGIEEPAKSNDASSPSDDSKSTDSKADARK 213
hs DCEDIDCKLMPPPPPPGPMKKDKDQDSITGEKVDSSSDSESEMGPQEAATQAESDQK 206
ce SNGGSKSVKMEPKVEKNEEFEEYIG-----DPVRLDMEPFARPSLR 89
sc LPDAVDFEDELADDDDLPEESD-----ANLHPAMMTMG-AYD 89
sp DLTDLFKQNGTDMSVINSLGDTNN-----PGMNESPKILDSSF 61
      : . .

dm LDTPLADILPSKYQN--VDVRELFDFRPQKVLRFSLFGPGKPTSLPQIWRHVRKRRRK 271
hs LTLPLAGIMQHDATAKLLPSVTELFPEFRPGKVLRFRLRFGPGK--NVPSVWRSARRKRRK 264
ce DDAPLSSILHPDLG--IDPRIFFKDFNPNKTLRF'SRLFAQNIKHTSRAEIWWASRTFSK 147
sc DVNENGAVLGI DSNS----LNMQLPEINGDLSQQFILEDDGGTPATSNALFMGMMDANEIH 145
sp NSNPQDGPNYEDFDF----MGSIHKEFGNNINEMDDMEDVSDDNLPPEEQAVNYTGDKDD 117
      . : : .

dm RNQSRDQKTTNTGGSDSPDTEEPKRKGFSLHYAAEPTPAECMSDDEDKLLGDFNSEDVR 331
hs KHRELIQEEQ-IQEVECSVESEVSQKSLWNYDYAPPPPEQCLSDDEITMMAPVESKFSQ 323
ce HQRKKEPEEP-----LADDVIVGAKKLKLNIEKVPRVMLADDEEERMRRPILT---- 196
sc LATETGVLDGSGANEIGHSQLSIGGVNGNDMSINGGFIMEPDMSDGKHKKATKLD---- 200
sp EDFGKLLAKEMGEEAAGQVLSGVG-----FSIPSGLVPPSEPKTVSSTTEELQN---- 167
      . . .

dm PEGPDNGENSQPKPVADWRFGPAQIWIYDMLVDPDSGEGFNYGFKTKAASTSSQPQLKDE 391
hs STG-DIDKVTDTKPRVAEWRYGPARLWYDMLGVPEDGSGFDYGFKLKRTHEHEPVIKSRMI 382
ce -DAEEMAKKNEEGTVVQPWRTGPAKIWYDMMNLPMTSQAVNYGFKLKKSPQKVSIRSGKP 255
sc ---LINHEKYLKLYFPDFEKGLKWNKLIYRRSVPYHWS-----EISRKVPFPLN 252
sp ---EAQIRESIVKTFPPTFERGVLLNFSELFKPK--PVKLAP----PKKKT'PKVCVPGR 217
      . . : . * : : :

dm RRVKSPEDDVEDPS-----IADDAFLMVSQLHWEDDVVWDGN 428
hs EEFKLEENNGTDL-----LADENFLMVTQLHWEDDIWIDGE 419
ce LNYRTPDDLPTSSGPAPNSAPFLDKVEVIDKSCEASTSEDILLPYQVIEWENDVILDGE 315
sc LKFKVQDDKRLFN-----SRTISYVAPIYQGKNNLLQSNS 288
sp LTLEVDTDYAIFN-----SKKS-----LPLKRNVPSPIS 247
      . : : : :

dm DIKAKVLQKLSKNTAAGWLPSSGSRTAGAFSQPGKPSMPVSGSGSSKQSGASSKKAQQN 488
hs DVKHKGTQKQ--RASLAGWLPSSMTRNAMAYN-----VQQGFAATLD---- 459
ce EVKDQLLEEF--NGRGCWIP'QYTRTYEHFVYAAN-----NNAFEPQMPDGKSAPIN 366
sc SASRRGLIHVSI DELFP'IKEQQKKRKIIHDEK----- 320
sp THTTK-----RRRTANTSQRND----- 264
      . : .

dm AQAKPAEAPDDTWYSLFPVENEELIYKWEDEVIWDAQVSKVPKPKVLTLDPNDENIIL 548
hs -----DDKPWYSIFPIDNEDLVYGRWEDNI IWDAQAMPRLLEPPVLTLDPNDENLIL 511
ce LTGPDSAILPTPGHSIFPSAPCDLDILPWETNI IWADAMPSTLEPIDFLVDFQDDPLIY 426
sc -----TISEDLLIATDDWDQEKIINQGTSSSTATLADSSMTPNLKFSGGYKLSLIE 371
sp -----GLDLNTVFTTNDWKNIIYDESVDNKTNQSS-----FFID 299
      : : . : : : : *

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```

dm  LLDDDDQVALSFIFDKLHSQ-IKQLPESWPFLLKPVNKKQVKDYTYVIKRPMDLETIGKNI 1646
hs  LLDDDDQVAFSFI LDNI V T Q K M M A V P D S W P F H H P V N K K F V P D Y Y K V I V N P M D L E T I R K N I 1557
ce  LLDTNDLIGFSYLLGEIVQK-MKNIPKSALFHTRVDPKKIPAYYLKISDPMDLSIMEQKS 1632
sc  -----
sp  -----

dm  EAHRYHSRAEYLADIELIATNCEQYNGSDTRYTKFSKKILEYAQTQLIEFSEHCQLENN 1706
hs  SKHKYQSRESFLDDVNLILANSVKYNGPESQYTKTAQEIVNVCYQTLTEYDEHLTQLEKD 1617
ce  KSQEYKSIDFLKDAEKIYTNSVVFNGAESVYSLKAKEMFEMAEMLVKDQMDTLGELERN 1692
sc  -----
sp  -----

dm  IAKTQERARENAPPEFDEAWGNDDYNFDRGSRASSPGDDYIDVEGHGGHASSSNSIHRSMG 1766
hs  ICTAKEAALEEA-----ELES L D P M T P G P Y T P Q P P D L Y D T N T S L S M S R D A S 1663
ce  INPSAINDAAAA----- 1704
sc  -----
sp  -----

dm  AEAGSSHTAPAVRKPAPPGPGEVVKRGRGRPRKQRPVEEDLQ CST D D E D D D E E E D F Q E V S 1826
hs  VFQDESNSVLDIPSATP-----EKQ---VTQEGEDGDGLADEEEGT V Q Q P Q 1708
ce  -----QRGLAMDSDDHMEDEMDHPTEE 1726
sc  -----
sp  -----

dm  EDENNAASILDQGERINAPADAMDGMPKNIKTEIDLEAHQMADESMDVDPNYDPSDFL 1886
hs  ASVLYEDLLMSEGEDDEEDAGSDEEGDNP-----FSATQLSESGSDSDVVGSGG---- 1756
ce  EEEEDDDEIMDDMDIDATGYSYDH----- 1751
sc  -----
sp  -----

dm  AMHKQRQSLGEPSSLQGAFTNFLSHEQDDNGPYNPAEASTSAASGADLGMDASAMQMAP 1946
hs  IRPKQPRMLQENTRMDMENEESMMSYEGDGG----EASHGLED----SNISYGSYEYP 1806
ce  -----DDNVAVGQIFNDLAMS D S D E D E -----RAEDV 1778
sc  -----
sp  -----

dm  EMPVNTMNGMGIDDDLDISESEDEDDGSRVRIKKEVFDDGDYALQHQQMGQAASQSQIY 2006
hs  DPKSNTQDTSFSSIGGYEVSEEEDEE-----EEEQRSGPSVLSQVH 1848
ce  KRPANGDDNLLDSF----- 1792
sc  -----
sp  -----

