DISSERTATION

REGULATION OF TRANSCRIPTION BY FACTORS INTERACTING WITH THE TATA BINDING PROTEIN

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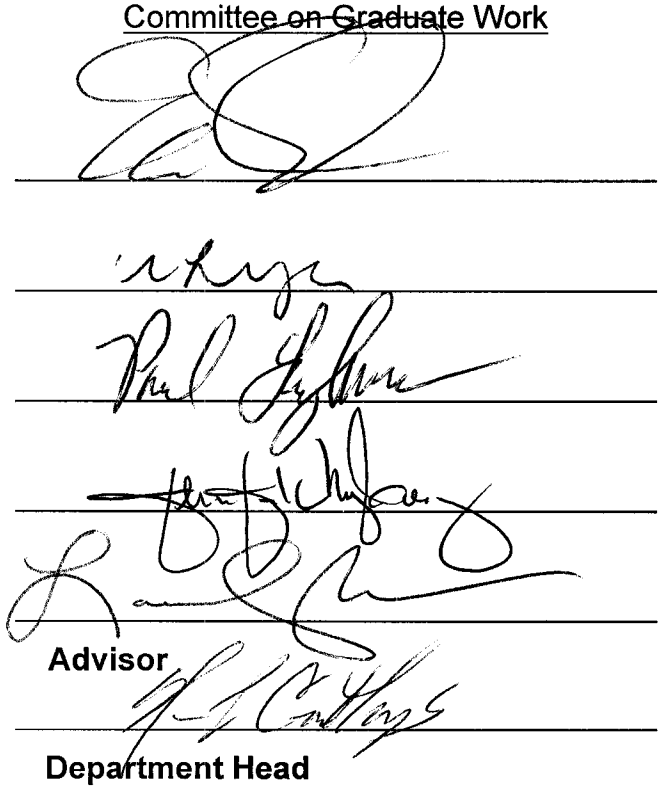
WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GAYATRI YATHERAJAM ENTITLED REGULATION OF TRANSCRIPTION BY FACTORS INTERACTING WITH THE TATA BINDING PROTIEN BE ACCEPTED AS FULLFILING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee ~~on Graduate~~ Work



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ABSTRACT OF DISSERTATION

REGULATION OF TRANSCRIPTION BY FACTORS INTERACTING WITH THE TATA BINDING PROTEIN

Transcription is an essential cellular process to maintain cell growth, differentiation and development. Most of these developmental processes require proteins and thus the process of decoding proteins from DNA (transcription) is a critical phenomenon. Several factors and multi-subunit complexes play essential functional roles in transcription. Among all these factors, the TATA binding protein (TBP) has a central and essential function. The binding of TBP to the TATA box of the DNA is the first and often the rate-limiting step of transcription initiation. There for this step is highly regulated.

TFIID is a transcription factor that is constituted of TBP and 14 different TBP associated factors (TAFs). TAFs are known to have gene specific functions. In yeast, TFIID is approximately 1.2 MDa in size. To get a better understanding of the molecular organization of TFIID, we used a yeast two-hybrid approach to detail the TBP-TAF and TAF-TAF interactions. Every TAF exhibited a unique interaction profile. This study furthered our understanding of the organization of TFIID. Finally we delineate the TAF interaction regions within the scaffolding TAF, TAF1.

TFIID interacts with TFIIA. However, the specifics of this interaction were not clear until we establish that TAF11 makes functional contacts between the rest of TFIID and TFIIA functioning as an essential link between these two transcription factors. Using point mutants of TAF11 and TFIIA, we show an *in vivo* compensatory interaction between these two factors thus establishing that TAF11 acts as a mediator between TFIID and TFIIA.

MOT1 is a TAF known to have activation and repression function in transcription regulation. Not much is known about this intriguing protein and how it accomplishes this dual function. We tethered MOT1 to the DNA binding domain of a transcription factor and recruited it to the DNA to study its effects on transcription. Our findings clearly establish the repressive function of MOT1 *in vivo*.

The studies presented here give us a better understanding of various facets of transcription regulation by factors that associate with TBP.

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Introduction to eukaryotic RNA polymerase II mediated transcription

I.1 Eukaryotic RNA polymerase II mediated transcription

Eukaryotes have evolved elaborate mechanisms to control the spatial and temporal patterns of transcription, which maintain cell growth, differentiation and development. Proteins are the fundamental molecules that control many processes of development. Expression of protein-coding genes is carried out by the RNA polymerase II (Pol II) mediated system (42, 97, 204). Studying transcription helps us to understand any aberrations that might result in diseases like cancer. It is essential to have a clear view of transcription and the functions of multiple factors that are involved in this mechanism.

Since the purification of RNA Pol II, much has been discovered in this area. Transcription can be loosely classified into three different stages: Initiation, elongation and termination. Transcription activators, which bind specific upstream activating sequences (UAS) on the promoter of protein coding genes, specifically bind and recruit the general transcription machinery and Pol II (98, 142, 162). Transcription initiation can occur in a stepwise fashion (17, 70, 298) or in a single step, since most of the transcription factors were found associated

with Pol II (27, 34, 142, 148, 158). A conflict in opinion is prevalent among researchers regarding the theories of stepwise assembly and the holoenzyme concept. Gene activation at any given promoter might involve the entire spectrum of these two mechanistic possibilities. However, for simplicity we will adopt the concept of stepwise assembly of transcription factors.

Transcription by RNA Pol II requires a set of at least six general transcription factors (TFIID, TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) in addition to the Pol II enzyme itself (298). Binding of TFIID to the TATA box sequence is the first and often the rate-limiting step in transcription initiation (17, 45, 269). This complex nucleates the assembly of the pre-initiation complex (PIC) (figure I.1) (45, 70, 173). The PIC melts a 12-15 bp region in the promoter DNA and initiation continues with the formation of the first few phosphodiester bonds. After a few probable abortive initiation attempts, the polymerase eventually generates longer RNA and these stages are followed by progressive elongation and termination. This RNA is further processed including the addition of a 5' cap and a 3' Poly-A tail resulting in a fully mature messenger RNA (mRNA). This mRNA is translocated to the cytoplasm and translated into a protein. Thus, sequence information from DNA is decoded into a protein.

A typical promoter regulating region, encompasses upstream activator sequences (UAS), enhancer and repressor sequences, and transcription silencers. A core promoter generally contains a transcription initiation site (Inr), and a TATA box (AT rich) that is located 25-30 bp upstream of the initiation site in higher eukaryotes or 40-120 bp in yeast (250).

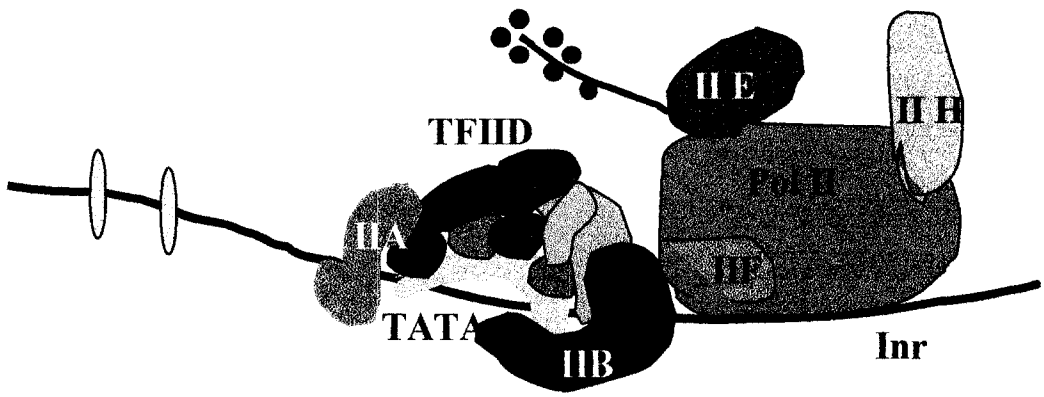

Figure I.1: Schematic of pre-initiation complex assembly in RNA Pol II mediated transcription. Basal transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH are indicated. Polymerase II (Pol II) is also shown. TATA sequence, transcription initiator sequence and nucleosomes are represented on the DNA.

In most cases, a transcription initiation site has initiator elements (243, 244), which are the RNA start site and also encompasses sites that are bound by regulatory factors (129, 225, 270). These core promoter elements affect the lineage-specific, temporal and spatial regulation of gene expression (60, 76, 99, 236). In addition to transcription regulation by these core promoter elements, the activator and repressor sequences, silencers and enhancers by interaction with the transcription activators and other regulators mediate transcription.

A more recent focus in the field of transcription is chromatin (DNA packaged into a nucleo-protein complex), which is an essential component for fitting the DNA into the confines of the nucleus. The organization of the nucleosomes into higher order structures, restrict the access of proteins involved in transcription to the DNA. Thus, chromatin generally acts as a negative regulator of gene expression (135, 170, 218, 287).

Eukaryotic transcription is one of the most highly regulated processes. Gene regulation broadly consists of activation and repression, wherein regulators effect the recruitment of transcription machinery and chromatin modifying complexes (11, 21, 97, 138, 181, 214, 271, 289). These factors now further influence the activity of transcription apparatus (8, 222, 291) and also modify the re-initiation of the transcription (94, 244, 297).

In spite of having such abundance of knowledge in the area of transcription initiation and regulation there is much to be learned about how various transcription factors and other multi-subunit complexes modulate

transcription. Further detailed studies will provide insights into combinatorial control and regulatory mechanisms that result in differential gene expression.

I.2 Pol II basal transcription machinery in *Saccharomyces cerevisiae*

S. *cerevisiae* is an extraordinary tool for the study of transcription. RNA Pol II is highly conserved among eukaryotes and so we can take advantage of this fact and pursue studies in much smaller and easily cultivatable organism like S. *cerevisiae*. The information we gather from here can be applied directly to more complex eukaryotes. Yeast provides us the advantage of powerful genetics for investigating many fundamental issues. In the thesis a special emphasis shall be placed on discussing the yeast system as all the studies were done in yeast. However, an effort shall be made to integrate the information from yeast and metazoans. Special mention to 'eukaryotes,' shall be made, when I make general statements. Since, the transcription mechanism and machinery is highly conserved from lower to higher eukaryotes, information provided here is pertinent to all species except for minor differences.

Initial attempts to reconstitute promoter directed transcription *in vitro* by Pol II alone resulted in no transcription (224, 280). Selective initiation was achieved upon the addition of crude cell extracts (176). Since then, several factors required for basal transcription (general transcription factors- GTFs) have been identified and biochemically purified from a wide variety of organisms and were named based on their chromatographic elution profiles and order of identification as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH (176). Based on the

order of recruitment and binding to the core promoter of these GTFs, arose the concept of stepwise assembly for the formation of PIC. Many of the GTF's and Pol II subunits and their functions are conserved from lower to the higher eukaryotes. Subunits from human Pol II can replace the yeast Pol II in transcription assays (179). Although yeast TBP can replace human TBP in *in vitro* experiments (18, 26, 96), yeast TFIIB cannot replace human TFIIB (240). TFIIB determines the spacing between TATA and accurate start site selection (210). Therefore, the differential spacing between TATA and start sites in human (25-30 bp) and yeast (40-120 bp) accounts for the incompatibility of human TFIIB in yeast. Each of the general transcription factors has a certain defined role in transcription (please refer to the table I.1 on Pol II basal transcription machinery in S. *cerevisiae*).

I.3 Transcription factors IID and IIA, and TAFs as interaction mediators

RNA Pol II cannot accurately function without the assistance from a large group of auxiliary general transcription factors. Of all the transcription factors, TFIID and TFIIA command much attention. The reason being that, not only are they involved in the rate-limiting step of transcription initiation (exception *HSP1* and *CYC1*, e.t.c.) and in co-activation of transcription. In addition, TFIIA influences the way TFIID binds at the core promoter and the formation of the PIC. So in short these two are important because one (TFIID) is a rate limiting factor and other (TFIIA) influences this rate limiting step in transcription.

Table I.1: Pol II basal transcription machinery in S. *cerevisiae*

Factor	Subunit molecular weights	Genes	Protein-Protein Protein-DNA interactions	Functions & Characteristics
TFIIA	Toa1 (32 KDa) Toa2 (13.5 KDa)	*TOA1* *TOA2*	TFIID TBP-DNA (48, 137, 164)	Transcription activator (167) Anti-repressor (6, 78, 87, 121, 185) Transcription initiation (48, 137, 164)
TFIIB	Monomer 38 KDa	*SUA7* (210)	TFIID-DNA (17, 173) TFIIF (69) Pol II (62, 93) TBP-DNA (197) Promoters (149)	Links TFIID to the promoter with Pol II/IIF Specification of start site (101, 163, 210, 252)
TFIID	TBP (27 KDa) TBP-associated factors (TAFs) (45, 59, 70, 173)	SPT15 and other genes	TFIIA (48, 137, 164)	Rate limiting step in transcription initiation Co-activator properties Recognition of TATA Recruitment of GTF's Nucleation of PIC
TFIIE	α, β dimer in yeast tetramer in human (156)	*TFA1* *TFA2* (63)	Pol II (71, 177) TFIIF (71, 177) TFIIH (202)	Recruits TFIIH to the PIC Stimulates TFIIH dependent phosphorylation of Pol II CTD (171, 202, 203)
TFIIF	105KDa 54 KDa 80KDa (non-essential) (40, 132, 213)	*TGF1* *TGF2* *TFG3* (102, 103)	Pol II (42, 90)	Suppression of non-specific binding of pol II to DNA and PIC stabilization (42, 90) Escorts Pol II (42, 90) Increases the rate of elongation (12, 71, 124) Inhibits spurious initiation sites (41, 130)
TFIIH	95 KDa 85 KDa 73 KDa 59 KDa 50 KDa 47,45 KDa 37 KDa 32 KDa 33 KDa	*SSL2* *RAD3* *TFB1* *TFB2* *SSL1* *CCL1* *TFB4* *TFB3* *KIN28*	TFIIF	DNA dependent ATPase activity (43, 226) ATP helicase activity (125, 276) CTD kinase activity (63, 171, 238) Open promoter complex formation (58, 114, 125, 276) Phosphorylation of CTD (47, 267) Stable elongation stage (57) Nucleotide excision repair & Cell cycle progression (64, 83, 277, 295)
Pol II	12 subunits (refer to (97))	*RPB* (1-12)		Enzymatic property for transcription Transcription activation (14) Pre-mRNA processing 5' Capping & 3'end formation (33, 178, 296)

Modified from reference (97). All the references are shown in red lettering.

TFIID is constituted of TBP and 14 different TBP associated factors (45, 59, 70, 173). Not much was known about the TFIID architecture, but recent surge of literature has given us important insights as to its appearance and the organization of the sub-units in this 1.2 MDa complex. Three-dimensional imaging performed by using electron microscopy and image analysis software shows the structure of TFIID to be tri-lobed (2, 15). Using immuno-localization, a few of the TAFs that contain histone fold domain (HFD) have been mapped in this structure (155). Several studies have identified TBP-TAF and TAF-TAF interactions in TFIID to better understand its structure and organization (155, 231, 237, 292). The functional role of TFIID can be ascertained to the individual roles of its sub-components. To get a better understanding of the molecular organization of TFIID, I chose to study the protein-protein interactions using two-hybrid analysis of its sub-components and develop an interaction map (292). This study gave us insights into TBP and TAF organization .It was interesting to note that each subunit had a unique interaction profile. This study is further discussed at length in chapter II.

TFIIA is generally known as a co-activator of transcription. It was originally identified as a general transcription factor that is necessary for accurate transcription initiation. Yeast TFIIA is composed of two subunits, Toa1 and Toa2, which are 32 and 13.5 KDa in molecular weights respectively. The two subunits form a heterodimeric structure (80, 279). TFIIA is known to make extensive contacts with TBP and DNA upstream of the core promoter (126, 167). TFIIA stabilizes TFIID on the TATA element (137), which is critical for gene activation

(167). TFIIA is important for accurate transcription initiation *in vitro* as well (220). It activates transcription *in vitro* in presence of TAFs (45, 52, 99, 206). TFIIA acts as an anti-repressor by inhibiting certain repressors associated with TBP (6, 78, 87, 121, 185).

TFIIA is required for the stimulation of transcription (52, 206, 251, 293). TFIIA functions as a co-activator of transcription by interacting with activators and it facilitates the recruitment and stable formation of the TFIIA-TFIID-DNA complex (48, 137, 164). The association of TFIID with DNA is TFIIA-dependent (232). TFIIA contacts TFIID via its interactions with TAFs (91, 145, 294). TFIIA is also known to remove the inhibitory interactions of certain TAF's and other factors associating with TBP (7, 91, 141, 205). Thus, it is also known as an anti-repressor.

Our laboratory has identified a direct interaction between TFIIA and TAF11 (145). In chapter III, TAF11 is shown to enhance the TFIIA-TBP-DNA complex formation, TFIIA mutants defective for interaction with TAF11 were shown to have conditional growth defects and phenotypes associated with activated transcriptional defects (145). We also show compensatory interactions between TAF11 and TFIIA. The significance of the N-terminal domain of TAF11 for its interaction with TFIIA is also discussed at length.

I.4 TBP, a universal transcription factor

Eukaryotic transcription is controlled by three different polymerases I, II and III, all of which are dedicated to transcribe different sets of genes. Genes transcribed

by these three different polymerases have different promoter structures, which attract the appropriate basal transcription machinery specific to them and further aid in recruitment of the correct polymerases (176, 230). In spite of the wide variability in transcription factors and polymerases in these three systems, a commonality is the TATA binding protein (TBP). In yeast TBP was first identified in a genetic screen looking for suppressors of retrotransposon TY insertion at certain promoter elements (61, 95). In Pol II mediated systems, the transcription factor TBP was first shown to be present at a large number of promoters (19, 234, 298). In yeast, TBP could be purified to a single polypeptide of 27 KDa (24, 95, 116, 117). It was isolated from many other species as well (107, 110, 112, 127, 192, 208). TBP mediates transcription *in vivo* but does not support activator-mediated transcription (107, 112, 208, 215, 245). Later biochemical analysis revealed that TBP was present in a large complex that is constituted of TBP and a number of TBP associated factors (TAFs) (59, 257). This complex can be isolated by using less stringent conditions than used earlier. After the initial discovery and isolation connecting TBP to Pol II, it was subsequently shown both by biochemistry and genetics that TBP is involved in RNA Pol I and RNA Pol III systems as well. TBP plays a major role in transcription of Pol I as a subunit of SL1 transcription factor (39) and in the Pol III mediated transcription of the TATA less promoters as a subunit of TFIIIB (119, 128, 168, 242, 254, 284). Mutations in TBP affected the transcription of genes by all the three polymerases (44, 235). TBP plays a role in all the three transcription systems, both at TATA containing

and TATA-less promoters by its presence in TFIID, SL1 and TFIIIB. Thus, it is a true universal transcription factor.

I.5 TBP, its functions and characteristics

In higher eukaryotes, promoters of the genes transcribed by Pol II either have a conventional TATA box and initiator or just the initiator sequences. TBP contained in TFIID is recruited to these promoters. TBP can replace TFIID in directing basal levels of transcription from TATA containing promoters *in vivo*.

TBP is a highly conserved protein and plays a major role in transcription. It has a bipartite structure with a highly conserved C-terminal domain, which is more than 75% identical across species. The core domain contains a large DNA binding domain that interacts with DNA as a monomer (118) in minor groove of the DNA helix (152, 248). The crystal structure of TBP has provided us with important clues towards its appearance. TBP resembles a horse saddle (198). The symmetric structure differs in sequences on either side, the half concave underside of the saddle fits into the minor groove of the DNA.

TBP is a target for both positive and negative regulation. In spite of its small size, it has the ability to bind many proteins like the GTFs, TAFs, activators and inhibitors. These interactions are mostly accomplished by interaction with its large convex surface. TBP generally has a positive role in transcription. However, via its interactions with some factors like NC1, NC2, Dr1 and MOT1, the activity of TBP can also affect transcription negatively (6, 121, 180). Most of the factors

interacting with TBP are likely to be dynamic and not all the possible partners will be engaged in interactions at any one time.

The N-terminus of TBP is well conserved in vertebrates where it has characteristic glutamine repeats that are subject to allelic variations in humans. The expansion of the glutamine repeats from 28-42 in normal individuals to 47-56 lead to cerebellar ataxia and other neurodegenerative diseases (72, 194). The N-terminus of TBP also influences DNA binding by the core domain. N-terminus inhibits the formation of a stable bent DNA complex by interacting with the inhibitory DNA binding surface on the TBP saddle (IDB) (299). In spite of this inhibitory role, it was surprising that the deletion of the first 24 residues resulted in embryonic lethality in mice (105). The molecular mechanism underlying this observation is still unclear but this observation uncovers a specialized role for TBP in fetal development. In some vertebrates, TBP levels were shown to control cell cycle and apoptosis (266). Thus, TBP has a multifaceted role in overall cell growth and development.