dm  MVDSSNEPTLDYQPPQLDFQQVQEMEQLQHQMPPMQSEQLQQQQTPQGDNDYAWTF 2065
hs  LSEDEEDSE-----DFHSIAGDSDLDSDE----- 1872
ce  -----
sc  -----
sp  -----

```

Figure 3-1: Homology of TAF1 functional regions.

Protein alignment of TAF1 homologues. Human (hs), *Drosophila melanogaster* (dm) *Saccharomyces cerevisiae* (sc), *Schizosaccharomyces pombe* (sp), *Caenorhabditis elegans* (ce) protein sequences were aligned using ClustalW (Thompson, Higgins et al. 1994). Functional regions TAND (DT1), Taf interaction (TF1), histone acetyltransferase (HT4) and promoter binding (PB1) are underlined in blue, red, green and yellow respectively. “\*”, “:”, “.” denote residues that are identical in all species, conserved substitutions, and semi-conserved substitutions, respectively.

Figure 3-2

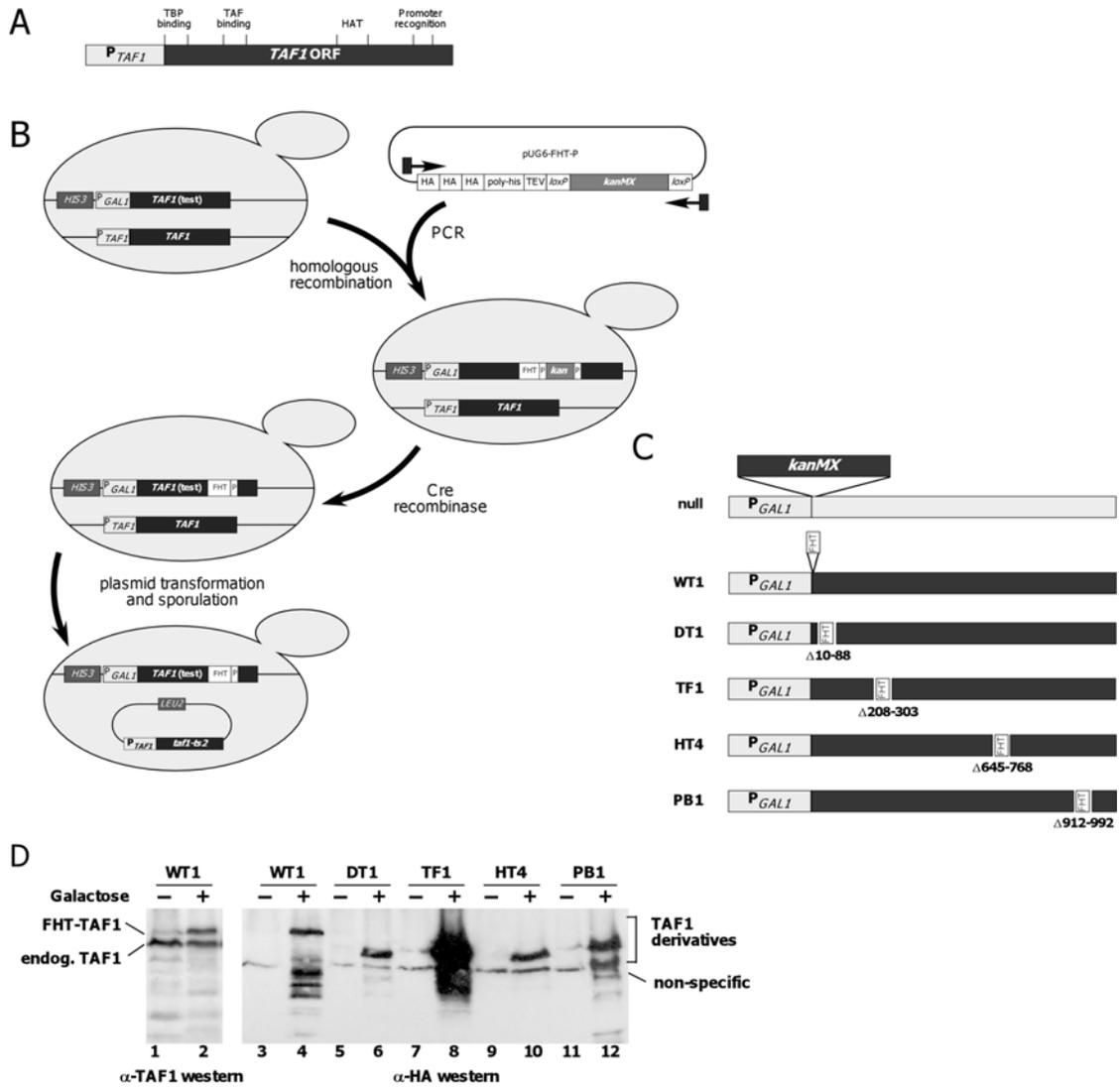


Figure 3-2: Design of galactose-inducible epitope-tagged TAF1 mutants.

A, A schematic of the TAF1 gene is shown, along with the approximate location of four essential functional domains. B, The TAF1 open reading frame was placed under control of the GAL1 promoter (PGAL1) in a diploid strain. The kanMX gene was used to select for the delivered FHT epitope tag inserted at the locations indicated in panel C. kanMX was subsequently removed using the Cre-Lox recombination system, leaving behind the FHT tag. These strains were transformed with TAF1/URA and sporulated. Ultimately, TAF1/URA was shuffled out and replaced with taf1ts2/LEU (as shown). C, Schematics of the constructed mutants. WT1, FHT-tagged WT; DT1, deletion of TAND domain, TF1, deletion of TAF interacting region (residues 208-303); HT4, deletion of HAT region (residues 645-768); PB1, deletion of promoter-binding region (residues 912-992). The “null” control represents WT1 in which the kanMX selection gene was not removed. D, Galactose induction of TAF1 derivatives. An immunoblot probed with TAF1 polyclonal antibodies is shown in lanes 1 and 2, revealing the endogenous untagged wild type TAF1 (lower band) and an FHT epitope tagged derivative (upper band). Lanes 3-12 were probed with HA antibodies recognizing the FHT tag. 2% galactose (+) was added to raffinose-grown cells for 45 min. prior to harvesting.

### 3.3.1 Growth Phenotypes

Diploid recombinants were transformed with a *TAF1/URA3* plasmid and sporulated. Haploid spores were germinated and the ability of the TAF1 mutants to support cell viability was measured by selecting for the loss of the *TAF1/URA3* plasmid on media containing 5-FOA. As shown in Figure 3-3, none of the mutants nor wild type TAF1 supported cell viability when *TAF1* was placed under *GALI* control and cells grown in dextrose, which confirms that *TAF1* expression is under tight *GALI* control. In the presence of galactose, cells containing wild type, epitope tagged wild type, or TAND deleted (DT1) *TAF1* grew at 25°C. DT1 did not grow at 37°C; similar TAND deletion mutations also display a temperature-sensitive phenotype (Bai, Perez et al. 1997; Kokubo, Swanson et al. 1998; Chitikila, Huisinga et al. 2002). All other mutants failed to

grow at either temperature, reflecting the essential function of the TAF interaction domain (TF1), histone acetyltransferase activity (HT4), and promoter binding domain (PB1), as determined previously (Bai, Perez et al. 1997).

Figure 3-3

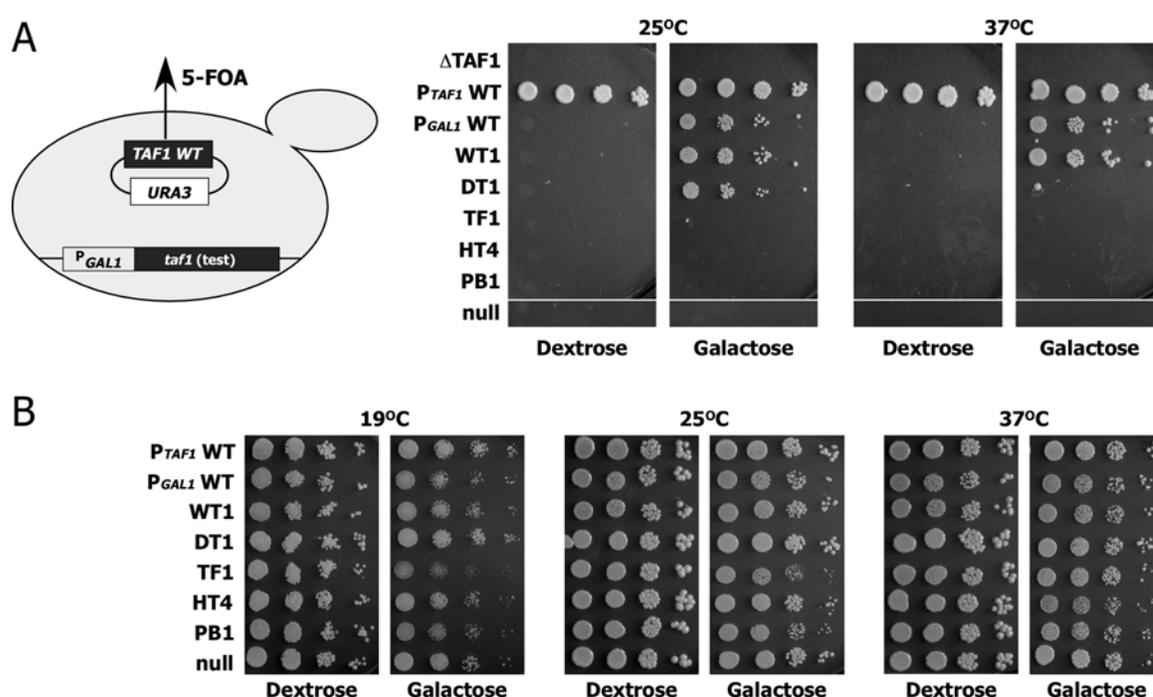


Figure 3-3: Effects of TAF1 mutants on cell growth.

A, Viability assay. Strains carrying the TAF1/URA plasmid were cultured in YPG media, then ten-fold serially diluted onto CSM plates containing 5-FOA and either 2% dextrose (-) or galactose (+). Plates were incubated at either 25 °C or 37 °C for 4 days. B, Toxicity assay. Strains carrying the TAF1/LEU plasmid were cultured in CSM-LEU media + 2% raffinose, then serially diluted onto CSM-LEU plates containing either 2% dextrose (-) or galactose (+) for 4 days (5 days for plates grown at 19 °C). WT, wild type; WT1, FHT-tagged WT; DT1, deletion of TAND domain; TF1, deletion of TAF interacting region (residues 208-303); HT4, deletion of HAT region (residues 645-768); PB1, deletion of promoter-binding region (residues 912-992). P<sub>TAF1</sub> WT, WT TAF1 expressed chromosomally from the TAF1 promoter; P<sub>GAL1</sub> WT, WT TAF1 expressed chromosomally from the GAL1 promoter.

It is clear that TAF1 has a number of essential functional domains. Conceivably, destruction of any one domain could generate a dominant negative that is capable of engaging in certain essential interactions but not others, and as a result competing out the wild type function. To test this, mutant TAF1 derivatives were expressed in the presence of wild type *TAF1*. As shown in Figure 3-3, mutant TF1 significantly inhibited growth at 19°C and 25°C, and PB1 displayed a more modest inhibitory effect. The latter is consistent with previous findings (Mencia and Struhl 2001; Singh, Bland et al. 2004). Not surprisingly, these results suggest that TAF-TAF interactions are important for TAF1 function. Without these interactions TAF1 might be able to engage other parts of transcription machinery, thereby blocking wild type TAF1 function. In light of the observation that TF1 is particularly overexpressed compared to the other mutants, we cannot exclude the possibility that a large overproduction of TF1 is toxic to cells in a way that is unrelated to transcription. For the PB1 mutant, loss of the promoter binding region of TAF1 might allow other parts of TAF1 to engage the transcription machinery, thereby competing out wild type TAF1. Surprisingly, HT4 displayed little or no dominant negative behavior, despite having intact TAF-TAF and promoter binding domains. Possibly this region might be important for overall TAF1 stability and/or function.

### **3.3.1 Subcellular Localization**

Any potential defects in the function of TAF1 mutants might be attributable to subcellular mis-localization of the mutants. To address this possibility,

immunofluorescence was conducted on the FHT epitope tag engineered into the TAF1 mutants. As shown in Figure 3-4, all epitope-tagged wild type and mutant TAF1 proteins, except TF1, were properly localized to the nucleus as demarcated by DAPI staining. Cells lacking an epitope tagged TAF1 showed little or no staining. TF1 appeared throughout the cell, suggesting that its nuclear translocation signal was disrupted. Indeed, the amino acid sequence located between residues 230 and 246 corresponds to a potential nuclear localization signal (Dingwall and Laskey 1991; Ruzzi, Marconi et al. 1997). The finding that TF1 is mislocalized to the cytoplasm does not exclude the possibility that some portion of TF1 enters the nucleus, where it could interfere with gene expression. Alternatively, the high level of expression and mislocalization of the TF1 mutant to the cytoplasm could contribute to the observed dominant negative toxicity.

Figure 3-4

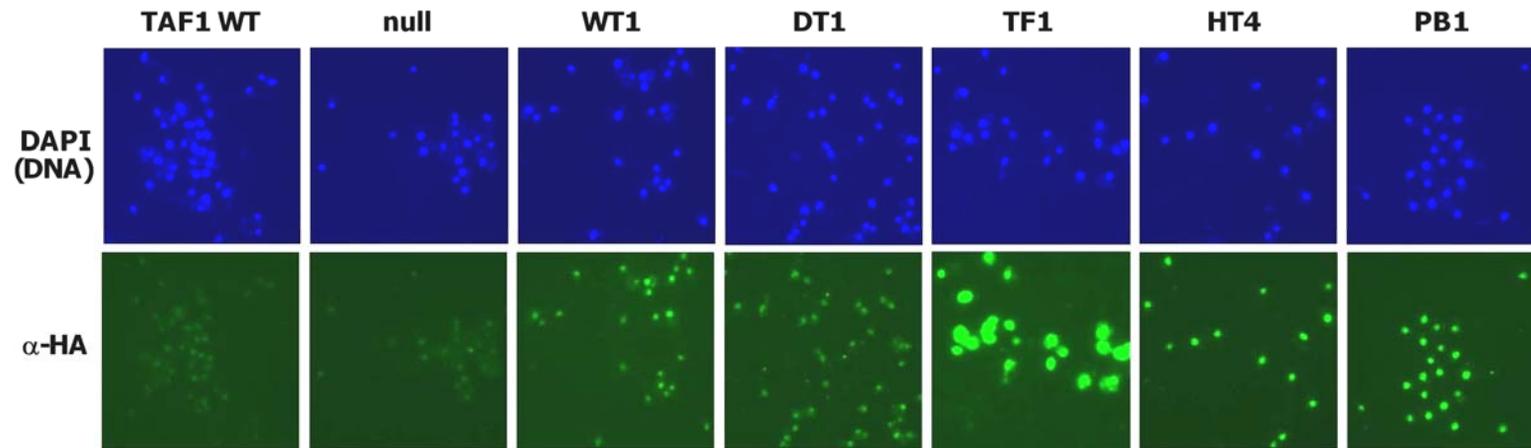


Figure 3-4: Subcellular localization of TAF1 mutants.

TAF1 strains were induced with 2% galactose for 3.5 hours, mounted on polylysine coated slides, and visualized by light microscopy. The top panels show staining of nucleic acids with DAPI. The bottom panel is immunofluorescence of TAF1 mutants detected with HA primary antibodies and mouse IgG-Alexa Fluor 488 secondary antibodies. WT, wild type; WT1, FHT-tagged WT; DT1, deletion of TAND domain; TF1, deletion of TAF interacting region (residues 208-303); HT4, deletion of HAT region (residues 645-768); PB1, deletion of promoter-binding region (residues 912-992).

### 3.3.1 Protein Stability

The ultimate goal of these studies is to transiently expose the cells to the TAF1 mutants while at the same time eliminate the functional copy of TAF1 that maintains cell viability. As the next step in this direction, we compared the expression level of the TAF1 mutants at 25°C and 37°C, the latter being the restrictive temperature that inactivates the otherwise functional copy of *taf1<sup>ts2</sup>*. As shown in Figure 3-5 Figure A, all HA-tagged mutants were inducible and present at 37°C.

Using dextrose to shutoff *GALI*-driven expression, we examined the stability of the various TAF mutants at 37°C (Figure 3-5 B, and quantitated in Figure 3-5 C). Epitope-tagged wild type TAF1 had a turnover half-life of approximately 60 min., whereas the TAF1 mutants, with the exception of TF1, had a significantly more rapid turnover with an apparent half-life of ~30 min. The actual half-life is expected to be somewhat shorter given that the approximate mRNA half-life is about 20 min. (Holstege, Jennings et al. 1998). Thus, defects in regions of TAF1 that are required for interactions with TBP and promoters or contain the putative HAT domain lead to rapid TAF1 turnover. The decay rate for the TF1 mutant was biphasic, in which about half of the protein was degraded with a similar profile as wild type TAF1, and the remaining half being very stable. Given that much of the TF1 mutant accumulates in the cytoplasm, unlike the other mutants, it might be less susceptible to protein degradation enzymes that target nuclear proteins. The fraction of TF1 that is degraded could be nuclear, although this is not known.

Figure 3-5

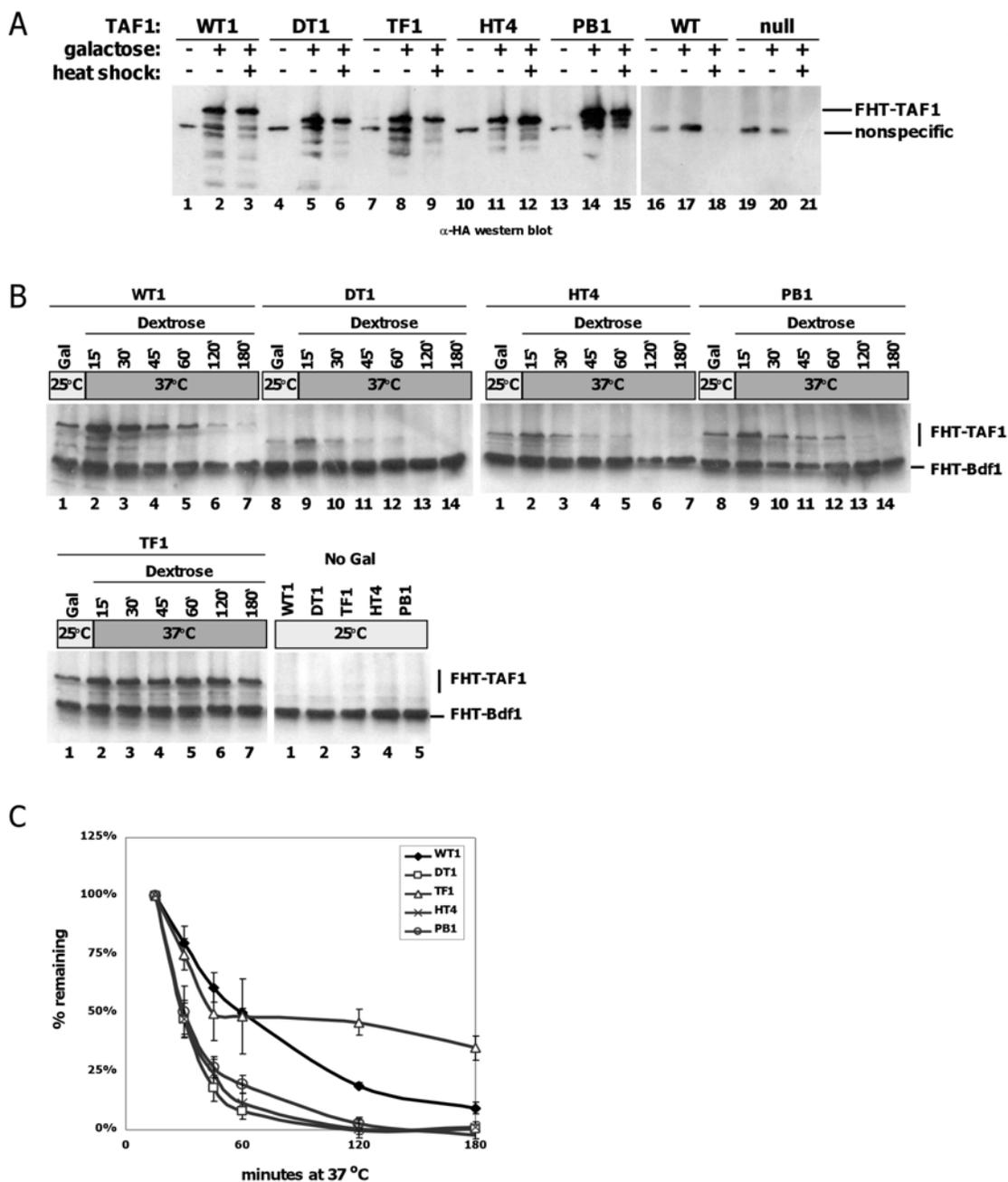


Figure 3-5: Stability of TAF1 mutants at 37 °C.

A, Expression of FHT-TAF1 mutants at 37°C. WT, wild type; WT1, FHT-tagged WT; DT1, deletion of TAND domain; TF1, deletion of TAF interacting region (residues 208-303); HT4, deletion of HAT region (residues 645-768); PB1, deletion of promoter-binding region (residues 912-992). 1 mL of 0.5 OD600 cells grown in YPR media at 25°C were removed at three stages during growth, as indicated above each lane: 1) prior to galactose induction and heat shock (-, -), 2) after 30 min. in 2% galactose but immediately prior to heat shock (+, -), and 3) same as stage 2 but 45 min. after 37°C heat shock. Cells were extracted in protein sample buffer and analyzed by 7.8% SDS-PAGE and immunoblotting with HA antibodies. B, Turnover kinetics of FHT-TAF1 mutants at 37°C. TAF1 strains were grown in YPR at 25°C to an OD600 ~0.8. Galactose was then added to 2% for 45 min. Induction was halted by addition of 2% dextrose and the cultures were shifted to 37°C. Equal volume aliquots were removed at 15, 30, 45, 60, 120, and 180 min. after addition of dextrose. To control for protein extraction, recovery, and detection, whole cells constitutively expressed FHT-tagged Bdf1 were spiked into each sample. Blots were probed with HA antibodies. Four independent replicates of the shutoff were performed, and representative data shown. C, Quantitation of repeats from panel B. Quantitation of film exposures was performed using Molecular Dynamics densitometer and ImageQuant software. Exposure times were chosen so that all signals were in the linear range of detection. Local background was subtracted from signal band at each time point. The value at the 15 min. time point was set to 100% for each TAF1 mutant, and the percent of FHT-TAF1 remaining over time was plotted.

### 3.3.1 Genome-wide Expression

TFIID, including TAF1, contributes to the expression ~90% of the yeast genome. Inasmuch as different TFIID subunits and different portions of TAF5 and TAF10 have been ascribed gene-specific function (Durso, Fisher et al. 2001; Kirchner, Sanders et al. 2001; Kirchner, vom Baur et al. 2002; Shen, Bhaumik et al. 2003), we sought to examine whether the four functional domains make gene-specific contributions to gene expression on a genome-wide scale using microarray analysis. To minimize potential indirect effects where changes in the expression of certain genes (like transcriptional regulators) alter the expression of a large number other genes, we sought a means to

rapidly replace the functional copy of *TAF1*. To do this we used a temperature-sensitive *taf1<sup>ts2</sup>* allele as the functional copy (Figure 3-2 C). We developed a two-part strategy. First, *GALI*-driven TAF1 mutants were induced with galactose, then the *taf1<sup>ts2</sup>* allele was inactivated by abruptly shifting the culture temperature to 37°C for 45 min. The *GALI* promoter is TFIID-independent (Huisinga and Pugh 2004) and thus expression of the TAF1 mutants should continue when *taf1<sup>ts2</sup>* is inactivated at the restrictive temperature. As a first step in this approach, it was necessary to determine whether galactose-induced TAF1 could replace the resident TAF1 on the short time scale of the experiment. This is crucial since TAF1 is part of the multi-subunit TFIID complex, and the degree of subunit exchange is not known. Second, to achieve maximal impact on a minimal time scale (to minimize indirect effects) we sought to determine the minimal amount of time needed to synthesize the TAF1 derivatives and have it functionally replace the resident functional copy of TAF1.

To determine the optimal timing for galactose induction, we first examined the kinetics of galactose-induced TAF1 expression, shown in Figure 3-6 A is achieved ~30 min. after addition of galactose. This sets the minimal length of time over which expression profiling can be conducted. Next, we performed galactose induction at varying times prior to heat inactivating the *taf1<sup>ts2</sup>* allele, as illustrated in Figure 3-6 B. After 45 min. at 37°C, cells were harvested and the level of isolated mRNA was measured for over 5000 yeast genes and compared to a non-inducible wild type TAF1 strain subjected to the same conditions. Thus, a gene-by-gene impact of galactose-induced TAF1 vs. a constitutively expressed TAF1 reference was determined. These ratios were binned and plotted as a smoothed frequency distribution, Figure 3-6 C.

Figure 3-6

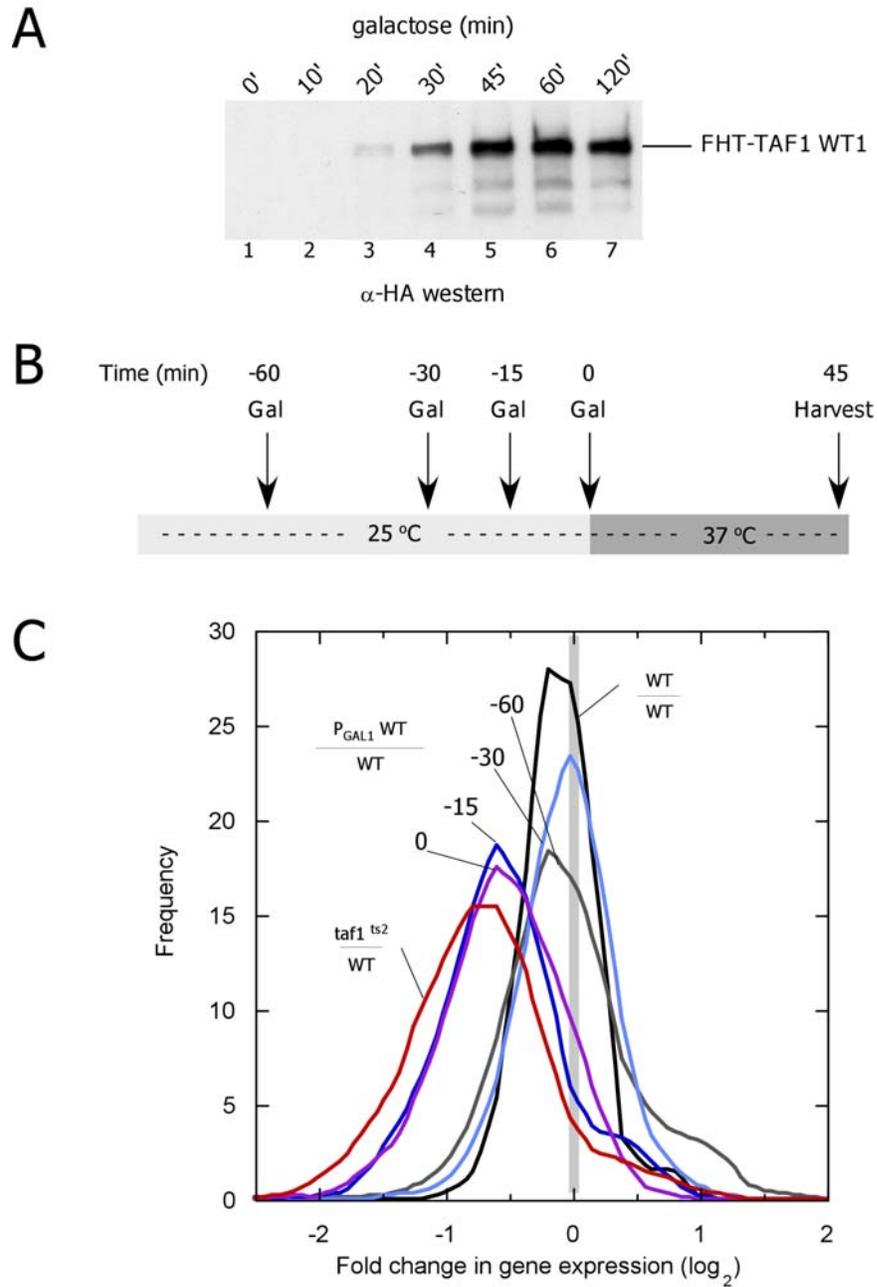


Figure 3-6: Replacement of endogenous TAF1 with galactose-induced TAF1.

A, Kinetics of GAL1 induction of TAF1. Cultures were grown in CSM-LEU raffinose media at room temperature and induced with 2% galactose. 0.5 OD600 aliquots were removed at the indicated time intervals after addition of galactose. TAF1 was detected by immunoblotting with HA antibodies. B, Schematic illustrating the time points at which TAF1 mutants were galactose-induced prior to heat shock (defined as time = 0). C, Frequency distribution of log<sub>2</sub> changes in gene expression, conducted at various galactose induction time points. Fold changes in gene expression for >5000 genes were determined for galactose-induced TAF1 (strain yjdi363) relative to identically treated wild type TAF1 which was under the control of its own promoter (strain yjdi375). Log<sub>2</sub> fold changes in expression were binned in increments of 0.03. The membership number in each bin was plotted as a function of the average fold change in gene expression in each bin, and fit to a smooth curve. Two independent homotypic hybridizations of yjdi375 representing no change is shown as “WT/WT”. yjdi381/yjdi375 (*taf1ts2* /WT) represents the maximal drop in expression (maximal leftward shift of the distribution).

As a control representing complete replacement of the resident TAF1 with a galactose-induced TAF1, two independent reference experiments were compared (“WT/WT”, black curve). The shape, width and peak location of this curve represents no change in expression and thus is diagnostic of complete substitution. At the other end of the spectrum, use of a galactose-induced null TAF1 (“*taf1<sup>ts2</sup>*/WT”, red curve) is diagnostic of no replacement, and represents the maximal decrease in mRNA expression. As shown in Figure 3-6 C, induction of TAF1 either 30 or 60 minutes prior to heat shock resulted in no decrease in mRNA levels. However, induction 15 min. prior to heat shock or at the same time that the cells were heat shocked (“-15” or “0” curves) lead to a distribution profile that was more similar to the null control, suggesting that approximately 30 min. is required for sufficient levels of TAF1 to be expressed and incorporated into a functional TFIID complex.