In yeast, Pol II mediated transcription has two additional TBP containing complexes in addition to TFIID. In B-TFIID, TBP is associated with BTAF1 (268). In TBP –TFIIA containing complex (TAC), TBP is associated with an unprocessed form of general transcription factor TFIIA (187). The TBP containing complexes were identified biochemically. Little is really known about their *in vivo* functionality.

Universal requirement for TBP in transcription was questioned, when first TBP related factor (TRF1) was identified in *Drosophila* (46). The TRF1 primary

structure is highly related to the conserved core domain of TBP and is expressed in embryos, adult nervous system and male germ cells. TRF-1 localizes to Pol III transcribed loci and also has a role in transcription of Pol II transcribed genes (113). Later, TRF-2 was identified and was shown to participate and have a role in chromatin remodeling (106, 201, 217, 264).

TBP can be dispensable under some circumstances (285). A complex which was termed as 'TBP free TAF containing complex' (TFTC) was isolated. TFTC, both in crude as well as highly purified forms, could support transcription of a promoter from both TATA containing and TATA less promoter and also responded to transcription activators. However, if TFTC is a bonafide complex it would be present in crude transcription extracts and also would support the activity of promoters mutated at the TATA (3). To reconcile the facts that TBP can be dispensable at least under certain circumstances, and at the same time play essential roles in promoter recognition and pre initiation complex assembly at most of the promoters, the following arguments are proposed. Several of the TBP associated factors-TAFs could be making essential contacts with promoter and initiator sequences at genes where TBP is dispensable (199). Such interactions between TAFs and the core promoter may also be the basis by which TAFs can directly select for core promoters *in vivo* (97). At promoters that lack a conventional TATA where there is a larger role for the TAFs and TBP might perhaps play part in assembly of the PIC complex rather than promoter recognition.

Transcription is controlled not by any one single factor or a multi-subunit complex, but is a combined effort by many factors, which precisely bring about differential gene expression by a combinatorial effect. This reinforces the fact that each of these complexes represents a precise combination of common and unique components and that much more research has to be conducted to answer many major questions in the area of transcription.

I.6 TBP associated factors (TAFs)

TBP, in most of the cases is associated with other protein factors. In metazoans the TBP containing complexes include TFIID, SL1 (in yeast; CF), TFIIIB, B-TFIID, and SNAPc (absent in yeast). Since our primary interest is on Pol II mediated transcription we shall discuss in detail the TAFs that are specific to this system.

Historically, TBP was thought to be able to replace TFIID in transcription assays (208). While TBP could replace TFIID as the nucleating factor for PIC formation (208), it was unable to respond to most upstream regulatory activators similar to TFIID. Further biochemical fractionation of TFIID revealed that at least some of the proteins were tightly associated with TBP to form a large stable multi-subunit complex (59). These TBP containing complexes could replace TFIID for activated transcription (191, 211, 255, 257, 301). Later, several groups cloned these TBP associated factors (TAFs) from *Drosophila*, humans and yeast. It is interesting to note that they were all conserved from lower to the higher eukaryotes (21, 153). Collectively there are now 14 different TAFs in TFIID alone and 13 of them have essential functions. In yeast, the molecular weights of these

TAFs range from about 17-170 KDa. Initially, the nomenclature was based on their electrophoretic mobility. Recently, a unified nomenclature for the TAFs has been derived to accommodate cross species comparisons and avoid confusion (263).

I.7 TAFs, as transcription regulators

The majority of TAFs are shown to be essential. Nearly all the yeast TAF mutations are lethal (211, 219) and a few undergo cell cycle arrest (4). In spite of this essentiality, some studies have shown that TAFs are dispensable for transcription in yeast at certain promoters (4, 189, 274). However, recent microarray analysis has shed much light on the requirement for TAFs (241). In this study the researchers made temperature sensitive mutations in all the TAFs and did a global check for the genes being affected. This study shows that in yeast the genes have TAF dependence from 2%-80%. It was interesting to note that TAF2 has a very small impact factor on genes (2%) in comparison to TAF9, which has a larger effect (59-61%) on genes. The general contention is that TAFs have gene specific functions and their requirement is not universal, but is broadly required.

TFIID is the principle TAF containing complex in Pol II-mediated transcription. Immunodepletion of yeast TFIID by TAF-specific antibodies blocks *in vitro* transcription by RNA Pol II but not by RNA Pol I or Pol III. In many purified *in vitro* transcription systems, it was shown that TAFs are essential for stimulation of transcription by activators. Evidently, TAFs have direct sites of interaction for

activation domains of various targets (271). These TAFs serve as co-activators targeted by DNA binding transcription factors (59, 108, 257). Several studies have shown that specific TAF-DNA contacts occur within the context of assembled TFIID (20, 253, 270).

Certain TAFs like, TAF4, TAF6, TAF9 and, TAF12, have histone fold domains and thus resemble core histones. Studies show that the histone-like TAFs have more central roles in transcription activation than others (5, 186, 190, 195). A few TAFs, like TAF1, have more specialized roles in transcription. TAF1 has enzymatic activities like HAT and kinase activities and is also generally referred to as scaffolding TAF. More importantly, TAF1 is required for transcription from promoters lacking consensus TATA sequences (154). To summarize, the important TAF functions include acting as co-activators, coupling activator-mediated signals to the basal transcription machinery, acting as promoter selectivity factors, and stabilizing TFIID on the core promoter. However, not all TAFs have every function mentioned above and they do not elicit their effects at all the promoters.

I.8 TAFs and their presence in different multi-subunit complexes

In conjunction with the Pol II mediated transcription, subsets of Pol II TAFs are also present in yeast SAGA (Spt6, Ada, Gcn5 and acetyl transferase complex) and the human PCAF (histone acetylase complexes) (88, 200). Interestingly SAGA has five TAFs, four of which are the histone fold containing TAFs. This discovery opened new functional avenues for TAFs outside of the context of

TFIID, since both these complexes have been implicated in chromatin modifications. There is another TBP free TAF containing complex known as TFTC, which has a subset of TAFs (285). This TFTC complex was shown to support transcriptional activation on both TATA containing and TATA less promoters. It was interesting to note that TAF1, and Gcn5 of TFIID and SAGA respectively affect the expression of overlapping fractions of yeast genes that can be regulated through either complexes, indicating that *in vivo* these two complexes might have redundant functions (241). The TFIID and SAGA-shared TAFs are required for normal expression of 70% of the yeast genome (241).

The presence of TAFs in several complexes like SAGA, STAGA (Spt3-Taf31-Gcn5) (175), PCAF (200), TFTC (15), has complicated our interpretation of the functional significance of the TAFs. If a TAF is found to have a particular activity, it is difficult to interpret the TAFs functional activity from the perspective of a single multi-subunit complex. It also raises questions regarding the uniqueness of the TAFs and whether they have overlapping functions. However, so far we know the answer for the TFIID and SAGA complexes and further microarray analysis will also reveal new insights into TAF functions in different sub-complexes. Recent studies on TAF function specific to a particular complex use mutations which disrupt the complex in question, but have no affect on the other complexes containing TAFs. Thus, the specific function of a TAF particular to a complex can be addressed.

I.9 TAF and its interactions with the basal transcription machinery

TAFs make essential contacts with the basal transcription machinery. TAF-GTF interactions might be useful for the formation of the PIC, recruitment of the GTFs and Pol II, and also induce conformational changes in TFIID. Several studies have identified direct interactions between TAFs and GTFs by both biochemical and genetic methods. The TAF-GTF interactions include dTAF4 (and its homologs), and yTAF11 with TFIIA (145, 294), dTAF9 (and its homolog in human) with TFIIB (85, 134), dTAF5 and hTAF6 with TFIIE (104, 211, 219), and yTAF13 with TFIIF (102).

It is clear that many of the TAFs have direct interactions with the general transcription factors but not in all cases is there a definite correlation between their interaction and functional significance. The general contention is that these interactions help in recruiting the GTFs and aid in the formation of the PIC.

I.10 TAF-DNA interactions

In yeast, promoters have been grouped into two classes: TAF-dependent promoters and TAF-independent promoters (147, 159). More recently a new class of promoters has been identified that have an intermediate requirement for the TAFs (241). At TAF-dependent promoters TBP and TAFs are present at comparable levels. The UAS seems to mediate the selective recruitment of TAFs to these promoters (160). However, at TAF-independent promoters, TAFs are not present or present at levels far below to that of TBP. A variety of studies have suggested interaction between TAFs and DNA. TBP gives rise to a discreet footprint that just covers the TATA box, while DNAse I footprint of TFIID on some

core promoters was substantially larger (21). This occurs only at certain promoters, suggesting the DNA specific contacts made by TAFs are not general. There have been several studies showing direct interactions by the TAFs with DNA both upstream and downstream of the TATA box. In particular, studies have shown that TAF1 makes essential and critical contacts with the promoter upstream of the DNA binding site (183), whereas TAF2 was shown to make contacts with DNA downstream of the TATA box and the initiator elements (272). Depletion of individual TAFs resulted in reduction of transcription from promoters lacking a canonical TATA box (189, 190). Thus, it is clear that a few TAFs have essential roles in promoter recognition and stabilization of TFIID at the core promoter.

I.11 MOT1, an unusual TBP associated factor

There are several transcription repressors that form complexes with TBP or interact with TBP and that repress activated as well as basal transcription (121, 180, 184). MOT1 belongs to the category of repressors that does interact with TBP causing it to dissociate from the DNA.

MOT1 stands for Modifier Of Transcription 1 (51). MOT1 was isolated in genetic screens as a mutation that led to an increase in the basal level expression of several pheromone responsive genes in the absence of a trans-activator and pheromones (51). In another genetic screen, it was isolated as a trans-acting factor that affects DNA polymerase genes (209). MOT1 is an

essential gene and in yeast it encodes a 210 KDa protein, which is conserved from yeast to mammals (51).

MOT1 belongs to the Snf2/Swi2 family of ATPases (51), all of which contain a large conserved domain of approximately 550 amino acid residues. Snf2/Swi2 proteins are present in highly diverse organisms from bacteria to humans. Many studies in yeast show that Snf2/Swi2 affect the expression of genes by counteracting adverse effects of the nucleosomes (22, 100) and their ATPase activity is essential for their function. In spite of MOT1 being grouped into this family of genes, it affects transcription not by nucleosome remodeling but by interaction with TBP. MOT1 utilizes its ATPase activity to dissociate TBP from DNA (7) whereas Snf2 primarily utilizes its ATPase energy to model nucleosomes on DNA (286).

Biochemically, MOT1 was identified as a factor in nuclear extracts that inhibited TBP binding to the DNA in an ATP-dependent fashion (6). The *In vitro* activity of MOT1 could be counteracted by TFIIA and to a small extent by TFIIB. *In vitro* studies also show that the displacement of TBP by MOT1 was not promoter specific (6). Over-expression of MOT1 resulted in negative growth effects, which could be suppressed by over-expression of TBP and TFIIA (7). Thus, a connection between MOT1 function, TBP and TFIIA was established.

A functional connection between MOT1, NOT1 (negative on TATA) and Spt3 was also shown. MOT1 was also identified in genetic screens looking for factors that functionally interact with Spt3 (172). MOT1 and NOT1 (37, 38) seem to exert opposite effects on transcription from TATA less promoters. At a TATA

less promoter NOT1 mutation increased transcription, whereas MOT1 mutation decreased transcription. Thus, NOT1 has negative and MOT1 has positive affects at transcription from TATA less promoters. However, if this is true it does not fall in line with the general contention that MOT1 dissociates TBP from non-conventional TATA elements and thus redistributes the limiting pool of TBP to TATA containing promoters and negatively affecting the TATA-less promoter transcription.

I.12 MOT1 in B-TFIID

In mammalian cell extracts, an alternate form of TFIID was isolated known as B-TFIID (261, 262). In contrast to TFIID, B-TFIID did not respond to transcription activators (262). This B-TFIID complex possessed ATPase activity (261). Upon sequence analysis it was shown that one component in the complex resembled yeast MOT1. Thus B-TFIID is a mammalian homolog of yeast TBP-MOT1 complex. To avoid confusion MOT1 was renamed as B-TAF1 along with other TAFs in the new nomenclature (263). However, in this thesis I shall call this factor as MOT1, which very aptly describes its functional role *in vivo.*

I.13 MOT1 interactions with TBP and its implications for transcription regulation

In vitro studies have shown that MOT1 forms a stable complex with TBP in solution and on promoter DNA (6). MOT1 utilizes the energy from ATP hydrolysis to dissociate TBP from DNA (7). This ability to dissociate TBP is associated with

repression of basal and activated Pol II gene transcription (7). Classical role of MOT1 is of a transcription repressor. However, recent microarray analyses suggest that MOT1 regulates transcription both positively and negatively (50, 82). In addition to these large scale profiling studies, others have also shown that at lower levels MOT1 activates transcription (193). A few genetic studies have shown that mutations in MOT1 resulted in decreased transcription of certain genes. Even more exciting are chromatin immunoprecipitation analysis that show MOT1 co-localized along with TBP at the genes which it regulates both positively and negatively (82). It seems like there is no simple 'yes' or 'no' answer for the presence of TBP at genes both positively and negatively regulated by MOT1. However, these studies do tell us that both positive and negative functions of MOT1 are direct effects of its action (50, 82). I focus on studying the *in vivo* function of MOT1 in chapter IV to further investigate its function. This chapter also provides much more information from the MOT 1 literature and has been written in the format of a manuscript.

I.14 Statement of significance and thesis layout

For my dissertation research, I have chosen to answer separate but related questions concerning factors that are physically associated with TBP or factors that are genetically shown to interact with TBP in S.*cerevisiae.* First, TFIID is constituted of TBP and 14 different TBP associated factors known as TAFs. TFIID exists as a 1.2 MDa complex in yeast. Not much is known about the structure or organization of TFIID. I chose to study the organization of sub-units

within TFIID, by systematically identifying all the protein-protein interactions using yeast two-hybrid analysis. We used the results to create a TFIID interaction map (292). This study provides us with a better understanding of TFIID complex and its organization. It also tells us that each one of the TAFs had a unique interaction profile which was non-overlapping.

In addition to identifying all the TBP-TAF and TAF-TAF interactions, I have specifically focused on TAF1, a 130 KDa protein in yeast. TAF1 was interesting for several reasons. One, it is known as the scaffolding TAF in TFIID. Two, it has several interesting enzymatic activities. We wanted to utilize TAF1 for understanding two different questions. First, to identify a smaller region in TAF1 that would encompass most of the interactions representative of the full-length protein. This would allow us to do more biochemical manipulations to understand TAF1 further. Second, a few TAFs in humans were shown to contact TAF1 at specific domains and modify the enzymatic activities of TAF1. We wished to known if these specific interactions were also conserved in yeast. We identified smaller regions of TAF1 that were active 'interaction wise' and we show that the most of the interactions previously identified in humans are conserved in yeast. This study has provided valuable information regarding TAF1 function and its interactions with other TAFs has been mapped to specific domains within it.

There is a large body of work suggesting that there are functional contacts between TFIID and TFIIA. However, not much is known about the manner of these contacts and their significance. Our laboratory has previously shown that TAF11 makes functional contacts with both TFIID and TFIIA, thus acting as a

mediator for these two transcription factors. To further understand the functional correlation between this mediator function of TAF11 between TFIID and TFIIA, I looked at a specific allele of TAF 11 known as ΔN-TAF11. This allele exhibited temperature sensitive phenotype. More importantly it was defective for interactions with TFIIA. I was also interested in another allele of TAF11 known as E182G that exhibited several interesting phenotypes and was isolated in a screen to suppress the defects of TFIIA. By looking at these two alleles in closer detail we have learned about TAF11's functional significance in mediating interactions between TFIID and TFIIA. This was a collaborative project with a previous graduate student in the laboratory and is being submitted as a manuscript for publication (described in chapter III).

MOT1 is a very interesting TBP associated factor. It interacts with TBP to regulate its function negatively. Most other TAFs have positive roles in transcription. However, MOT1 is not present in the conventional TFIID complex but is present with TBP in a separate complex known as B-TFIID. MOT1 regulates transcription both positively and negatively. Up to now there has been no direct *in vivo* assays that could differentiate the functional ability of MOT1 to activate and repress transcription. We developed an *in vivo* assay allowing us to focus on the repression function of MOT1. We recruit MOT1 to the test promoters and study its effect on TBP and transcription. This study has provided us with valuable insights into the function of MOT1 action and particularly on transcription repression. This work was done in collaboration with Dr. David

Auble (Virginia Medical School), a pioneer in the area of MOT1. The work done in chapter IV is presented in a manuscript form and is ready for submission.

So far, I have looked at TBP associated factors, which were present along with TBP in a complex. However, in the appendix of my dissertation, I focus on *SPN1*, which was shown by our laboratory to genetically interact with TBP and was isolated as suppressor for post recruitment defects in TBP. Not one or few factors carry out regulation of transcription. Several factors either individually or present in complexes do so. I probe deeper into the question of occupancy of transcription factors at a gene that is post recruitment regulated and whose transcription is affected by *SPN1*. This study is done as an extension of the technique (chromatin immunoprecipitation assays) that I had learned and have applied to understand some pertinent question regarding an ongoing project about *SPN1* in our laboratory. This study gives us valuable insights not only on *SPN1* function but also how the transcription factors and chromatin remodeling factors occupy genes in general. This section is included in appendix of my dissertation, as it is still an ongoing effort in our laboratory to understand *SPN1* function.

In chapter V, I highlight the future directions for each of the projects that I have been involved in. I see a lot of potential work, which can be started up from these future directions, especially in the case of MOT1 and *SPN1.*

Chapter II

Protein-Protein interaction map for TFIID

This chapter was published in Nucleic Acids Research. The text of this manuscript is present as it appears in the journal and is formatted to fit the guidelines in this dissertation. All the figures included are the same. This work was done in collaboration with Lei Zhang and Susan M. Kraemer from our laboratory. Sue Kraemer created some of the clones and Lei Zhang and I created the rest. I wrote the manuscript and created all the figures. Literature citation for this chapter is:

Yatherajam,G., Zhang,L., Kraemer,S.M., and Stargell,L.A. Protein-Protein Interaction map for TFIID, Nucleic Acids Res. 2003 Feb 15;31(4):1252-60.

II.1 Abstract

A major rate-limiting step in transcription initiation by RNA polymerase II is recognition and binding of the TATA element by the transcription factor TFIID. TFIID is composed of TATA binding protein (TBP) and approximately a dozen TBP-associated factors (TAFs). Emerging consensus regarding the role of TAFs is that TFIID assumes a gene specific activity that is regulated by interaction with other factors. In spite of many studies demonstrating the essential nature of TAFs in transcription, very little is known about the subunit contacts within TFIID. To understand fully the functional role of TAFs, it is imperative to define TAF-TAF interactions and their topological arrangement within TFIID. We performed a systematic two-hybrid analysis using the thirteen essential TAFs of the *Saccharomyces cerevisiae* TFIID complex and TBP. Specific interactions were defined for each component, and the biological significance of these interactions is supported by numerous genetic and biochemical studies. By combining the interaction profiles presented here and the available studies utilizing specific TAFs, we propose a working hypothesis for the arrangement of components in the TFIID complex. Thus these results serve as a foundation for understanding the overall architecture of yeast TFIID.

II.2 Introduction

Transcription initiation by eukaryotic RNA polymerase II (pol II) requires the concerted action of various general transcription factors (GTFs) like TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH in addition to pol II (204, 221, 223). The first step in forming a functional pre-initiation complex is the interaction of TFIID with the promoter. TFIID is a multi-protein complex that contains TATA binding protein (TBP) and over a dozen different TBP associated factors (TAFs) (21, 86, 89, 233, 271). In yeast, 13 of these TAFs are essential for cell viability, and as such, must play critical and non-redundant roles in the regulation of gene expression.