Based upon this study we chose to induce the TAF1 mutants 30 min. prior to heat shock. After an additional 45 min. at 37 °C, cells were harvested and microarray analyses performed. Figure 3-7 displays a cluster plot of the genome-wide changes in expression relative to an untagged galactose-inducible wild type TAF1. Only those genes that contained quality data in six of the seven experiments and showed greater than 1.7 fold change in gene expression in at least one experiment are shown. Each row corresponds to a gene and each column a particular mutant. Decreases in expression are indicated by green, increases by red, black is no change, and grey is no data. Data were clustered using the K-means algorithm into five clusters. Since three of the clusters were visually similar, they were merged to form a single large cluster (Figure 3-7, Cluster 1). At nearly all genes, expression of epitope tagged wild type TAF1 (WT1) functionally replaced the *taf1<sup>ts2</sup>* allele, yielding no changes in gene expression, as expected. Galactose induction of a null TAF1 allele lead to substantial decreases in expression at about 90% of all genes (Cluster 1), reflecting the previous determination that TFIID contributes to the expression of ~90% of the yeast genome (Huisinga and Pugh 2004). Similar drops in expression for these genes were observed for all other TAF1 mutants, suggesting that virtually all TAF1-dependent genes require all four functional domains of TAF1 for proper expression.

Figure 3-7

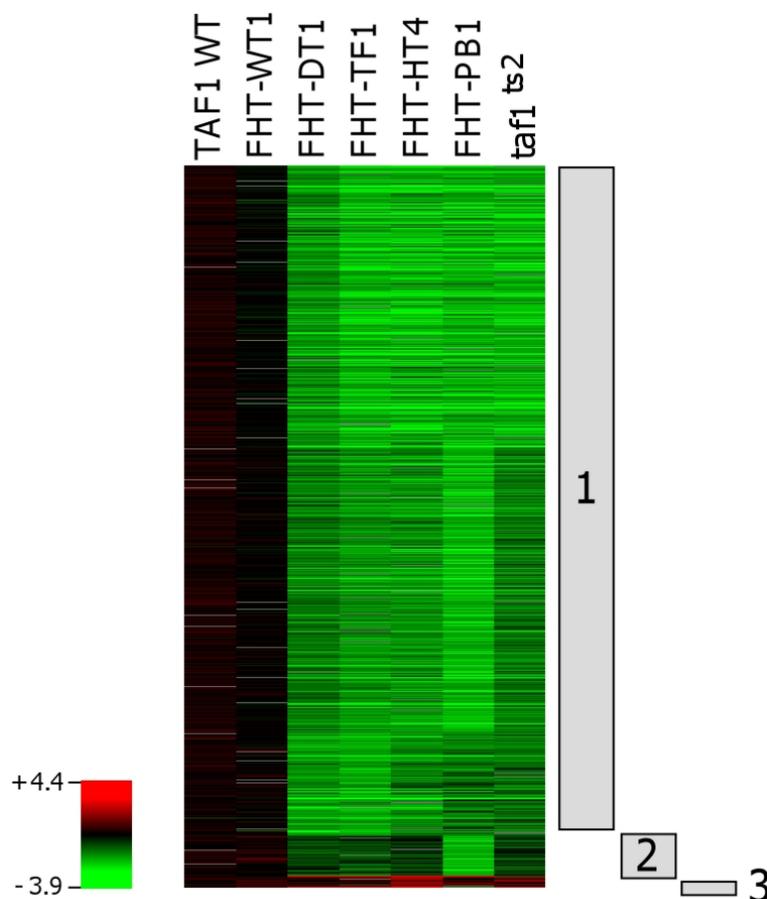


Figure 3-7: All domains of TAF1 are essential for nearly all TFIID regulated genes.

Strain harboring each of the indicated TAF1 mutants were induced with galactose 30 min prior to heat shock, then harvested 45 min. later. mRNA was isolated and co-hybridized along with a galactose-induced untagged wild type reference. Data were normalized to externally spiked *B. subtilis* controls based upon OD600 cell density measurements. Fold changes in gene expression (log<sub>2</sub> scale) were K-means clustered using Cluster software and visualized with Treeview (Eisen, Spellman et al. 1998). 2103 ORFs are present in the clusters. Membership required a log<sub>2</sub> absolute value of greater than 0.76 in at least one experiment and data in six of the seven clustered experiments. K=5 was used for the clustering, but visually similar clusters were merged to result in three clusters (1, 2, 3; right side). Rows represent individual genes and fold changes in gene expression are reflected in color intensity with red, green, black, and grey reflecting increase, decrease, no change, and no data, respectively. Columns represent data sets from each mutant. WT, wild type; WT1, FHT-tagged WT; DT1, deletion of TAND domain; TF1, deletion of TAF interacting region (residues 208-303); HT4, deletion of HAT region (residues 645-768); PB1, deletion of promoter-binding region (residues 912-992).

There are caveats to this conclusion. First, the TF1 mutant, which is defective in TAF-TAF interactions, is overexpressed and mislocalized to the cytoplasm, so it might not be available in the nucleus in sufficient quantities to rescue the *taf1<sup>ts2</sup>* allele. Nevertheless, since most genes require one or more TAFs for function, the genome-wide expression profile derived from the TF1 mutant is expected to be no different than the *taf1<sup>ts2</sup>* allele alone. A second caveat is that all nuclear-localized mutants degrade more rapidly than wild type TAF1. This rapid turnover could limit the amount of TAF1 mutants available for mediating gene expression, and thus the expression profile might be similar to the *taf1<sup>ts2</sup>* allele. However, if the TAF1 functional regions demarcated by the DT1, HT4, and PB1 mutations play genes-specific roles, there should be sufficient levels of these mutants present in the nucleus to show gene-specific patterns, which with one exception discussed below, was not observed. A third caveat is the possibility that deletion of each of these domains structurally destabilizes the entire protein thereby rendering it nonfunctional. While this possibility cannot be excluded, previous studies using similar mutations have demonstrated that at least three of the functional domains (TAND, TAF-TAF interaction domain, and the HAT domain) are functional in the absence of the remainder of the protein, suggesting that each domain is not structurally dependent upon the other (Mencia and Struhl 2001; Singh, Bland et al. 2004). It is possible that the common FHT tag placed N-terminal to each deletion contributes to the similarity of the expression data. However, this tag is also present in the WT1 strain (FHT tagged WT), which is dramatically different from the deletion mutations. The

deletion mutations are very similar to the null/*taf1<sup>ts2</sup>* strain, which does not express a functional copy of *TAF1* from the *GALI* promoter.

Both Clusters 2 and 3 largely consist of TAF-independent, SAGA-dominated genes (Huisinga and Pugh 2004). Cluster 3 is quite small, being composed of 34 genes. This cluster of genes was slightly negatively regulated by TAF1, resulting in up-regulation (red bars) in the *taf1<sup>ts2</sup>* strain. Interestingly, in the presence of the HT4 mutant these genes were strongly up-regulated, indicating that the histone acetyltransferase domain of TAF1 might negatively regulate these genes. At these SAGA-dominated genes, an activity within the HAT domain of TAF1 might antagonize the SAGA assembly pathway, without contributing substantially to transcriptional output. This activity might be an acetyltransferase activity or some other function residing in this domain.

Surprisingly, Cluster 2 genes were down-regulated by the promoter-defective PB1 mutant, whereas all other mutations had minor effects on these genes. Apparently, TAF1 that lacks the promoter recognition capability interferes with the expression of genes that are normally regulated by SAGA rather than TFIID. The basis for this is unclear, but it raises the intriguing possibility that the promoter recognition activity of TAF1 not only helps target TFIID to TAF-dependent promoters as previously suggested (Mencia and Struhl 2001), but also inhibits TFIID from interacting with TAF-independent/SAGA-dominated promoters. When this domain is removed TAF1 might then bind to these promoters in a manner that interferes with TAF-independent/SAGA-regulated transcription. Alternatively, other intact domains in the PB1 mutant (e.g. TAND or the

TAF interaction domain) could bind to and sequester components of the TAF-independent transcription pathway such as TBP.

Taken together, the findings suggest at 37 °C, conditions where yeast grow normally, the four functional domains of TAF1 are essential at virtually all TAF1-regulated genes. Thus, in order for TFIID to function properly, TAF1 must interact with TBP, TAFs, and promoter DNA at all TAF1-dependent genes, and utilize the function(s) associated with the histone acetyltransferase domain.

### **3.3.1 Future Directions**

To fully dissect the nature of the TAF1 mutants created in this study, additional experiments could be performed on similar mutants. To address the potential that the FHT tag is interfering with proper protein folding and therefore gene expression, the same deletion mutants could be constructed without the epitope tag. The original mutants and any others could be tested in microarray experiments in the context of a wild-type TAF1 and without the temperature shift described here. Effects on gene expression would be due to dominance of the TAF1 mutants over the endogenous TAF1 WT.

The TAF-interaction mutant ( $\Delta 208-303$ ) deletes a potential nuclear localization signal. This mutant displays interesting properties, including slight toxicity, inability to support viability, a high level of protein expression, and this mutant derivative of TAF1 is very stable. It would be interesting to place a functional nuclear localization signal onto this mutant and determine if the properties observed are due to the loss of interaction with the other TAFs, or mis-localization of the TAF1 mutant.

Lastly, it will be necessary to address if the FHT-TAF1 mutants are assembled into TFIID complexes under the microarray conditions tested. The experimental design requires that the FHT-TAF1 mutants are expressed from the *GALI* promoter and exchanged for the endogenous *taf1<sup>ts2</sup>* into TFIID. Additionally, since the cultures are shifted to 37 °C and we have previously demonstrated the instability of the FHT-TAF1 mutants, it is important to determine if the FHT-TAF1 mutants are stably associated with the other TAF subunits and TBP in TFIID. Epitope tagging of the TAF1 mutants provides a uniform method for immunoprecipitation of TAF1 and associated factors.

### 3.4 Materials and Methods

#### 3.4.1 Plasmids

YCp50 (*TAF1* WT, *URA3*), and pRS313 *taf1<sup>ts2</sup> HIS3* (Reese, Apone et al. 1994) were gifts from Joe Reese. pYN2 (*TAF145* WT, *TRP1*) (Kokubo, Swanson et al. 1998) was a gift from Tetsuro Kokubo. pJI11 (pRS315 *taf1<sup>ts2</sup> LEU2*) and pJI12 (pRS315 *TAF1* WT *LEU2*) were created by amplifying the *TAF1* gene from pRS313 *taf1<sup>ts2</sup>* or pYN2 respectively with *taf1 ts2* oligonucleotides (Table 3-1 ) containing Not I and Sal I restriction sites. The 4456 base pair (bp) PCR products were digested with Not I and Sal I (New England Biolabs) (4442 bp) and ligated into digested pRS315 (5953 bp) to create 10396 bp pJI11, pJI12. PCR product contains 494 bp upstream of *TAF1* ORF and 741 bp downstream. To confirm *taf1<sup>ts2</sup>* temperature sensitive (ts) phenotype pJI11 and pJI12 were transformed into Y13.2 (Kokubo, Swanson et al. 1998) and plated on CSM-LEU.

pYN1 (*TAF1* WT *URA3*) was shuffled out of Y13.2 by plating cells on CSM-LEU+5-FOA (Zymo Research). Cells were then grown at 23 °C or 37 °C for 3 days on CSM-LEU (dex) plates to confirm its phenotype. The temperature sensitive phenotype was also confirmed in the *taf1::KanMX* null strain (yjdi381) on CSM-LEU (dex).

pCALF-T(PGK) (Kou, Irvin et al. 2003) was converted to pCALF-FHT-T(PGK) 2.2 by inserting a 66bp bp HIS-TEV oligo into a Nde I site downstream of the coding sequence for HA. pUG6-FHT-P (4170 bp) was made by PCR amplifying 259 bp containing the FHT sequence from pCALFHT-T(PGK) 2.2 plasmid. PCR product was digested with Sal I and 161 bp was ligated into Sal I-digested pUG6 plasmid (4009 bp) such that the orientation is *FHT-loxP-KanMX-loxP*.

### 3.4.1 FHT-TAF1 mutant strains

*Saccharomyces cerevisiae* strain BY4743 (Brachmann, Davies et al. 1998) (Invitrogen) was used as the parental strain. Initially, the strain was transformed with pSH47 (*URA3*) (Guldener, Heck et al. 1996) encoding galactose inducible Cre recombinase. 70-mer oligonucleotides F4 and R2 (Table 3-1; regions of homology to *TAF1* are in bold) were used to PCR amplify 1991 bp of pFA6a-His3MX6-PGAL1 containing the *HIS3* gene and *GALI* promoter (Longtine, McKenzie et al. 1998). The PCR product was transformed into BY4743 using the high-efficiency lithium acetate method (Guldener, Heck et al. 1996) to replace 550 bp of the endogenous *TAF1* promoter with the *GALI* promoter, creating strain yLAC3. His<sup>+</sup> homologous recombination

transformants were selected on CSM-HIS-URA (dextrose) media and verified with colony PCR.

Regions of *TAF1* were deleted by replacing coding sequences with an FHT tag. The FHT tag encodes three HA (FH) repeats, a hexa-histidine (HH) sequence and the TEV protease sequence (TH). The kanamycin resistance region of pUG6-FHT-p was PCR amplified with 68-mer oligonucleotides with 50 bp homology to distinct regions of *TAF1* (Table 3-1, Figure 3-2). 1826 bp PCR products were transformed into yLAC3 and were selected on CSM-HIS-URA (dex) plates containing 500 ug/mL G418 (Gibco). The Kanamycin resistance cassette flanked by *loxP* sites was removed by induction of Cre recombinase with 2% galactose for 4 hours, leaving the FHT tag N-terminal to the mutation in *TAF1*. Kanamycin sensitive colonies were identified by replica plating on media containing and lacking G418. Additionally, mutations were verified by colony PCR with primers specific to each mutation.

#### 3.4.1.1 Haploids

Kanamycin sensitive FHT-TAF1 strains (Table 3-2) were plated on CSM-HIS + 5-FOA to select cells having lost pSH47 (Adams, Gottschling et al. 1997) and verified by replica plating on CSM-HIS and CSM-HIS-URA. Strains were then transformed with YCp50 (*TAF1* WT, *URA3*) and transformants selected on CSM-HIS-URA media. Strains were plated on pre-sporulation media (1% yeast extract, 2% peptone, 10% dextrose) for 2 days at 30 °C. Cells were cultured in sporulation media (0.3% potassium acetate, 0.02% raffinose) for 3 days at 30 °C. 200 uL of the culture was pelleted, resuspend in 1.2 M

sorbitol, 10 mM Tris pH 7.4, and treated with 20T (1 mg/mL) zymolyase (ICN) at room temperature (RT) for 20 minutes. Tetrads were dissected according to standard yeast techniques on YPD plates. Spores were replica plated onto CSM-HIS-URA media to select for *HIS3* (and therefore *GAL1* promoter). Mating type of the *taf1* strains were confirmed with *MAT A* and *MAT alpha* sex tester strains. *MAT alpha leu<sup>-</sup> HIS<sup>+</sup> LYS<sup>+</sup>* tetrads were selected. Strains were then transformed with pJI11 (*taf1 ts2, LEU2*) or pJI12 (*TAF1 WT, LEU2*) and selected on CSM-LEU media. Cells which lost YCp50 (*TAF1 WT, URA3*) were selected by plating on CSM-LEU + 5-FOA.

### 3.4.1 PCR

1X 25 mM magnesium chloride (Gene Choice), 2.5U Taq Polymerase (Gene Choice), 0.0002U Pfu Polymerase (Stratagene), 0.4mM dNTPs, and 0.2uM of each primer was used per 50 uL reaction for 32 cycles.

Table 3-1

Table 3-1: Oligonucleotides used in this study.

Primer name	Primer sequence <sup>3</sup> (5'–3')							
	10	20	30	40	50	60	70	
TAF1 F4	AGATA	ACCCAGGAATGGCTTCACTATTACCGACAGCGCCTCTTATATCAT						gaattcgagctcgtttaaac
TAF1 R2	TCATCTTCGTTGGCCAAGTTGGTCTTGCCGGATCCCTGCTGCTTTACCAT							cattttgagatccgggtttt
WT1F1	TAAACACAGAGAGAAAAAGAAGTACAACAGGAGTATAAGGCGATATGGTATA							ACCCATACGATGTTCCCT
WT1R2	TATGCTTCATCTTCGTTGGCCAAGTTGGTCTTGCCGGATCCCTGCTGCTTAGTGGATCTGATATCACC							
DT1F1	GTACAACAGGAGTATAAGGCGATATGGTAAAGCAGCAGGGATCCGGCAAGTACCCATACGATGTTCCCT							
DT1R2	AAACTATTTGAGTCGATACCGAGTACGGCACCGTTCTCGTTTACATCATCAGTGGATCTGATATCACC							
TF1F1	GCAAGCATAAGAAAGCCACCAAATTAGACTTGATAAACCATGAGAAGTATTACCCATACGATGTTCCCT							
TF1R2	TTTTTCATCATGAATAATCTTTCTTTTTTTTTGTTGCTCTTTAATAGGGAAAGTGGATCTGATATCACC							
HT4F1	TATCAGGAACAGATTTTCTTCTGACAAAAAGTTCCGGATTTGGTATAAGCTACCCATACGATGTTCCCT							
HT4R2	TTTAGCTTAGAATCAAATTATATGCTTCATTATCTTCTTGGAATTGTAAAGTGGATCTGATATCACC							
PB1F1	CTGATGAGATTAATCAGACCAACAAGCATGTTAAGACGGATAGAGATGATTACCCATACGATGTTCCCT							
PB1R2	TCTGTTCTCGTGGCACCACCATTTCTCTTCGAATTTTGCCTTGCTGCTCTAGTGGATCTGATATCACC							
taf1 ts2 Not I	GTGGG <b>GCGGCCG</b> CGTATCACCCCTTAGTGTGC							
taf1 ts2 Sal I	GAGGG <b>TCGAC</b> AGCGGCCAAAGAAGA							
FHT 5'	GGTCTTTTCTAATTCGTAG							
FHT 3'(Sal I)	CCTCCT <b>GTCGAC</b> TTACCTTGAAAATACAA							

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<sup>3</sup> Restriction sequence in **bold**.

TAF1 F4 and TAF1 R2 (70mer) primers were used to PCR amplify the His3MX6-PGAL1 cassette from pFA6a-His3MX6-PGAL1 (Longtine, McKenzie et al. 1998). These primers contain 50 nucleotides of homology to *TAF1* ORF and upstream of *TAF1* for homologous recombination. 68mer primers were used to PCR amplify the *kanMX* cassette from pUG6-FHT-P. These primers contain 50 nucleotides of homology to regions in *TAF1* for homologous recombination.

Table 3-2

Table 3-2: Yeast Strains used in this study

Strains	Promoter	TAF1 allele	Plasmid	MAT	Reference
BY4743	TAF1	WT		diploid	<sup>4</sup>
yLAC3	GAL1	WT	pSH47	diploid	this study