TAFs are not restricted to the TFIID complex, and can also be found in the yeast SAGA complex (88) and mammalian PCAF complex (200). *In vivo* depletion of these shared TAFs results in broad defects on transcription by pol II (5, 186, 190). In contrast, depletion of TAFs specific to the TFIID complex appears to affect transcription in a promoter-specific manner (4, 5, 189, 190). In keeping with gene-specific roles in transcriptional regulation, certain TAFs are critical for TATA-less promoter transcription due to important TAF interactions with initiator and downstream promoter elements (20, 25). Finally, although activated transcription is not generally dependent on TAFs in the yeast system (4, 189), activator-specific recruitment of TFIID is important for the regulation of ribosomal protein gene transcription in yeast (182), as well as the derepression of DNA damage-regulated genes (157). Likewise, activated transcription in higher eukaryotic systems can be dependent on TAFs, presumably due to direct

interactions between TAFs and activators (111, 204, 223, 301). Thus, TAFs play important roles in many different aspects of the regulation of gene expression.

Although the stoichiometry of the various TFIID components has been characterized (231, 232), very little is known about the overall architecture of the TFIID complex. Electron microscopy and digital image analyses of single TFIID particles indicate a C-shaped form with a central cavity (2, 15), and some of the individual TAFs have been mapped within the complex using TAF-specific antibodies (155). However, a complete understanding of the TFIID complex necessitates a cataloging of the specific TAF-TAF interactions within the complex. Here we describe an extensive two-hybrid analysis of protein-protein interactions between all of the essential components of yeast TFIID. A majority of these interactions are consistent with previously reported biochemical and/or genetic data dealing with the individual components. The two-hybrid results, coupled with the large volume of biochemical and genetic data regarding the individual subunits of TFIID, were used to construct a model of the topological arrangement of TAFs in the TFIID complex. This TFIID interaction map serves as an important step in a comprehensive analysis of TFIID.

II.3 Materials and Methods

II.3a DNA constructs: Activation domain (AD) hybrids were cloned into the 2μ LEU2 marked vector, pACT2.2 (55), which contains the ADH1 promoter, a nuclear localization sequence, the hemagglutinin (HA) epitope, and the Gal4 activation domain (residues 768 to 881). All the AD-TAF constructs were created using PCR and homologous recombination cloning in yeast. Full length DNA

binding domain (DB)-TAF hybrids were created by subcloning from the respective AD construct into the pPC97-TRP1 vector (CEN, TRP1), which contains the ADH1 promoter, a nuclear localization sequence, and the Gal4 DB (residues 1 to 147) (273). For those DB-TAF fusions that were positive for transcriptional activation when expressed in yeast cells (i.e. that could artificially recruit the transcription machinery), deletions in the open reading frame of the particular TAF were cloned into the DB fusion vectors. Three derivatives were designed for TAF1, corresponding to amino acids 1-208, 208-367 and 367-1066. For TAF12, two regions containing amino acid residues 1-280 and 280-539 were tested. Deletions for TAF3 were created by cloning amino acids 1-81 and 81-353 into pPC97-TRP1 vector.

II.3b Yeast strains: All Saccharomyces cerevisiae strains used were transformants of either MaV103 (273) or CG1945 (Clontech). MaV103 contains the GAL1 promoter (with four Gal4 binding sites) fused to the HIS3 TATA element and coding sequence; GAL4 and GAL80 are both deleted in the strain. CG1945 contains the GAL1 promoter and the GAL1 TATA element fused to the HIS3 coding sequence; GAL4 and GAL80 are both deleted in the strain. CG1945 cells have much tighter control of background levels of expression from the HIS3 gene.

II.3c Protein expression of two-hybrid constructs: Extracts were prepared from strains harboring the indicated TAF fusion constructs essentially as described in (44). Approximately 25-30 μg of protein was loaded onto the SDS-PAGE gels. Immunoblots were performed using monoclonal antibodies directed

against HA (BAbCO) at 1:1000 dilution, and the anti-mouse secondary antibody (Promega) was prepared at 1:20,000. Signals were developed using a chemiluminiscence kit (Pierce).

II.3d Artificial recruitment and two-hybrid assays: Each DB-TAF fusion was transformed into yeast strain MaV103 or CG1945 using a standard lithium acetate transformation. The ability to activate transcription by each DB-TAF fusion was tested by streaking the strain onto media containing 3-aminotriazole (AT), at a concentration of 40 mM for MaV103 or 5 mM for CG1945. Cells were grown at 30 ^{0}C for 3-7 days. Growth on this media indicated the ability to artificially activate the transcription of a HIS3 reporter gene. For those DB-TAF hybrids that were positive for artificial recruitment in both MaV103 and CG1945, truncations were designed in the open reading frames of the TAF and these constructs were tested in the artificial recruitment assay. Fusions that were negative in the artificial recruitment assay were transformed with the AD-TAF fusions and assayed for two-hybrid interactions by streaking to media containing AT. Cell growth on AT-containing media indicated a positive two-hybrid interaction between the two TAFs that were assayed. Scoring from + to +++ was based on the relative amount of cell growth for each DB-TAF strain containing the different AD-TAF derivatives. One plus (+) indicates growth in the primary streak only, and denotes a weak two-hybrid interaction which was significantly more than the growth level observed with the AD vector alone. Two pluses (++) indicates intermediate level of growth which is indicative of a stronger interaction, whereas three pluses (+++) indicates robust growth and the strongest interaction.

TAF2 and the deletion constructs for TAF1 and TAF3 were assayed in the strain MaV103 for the two-hybrid studies. The remaining TAFs were assayed in the strain CG1945.

II.4 Results

II.4a Characterization of TFIID components in an artificial recruitment assay: Two-hybrid analysis is a useful tool for mapping protein-protein interactions between subunits of transcription-related complexes (68), and defining and disrupting interactions between transcription factors (273). We set out to map the protein-protein interactions in the yeast TFIID complex using a systematic two-hybrid assay with all of the essential components of this complex. The thirteen essential yeast TBP-Associated Factors (TAFs; table II.1) and TBP were individually fused to the Gal4 DNA binding domain (DB; residues 1-147 of Gal4) and the Gal4 activation domain (AD; residues 768-881). The fusions were tagged with the HA epitope and expression of each was confirmed by immunoblot analyses (figure II.1).

DB-fusions were tested for activation by artificial recruitment in the strain MaV103 (273), in which binding sites for Gal4 control the transcription of the *HIS3* gene. Cells can be assayed for *HIS3* expression by growth on 3-aminotriazole (AT), a competitive inhibitor of the *HIS3* gene product. Like DB-TBP (28, 133, 290), 11 of the DB-TAF constructs conferred the ability to grow on AT (table II.2 and figure II.2).

Table II.1: Pol II TAF nomenclature used in the present study

Name	*S. cerevisiae*	*H. sapiens*
TAF1	TAF145/130	TAF250
TAF2	TAF150	TAF150
TAF3	TAF47	TAF140
TAF4	TAF48	TAF130/135
TAF5	TAF90	TAF100
TAF6	TAF60	TAF80
TAF7	TAF67	TAF55
TAF8	TAF65	(BAB71460)
TAF9	TAF17	TAF32/31
TAF10	TAF25	TAF30
TAF11	TAF40	TAF28
TAF12	TAF61/68	TAF20/15
TAF13	TAF19	TAF18

Reference (263)

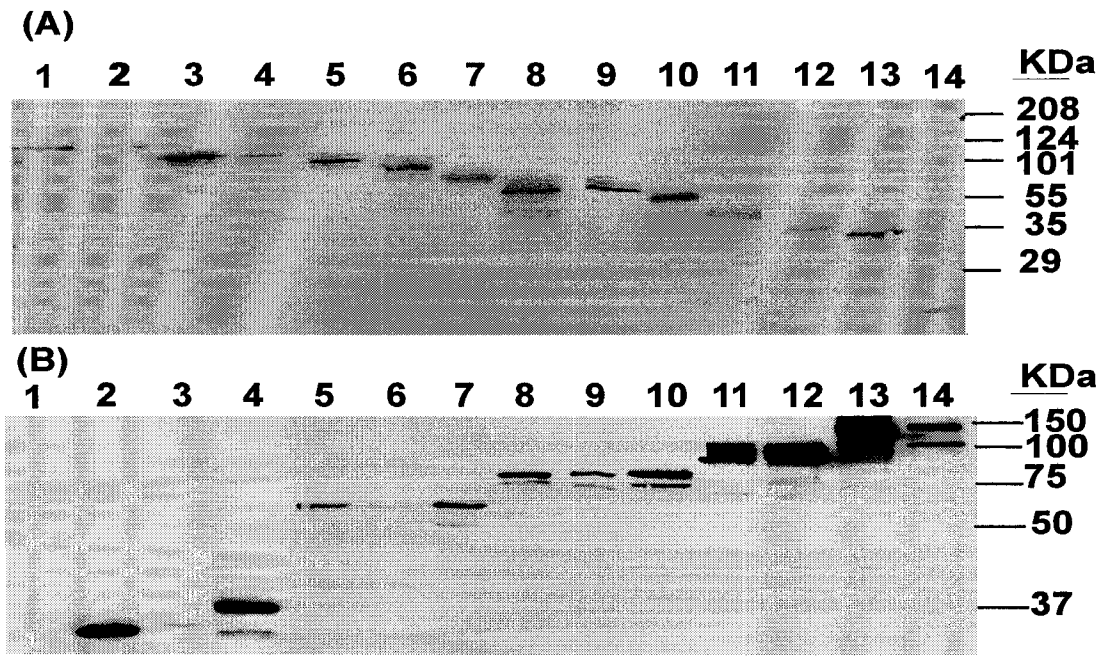

Figure II.1: Expression of two-hybrid fusion constructs. (A) Expression of AD-TAF fusions in vivo. Protein extracts (25μg) from strains harboring the indicated derivatives were loaded on 10% SDS-PAGE gels, and AD fusions were detected using antibodies directed against the HA epitope. Lanes (1-14) show the AD-TAF fusion constructs in descending order of molecular weight. (B) Expression of DB-TAF fusions in vivo. Protein extracts (25μg) from strains harboring the indicated derivatives were loaded on 12% SDS-PAGE gels, and the DB fusions were detected using antibodies directed against the HA epitope. Lanes (1-14) show the DB-TAF fusion constructs in ascending order of molecular weight.