yjdi307	TAF1	WT	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi302	GAL1	WT	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi270	GAL1	FHT-WT1	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi275	GAL1	FHT-DT1 ( $\Delta$ 10-88)	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi280	GAL1	FHT-TF1 ( $\Delta$ 208-303)	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi288	GAL1	FHT-HT4 ( $\Delta$ 645-768)	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi295	GAL1	FHT-PB1 ( $\Delta$ 912-992)	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi352	GAL1	FHT-WT1	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi353	GAL1	FHT-WT1	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi354	GAL1	FHT-DT1 ( $\Delta$ 10-88)	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi355	GAL1	FHT-DT1 ( $\Delta$ 10-88)	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi356	GAL1	FHT-TF1 ( $\Delta$ 208-303)	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi357	GAL1	FHT-TF1 ( $\Delta$ 208-303)	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi358	GAL1	FHT-HT4 ( $\Delta$ 645-768)	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi359	GAL1	FHT-HT4 ( $\Delta$ 645-768)	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi360	GAL1	FHT-PB1 ( $\Delta$ 912-992)	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi361	GAL1	FHT-PB1 ( $\Delta$ 912-992)	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi362	GAL1	WT	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi363	GAL1	WT	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi366	TAF1	WT	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi367	TAF1	WT	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
yjdi375	GAL1	<i>taf1::kanMX6</i>	pJI12 <i>TAF1</i> WT <i>LEU2</i>	alpha	this study
yjdi379	GAL1	<i>taf1::kanMX6</i>	YCp50 <i>TAF1</i> WT <i>URA3</i>	alpha	this study
yjdi381	GAL1	<i>taf1::kanMX6</i>	pJI11 <i>taf1</i> ts2 <i>LEU2</i>	alpha	this study
Y13.2	TAF1	$\Delta$ TAF1	pYN1 <i>TAF1</i> WT, <i>URA3</i>	alpha	<sup>5</sup>

<sup>4</sup> Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter and J. D. Boeke (1998). "Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications." *Yeast* **14**(2): 115-32.

### 3.4.1 Cell Growth/Lethality/Dominant Toxicity Assays

**Viability:** *MAT* alpha haploid strains carrying YCp50 (*TAF1* WT, *URA3*) were grown at 25 °C in YPD. Cells were diluted into YPR + 0.2% galactose and grown to mid log phase. 0.5 OD<sub>600</sub> Units of cells were removed and 5 uL of 10-fold serial dilutions were plated. Cells having lost the YCp50 plasmid were selected by growing on CSM + 5-FOA with 2% dextrose or 2% galactose at 25 °C or 37 °C.

**Toxicity:** *MAT* alpha haploid strains carrying pJII2 (*TAF1* WT *LEU2*) plasmids were grown in CSM-LEU raffinose to mid log phase. 0.5 OD<sub>600</sub> of cells were serially diluted 10fold and 5 uL was plated on CSM-LEU +2% dextrose or 2% galactose at 19 °C, 25 °C, and 37 °C. Photographs were taken after 96 hours for 25 °C and 37 °C plates and after 120 hours for 19 °C plates.

### 3.4.1 Galactose Shutoff

FHT-TAF1 strains were grown in YPR at 25 °C until OD<sub>600</sub> ~0.8. Galactose was added to 2% and strains were incubated at 25 °C for 45 minutes. Dextrose was then added to 2% and the cultures were placed in 37 °C water bath. Equal volume aliquots were removed at 15, 30, 45, 60, 120, and 180 minutes after addition of dextrose. After western blotting, quantitation was performed on four independent replicates using Molecular Dynamics densitometer and ImageQuant software. Exposure times were chosen so that all signals were in the linear range of detection. Local background was

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<sup>5</sup> Kokubo, T., M. J. Swanson, J. I. Nishikawa, A. G. Hinnebusch and Y. Nakatani (1998). "The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA- binding protein." *Mol Cell Biol* **18**(2): 1003-12.

subtracted from signal band at each time point. Intensity peaked at 15 minutes in dextrose (at 37 °C) for all strains. This value was set to 100%, and the percent of TAF1 remaining over time was plotted. Whole cells containing constitutively expressed HA-Bdf1 were loaded in each lane of the western blots as an internal control for protein extraction, recovery, transfer, and immunodetection, but were not included in the quantitation.

### **3.4.1 Immunofluorescence**

Cultures were grown in YPR overnight at 30 °C, diluted to 0.2 OD and grown for 3.5 hours in YPR + 2% galactose at 30 °C. 2 OD Units were fixed in 3.7% formaldehyde, treated with zymolyase and bound to polylysine coated slides. FHT-tagged TAF1 proteins were visualized by incubating with  $\alpha$ -HA.11 monoclonal antibodies (1:1000, Babco) and then with goat anti-mouse IgG-Alexa Fluor 488 (Molecular Probes). 4',6-Diamidino-2-phenylindole (DAPI) was used to visualize nucleic acids (Manzini, Barcellona et al. 1983). Samples were viewed on a ZEISS Axioplan epifluorescence microscope (Carl Zeiss, Inc. Thornwood, NY). TIFF images were collected using a Spot2 cooled CCD digital camera (Diagnostic Instruments, Sterling Heights, MI).

### 3.4.1 Western Blotting

Cells were washed in 0.5 mL 0.1 M sodium hydroxide for 5 minutes at room temperature, spun and resuspended in 2x protein sample buffer, and heated to 95 °C (Kushnirov 2000). HA-TAF1 mutants were electrophoresed in 7.8% Bis-acrylamide gels (PAGE) and transferred to nitrocellulose in 80% Tris-Glycine-sodium dodecyl sulfate/20% methanol for 120 minutes at 1.0 Amps. FHT-TAF1 mutants were detected with a-HA (HA.11, Babco) and a-mouse-HRP antibodies (Amersham), exposed to Hyperfilm (Amersham) with ECL (Amersham).

### 3.4.1 Galactose Additiong Timing

A) TAF1 production after galactose induction. Cultures were grown in CSM-LEU raffinose at room temperature and induced with 2% galactose. 0.5 OD aliquots were removed in 10 minute intervals after addition of galactose and immunoblotted. B) TAF1 induction in microarrays. Microarray cultures were grown in CSM-LEU raffinose at room temperate until OD ~0.8. Galactose was added to 2% at 0, 15, 30 or 60 minutes before shifting culture to 37 °C with warm CSM-LEU + 2% galactose. After shift to 37°C, the cultures were incubated for 45 minutes before harvesting and use in expression profiling. C) Histogram of log<sub>2</sub> expression profile as function of time in galactose before temperature shift. Strain yjdi375 ( $P_{TAF1}$ -TAF1 WT +  $taf1^{ts2}$ ) was used as reference. Test strains were yjdi363( $P_{GALI}$ -TAF1 WT +  $taf1^{ts2}$ ) and yjdi381 ( $P_{GALI}$ - $taf1::kanMX$  +  $taf1^{ts2}$ ).

### 3.4.1 Microarray Analysis

Microarrays were performed essentially as described (Chitikila, Huisinga et al. 2002; Huisinga and Pugh 2004). Briefly, cultures were grown at ~24 °C in CSM-LEU +3% raffinose to an OD<sub>600</sub> of ~0.8. FHT-TAF1 mutants were induced by adding galactose to 2% 30 minutes prior to temperature shift. Cultures were shifted to 37 °C by adding an equal volume of warm CSM-LEU + 2% galactose and placed in 37 °C incubator for 45 minutes to inactivate *taf1*<sup>ts2</sup>. Cells were harvested by centrifugation at room temperature, washed in RNase free (DEPC treated) ddH<sub>2</sub>O and frozen in liquid nitrogen.

Total RNA and poly(A<sup>+</sup>) mRNA purification, reverse transcription and labeling with fluorescent dyes (Cy3, Cy5 (Amersham)), hybridization, and scanning were all performed as described (Chitikila, Huisinga et al. 2002; Huisinga and Pugh 2004). 4 ug of mRNA was used for hybridizations. Slides were treated with Dye Saver<sup>2</sup> (Genisphere) according to manufacturer's instructions to preserve signal intensity. R software was used to mode-center replicates (dye swaps) (Ihaka 1996). *B. subtilis* transcripts (*Phe*, *Lys*, *Dap*, *Thr*, *Trp*) were added to each culture prior to total RNA isolation based on OD<sub>600</sub> units. These hybridize to cognate spots on each of the 16 grids per microarray slide. R output (mode centered) data was normalized by the spiking controls.

Genes were filtered using several criteria to minimize false positives. 1) Genes were eliminated if their signal on the array was greater than 25% saturated. 2) The mean foreground signal minus the median background signal had to be greater than standard deviation of background signal. 3) Quality data was needed from both replicates of the

dye swap. 4) The directional change of the mutant's signal (relative to reference) had to be equivalent in the replicates.

K-means clustering was performed using Cluster (Eisen, Spellman et al. 1998) on 2103 genes that contained data in 80% of the experiments and had a change of at least 1.7 fold in one of the mutants. K was chosen to equal 5 clusters (K=5), but three clusters were later merged due to a high degree of similarity. Clustering information was visualized using Treeview (Eisen, Spellman et al. 1998).

### 3.5 Bibliography

- Adams, A., D. E. Gottschling, C. A. Kaiser and T. Stearns (1997). Methods in Yeast Genetics. Cold Spring Harbor, Cold Spring Harbor Laboratory.
- Bai, Y., G. M. Perez, J. M. Beechem and P. A. Weil (1997). "Structure-function analysis of TAF130: identification and characterization of a high-affinity TATA-binding protein interaction domain in the N terminus of yeast TAF(II)130." Mol Cell Biol **17**(6): 3081-93.
- Basehoar, A. D., S. J. Zanton and B. F. Pugh (2004). "Identification and distinct regulation of yeast TATA box-containing genes." Cell **116**(5): 699-709.
- Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter and J. D. Boeke (1998). "Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications." Yeast **14**(2): 115-32.
- Chen, J. L., L. D. Attardi, C. P. Verrijzer, K. Yokomori and R. Tjian (1994). "Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators." Cell **79**(1): 93-105.
- Chitikila, C., K. L. Huisinga, J. D. Irvin, A. D. Basehoar and B. F. Pugh (2002). "Interplay of TBP Inhibitors in Global Transcriptional Control." Molecular Cell.
- Dingwall, C. and R. A. Laskey (1991). "Nuclear targeting sequences--a consensus?" Trends Biochem Sci **16**(12): 478-81.

- Durso, R. J., A. K. Fisher, T. J. Albright-Frey and J. C. Reese (2001). "Analysis of TAF90 mutants displaying allele-specific and broad defects in transcription." Mol Cell Biol **21**(21): 7331-44.
- Eisen, M. B., P. T. Spellman, P. O. Brown and D. Botstein (1998). "Cluster analysis and display of genome-wide expression patterns." Proc Natl Acad Sci U S A **95**(25): 14863-8.
- Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein and P. O. Brown (2000). "Genomic expression programs in the response of yeast cells to environmental changes." Mol Biol Cell **11**(12): 4241-57.
- Grant, P. A., D. Schieltz, M. G. Pray-Grant, D. J. Steger, J. C. Reese, J. R. Yates, 3rd and J. L. Workman (1998). "A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation." Cell **94**(1): 45-53.
- Green, M. R. (2000). "TBP-associated factors (TAFII)s: multiple, selective transcriptional mediators in common complexes." Trends Biochem Sci. **25**: 59-63.
- Guldener, U., S. Heck, T. Fielder, J. Beinhauer and J. H. Hegemann (1996). "A new efficient gene disruption cassette for repeated use in budding yeast." Nucleic Acids Res **24**(13): 2519-24.
- Holstege, F. C. P., E. G. Jennings, J. J. Wyrick, T. I. Lee, C. J. Hengartner, M. R. Green, T. R. Golub, E. S. Lander and R. A. Young (1998). "Dissecting the regulatory circuitry of a eukaryotic genome." Cell **95**: 717-728.

- Huisinga, K. L. and B. F. Pugh (2004). "A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*." Mol Cell **13**(4): 573-85.
- Ihaka, R. G., R (1996). "R: A Language for Data Analysis and Graphics." Journal of Computational and Graphical Statistics **5**(3): 299-314.
- Kirchner, J., S. L. Sanders, E. Klebanow and P. A. Weil (2001). "Molecular genetic dissection of TAF25, an essential yeast gene encoding a subunit shared by TFIID and SAGA multiprotein transcription factors." Mol Cell Biol **21**(19): 6668-80.
- Kirschner, D. B., E. vom Baur, C. Thibault, S. L. Sanders, Y. G. Gangloff, I. Davidson, P. A. Weil and L. Tora (2002). "Distinct mutations in yeast TAF(II)25 differentially affect the composition of TFIID and SAGA complexes as well as global gene expression patterns." Mol Cell Biol **22**(9): 3178-93.
- Kokubo, T., M. J. Swanson, J. I. Nishikawa, A. G. Hinnebusch and Y. Nakatani (1998). "The yeast TAF145 inhibitory domain and TFIIA competitively bind to TATA-binding protein." Mol Cell Biol **18**(2): 1003-12.
- Kotani, T., K. Banno, M. Ikura, A. G. Hinnebusch, Y. Nakatani, M. Kawaichi and T. Kokubo (2000). "A role of transcriptional activators as antirepressors for the autoinhibitory activity of TATA box binding of transcription factor IID." Proc Natl Acad Sci U S A **97**(13): 7178-83.
- Kou, H., J. D. Irvin, K. L. Huisinga, M. Mitra and B. F. Pugh (2003). "Structural and functional analysis of mutations along the crystallographic dimer interface of the yeast TATA binding protein." Mol Cell Biol **23**(9): 3186-201.

- Kushnirov, V. V. (2000). "Rapid and reliable protein extraction from yeast." Yeast **16**(9): 857-60.
- Lee, T. I., H. C. Causton, F. C. Hostege, W. C. Shen, N. Hannett, E. G. Jennings, F. Winston, M. R. Green and R. A. Young (2000). "Redundant roles for the TFIID and SAGA complexes in global transcription." Nature **405**: 701-704.
- Longtine, M. S., A. McKenzie, 3rd, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen and J. R. Pringle (1998). "Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*." Yeast **14**(10): 953-61.
- Manzini, G., M. L. Barcellona, M. Avitabile and F. Quadrifoglio (1983). "Interaction of diamidino-2-phenylindole (DAPI) with natural and synthetic nucleic acids." Nucleic Acids Res **11**(24): 8861-76.
- Mencia, M. and K. Struhl (2001). "Region of yeast TAF 130 required for TFIID to associate with promoters." Mol Cell Biol **21**(4): 1145-54.
- Mizzen, C. A., X. J. Yang, T. Kokubo, J. E. Brownell, A. J. Bannister, T. Owen-Hughes, J. Workman, L. Wang, S. L. Berger, T. Kouzarides, Y. Nakatani and C. D. Allis (1996). "The TAF(II)250 subunit of TFIID has histone acetyltransferase activity." Cell **87**(7): 1261-70.
- Naar, A. M., B. D. Lemon and R. Tjian (2001). "Transcriptional coactivator complexes." Annu Rev Biochem **70**: 475-501.
- Reese, J. C., L. Apone, S. S. Walker, L. A. Griffin and M. R. Green (1994). "Yeast TAFIIs in a multisubunit complex required for activated transcription." Nature **371**(6497): 523-7.

- Ruzzi, M., A. Marconi, M. Saliola, L. Fabiani, F. Montebove and L. Frontali (1997). "The sequence of a 8 kb segment on the right arm of yeast chromosome VII identifies four new open reading frames and the genes for yTAFII145." Yeast **13**(4): 365-8.
- Sanders, S. L., K. A. Garbett and P. A. Weil (2002). "Molecular characterization of *Saccharomyces cerevisiae* TFIID." Mol Cell Biol **22**(16): 6000-13.
- Shen, W. C., S. R. Bhaumik, H. C. Causton, I. Simon, X. Zhu, E. G. Jennings, T. H. Wang, R. A. Young and M. R. Green (2003). "Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly." Embo J **22**(13): 3395-402.
- Singh, M. V., C. E. Bland and P. A. Weil (2004). "Molecular and genetic characterization of a Taf1p domain essential for yeast TFIID assembly." Mol Cell Biol **24**(11): 4929-42.
- Thompson, J. D., D. G. Higgins and T. J. Gibson (1994). "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." Nucleic Acids Res **22**(22): 4673-80.
- Walker, S. S., W. C. Shen, J. C. Reese, L. M. Apone and M. R. Green (1997). "Yeast TAF(II)145 required for transcription of G1/S cyclin genes and regulated by the cellular growth state." Cell **90**(4): 607-14.
- Zanton, S. J. and B. F. Pugh (2004). "Changes in genomewide occupancy of core transcriptional regulators during heat stress." Proc Natl Acad Sci U S A **101**(48): 16843-8.

## Chapter 4

### Genome-wide Analysis of Functional Domains of Yeast Transcription Factor MOT1

#### 4.1 Abstract

The ~210KDa yeast transcription factor MOT1 plays both positive and negative roles in gene expression. A N-terminal portion of MOT1 binds to TBP, and through the action of the C-terminal ATPase domain, removes TBP from DNA. A large central region of the protein has no known function. It has been proposed that MOT1 regulates the level of TBP bound to promoter DNA, and can redistribute TBP through the genome, thereby playing both positive and negative roles in expression. Functional analysis of separate regions of MOT1 is limited due to cellular requirement of MOT1 for viability. To study separate regions of MOT1, we introduced galactose-inducible deletion derivatives into a temperature sensitive (*mot1-42*) yeast strain. After galactose induction and temperature inactivation of the temperature sensitive *mot1-42*, we examined the effects of these mutants on genome-wide expression. The requirements for each region of MOT1 varied across the genome. TFIID dominated genes are positively regulated by MOT1 and generally required all functions of MOT1 for proper expression. Stress response genes, which are typically regulated by the SAGA complex, are negatively regulated by MOT1. The N-terminal region of MOT1 necessary for interaction with TBP is critical for MOT1's ability to repress these genes. We also find that genes that are most negatively regulated by MOT1 tend to have high MOT1 occupancy at their

promoters. Additionally, at genes where MOT1 acts positively, NC2 acts positively. Likewise, where MOT1 acts to repress transcription, NC2 also acts negatively. These data suggest a dual method of TBP repression by NC2 and MOT1 at SAGA dominated genes, governed largely by MOT1-TBP interactions.

#### 4.2 Introduction

MOT1 (Bur3/BTAF1) is an essential yeast protein in the Swi2/Snf2 family of ATPases that operates both as a repressor and a co-activator. MOT1 dissociates TATA bound TBP from DNA in an ATP-dependent manner, but has no DNA sequence specificity and does not use helicase activity for TBP removal. MOT1 acts specifically at pol II genes, but only regulates less than 10% of the yeast genome. MOT1's role in transcription has been demonstrated as both positive and negative, depending on particular subsets of genes and conditions studied. It has been proposed that MOT1 recycles non-complexed TBP from inactive promoters, allowing formation of active pre-initiation complexes. Alternatively, MOT1 has been thought to deliver TBP to inactive promoters, thereby increasing transcription of these genes.

Many of the previous studies have concluded that MOT1's activities are diverse and often gene-specific. MOT1 can act positively at some genes and negatively at others. While a few MOT1 activated and repressed genes have been described under specific conditions, much is unclear about MOT1's genome-wide role in gene expression. Additionally, different alleles of *MOT1* have given surprisingly different results (Andrau et al., 2002; Dasgupta et al., 2002).

MOT1 is required for nucleosomal remodeling and activation at some promoters (*GALI*), while at other promoters (*BNAI*, *URAI*) there is no detectable chromatin structure change (Dasgupta et al., 2005; Topalidou et al., 2004). The demonstration that MOT1 does not remodel chromatin to allow more permissive TBP-DNA binding on some promoters (*BNAI*, *URAI*) (Dasgupta et al., 2005) strengthens the model that MOT1's method of action is to redistribute TBP from one promoter to another through the genome, or similarly, to clear TBP off of a promoter, allowing a functional TBP/PIC to bind (Adamkewicz et al., 2001; Muldrow et al., 1999).

Reconfiguration of the yeast genome following acute stress, such as heat shock, involves dramatic changes in transcription factor binding and transcriptional activity (Gasch et al., 2001; Gasch et al., 2000; Gasch and Werner-Washburne, 2002; Zanton and Pugh, 2004). Several studies show that MOT1 operates at stress induced genes, whose regulation is typically dominated by the SAGA-coactivator complex instead of TFIID (Gumbs et al., 2003; Huisinga and Pugh, 2004) (Geisberg and Struhl, 2004; Hahn et al., 2004; Kim and Iyer, 2004; Zanton and Pugh, 2004). The SAGA-dominated genes are characterized as stress response genes, containing a consensus TATA box and are regulated by a large number of factors, including MOT1 (Basehoar et al., 2004; Dasgupta et al., 2005; Gasch et al., 2000; Geisberg et al., 2002; Geisberg and Struhl, 2004; Gumbs et al., 2003; Huisinga and Pugh, 2004; Zanton and Pugh, 2004). MOT1 has been linked to the SAGA complex subunit Spt3 through genetic studies (Collart, 1996; Madison and Winston, 1997). There are conflicting reports on the association of MOT1 and TFIID on promoters *in vivo* (Andrau et al., 2002; Dasgupta et al., 2005; Geisberg and Struhl, 2004). Following environmental stress, MOT1 is co-incident with TFIIB and RNA polymerase

(Geisberg and Struhl, 2004). However, the physiological relevance of TBP redistribution by MOT1 at all genes under stressed and non-stressed conditions is not known.

Early studies concluded that MOT1 required a naked DNA template upstream of TBP-DNA complex in order to bind to TBP (Darst et al., 2001), but this was later shown to be an artifact of the gel mobility shift assay conditions used (Adamkewicz et al., 2001). Fluorescence anisotropy spectroscopy measurements show that MOT1 does not require a DNA template (“handle”) for efficient dissociation of TBP from DNA (Adamkewicz et al., 2001; Gumbs et al., 2003). Mutations to *MOT1* have been shown to increase transcription and to bypass the requirement for UAS element (i.e. *bur3-1*) (Prelich, 1997; Prelich and Winston, 1993). Additionally, *mot1* mutants alter the length of some known MOT1-dependent transcripts, indicating a role for MOT1 in start site location or RNA processing (Dasgupta et al., 2002).

The ATPase domain of MOT1 is required for removal of TBP from DNA and for viability, indicating that TBP regulation plays a key role in cellular physiology (Adamkewicz et al., 2001; Auble et al., 1994; Auble et al., 1997; Darst et al., 2003; Dasgupta et al., 2002; Davis et al., 1992). No helicase activity has been shown for MOT1 (Darst et al., 2001).

Overexpression of mutations to the C-terminus of MOT1 (ATPase domain) but not the N-terminus (TBP interaction) are dominantly toxic *in vivo*. Overexpression of TBP or creation of mutations to the TBP binding regions of MOT1 decrease the toxicity of MOT1 ATPase mutants (Adamkewicz et al., 2001; Darst et al., 2003). From both genetic evidence as well as *in vitro* biochemical data, it was proposed that catalytically inactive MOT1 proteins (i.e. D1408N) can still interact with TBP and bind TBP into an

inactive form (Adamkewicz et al., 2001). The toxicity seen in ATPase defective *mot1* mutants that interact with TBP could be due to MOT1 retaining TBP in an inactive complex.

In yeast, MOT1 acts only on genes transcribed by Pol II, and not pol I or III (Auble et al., 1994), even though TBP binds to these promoters as well (Cormack and Struhl, 1992). Protein-DNA crosslinking followed by immunopurification provides evidence that MOT1 associates at only six highly transcribed pol III genes (Geisberg et al., 2002). The ratio of MOT1/TBP at these genes is considerably lower than pol II genes, and therefore, MOT1 is not expected to play a major role in regulation of pol III transcripts in yeast (Geisberg et al., 2002).

To study MOT1's global role in gene expression we have employed a system of galactose inducible MOT1 mutants. Four regions of MOT1 containing the ATP-dependent TBP dissociation activity, TBP binding, and additional unknown functions that contribute to viability were deleted. After galactose induction of the mutants followed by a temperature shift (25C—>37C) to inactivate a *mot1-42* temperature sensitive allele, genome wide changes in transcription were monitored through microarray analysis. Comparison of expression data to FHT-MOT1 occupancy (ChIP on chip), and change in occupancy (dChIP on chip) were made. Additionally, the MOT1 expression data was compared to publicly available microarray and ChIP on chip data sets.

## 4.3 Results

### 4.3.1 Construction of FHT-MOT1 mutants

Since *MOT1* is an essential gene, it can not be constitutively deleted. Additionally, several distinct mutations to the *MOT1* gene are highly toxic when overexpressed (Adamkewicz et al., 2001). To study effects of functional domains of *MOT1*, mutations to the *MOT1* gene were placed under control of the inducible *GALI* promoter (Figure 4-1). 550bp upstream of the *MOT1* gene was replaced with the *GALI* promoter via homologous recombination (Figure 4-1). Growth in media containing dextrose or raffinose as the carbon source would prevent expression of the *mot1* deletion mutants. Prevention of constitutive expression would limit the possibility of secondary effects observed in microarray experiments and eliminate deleterious growth effects, as previously reported for C-terminal deletions to *MOT1* (Adamkewicz et al., 2001).

Deletions to the coding sequence for *MOT1* were created through homologous recombination of a PCR-amplified DNA fragment containing the selectable marker *KanMX* bracketed by loxP recombination sites. The *KanMX* marker system was chosen for mutagenesis because the heterologous *KanMX* gene would not spuriously recombine into the yeast genome (Guldener et al., 1996; Wach et al., 1994). The *KanMX* drug resistant marker did not conflict with the genetic background of the consortium deletion strain, BY4743 (Brachmann et al., 1998). Lastly, excision of the *KanMX* gene by induction of Cre-recombinase would allow the marker to be recycled and the disrupted/mutated gene would again become a single open reading frame.

To determine the functional effects of separate domains of MOT1, four separate internal deletions were created: TBP1 ( $\Delta$ 5-80), TBP2 ( $\Delta$ 307-432); UK1 ( $\Delta$ 1090-1259), ATP1 ( $\Delta$ 1403-1479), Figure **4-1**. The creation of these mutants, including replacing 550bp of the *MOT1* promoter with the *HIS3* gene and the *GAL1* promoter was identical to the method performed for *TAF1* in Chapter 3. To screen for correct insertion of the *KanMX* PCR product into the *MOT1* ORF for each mutation, and subsequent removal via Cre recombinase, a colony PCR reaction was used, Figure **4-2**. Additionally, mutagenesis of MOT1 was verified by immunoblot analysis for the presence of an appropriate size band that reacted with HA antibodies and derived from galactose-treated but not glucose-treated cells, Figure **4-3**.

Figure 4-1

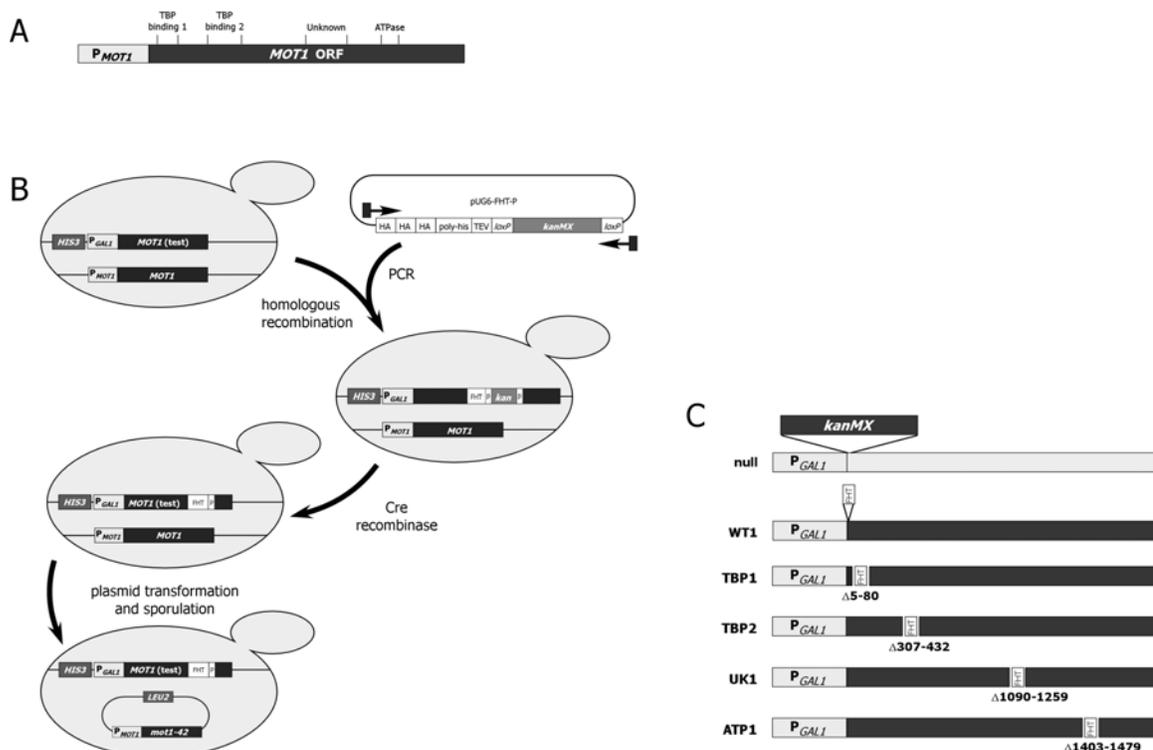
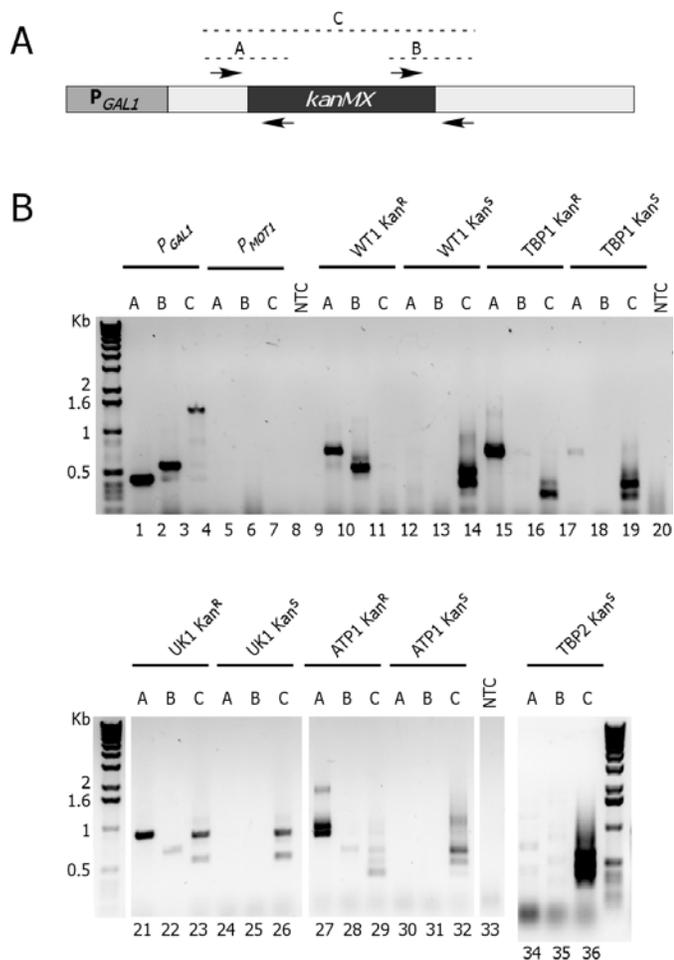


Figure 4-1: Schematic of MOT1 mutants used in this study.

(A) MOT1 was placed under control of the GAL1 promoter in a haploid yeast strain (BY4743 background). (B) Deletion derivatives of MOT1 were made via homologous recombination of a loxP-KanMX-loxP cassette. PCR-amplified cassette was transformed into diploid strain. After selection of transformants, KanMX was excised using Cre recombinase. Strains were sporulated to obtain haploid strains containing galactose inducible FHT-tagged *mot1* mutants. WT1, FHT tagged MOT1 WT; TBP1, deletion of TBP interaction region 1 (residues 5-80); TBP2, deletion of TBP-interaction region 2 (residues 307-432); UK1, unknown activity region (residues 1090-1259); ATP1, ATPase region (residues 1403-1479).

Figure 4-2

Figure 4-2: Colony PCR to screen *MOT1* mutants.

(A) Schematic of PCR reactions. For each mutation, the “A” and “B” PCR reactions were used to screen for correct position and orientation of the *KanMX* cassette, and subsequent removal after Cre recombinase. The “C” PCR reaction will detect endogenous *MOT1* WT as well as mutant *mot1*. The deletion mutations give slightly different products, corresponding to the amount of *MOT1* ORF that was deleted. (B) Colony PCR of select *MOT1* strains after *KanMX* homologous recombination (*Kan<sup>R</sup>*) and after Cre recombinase (*Kan<sup>S</sup>*). Expected PCR sizes for “A” reactions are ~825bp (depending on the individual primer pairs) and ~650bp for the “B” reactions.

Figure 4-3

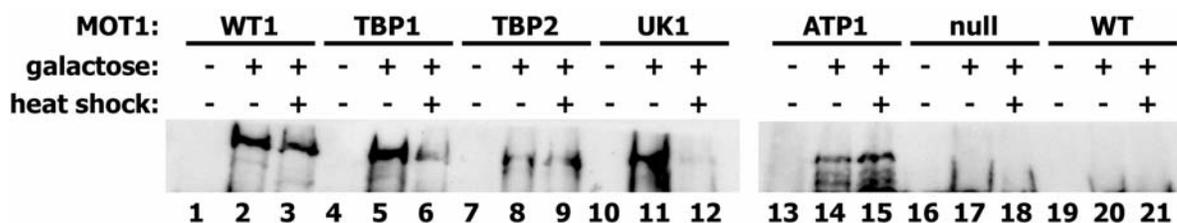


Figure 4-3: Stability of MOT1 mutants at 37 °C.

1.0 OD600 Units of cells grown in YPR media at 25 °C were removed at three stages, as indicated above each lane: 1) prior to galactose induction and heat shock (-, -), 2) after 60 min. in 2% galactose but immediately prior to heat shock (+, -), and 3) same as stage 2 but 45 min. after 37 °C heat shock. Cells were extracted in protein sample buffer, fractionated in 7.8 % SDS-PAGE denaturing gels and analyzed by immunoblotting with HA antibodies. The FHT-MOT1 derivatives are of similar size. No specific signal at the correct molecular weight was seen in the null or untagged strains (lanes 16-21). WT1, FHT tagged MOT1 WT; TBP1, deletion of TBP interaction region 1 (residues 5-80); TBP2, deletion of TBP-interaction region 2 (residues 307-432); UK1, unknown activity region (residues 1090-1259); ATP1, ATPase region (residues 1403-1479).

Mutations in the C-terminal portion of MOT1 (affecting the catalytic activity) are toxic when expressed, but mutations to the TBP interacting region (N-terminal) are not (Adamkewicz et al., 2001; Darst et al., 2003). We asked if our mutations would display similar toxic effects upon expression from the *GALI* promoter. The yeast strains (BY4743 background) used in this experiment all contained the *mot1-42* allele on a plasmid to support viability, and therefore, any deleterious effects on growth (toxicity) would be dominant. Because the *GALI* promoter is known to be MOT1 dependent (Topalidou et al., 2004), it was important to assess if these strains could grow on media containing galactose. Strain yjdi418 (*mot1::KanMX* + *mot1-42*) is able to grow on galactose plates at 20 °C and 30 °C, indicating that galactose metabolism is not significantly disrupted by the *mot1-42* allele at the permissive temperature.

Mutants were serially diluted and spotted onto complete minimal media containing dextrose or galactose. On dextrose media the mutants are not expressed, and as seen in Figure 4-5, left panel, all of the cell growth was indistinguishable at 20 °C and 30 °C. On media containing galactose all of the strains grew slightly slower, as had been seen previously, Figure 3-3. However, in contrast to previous studies (Adamkewicz et al., 2001), no toxicity was observed in any of the deletion mutants at 20 °C or 30°C. At 37 °C only the MOT1 WT and WT1 strains grew, but growth was slower than expected. None of the remaining FHT-MOT1 mutants grew, indicating they can not support viability at higher temperature. Alternatively, these mutant proteins could be toxic when overexpressed at 37 °C and this could inhibit growth.

Figure 4-4

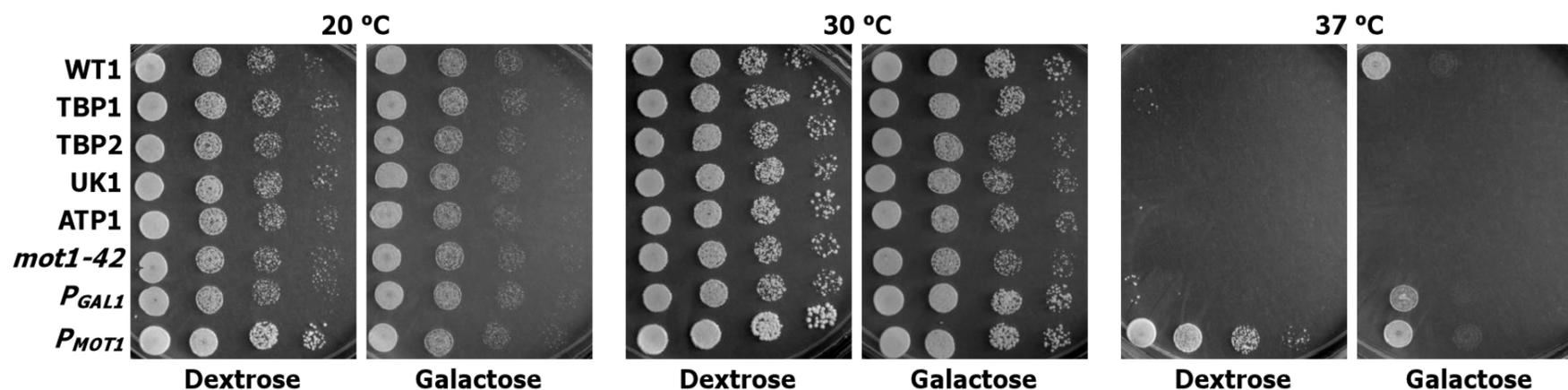


Figure 4-4: Cellular effects of expression of MOT1 mutants.

FHT-MOT1 mutant strains were grown in rich media containing raffinose as the carbon source (YPR). 10-1 fold serial dilutions were spotted onto minimal media (CSM) containing dextrose or galactose, and incubated at the indicated temperatures. WT1, FHT tagged MOT1 WT; TBP1, deletion of TBP interaction region 1 (residues 5-80); TBP2, deletion of TBP-interaction region 2 (residues 307-432); UK1, unknown activity region (residues 1090-1259); ATP1, ATPase region (residues 1403-1479). PMOT1, WT MOT1 expressed chromosomally from the MOT1 promoter; PGAL1, WT MOT1 expressed chromosomally from the GAL1 promoter.

It is unclear why deletions to the C-terminus of MOT1, which affect the catalytic ATPase activity, are very toxic at 30 °C when expressed from the *GALI* promoter (Adamkewicz et al., 2001), yet our deletion mutants UK1 and ATP1 do not show any detectable toxicity. The ATP1 deletion spans residues 1403-1479. A point mutation to this region, D1408N, which renders MOT1 catalytically inactive (Auble et al., 1994), was shown to be very toxic (Adamkewicz et al., 2001). The UK1 mutation ( $\Delta$ 1090-1259) affects the exact same residues as were previously tested (Adamkewicz et al., 2001).