Table II.2: Results of artificial recruitment assays

DB constructs	MaV103	CG1945
DB	- [a]	-
DB-TBP	+ [b]	-
DB-TAF1	+	+
DB-TAF2	-	-
DB-TAF3	+	+
DB-TAF4	+	-
DB-TAF5	+	-
DB-TAF6	+	-
DB-TAF7	+	-
DB-TAF8	+	-
DB-TAF9	+	-
DB-TAF10	+	-
DB-TAF11	+	-
DB-TAF12	+	+
DB-TAF13	-	-

[a] (-) Denotes no growth on aminotriazole and thus was negative for activated transcription from the *HIS3* reporter gene.
[b] (+) Denotes that DB-TAF fusion construct was positive for growth on aminotriazole and thus could activate transcription from the *HIS3* reporter gene.

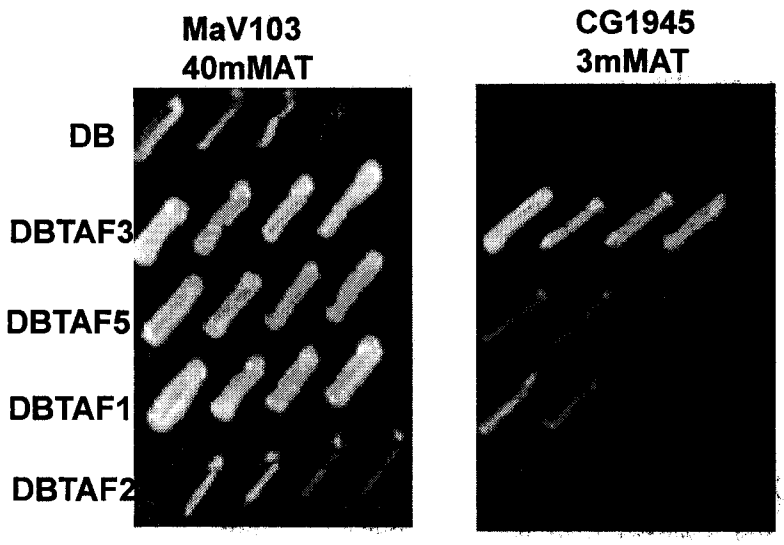

Figure II.2: Representative study of the artificial assay. Two strains of yeast, MaV103 and CG1945, that differ in promoter context for HIS3 reporter gene, were transformed with each of the indicated DB-TAF fusion constructs and assayed for the ability to grow on aminotriazole (AT) containing media. Cell growth on AT (40mM for MaV103 and 5mM for CG1945) is indicative of activated expression of HIS3 reporter gene. Yeast growth in each panel is a serial dilution of slashes from a single colony. All strains grew robustly on control plates of synthetic complete media lacking leucine and trytophan (not shown).

Thus, tethering these particular TAFs to a promoter allows for artificial recruitment of the transcription machinery, and expression of the *HIS3* gene. Although very useful for demonstrating functional activity of the DB-TAF fusions, inherent activation precludes the use of this strain background in a two-hybrid assay, since the DB-fusions are positive for growth in the absence of a Gal4 activation domain (AD) fusion.

We have previously shown that the activity of artificially recruited TBP is dependent on the reporter promoter (246). Others have also demonstrated that activation by artificial recruitment is highly sensitive to promoter sequence and context (77, 196, 229). For example, in MaV103 cells, in which the Gal4-driven promoter contains the *HIS3* TATA element and the *HIS3* structural gene, DB-TBP activates transcription (Table 2 and (246)). In contrast, DB-TBP fails to activate transcription in the yeast strain CG1945 (Clontech), which contains a Gal4-driven promoter with the *GAL1* TATA element fused to the *HIS3* structural gene. Due to the differential function of DB-TBP in the two strains, we tested the DB-TAF fusions in CG1945. Like TBP, ten of the TAFs failed to activate *HIS3* gene expression when recruited to the promoter in this strain (table II.2). Thus, these DB-TAF fusion strains were appropriate for use in a systematic two-hybrid analysis.

II.4b Delineation of regions competent for artificial recruitment in TAF1, TAF3 and TAF12: Three of the DB-TAF derivatives remained competent for activation in the yeast strain CG1945. In order to define, and attempt to excise, a region of each of these TAFs that conferred the potent artificial recruitment

function, we designed a set of deletion derivatives for each (figure II.3). Yeast TAF1 corresponds to an open reading frame of 1066 amino acids, and has several interesting domains including a TBP-interacting domain (9, 136, 141, 144), a central conserved region containing the histone acetyltransferase activity (HAT domain) (188) and a promoter-interacting region near the C-terminus (183). We found that the artificial recruitment function for TAF1 was restricted to the N-terminal 208 amino acids, whereas the more C-terminal regions (encompassing residues from 208 to 367 or from 367 to 1066) were negative for artificial recruitment. Yeast TAF3 is encoded by an open reading frame of 353 amino acids, and has a histone fold domain (HFD) located near the N-terminus (74). The artificial recruitment competent region of TAF3 was mapped to the region containing residues from 81-353, while the DB fusion to residues 1 to 81 of TAF3 failed to activate transcription. TAF12, a protein of 539 amino acids, has a non-conserved N-terminus, and a C-terminal region that is essential for cell viability and also contains a HFD (191). DB fusions to an N-terminal region (residues 1-280) and a C-terminal region (residues 280-539) of TAF12 were tested. We found that the N-terminal domain was positive for the artificial recruitment where as the C-terminal region containing the histone fold motif was negative in the artificial recruitment assays. Interestingly, the HFDs of TAF3 and TAF12 were negative for artificial recruitment, which could be a common characteristic of HFDs, since none of the other TAFs containing the HFDs (TAF4, TAF6, TAF8, TAF9, TAF10, TAF11, and TAF13) were positive in the artificial recruitment assay in the CG1945 strain background.

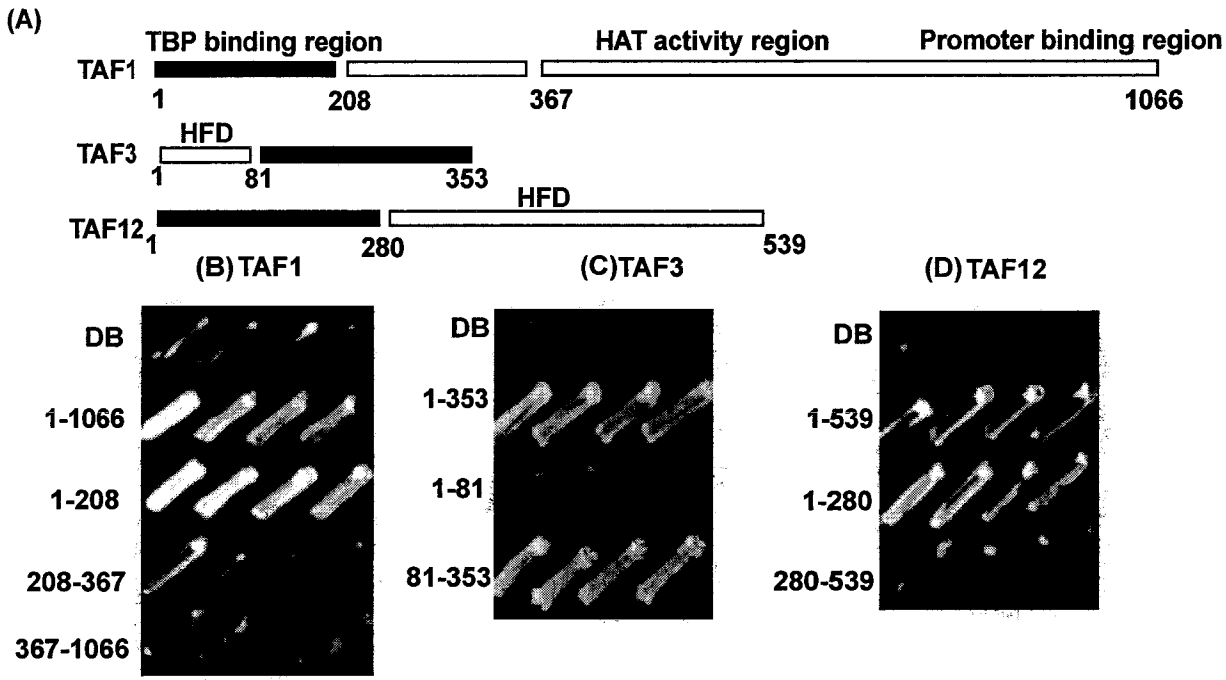

Figure II.3: Artificial recruitment assay for deletion derivatives of TAF1, TAF3 and TAF12. (A) Linear representation of each of the TAFs with the regions indicated that were used in the deletion constructs. For TAF1, the N-terminal TBP binding domain, a central conserved domain and a C-terminal promoter binding region are indicated. For TAF3 and TAF12, the location of histone fold domain (HFD) is indicated. The black box in each panel denotes the region that was positive in an artificial recruitment assay. (B) Artificial recruitment assay for deletion derivatives of TAF1. Strains containing vector alone with the DNA binding domain of Gal4 activator (DB) (control), full length DB-TAF1 (residues 1-1066) as well as DB fused deletions constructs of TAF1 as indicated were assayed for their ability to grow on aminotriazole (AT). Cell growth on AT is indicative of activated expression of *HIS3* reporter gene. (C) Artificial recruitment assay for deletion derivatives of TAF3. Strains containing the vector alone (control), full length DB-TAF3 (residues 1-353) as well as DB fused deletion constructs of TAF3 as indicated were assayed for their ability to grow on AT, indicative of activated expression of *HIS3* reporter gene. (D) Artificial recruitment assay for deletion derivatives of TAF12. Strains containing the vector alone (control), full length DB-TAF12 (residues 1-539) as well as DB fused deletions constructs of TAF12 as indicated were assayed for their ability to grow on AT, indicative of activated expression of *HIS3* reporter gene.

II.4c Mapping yeast TFIID interactions using a comprehensive two-hybrid approach: Strains expressing the DB-TAF derivatives (either full length or deletions) that were negative for activated transcription alone were independently transformed with each of the 13 AD-TAF fusion constructs, AD-TBP and the AD vector. These strains were assayed for expression of *HIS3* by growth on AT (figure II.4) and the results were scored for each individual strain. A total of 196 combinations were assayed (table II.3). We observed a total of 33 strong and intermediate interactions, which represents 17% of the interactions tested. If weak interactions are also included, 49 positive interactions were detected, which represents 25% of the assays. It is quite clear from the results that each TAF exhibited a unique interaction profile.

TAF1 showed strong interactions with a number of TAFs (TAF4, TAF5, TAF6, TAF7 and TAF9), which is concordant with its portrayal as a critical scaffolding TAF for TFIID architecture in higher eukaryotes (278). In addition, we found that the 208-367 region of TAF1 exhibited both distinct and overlapping interaction profiles when compared to the 367-1066 region. Residues 208-367 specifically interacted strongly with TAFs: TAF4, TAF5 and TAF9, whereas TAF6 interacted with both this region and the more C-terminal region of TAF1 (residues 367-1066). Interestingly, a TAF1 derivative with most of this region deleted (residues 208 to 303) is not competent for interactions *in vivo* with TBP or other TAFs (183), suggesting that the integrity of the TFIID complex is dependent on interactions specific to this region. TAF7, interacted exclusively with the more C-terminal region of TAF1 (residues 367-1066).

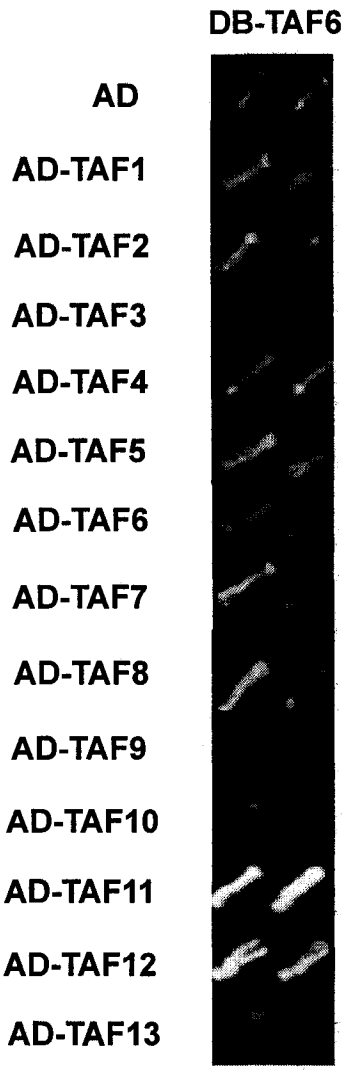

Figure II.4: Representative panel for the systematic two-hybrid assay. Yeast strains expressing DB-TAF6 were independently transformed with different AD-TAF constructs and their ability to interact and activate the reporter *HIS3* gene was assayed by growth on media containing amino triazole (5mM). Yeast growth in each panel is a serial dilution of slashes from a single colony. TAF6-TAF11 was scored as strongly positive (+++) and TAF6-TAF12 interaction was scored as (++). An interaction between TAF6 and TAF10 was detectable (+) when strains were streaked for single colony growth (data not shown).

Table II.3: Summary of two-hybrid interactions

Interaction Phenotype[a]

AD fusions

DB Fusions	AD	TAF1	TAF2	TAF 3	TAF4	TAF5	TAF6	TAF7	TAF8	TAF9	TAF10	TAF11	TAF12	TAF13	TBP
DB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TAF1[b]	+	na[c]	na	na	na	na	na	na	na	na	na	na	na	na	na
(1-208)	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na
(208-367)	-	-	-	-	+++	++	+++	-	-	++	-	-	-	-	-
(3671066)	-	-	-	-	+	-	+++	+++	-	-	-	+	-	-	-
DBTAF2	-	-	-	+	+++	-	-	-	+	-	-	-	-	-	-
DBTAF3[b]	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na
(1-81)	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-
(81-353)	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na
DBTAF4	-	-	-	-	-	-	-	-	-	-	+++	-	+++	-	-
DBTAF5	-	-	-	-	+++	-	-	-	-	-	+++	-	+++	-	-
DBTAF6	-	-	-	-	-	-	-	-	-	-	+	+++	++	-	-
DBTAF7	-	+++	+	-	++	-	-	+	+	-	-	+	-	-	+
DBTAF8	-	-	-	-	+++	++	-	-	-	-	-	-	+	-	-
DBTAF9	-	-	-	-	+++	-	+++	-	-	-	-	-	+	-	-
DBTAF10	-	-	+	-	+	++	+++	-	++	+	+++	++	+++	+	-
DBTAF11	-	-	-	-	+	-	-	-	-	-	-	-	+	+++	-
DBTAF12[b]	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na
(1-280)	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na
(280-539)	-	-	-	-	+++	-	-	-	-	-	++	++	++	-	-
DBTAF13	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-
TBP	+	na	na	na	na	na	na	na	na	na	na	na	na	na	na

[a] Relative growth rate on AT of strains harboring the indicated AD and DB fusions. Robust growth on AT is scored as '+++' and is result of activation of *HIS3* reporter gene. Intermediate and weak growths on AT are represented as '++' and '+', respectively.

[b] Deletion constructs were used instead of the full length TAFs. Numbers in the parenthesis indicate the amino acid residues that were used in the construct for the two-hybrid analysis.

[c] The DB-TAF fusion constructs that were not appropriate for use in the two-hybrid assay since these constructs themselves activated transcription in the absence of the AD-TAF fusions.

An intact C-terminal region of TAF1 is essential for interaction with promoter DNA (183), and it is interesting to speculate that interaction with this TAF may be important for the functional role of TFIID in promoter interactions. It should be noted that TAF1 interactions with TAF4, TAF5, and TAF7, have also been reported for their higher eukaryotic homologues (150, 259, 281).

The distinctive nature of the interaction profile for each TAF was especially striking for a few of the TAFs with a histone fold domain (HFD). For example, HFD-containing TAF13, interacted strongly with HFD-containing TAF11. The TAF11-TAF13 interaction is supported by genetic studies in yeast (143) and structural studies of the human homologues of these two TAFs (13). In contrast to the specificity of TAF13 for TAF11, TAF11 exhibited strong interactions with several other HFD-containing TAFs: TAF6, TAF10 and TAF12 and at least in the case of TAF12, this interaction maps to the region (residues 280-539) that contains the HFD (table II.3).

HFD-containing TAF4 (H2A-like) and TAF12 (H2B-like), and TAF9 (H3-like) and TAF6 (H4-like), exhibited strong reciprocating interactions. These interactions are entirely consistent with the predictive specificity from their histone counterparts (21), biochemical data describing an octamer complex containing these particular TAFs (237), and studies on their human counterparts (109, 283). Our results yield further information in that we detected cross interactions between TAFs that resemble core histones other than their predicted heterodimeric partners. We show that TAF4 interacts with TAF9, and TAF6 interacts with TAF12. In fact, the region of TAF12 harboring the HFD not only

interacts with its histone-like partner TAF4 (75, 283) but also with HFD-containing TAF10 and TAF11. Interestingly, the TAF12-TAF6 interaction appears to map to the N-terminal region of TAF12 lacking the HFD since elimination of this region results in elimination of the interaction observed when the two full-length TAFs are utilized. This result underscores the important consideration that just because two HFD-containing TAFs interact, this does not indicate that the interaction is mediated via the HFD.

II.4d TBP makes very few TAF interactions: Although DB-TBP does not artificially recruit the transcription machinery in the CG1945 strain, co-expressions of the Gal4 AD vector resulted in robust growth on AT (table II.3). We presume this effect is the result of an interaction between TBP and the activation domain. Since activation domains can interact with multiple targets (for review see (84), it is likely that a second surface of the AD is then recruiting the remainder of the transcription machinery. Because of this background activation, DB-TBP could not be assayed with the AD-TAF fusion constructs. When TBP was fused to the activation domain of Gal4 (AD-TBP) and assayed with the DB-TAF derivatives, an interaction was observed with TAF7 and no other DB-TAF derivatives. This suggests that TBP may not make extensive contacts within TFIID and/or that the truncation derivatives of certain TAFs have removed TBP-interacting regions (discussed below).

II.5 Discussion

We have used a systematic two-hybrid approach to map the interactions within the yeast TFIID complex. Each TFIID component has a unique interaction profile,

and a majority of these interactions have supporting evidence from other studies. For example, we observed interactions between yeast TAF5 and three other TAFs (TAF1, TAF4 and TAF12). In keeping with our observations, a number of studies with human TAF5 show that it interacts with human TAF1, TAF4, TAF6, TAF9, TAF11 and TAF12 (73, 259). Thus the human homologue appears to share some of the interaction properties of the yeast counterpart. A functional interaction between yeast TAF5 and TAF1 is also supported by biochemical studies in which temperature sensitive mutations in TAF5 exhibit a concomitant depletion of TAF1 (56). As another example, we found that yeast TAF6 interacts with yeast TAF1, TAF9 and TAF12 in the two-hybrid assay. Biochemical studies on the metazoan counterpart of yeast TAF6 show that it interacts with human TAF9 and TAF12 as well (104). Further support for the veracity of the interaction profiles we observed is also garnered from biochemical and genetic studies on the histone-fold domain (HFD) containing TAFs (13, 21, 109, 143, 237). The corroboration of existing genetic and biochemical data with our interaction profiles strongly supports the robustness of utilizing the two-hybrid assay to define the protein-protein interactions within the TFIID complex.

However, there are possible limitations of the two-hybrid assay for exploring the protein-protein interactions within the TFIID complex. As is common with this assay, there is a potential for false positives and/or false negatives. Since this is an *in vivo* assay and all the endogenous TAFs (and other transcription factors) are present, false positives might arise by the presence of bridging molecule acting between the two TAFs being tested. In this case, the

interaction between the two fusions would not be direct. For a number of reasons, we think this unlikely. Firstly, if bridging molecules play a major role then we would expect to see a plethora of TAF-TAF interactions. In fact, out of 196 combinations, we only observed 33 strong and intermediate interactions, which represents only 17% of those tested. Secondly, if bridging is a major confounding factor, we would predict to observe it when analyzing two TAFs for which there is a known intermediate. For example, we show that TAF11 interacts with TAF6, TAF12 and TAF13. An interaction between TAF11 and TAF13 has also been reported in other genetic and biochemical studies (13, 143). If bridging were a common occurrence, we would expect that endogenous TAF11 would act as an intermediate to facilitate TAF13 and TAF6, or TAF13 and TAF12 interactions. Yet, we detect no interactions between TAF13 and TAF6, or TAF13 and TAF12, in the assay. Thus we see no evidence supporting the contention that bridging is a major factor in the interpretation of our results. With regard to false negatives, there are certain to be interactions that are not detectable in the two-hybrid assay. These interactions could be too weak for detection or they may be transient. In addition, our fusion design, which places the DB or the AD at the N-terminus of the TAF, could interfere with certain TAF-TAF interactions. This could explain the observation that some of the reciprocal interactions are not detected in the two-hybrid assays. In spite of these limitations, our interaction profiles are a useful step in understanding the overall organization of the TFIID complex.