We tested whole cell extracts of galactose induced MOT1 cells to determine relative levels of MOT1 expression from the *GALI* promoter. Internal deletions of MOT1 all show similar steady state levels of expression (Adamkewicz et al., 2001). We observe that all of the FHT-MOT1 deletion mutations except TBP2 are expressed at uniform levels Figure 4-3.

The lack of growth on galactose media at 37°C in any of the deletion mutants demonstrates that several regions of MOT1 contribute essential functions. To determine the genome-wide effects caused by deletion of separate regions of MOT1, we performed microarray analysis on these strains.

#### **4.3.1 MOT1 Galactose Induction**

The *mot1-42* temperature sensitive allele is the result of a single amino acid substitution (L383P) (Darst et al., 2003). Galactose induction of the FHT-MOT1 mutants followed by heat inactivation of the *mot1-42* allele at 37 °C for 45 minutes and analysis

of transcription through microarrays allows us to study the individual effects of deleting domains of MOT1 on a genome-wide scale.

To determine a proper time point for galactose induction relative to temperature shift, we performed co-hybridization microarray analysis after various lengths of induction, similar to what was previously described for TAF1. This pre-induction (before heat inactivation) test was necessary since genes involved in galactose utilization are known to be MOT1 dependent (Topalidou et al., 2004). The growth of *mot1-42* in a *mot1* null strain on galactose media (see above, Figure 4-4) indicate that galactose induction is not disrupted by the *mot1-42* allele at the permissive temperature. A *mot1::KanMX* null strain (yjdi 418) carrying the *mot1-42* allele on a *LEU2* marked plasmid (pMOT244) was used as a negative control for induction, since it does not express detectable MOT1 when induced (see Table 4-1 and Figure 4-3). For this experiment the reference strain was constructed from the same *mot1::KanMX* null mutant, but carried *MOT1* WT on a plasmid (pAV20). Since the resulting phenotype of this strain is MOT1 WT, this strain should only show effects due to changing carbon source (raffinose to galactose) and heat shock, but not effects due to induction of MOT1. Therefore, this isogenic strain would be the ideal reference strain for the galactose induction test. MOT1 reference and test strains were grown in standard rich media plus raffinose (YPR), at room temperature (~20 °C), treated with 2% galactose for various lengths of time and then shifted to 37°C by adding an equal amount of appropriately heated YPR + 2% galactose and incubated for 45 minutes. The expression levels from galactose induction of P<sub>GALI</sub>-*MOT1* WT + *mot1-42* was compared to the null/*mot1-42* and null/*MOT1* WT strains.



There were two main concerns for the pre-induction test with MOT1. First, because MOT1 has both positive and negative effects on gene expression, but works on a limited number of genes, it would be hard to define conditions that would minimize indirect effects. Secondly, since expression from the *GALI* is controlled, at least in part, by MOT1 (Topalidou et al., 2004), heat inactivation of *mot1-42* might prevent galactose-induced expression of the FHT-MOT1 mutants. Determining the length of time in galactose to precede the temperature shift that minimized indirect effects and restored *MOT1* WT profile after heat shock was critical for defining conditions to be used for the FHT-MOT1 mutant microarray experiments (see below).

We took advantage of several key pieces of information to test the design for the addition of galactose. First, we knew from previous western blotting (see Chapter 3, Figure 3-5A) that induction from the *GALI* promoter would produce a detectable amount of protein (by western blotting) in approximately 20 minutes. From the FHT-TAF1 microarrays (Chapter 3), we had evidence that induction from the *GALI* promoter would allow restoration of a WT TAF1 phenotype in after roughly 30 minutes of galactose induction. While this was slightly slower than the rate of protein production, this data was particularly important, since we had not overexpressed the other subunits of TFIID. Since FHT-TAF1 WT1 was able to recapitulate the TAF1 WT activity after a 30 minute pre-induction, this indicated that FHT-TAF1 WT was associating with the other TAFs in TFIID and replacing the endogenous taf1<sup>ts2</sup> protein. It was likely that MOT1, which acts independently of a known complex, could restore a WT MOT1 phenotype after a similar induction.

For TAF1, the bulk of the genome decreased upon heat inactivation of *taf1<sup>ts2</sup>*, evident in a histogram plot of gene expression. Very few increases in gene expression were seen after inactivation of *taf1<sup>ts2</sup>* (Huisinga and Pugh, 2004; Shen et al., 2003). However, a smaller portion of the genome is controlled by MOT1 (Dasgupta et al., 2002). Additionally, since we observe both positive and negative effects on expression, a histogram could not be used to determine a WT gene expression profile. We therefore compared the genes decreasing the most in expression (lowest 10%) from the galactose induced null + *mot1-42* to a published *mot1-14* data set (Dasgupta et al., 2002) and used the CHITEST function in EXCEL to determine significance of overlap. Comparison of the *mot1::KanMX* (null) + *mot1-42* data to the *mot1-14* data shows a very high degree of similarity (P-value  $4 \times 10^{-78}$ ). For the  $P_{GALI}$ -MOT1 WT inductions of 0, 15 or 30 minutes produced very similar changes in gene expression (P-values  $3 \times 10^{-60}$ ,  $2 \times 10^{-91}$ ,  $3 \times 10^{-39}$  respectively). However, after a 60 minute induction, a marked decrease in similarity was observed (P-value only  $8 \times 10^{-3}$ ). This dissimilarity illustrates the situation where  $P_{GALI}$  MOT1 WT + *mot1-42* behaves more like MOT1 WT than *mot1-42*. These results are summarized in Table **4-3**.

A similar test of the top 10% of data sets (genes increasing in expression) was also performed to determine the similarity of genes increasing in expression, either directly or indirectly after extended inductions. There is very little correlation between these data sets, indicating that the same genes are not increasing in expression in both the *mot1-14* control experiment and our experiments. These results are summarized in Table **4-2** and indicate that overexpression of MOT1 WT prior to heat shock causes

increased expression of very few genes. From these data, the 60 minute induction was chosen before heat inactivation of the *motI-42* allele.

Table 4-2

Table 4-2: Comparison of lowest 10% of *mot1* null and P<sub>GAL1</sub> *MOT1* WT microarray data to the lowest 10% of *mot1-14* data<sup>6</sup> for varying galactose induction times

Induction Length	Test Data	Control Data <sup>1</sup>	P-value
0 minutes	<i>mot1::KanMX</i> (null) + <i>mot1-42</i>	<i>mot1-14</i>	4 x 10 <sup>-78</sup>
0 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	3 x 10 <sup>-60</sup>
15 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	2 x 10 <sup>-91</sup>
30 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	3 x 10 <sup>-39</sup>
60 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	8 x 10 <sup>-3</sup>

Table 4-3

Table 4-3: Comparison of top 10% of *mot1* null and P<sub>GAL1</sub> *MOT1* WT microarray data to the top 10% of *mot1-14* data<sup>1</sup> for varying galactose induction times.

Induction Length	Test Data	Control Data <sup>1</sup>	P-value
0 minutes	<i>mot1::KanMX</i> (null) + <i>mot1-42</i>	<i>mot1-14</i>	5 x 10 <sup>-2</sup>
0 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	7 x 10 <sup>-2</sup>
15 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	7 x 10 <sup>-2</sup>
30 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	7 x 10 <sup>-2</sup>
60 minutes	P <sub>GAL1</sub> <i>MOT1</i> WT + <i>mot1-42</i>	<i>mot1-14</i>	1 x 10 <sup>-1</sup>

<sup>6</sup> Dasgupta, A., Darst, R.P., Martin, K.J., Afshari, C.A. and Auble, D.T. (2002) Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. *Proc Natl Acad Sci U S A*, **99**, 2666-2671.

### 4.3.1 FHT-MOT1 Deletion Mutant's Effects on Genome-Wide Expression

After determining an appropriate length of time (60 minutes) for galactose induction before temperature inactivation of the *mot1-42* temperature sensitive allele, using a null strain and comparison to published data, I performed microarrays on the FHT-MOT1 deletion mutants. The reference strain for these experiments is  $P_{GALI}$ -*MOT1* WT + *mot1-42*, which was a test strain in the previous microarray experiments. As previously described, induction of this strain for 60 minutes in 2% galactose provided ample time for production of MOT1 and restoration of transcription to a WT MOT1 level, as determined by comparison to data from *mot1-14* and WT *MOT1* expressed from the *MOT1* promoter. This 60 minute induction did not noticeably increase expression, demonstrating that induction of MOT1 did not lead to genome-wide indirect effects on transcription. The galactose inducible haploid test strains used were FHT-tagged WT *MOT1* (WT1), TBP1 ( $\Delta$ 5-80), TBP2 ( $\Delta$ 307-432), UK1 ( $\Delta$ 1090-1259), and ATP1 ( $\Delta$ 1403-1479), all carrying the *mot1-42* temperature sensitive allele on the plasmid pMOT244. See Table 4-8 for list of strains, including relevant genotype and plasmids.

Microarray data from two independent biological repeats, each labeled with a different Cy-dye (dye swap) were required to pass four filters to be included in analysis as quality data. For all data sets ~3500 genes passed the filtering criteria and were used in data analysis.

K-means clustering was used to organize patterns of similarly expressed genes (Eisen et al., 1998), Figure 4-5. Data was required to be present in 80% of the data sets

and a minimum inclusion criteria of change of at least 1.7 fold (increase or decrease) in one experiment was also required. These criteria are more relaxed than the absolute 2-fold cutoff levels that are reported in the literature (Andrau et al., 2002; Dasgupta et al., 2002), and selected 946 genes, or ~ 15% of the yeast genome. Seven clusters (K) were chosen as the maximum number of visually non-redundant clusters. These 946 genes were also hierarchically clustered to arrange the most similar mutants (data columns). Similarity between experiments is represented by dendrogram branch length.

Interestingly, the TBP1 mutation is not similar to TBP2. While the *mot1-42* allele is the result of a point mutation (L383P) (Darst et al., 2003) that is deleted in the TBP2 mutation ( $\Delta$ 307-432), the expression profile from the *mot1-42* strain was most similar to UK1 ( $\Delta$ 1090-1259). The N-terminal TBP2 deletion mutant was most similar to deletion of the catalytic ATPase activity, which is at the C-terminus of the protein.

Several important conclusions can be drawn from the clustering (Figure 4-5). First, the tagged WT (WT1, column 1) does not greatly impact gene expression, either positively or negatively. Since the N-terminus of MOT1 interacts with TBP, it is possible that this mutation could interfere with MOT1-TBP binding and MOT1's ATP-dependent removal of TBP from DNA. However, few changes in gene expression are observed after adding the FHT tag onto MOT1. Secondly, overexpression of the FHT-WT1 mutant, which supports viability, does not lead to increases in gene expression. This result shows that the conditions used for this microarray analysis do not lead to indirect effects.

Third, MOT1 has both positive and negative functions on gene expression. Expression of ~500 genes decreases after heat shock in the *mot1-42* strain (clusters 1 and

2 in Figure 4-5). This is a broader action than was previously reported with a different allele (*mot1-14*) and absolute 2-fold cutoff (Dasgupta et al., 2002). Inactivation of *mot1-42* results in modest increased expression in ~80 genes (cluster 6).

Fourth, distinct patterns of expression are seen in the FHT-*mot1* mutant strains. Deletion of the most N-terminal region of MOT1 (TBP1, column 3) does not decrease expression of genes in cluster 2 or 3 as was seen for the other mutants, most notably in TBP2. Deletion of two regions with the same activity, TBP interaction, show opposing effects on genes in cluster 4 (columns 3, 5). The TBP1 domain represses these genes (deletion of this domain leads to an increase in expression), where TBP2 acts positively at these genes (TBP2 deletion causes decrease in expression). Aside from ~25 genes (cluster 7) that increase in expression upon mutation to the ATPase domain, only the TBP1 mutation shows increases in expression. This indicates that functional interaction with TBP plays a key role in the repressive functions of MOT1. Deletion of TBP interacting region #2 (column 5) decreased expression of ~100 genes (cluster 3), while the other mutations only display modest decreases. Proper expression of genes in this cluster could heavily rely on MOT1 to deliver TBP to a promoter, but do not require the ATPase activity. Further study of MOT1 and TBP co-occupancy at these genes using chromatin immunoprecipitation is planned.

Figure 4-5

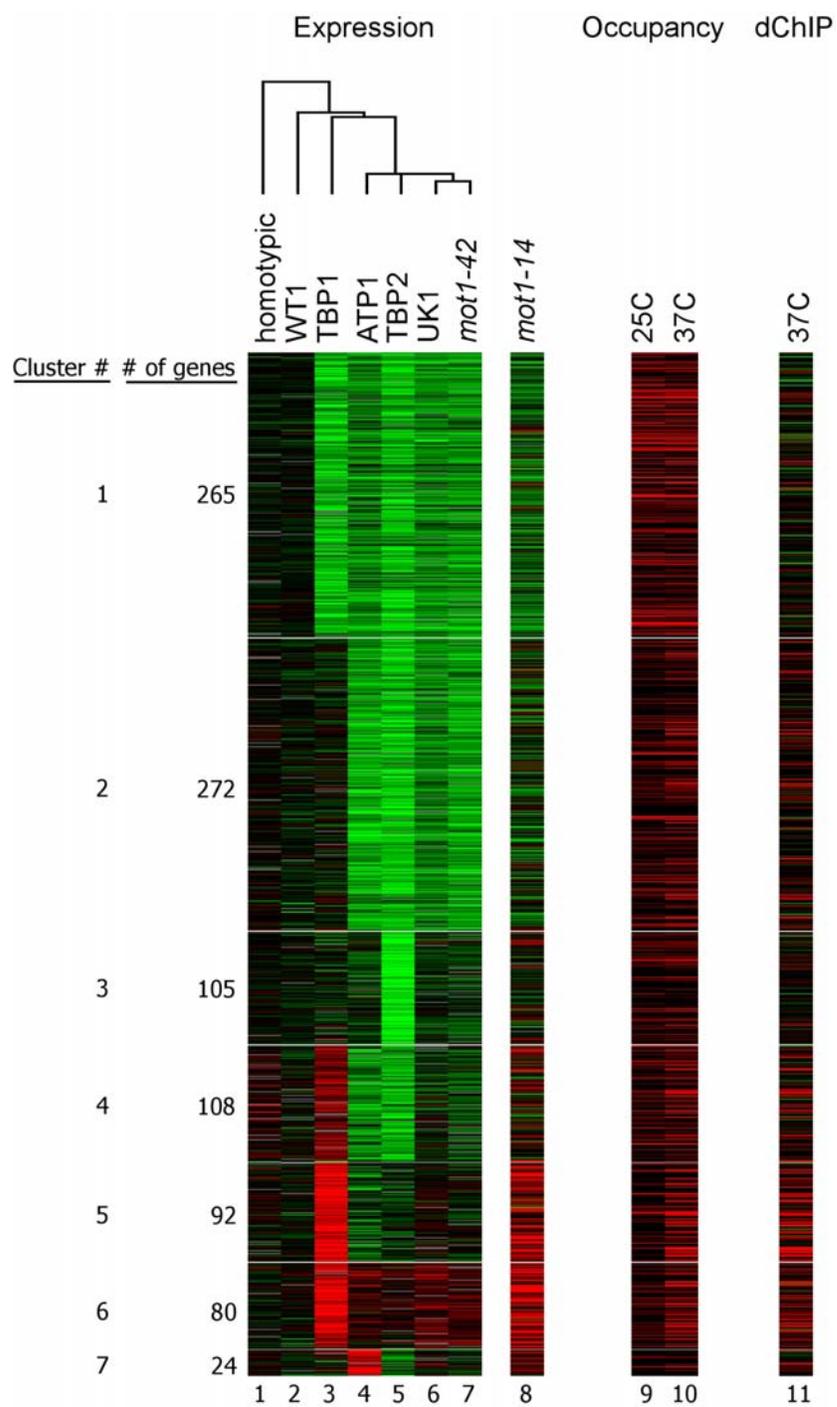


Figure 4-5: Effects of MOT1 mutants on genome-wide scale.

Comparison of MOT1 expression and MOT1 occupancy (ChIP) data. K-means clustering of 946 genes that were present in 80% of the experiments and had a log(2) absolute value change of greater than 0.76 in one experiment. Column 8 is *mot1-14* expression data (Dasgupta et al., 2002), and columns 9-11 are FHT-MOT1 ChIP data at 25 °C, 37 °C and change in occupancy (differential ChIP) after 25 °C → 37°C temperature shift, respectively (Zanton and Pugh, 2004). WT1, FHT tagged MOT1 WT; TBP1, deletion of TBP interaction region 1 (residues 5-80); TBP2, deletion of TBP-interaction region 2 (residues 307-432); UK1, unknown activity region (residues 1090-1259); ATP1, ATPase region (residues 1403-1479).

Since we observed striking differences in expression profiles in the different mutant strains, we sought to compare the expression data from the MOT1 deletion mutations to published data from a separate *MOT1* allele, *mot1-14* (Dasgupta et al., 2002). This data was aligned with the K=7 clusters as shown in Figure 4-5. This comparison includes data from the *mot1-14* expression study that did not meet the 2-fold cutoff (Dasgupta et al., 2002). Interestingly, the *mot1-14* data visually appears to be an average of the expression profiles from the deletion mutants in a particular cluster. This comparison shows that the expression profiles from the deletion mutant strains are generally similar to changes in expression seen after heat inactivating a well characterized *mot1* ts allele.

#### 4.3.1 Bioinformatic Analysis of Clustered Genes

To further characterize the genes in each cluster, we took a bioinformatic approach using published data sets of genome-wide expression, chromatin immunoprecipitation (ChIP on chip) studies, DNA motifs, and groups of genes with

similar features. Examples of ‘groups’ are TATA containing genes, genes affected by deletion or inactivation of a particular factor (i.e. *taf8-ts7*), or environmental stress response (ESR) genes. Genes in each cluster were compared to genes in the top or bottom 10% of other data sets. Analysis includes 544 ChIP on chip data sets, 238 microarray expression sets, and 195 groups and DNA elements data sets. The probability of overlap was calculated using CHITEST function in Microsoft EXCEL and log(10) of the P-values are listed in parenthesis for several key factors are listed below.

The wide-spread decreases in expression indicate genes in cluster one require all tested functions of MOT1 for proper expression. MOT1 positively regulates these genes—the four deletion mutants, the *mot1-42* and the *mot1-14* allele all display primarily decreasing expression. Cluster one genes were found to overlap with genes known to be environmental stress response (ESR) down-regulated genes (P-value  $10^{-196}$ ). They generally do not contain TATA boxes (Basehoar et al., 2004) and their expression is dominated by the TFIID pathway (Huisinga and Pugh, 2004). These genes are sensitive to TBP mutations ( $10^{-53}$ ), deletion of the TAND domain ( $10^{-16}$ ), and are positively regulated by MOT1 (*mot1-14* ;  $10^{-73}$ ) and by NC2 ( $10^{-5}$ ). Crosslinking indicates that TBP decreases at these genes upon heat shock ( $10^{-45}$ ). Coincident with the decreasing amount of TBP, the expression of these genes decreases after heat shock, and therefore these are heat-repressed genes. Previous studies that show MOT1 occupancy decreases at heat repressed genes (Zanton and Pugh, 2004). Analysis indicates that MOT1 decreases binding to cluster one genes ( $10^{-6}$ ), but Figure 4-5 shows that after a 15 minute heat shock, MOT1 is still detectable at a large number of cluster one genes.

While the genes that are decreasing in clusters 1 and 2, are heat shock repressed (Zanton and Pugh, 2004) the decreasing expression in Figure 4-5 is not due to heat shock, since the reference strain was also heat shocked. Since the microarray data is a ratio of expression in the test strain versus the reference strain, genes that decrease in both strains (i.e. heat repressed genes) are considered no change. The decreases seen in clusters 1 and 2 are therefore due to differences between the MOT1 mutants and the MOT1 WT reference strain.

Genes in cluster two do not depend on the most N-terminal portion of MOT1 for expression, but do require all other regions of MOT1. Like cluster one, MOT1 is acting positively at these genes. The expression pattern in cluster three is mainly due to loss of interaction between TBP and MOT1 deleted for residues 307-432. Comparison to 'groups' data shows these genes are TFIID dominated and TATA-less genes, which typically carry out housekeeping roles in yeast (Huisinga and Pugh, 2004). Cluster three shows no bias for either ESR or heat shock induced or repressed genes. The most intriguing results from the bioinformatic analysis of clusters two and three is that they are not enriched (top 10%) for binding by factors in any of the 544 ChIP on chip data sets tested. These genes could therefore reflect a portion of the genome whose primary positive regulation is from MOT1, and are repressed by TBP dimerization and TAND domain of TAF1.

Like clusters two and three, there was no significant enrichment in binding of the other factors analyzed to the genes in cluster four. Genes in cluster four show a slight bias towards the SAGA-dominated pathway (15% of genes in this cluster are SAGA

dominated compared to the genome wide average of 9%) (Basehoar et al., 2004; Huisinga and Pugh, 2004). However, unlike typical SAGA-dominated genes, there is no enrichment of TATA boxes in cluster four. An overrepresentation of genes involved in the diauxic shift response ( $10^{-21}$ ) and induced after heat shock ( $10^{-9}$ ) is observed in cluster four. Expression of cluster four genes is particularly sensitive to mutations in TBP that affect TBP-DNA interactions (V161R, V71R, N69R) and K145E, a mutation that disrupts interaction with MOT1 and TFIIA, which stabilizes TBP to promoter DNA (Coleman et al., 1999; Weideman et al., 1997).

Cluster five contains ESR-induced and SAGA dominated genes ( $10^{-97}$ ), and an enrichment of TATA boxes ( $10^{-26}$ ). The expression profile of the MOT1 mutant TBP1 is the major determinant of this cluster, indicating a relationship between MOT1, TBP, SAGA, and stress response pathways. Genes with the highest expression from a TBP(F182V) mutation, which eliminates interaction with NC2, are enriched in cluster five ( $10^{-15}$ ). Therefore, NC2 represses these genes. Mutation to MOT1 causes a strong increase in expression, so MOT1 is also acting to repress these genes.

Only a small number of ChIP data sets show significant enrichment in cluster six, and all seem to be stress response related factors. For example Rph1 (DNA damage), Nrg1 (glucose repression), Mot3 (repression of hypoxic genes), Rox1 (repression of hypoxic genes), Sut1 (repression of hypoxic genes), Met4 (sulfur biosynthesis), Cin5 (salt tolerance), and Msn2 (response to stress) were all enriched with P-values of  $\sim 10^{-5}$ . Similar to cluster five, this subset of genes are heat shock induced ( $10^{-57}$ ), SAGA dominated ( $10^{-44}$ ) and contain a TATA box ( $10^{-27}$ ). Like cluster five, genes increasing

expression in TBP(F182V) are enriched in cluster six ( $10^{-43}$ ), indicating that NC2 works to repress these genes as well.

Together clusters 5 and 6 comprise ~170 genes that are clearly stress response genes. They are SAGA-dominated, contain TATA boxes and known stress response motifs such as Msn2 (Kellis et al., 2003), and are bound by a number of stress response transcription factors. MOT1 and NC2's ability to interact with TBP aids in repression of these genes. Interaction between TBP and the SAGA complex is important for expression, since deletion of Spt3 causes expression of these genes to decrease. The subset of genes in the lowest 10% of expression in a *spt3* deletion strain are enriched in cluster five ( $10^{-30}$ ) and six ( $10^{-14}$ ). Genes decreasing the most in a TBP(V161E) or TBP(V71E) mutants are enriched in cluster 5 ( $10^{-38}$ ,  $10^{-29}$ ) and cluster six ( $10^{-38}$ ,  $10^{-20}$ ).

MOT1 may be functioning at cluster 5 and 6 stress response genes to restore transcription to a basal level after stress conditions have abated. Loss of TBP-MOT1 interaction due to the TBP1 deletion may prevent MOT1 from effectively clearing TBP from these promoters, as has been suggested as a mechanism of MOT1 mediated repression (Topalidou et al., 2004). Alternatively, MOT1 might normally compete with TFIIA interaction at these genes, leading to repression. However, the TBP1 mutant may be deficient for this competitive action and therefore contribute to the high level of expression of these genes.

Cluster seven genes have lower than expected levels of histone modifications, including H3 methylation at lysine 4 ( $10^{-12}$ ), acetylation of histone H3 tail at residues 9, 14 ( $10^{-10}$ ), and histone H4 acetylation at lysines 5, 8, 12 and 16 ( $10^{-10}$ ). They overlap with genes that we previously identified (Chitikila et al., 2002) as being lowly expressed

and repressed by both TBP dimerization TAND domain of TAF1. These genes were found to cluster in the subtelomeric regions of chromosomes. Their location and low expression level is in consensus with lack of histone tail acetylation. Cluster seven genes contain a TATA box, but are not ESR genes, nor are they dominated by the SAGA pathway. All of this data indicates that these genes are mainly quiescent and repressed in large part by residence in TBP inaccessible regions of the genome.

The clusters that are most repressed by MOT1 (clusters 4, 5, 6, and 7) show the highest amount of MOT1 bound (columns 9, 10). The genes where a mutation to MOT1 has the largest decreases in expression (positively regulated by MOT1) do not show high MOT1 occupancy. The reason for this could be that MOT1 is not stably associated at these promoters. A brief association at these genes could still allow MOT1 to enzymatically dissociate inactive TBP/PIC from these promoters.

#### **4.3.1 Future Directions**

The interesting findings from the MOT1 mutants require additional testing to fully appreciate their biological importance. Potential future experiments on these MOT1 mutants include genome-wide localization tested through chromatin immunoprecipitation (ChIP on chip) under conditions used for the microarray experiments. This data can be compared to the expression profiles from these mutants to address the positive and negative functions of MOT1. ChIP on chip data from TBP, TFIIA (*TOA1*, *TOA2*), TFIIB, TFIID, and NC2 will also help determine the context of MOT1-mediated gene regulation on a genome wide scale.

Data from the human homologue of MOT1, TAF172, indicates that free TBP and not TFIID complexes are the major targets of MOT1/TAF172 (Chicca et al., 1998). The ability of MOT1 to remove TFIID from promoter DNA through ATP hydrolysis will need to be measured directly. The interaction of MOT1 and NC2 biochemically can be tested through immunoprecipitation/pulldown assays. Additionally, sequential ChIP on chip can investigate if MOT1 and NC2 co-occupy the same promoter *in vivo*. These same experiments can also shed light on the potential cooperativity of MOT1 and RAP1, which also plays both positive and negative roles in gene expression.

Table 4-4

Table 4-4: Properties of Clusters.

Cluster		TATA Box	SAGA dominated	ESR down	ESR up	Ribosomal Protein Gene	NC2 induced	NC2 repressed	Lowest 10% intensity	Diauxic shift
1	N	11	6	171	0	73	83	1	2	0
	%	4.4	2.6	64.5	0.0	27.5	35.5	0.4	0.8	0.0
	P-value	-7	-3	-196	-3	-185	-36	-6	-7	0
2	N	28	10	32	4	10	27	7	8	2
	%	10.9	3.8	11.8	1.5	3.7	10.9	2.8	2.9	0.7
	P-value	-3	-2	-1	-2	-1	0	-4	-5	-1
3	N	28	10	32	4	10	27	7	8	2
	%	30.4	10.2	30.5	3.8	9.5	32.1	8.3	7.6	1.9
	P-value	-1	-1	0	-1	0	0	-1	-1	0
4	N	24	16	4	13	1	4	5	7	5
	%	24.2	15.2	3.7	12.0	0.9	4.5	5.7	6.5	4.6
	P-value	-1	-1	-1	-4	0	-1	-1	-1	-20
5	N	59	43	0	42	0	1	31	10	0
	%	70.2	50.0	0.0	45.7	0.0	1.2	36.9	10.9	0.0
	P-value	-26	-36	-3	-77	-1	-2	-15	0	0
6	N	53	43	1	27	0	0	44	6	1
	%	74.6	58.1	1.3	33.8	0.0	0.0	60.3	7.5	1.3
	P-value	-26	-44	-2	-34	-1	-2	-42	-1	-1
7	N	20	2	1	0	0	0	3	3	0
	%	83.3	9.1	4.2	0.0	0.0	0.0	17.6	12.5	0.0
	P-value	-12	0	0	-1	0	-1	-1	0	0
	G.W.avg (%)	19.1	9.2	9.4	4.5	2.1	10.0	9.9	12.1	0.2

The number of genes (N) that are resident in a cluster and have a genomic property are listed in the top row for each cluster. The percent (%) of genes with this property from the given cluster is calculated in the second row for each cluster, and the genome-wide average (G.W. avg %) percentage of the individual properties (e.g. 19.1% of genes in the yeast genome contain a TATA box) is listed at the bottom of the table. The log<sub>10</sub> of the P-value of the number of genes in a cluster containing each property occurring randomly is also listed. Data displayed in this table does not include all properties, but is a representative of the data analysis.

The number of genes (N) that are resident in a cluster and have a genomic property are listed in the top row for each cluster. The percent (%) of genes with this property from the given cluster is calculated in the second row, and the genome-wide average (G.W. avg %) is listed at the bottom of the table. The P-value of the number of genes in a cluster containing each property occurring randomly is also listed.

When the entire data set for an individual MOT1 mutant (~3500 genes instead of 946 in the clustering) was compared, we find that genes that are decreasing the most in the FHT-MOT1 mutants have a high amount of Rap1 bound. Consistent with previous conclusions from microarray data analysis, this indicates that genes positively regulated by MOT1 are bound by Rap1, which is linked to the TFIID-dominated pathway (Huisinga and Pugh, 2004).

We also find that putative or transcription factors Gat3, Fhl1, Rgm1, Smp1, Gal1, Pdr1, Cin5 and Yap5 frequently crosslink to genes that decrease in expression in MOT1 mutants (genes where MOT1 acts positively). These factors are hallmarks for a set of stress response genes that are positively regulated by MOT1 and mainly are TFIID-dominated.

The data analysis indicates that MOT1 has two distinct mechanisms of action. MOT1 acts positively to regulate TFIID-dominated genes. These genes have been defined as housekeeping genes, are mainly expressed at low levels, and are not regulated by a large number of other factors. MOT1 may act at these genes by recycling TBP from inactive PIC complexes and allowing formation of functional transcriptional complexes. Interestingly, at subsets of genes where MOT1 functions positively, the NC2 complex

also plays a positive role. At the ~10% of the genome that is involved in stress response, which is generally SAGA-dominated, MOT1 may operate negatively by removing functional TBP complexes from the promoter to restore transcription to a pre-stressed level. At genes where MOT1 plays a negative role, NC2 also aids in repression (clusters 5, 6).

Table 4-5

Table 4-5: P-value of overlap between ChIP data set and MOT1 expression clusters.

Cluster	Factor	Log(10) P-value
1	Rbp1	-68
	Fhl1	-43
	Rap1	-29
	H3 AcK18	-14
	Gcn5	-17
	Esa1	-16
5	Bdf1	-13
	Msn2	-9
	Msn4	-11
6	Rph1	-7
	MOT3	-6
	Met4	-5
	Msn2	-5
	Cin5	-5
7	Rap1	-14

Significance was calculated between top 10% of factor occupancy (percent rank of ChIP) and gene membership in each cluster. Data displayed in this table does not include all properties, but is a representative of the data analysis. Clusters 2, 3 and 4 are not listed because no factors were determined to be significantly (p-value <  $10^{-5}$ ) enriched at these genes.

Table 4-6

Table 4-6: P-value of overlap between groups and MOT1 expression clusters.

Cluster	Condition	Log(10) P-value
1	Rap1	-186
	RP genes	-99
	ESR1 motif	-23
	ESR2 motif	-19
2	TFIID (ts1)	-6
3	chromosome 16	-9
4	HS induced/TFIID dominated	-9
	hs induced	-9
	Diauxic shift	-21
5	ESR induced and SAGA dominated	-97
	ESR	-77
	HS induced	-45
	TATA box	-43
6	HS induced	-33
	TATA box	-19
7	Group 4 genes <sup>7</sup>	-24

Significance of overlap for membership in group and each cluster. Data displayed in this table does not include all groups, but is a representative of the data analysis.

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<sup>7</sup> Genes in class four as described in Chitikila, C., Huisinga, K.L., Irvin, J.D., Basehoar, A.D. and Pugh, B.F. (2002) Interplay of TBP Inhibitors in Global Transcriptional Control. *Molecular Cell*.

#### 4.4 Materials and Methods

Construction of the MOT1 mutants, including homologous recombination, tetrad dissection, and sporulation were performed similarly to TAF1 (Chapter 3). Microarray analysis of FHT-MOT1 strains was performed essentially as for TAF1 in Chapter 3, with the following changes. For the analysis of the effects of the FHT-MOT1 deletion mutants a 60 minute pre-induction in YPR + 2% galactose at room temperature (~20 °C) was used (see above for rationale). After the induction, all cells were shifted to 37 °C for 45 minutes by mixing the cultures with an equal volume of warm YPR + 2% galactose to heat-inactivate the *mot1-42* allele. Oligo-dT cellulose (Ambion) was used to purify mRNA according to the manufacturer's instructions instead of the Oligotex (Qiagen) resin.

Table 4-7

Table 4-7: Oligonucleotides used in this study.

	10	20	30	40	50	60	70
WT1 R2	GAACCGg	TCTCTATTAATATTACCTGCCGGTCCAGCCTCGAAACTCGTGAAGTGGATCTGATATCACC					
WT1 F1	TCTATACTTTAACGTCAAGGAGAAAAACCCGGATCTCAAATGATGACGTACCCATACGATGTTTCCT						
ATP1 R2	AGTGCCTCCAAAGCCAGTACACCTGCTTCCTGCTCCTTAGATGATGTTTTAGTGGATCTGATATCACC						
ATP1 F1	CATCATATGACGTAGCTAGAAATGATCTCGCAGTCCTAAACAAAACCTGAATACCCATACGATGTTTCCT						
TBP2 R2	TTTTCTAAAAGGCCATGGGCAAATAGGAAATTTGTCTTTATGCTGACGAAAGTGGATCTGATATCACC						
TBP2 F1	AATTCCAAGGCATTTATGAACTACTTTTAGATAATCTAATGAGCGAAAATTACCCATACGATGTTTCCT						
UK1 F1	ATGAGGAAGCAAGTAAAGTTGACAACGAGCAAGGTCAAAAAATAGTCGACTACCCATACGATGTTTCCT						
UK1 R2	TGATATTTTCTTAAAGTCGCCTTGATGGCAATGGGTAAATTTAAATGGCTTAGTGGATCTGATATCACC						
TBP1 R2	TTATCCAATGGAGAACCTTCATTTGTACCACCTACCAAGTCAGATTCAATAGTGGATCTGATATCACC						
TBP1 F1	CTTTAACGTCAAGGAGAAAAACCCGGATCTCAAATGATGACGTCACGATAACCCATACGATGTTTCCT						
MOT1 F4	GGCAAGACACATTTACCTGATATTGAAGAATGCAACCTCTTTCATAATGGAATTCGAGCTCGTTTAAAC						
MOT1 R2	GTCTCTATTAATATTACCTGCCGGTCCAGCCTCGAAACTCGTGACGTCATCATTTTGAGATCCGGGTTTT						

Table 4-8

Table 4-8: MOT1 yeast strains used in this study.

Strain	promoter	FHT MOT1	Deletion	plasmid	MAT
yjdi408	<i>GALI</i>	WT1	--	<i>mot1-42</i>	alpha
yjdi410	<i>GALI</i>	TBP1	5-80	<i>mot1-42</i>	alpha
yjdi412	<i>GALI</i>	TBP2	307-432	<i>mot1-42</i>	alpha
yjdi414	<i>GALI</i>	UK1	1090-1259	<i>mot1-42</i>	alpha
yjdi416	<i>GALI</i>	ATP1	1403-1479	<i>mot1-42</i>	alpha
yjdi418	<i>GALI</i>	null	<i>mot1::KanMX</i>	<i>mot1-42</i>	alpha
yjdi419	<i>GALI</i>	null	<i>mot1::KanMX</i>	<i>MOT1</i> WT	alpha
yjdi420	<i>GALI</i>	WT	--	<i>mot1-42</i>	alpha
yjdi424	<i>MOT1</i>	WT	--	<i>mot1-42</i>	alpha

The *mot1-42* allele on plasmid pMOT244 (*LEU2*) and *MOT1* WT (pAV20, *LEU2*) were gifts of David Auble (University of Virginia).

#### 4.5 Bibliography

- Adamkewicz, J.I., Hansen, K.E., Prud'homme, W.A., Davis, J.L. and Thorner, J. (2001) High affinity interaction of yeast transcriptional regulator, Mot1, with TATA box-binding protein (TBP). *J Biol Chem*, **276**, 11883-11894.
- Andrau, J.C., Van Oevelen, C.J., Van Teeffelen, H.A., Weil, P.A., Holstege, F.C. and Timmers, H.T. (2002) Mot1p is essential for TBP recruitment to selected promoters during in vivo gene activation. *Embo J*, **21**, 5173-5183.
- Auble, D.T., Hansen, K.E., Mueller, C.G., Lane, W.S., Thorner, J. and Hahn, S. (1994) Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes & Development*, **8**, 1920-1934.
- Auble, D.T., Wang, D., Post, K.W. and Hahn, S. (1997) Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP- driven enzyme that dissociates TATA-binding protein from DNA. *Mol Cell Biol*, **17**, 4842-4851.
- Basehoar, A.D., Zanton, S.J. and Pugh, B.F. (2004) Identification and distinct regulation of yeast TATA box-containing genes. *Cell*, **116**, 699-709.
- Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, **14**, 115-132.

- Chicca, J.J., 2nd, Auble, D.T. and Pugh, B.F. (1998) Cloning and biochemical characterization of TAF-172, a human homolog of yeast Mot1. *Mol Cell Biol*, **18**, 1701-1710.
- Chitikila, C., Huisinga, K.L., Irvin, J.D., Basehoar, A.D. and Pugh, B.F. (2002) Interplay of TBP Inhibitors in Global Transcriptional Control. *Molecular Cell*.
- Coleman, R.A., Taggart, A.K.P., Burma, S., Chicca II, J.J. and Pugh, B.F. (1999) TFIIA regulates TBP and TFIID dimers. *Molecular Cell*, **4**, 451-457.
- Collart, M.A. (1996) The NOT, SPT3, and MOT1 genes functionally interact to regulate transcription at core promoters. *Mol Cell Biol*, **16**, 6668-6676.
- Cormack, B.P. and Struhl, K. (1992) The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. *Cell*, **69**, 685-696.
- Darst, R.P., Dasgupta, A., Zhu, C., Hsu, J.Y., Vroom, A., Muldrow, T. and Auble, D.T. (2003) Mot1 regulates the DNA binding activity of free TATA-binding protein in an ATP-dependent manner. *J Biol Chem*, **278**, 13216-13226.
- Darst, R.P., Wang, D. and Auble, D.T. (2001) MOT1-catalyzed TBP-DNA disruption: uncoupling DNA conformational change and role of upstream DNA. *Embo J*, **20**, 2028-2040.
- Dasgupta, A., Darst, R.P., Martin, K.J., Afshari, C.A. and Auble, D.T. (2002) Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. *Proc Natl Acad Sci U S A*, **99**, 2666-2671.
- Dasgupta, A., Juedes, S.A., Sprouse, R.O. and Auble, D.T. (2005) Mot1-mediated control of transcription complex assembly and activity. *Embo J*, **24**, 1717-1729.

- Davis, J.L., Kunisawa, R. and Thorner, J. (1992) A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Molecular & Cellular Biology*, **12**, 1879-1892.
- Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A*, **95**, 14863-14868.
- Gasch, A.P., Huang, M., Metzner, S., Botstein, D., Elledge, S.J. and Brown, P.O. (2001) Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. *Mol Biol Cell*, **12**, 2987-3003.
- Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D. and Brown, P.O. (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell*, **11**, 4241-4257.
- Gasch, A.P. and Werner-Washburne, M. (2002) The genomics of yeast responses to environmental stress and starvation. *Funct Integr Genomics*, **2**, 181-192.
- Geisberg, J.V., Moqtaderi, Z., Kuras, L. and Struhl, K. (2002) Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **22**, 8122-8134.
- Geisberg, J.V. and Struhl, K. (2004) Cellular stress alters the transcriptional properties of promoter-bound Mot1-TBP complexes. *Mol Cell*, **14**, 479-489.
- Guldener, U., Heck, S., Fielder, T., Beinhauer, J. and Hegemann, J.H. (1996) A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res*, **24**, 2519-2524.

- Gumbs, O.H., Campbell, A.M. and Weil, P.A. (2003) High-affinity DNA binding by a Mot1p-TBP complex: implications for TAF-independent transcription. *Embo J*, **22**, 3131-3141.
- Hahn, J.S., Hu, Z., Thiele, D.J. and Iyer, V.R. (2004) Genome-wide analysis of the biology of stress responses through heat shock transcription factor. *Mol Cell Biol*, **24**, 5249-5256.
- Huisinga, K.L. and Pugh, B.F. (2004) A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell*, **13**, 573-585.
- Kellis, M., Patterson, N., Endrizzi, M., Birren, B. and Lander, E.S. (2003) Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature (London)*, **423**, 241-254.
- Kim, J. and Iyer, V.R. (2004) Global role of TATA box-binding protein recruitment to promoters in mediating gene expression profiles. *Mol Cell Biol*, **24**, 8104-8112.
- Madison, J.M. and Winston, F. (1997) Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TATA-binding protein to confer promoter-specific transcriptional control in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **17**, 287-295.
- Muldrow, T.A., Campbell, A.M., Weil, P.A. and Auble, D.T. (1999) MOT1 can activate basal transcription in vitro by regulating the distribution of TATA binding protein between promoter and nonpromoter sites. *Mol Cell Biol*, **19**, 2835-2845.
- Prelich, G. (1997) *Saccharomyces cerevisiae* BUR6 encodes a DRAP1/NC2alpha homolog that has both positive and negative roles in transcription in vivo. *Mol Cell Biol*, **17**, 2057-2065.

- Prelich, G. and Winston, F. (1993) Mutations That Suppress the Deletion of an Upstream Activating Sequence in Yeast - Involvement of a Protein Kinase and Histone-H3 in Repressing Transcription In vivo. *Genetics*, **135**, 665-676.
- Shen, W.C., Bhaumik, S.R., Causton, H.C., Simon, I., Zhu, X., Jennings, E.G., Wang, T.H., Young, R.A. and Green, M.R. (2003) Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly. *Embo J*, **22**, 3395-3402.
- Topalidou, I., Papamichos-Chronakis, M., Thireos, G. and Tzamarias, D. (2004) Spt3 and Mot1 cooperate in nucleosome remodeling independently of TBP recruitment. *Embo J*, **23**, 1943-1948.
- Wach, A., Brachat, A., Pohlmann, R. and Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*, **10**, 1793-1808.
- Weideman, C.A., Netter, R.C., Benjamin, L.R., McAllister, J.J., Schmiedekamp, L.A., Coleman, R.A. and Pugh, B.F. (1997) Dynamic interplay of TFIIA, TBP and TATA DNA. *J Mol Biol*, **271**, 61-75.
- Zanton, S.J. and Pugh, B.F. (2004) Changes in genomewide occupancy of core transcriptional regulators during heat stress. *Proc Natl Acad Sci U S A*, **101**, 16843-16848.

## Chapter 5

### Regulation of TBP by MOT1 and TAF1

In a cell many factors have direct and indirect contributions on gene expression. The transcriptional output of any gene is the sum of all the positive and negative functions. TBP plays a central role in polymerase II expression, and is heavily regulated both positively and negatively through a variety of different mechanisms. To gain understanding of the communication between transcription factors inside a yeast cell, I studied two transcription factors, TAF1 and MOT1, which are both known to interact with TBP.

Direct measurements of the binding of 28 TBP derivatives to three different versions of a portion of the N-terminus of TAF1 (TAND) were made *in vitro*. Our results provide a high resolution map of the interaction surfaces of TBP and the TAND region. We find that the interactions between TAND and TBP are largely electrostatic. TBP mutants that display unique physical properties, most notably increases in transcription, show a wide range of TAND binding. Several of these TBP mutants severely disrupt the *in vitro* interaction with the TAND domain. However, loss of repressive functions associated with the TAND domain, such as competitive binding with DNA and TFIIA, due to the TBP mutants can not fully explain their *in vivo* and *in vitro* characteristics. It was concluded that other repressive interactions are destabilized in these mutants. The data indicates that the TBP mutations cause defects in TBP self association. TBP

dimerization plays a larger physiological role in repressing transcription than does the TAND domain.

To further define the relationship between TBP and TAF1, pre-defined functional regions of TAF1 were deleted. The TAF1 mutants were epitope tagged and placed under control of an inducible promoter to regulate expression. The TAF1 derivatives created are deletion of: the TAND domain; a region that interacts with the other TAFs in TFIID; enzymatic HAT activity; and promoter DNA binding. Using these mutants on a genome-wide scale allowed us to study TAF1-mediated control of transcription in yeast.

The derivatives of TAF1 exhibit *in vivo* phenotypes including temperature sensitivity, toxicity, and inability to support viability. With one exception, these mutant proteins all localized into the nucleus. A tagged wild-type maintains a WT level of transcription and supports viability in a temperature-sensitive strain. These effects show that the epitope tagged, inducible TAF1 (WT1) protein could be incorporated into TFIID and complement normal cellular processes. However, none of the deletion derivatives possess these capabilities.

These mutant TAF1 proteins show increased degradation *in vivo*. Mutations to any of the TAFs in TFIID that destabilize the complex are known to be degraded rapidly. These results indicate that the TAF1 deletion derivatives are indeed defective for important relationships in TFIID. Other researchers have created mutations in TAF1 similar to those used here and see association of other TAFs and TBP (except in the  $\Delta 208-303$  mutant). However, it is unclear to what extent the TAFs and TBP associate with our mutants under the conditions used. Further experiments are planned to determine the integrity of TFIID containing TAF1 deletion derivatives.

All of the epitope tagged TAF1 derivatives could not support WT levels of transcription as the sole copy of TAF1 in these yeast strains. Interestingly, the distinct mutations create similar patterns of expression, indicating that all functions of TAF1 are essential for proper gene expression. A deletion of the promoter binding domain interferes with the SAGA-dominated pathway of gene expression. This indicates that TFIID is directed to the majority of promoters, but is withheld from SAGA genes due to a small domain at the C-terminus of TAF1.

To further our understanding of TBP regulation, a similar epitope tagging and deletion strategy was performed on the transcription factor MOT1. MOT1 regulates the level of TBP at promoters through ATPase dependent dissociation of TBP from DNA. MOT1 regulates a small fraction of the genome, is associated with the SAGA complex, stimulates and represses transcription.

In contrast to the TAF1 results and previous studies on similar mutants of MOT1, our derivatives do not display a range of phenotypes. None of the mutants are toxic when overexpressed from the *GALI* promoter, and none can support viability at 37 °C. Strikingly, the MOT1 derivatives cause diverse effects on transcription. At TFIID dominated genes, where MOT1 acts positively, all functions of MOT1 are required for proper gene expression. The NC2 transcription complex increases expression of these genes.

A subset of genes where MOT1 regulates TBP largely independently of other factors also requires all domains of MOT1. SAGA-dominated genes are involved in response to stress. At these genes MOT1 plays a repressive role, in large part mediated

by the N-terminal region which interacts with TBP. The NC2 complex associates with, and represses these genes. The different patterns of gene expression in the MOT1 deletion derivatives shows that MOT1 possess separable activities which contribute to distinct classes of genes.

These results provide a greater understanding of regulation of the TATA box protein in terms of interaction with TAND and genome-wide regulation imparted by TAF1 and MOT1 functional domains. The results presented here indicate that TAND makes substantial contacts to the underside of TBP *in vitro*, but these interactions are largely masked by other factors *in vivo*. All regions of TAF1 tested are required for expression of TFIID-dominated genes. MOT1 regulates TFIID dominated genes positively, SAGA-dominated genes negatively, and may be the factor controlling expression of a small subset of the yeast genome.

While the expression microarray studies were performed *in vivo*, direct relationships to the remainder of the transcription machinery was not made. It is unclear how MOT1 and TFIIA coordinate regulation of the entire yeast genome. Additionally, nucleosome remodeling and histone modification make major contributions to gene expression. Future experiments on MOT1 and these factors would increase our knowledge of the nature of chromatin at all MOT1 regulated promoters, and if MOT1 contributes to changes in chromatin structure across the entire genome. From the bioinformatic analysis we see that MOT1 and NC2 have similar directional effects on transcription at TFIID and SAGA-dominated genes. However, it is unclear if both of

these factors are operating on a gene at the same time, and what impact they have on TBP association with a given promoter. Sequential chromatin immunoprecipitation at the different classes of genes performed on pairs of these factors, especially in the MOT1 mutants, will likely be performed in the near future. Additional experiments will increase our understanding of how these complexes coordinate their genome-wide regulation of TBP, especially in the context of chromatin and other transcription factors.

## VITA

Jordan Davis Welch Irvin

### Education:

Ph.D., The Pennsylvania State University, University Park, PA., 2005. Major in Biochemistry and Molecular Biology.

Thesis Title: Genome-wide Functional Analysis of TAF1 and MOT1, Regulators of the TATA Box Binding Protein in *Saccharomyces cerevisiae*. Advisor: Dr. B. Frank Pugh, Ph.D., Professor of Biochemistry, Microbiology and Molecular Biology.

B.S., Indiana University, Bloomington, IN., 1998. Major in Biology.

Honors Thesis Title: Gene Gun Transformation of *Poa pratensis* (Kentucky Bluegrass) with pUC18 plasmid DNA coated tungsten particles.

Advisor: Dr. Richard Wagner, Ph.D.

### Publications:

**Irvin JD** and Pugh BF. "Genome-wide Transcriptional Dependencies on TAF1 Functional Domains." Submitted, *Journal of Biological Chemistry*, July 2005.

Kou H, **Irvin JD**, Huisinga KL, Mitra M, Pugh BF. "Structural and functional analysis of mutations along the crystallographic dimer interface of the yeast TATA binding protein." *Mol Cell Biol*. 2003 May; 23(9):3186-201.

Chitikila C, Huisinga KL, **Irvin JD**, Basehoar AD, Pugh BF. "Interplay of TBP inhibitors in global transcriptional control." *Mol Cell*. 2002 Oct;10(4):871-82.

### Awards:

Paul Berg Graduate Travel Award, August 2005. Pennsylvania State University Department of Biochemistry and Molecular Biology.

Braddock Graduate Fellowship 1999 - 2000 and 2000 - 2001 academic years. Pennsylvania State University Department of Biochemistry and Molecular Biology.