It is interesting to note that TBP interacts only with TAF7 in this assay. Interestingly, TAF7 is specific to the TFIID complex and not found in other TAF-containing complexes (32, 232). As such, this interaction would contribute to the specificity of the association of TBP with TFIID. One possibility for the paucity of interactions for TBP is that our truncation derivatives have eliminated not only the artificial recruitment competent regions of three of the TAFs, but these also may be direct interaction sites with TBP. For example, it is well established that there is an association between the N-terminus of TAF1 and TBP (9, 10, 183). We observed that this N-terminal region of TAF1 harbored the artificial recruitment functions of the full length TAF, thus we had to eliminate this region from the DB-TAF1 derivative used in the two-hybrid assay. Likewise, it is possible that the regions of TAF3 and TAF12, which were positive for activated transcription, may interact directly with TBP. Finally, since TBP-free TAF-containing complexes (285), and TAF-independent TBP-containing complexes have been described (147, 159, 187), it is also possible that TBP does not make extensive and strong contacts with TAFs in the yeast TFIID complex. This hypothesis is consistent with recent observations indicating that TBP dynamically associates with the stable TFIID-TAF complex and is freely exchanged with excess competitor TBP (231).

In a comparison of the interaction profiles, it is evident that several TAFs appear to occupy key positions in the TFIID complex. For example, HFD containing TAFs, TAF4, TAF6, TAF9, TAF10 and TAF12, make multiple and strong interactions with a significant number of other TAFs. Others have also shown multiple two-hybrid interactions for a subset of these TAFs (265), as well

as TAF10 interactions with TAF3 and TAF8 (74). Consistent with the pivotal role of these HFD-containing TAFs in the TFIID complex, previous studies have shown that mutations in TAF6, TAF9 or TAF12 (186), or TAF10 (131), result in defects in the overall integrity of TFIID.

Based on the results presented here and evidence from other biochemical and genetic studies, we present an interaction map for TFIID of *Saccharomyces cerevisiae* (figure II.5). Since in vitro studies have indicated the presence of a core octamer-like substructure formed by TAFs that resemble histones (109, 237), we nucleated our model using these TAF-TAF interactions, and formed the rest of the map using the strong and intermediate interactions observed.

There are several striking aspects of the proposed TFIID model. First, though we found no interactions between TBP and TAF1 in the two-hybrid assay, these two components fall in close proximity to each other in the interaction map. Thus, the known interaction between TBP and the N-terminal domain of TAF1 (9, 136, 141, 144), can be easily accommodated in the model. In fact, since the more C-terminal regions of TAF1 interact with TAF4, TAF6 and TAF9, this further orients the N-terminal domain of TAF1 toward the proposed location of TBP. It is important to note that we did not consider the interaction between the N-terminal domain of TAF1 and TBP when building the map since this domain of TAF1 activated transcription on its own, which precluded its use in the two-hybrid assay. A second interesting feature of the interaction map is that TBP interacts with TAF7, and TAF7 interacts strongly with TAF1.

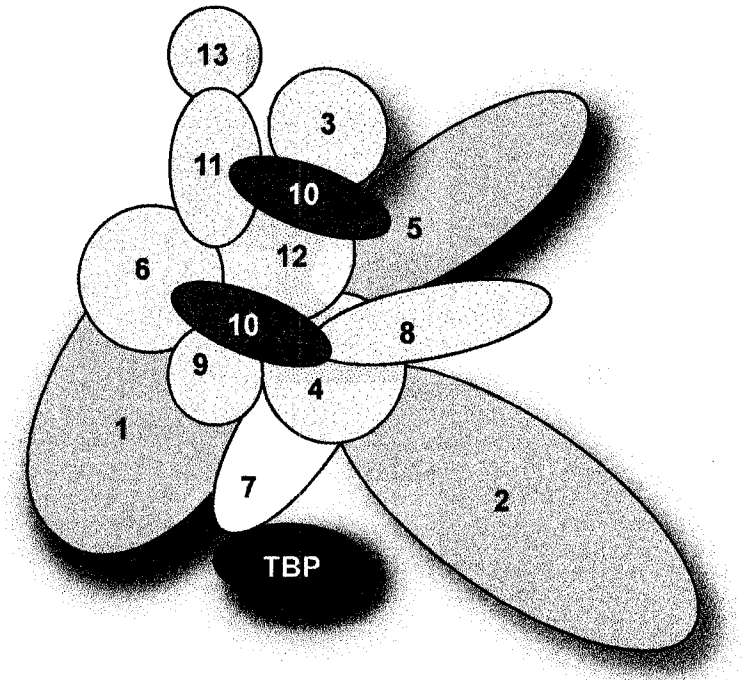

Figure II.5: TFIID interaction map. Each oval represents a different TAF as indicated. The histone-like TAFs implicated in an octamer-like complex are represented in pink. Other histone fold domain containing TAFs are shown in tan, except for TAF10 (shown in green). TAFs in the background are represented in light blue, TBP in dark blue. To keep the model simple, homodimeric interactions for TAF10 and TAF12 were excluded. The strongest interactions (denoted by +++ or ++) were most heavily weighted for inclusion in the interaction map. Exceptions to this were in the cases where reciprocal interactions were strong, or for interactions with TBP. For TBP there were few total interactions, thus all interactions were accomodated in the model.

Thus, there is the potential for three-way interactions between TAF1, TAF7 and TBP. Since TAF1 and TAF7 are specific to the TFIID complex (32, 232), this would ensure that TBP is specific for TFIID and not other TAF-containing complexes. In keeping with this idea, TAF7 interacts with HFD-containing TAF4, and although it is partnered with TAFs shared between TFIID and SAGA, TAF4 itself is TFIID-specific (233). Third, TAF2 is also situated nearby TBP, and it is tempting to speculate that this would be the region of TFIID that contacts the promoter. Studies have shown that in addition to TBP, both TAF1 and TAF2 make intimate contacts with the promoter DNA, play roles in core promoter discrimination, and aid in recognizing the initiator sequences (25, 253, 270, 272).

There were challenges in deriving the TFIID interaction map. For example, TAF10 was a complicated TAF to incorporate in the model. It interacts strongly with five other TAFs, and it also self-associates. In addition, it is known to be present in more than one copy in the TFIID complex (231), and appears to be located in two distinct lobes of TFIID (155). Thus, two copies of TAF10 were placed in the map to accommodate all of the strong interactions for TAF10. The fact that TAF10 has unique interaction properties is also evidenced by the observation that different mutations in TAF10 result in loss of distinct TAFs from the TFIID complex (131). As such, further studies regarding this particular TAF and its interaction partners will be required to understand fully the functional role of TAF10 in the TFIID complex.

In spite of the recent advances in biological techniques used to decipher microdetails of a protein complex, the organization of TFIID remains an enigma

to date. There is a growing understanding of the components within the TFIID complex (231, 232), and low-resolution images (35 Å) have been determined for TFIID (2, 15). Furthermore, the nine histone fold domain containing TAFs have been mapped on this image (155). However, there are some apparent differences between the stoichiometry of the individual subunits (231, 232), and the mapping results (155), as some TAFs appear to be present in multiple copies via one method but not the other. These minor differences simply serve to illustrate the point that multiple approaches using distinct techniques will be critical for a complete understanding of the TFIID complex. The study presented here provides a level of detail regarding the subunit interactions in TFIID that is novel and lacking from previous studies, and offers a unique insight into the overall architecture of the yeast TFIID complex.

II.6 Acknowledgements: We thank Karen Lehman for technical assistance with several of the two-hybrid assays. This work was supported by a grant to LAS from the National Institutes of Health (GM56884).

Supplement to Chapter II (S.II)

Delineation of TAF interacting surfaces on TAF1

This chapter was done in collaboration with an undergraduate student, Laurel Respicio, in our laboratory. This work was conducted to search for a region of TAF1, which would potentially encompass most of the functional region of the full-length protein. Our previous studies show that TAF1 participates in several TAF-TAF interactions. Present study is an effort to delineate a smaller region that would encompass most of the functional interactions of TAF1 and thus provide further insights into the domain nature of this large TAF. This study will be potentially submitted as a small research communication to either 'Yeast' or 'Gene'. When published, the literature citation would be as follows:

Delineation of TAF1 interaction surfaces for other TAFs and TBP. Yatherajam.G, Respicio.L and Stargell.LA.

S.II.1 Abstract

Transcription factor IID is a multi-subunit complex and is involved in core promoter recognition and activation of transcription by RNA polymerase II (Pol II) mediated gene expression. TAF1 is known as the scaffolding TAF and it interacts with several other factors including TBP, TBP associated factors, transcription regulators and other components of the basal transcription machinery. We have identified a smaller region of TAF1, which encompasses most of the interactions of full length TAF1. In addition to this, we show that the region of TAF1 that interacts with TAF7 in humans is also conserved in yeast. This region harbors the HAT activity in the human protein. Previous studies have shown that hTAF7 interacts with this region and inhibits its HAT activity. Here we establish that TAF1 and TAF7 interaction is conserved in yeast. Whether its function is also conserved requires further experimentation to show conclusively.

S.II.2 Introduction

TBP associated factors (TAFs) are important co-factors that mediate activated transcription by providing critical interaction sites for distinct activators as well as for the basal transcription machinery. As such, each TAF has a unique interaction profile with regards to TBP, TAFs, co-activators or basal transcription machinery. One such TAF, which is highly engaged in interactions with other factors, is TAF1. TAF1 interacts with TBP (104, 140, 228, 255, 281, 282, 300), transcription regulators (23, 81, 239), with other TAFs (139, 272, 281, 282, 292), and it also contributes to the PIC assembly (227). The N-terminus of TAF1 is sufficient for its interaction with TBP (9, 141, 166), and the C-terminus of TAF1 makes critical contacts with the promoter regions (183). TAF1 interacts with initiator sequences in both TATA box containing and TATA less core promoters (216, 253, 270, 288). TAF1 clearly has essential and non-redundant functions in transcription initiation and regulation either directly or indirectly.

Gene expression analysis has shown that, of the 5441 genes scored, 16% of the genes exhibited dependence for TAF1 (115). TAF1 encodes a 130 KDa protein in yeast and is also known as the scaffolding TAF. It harbors several enzymatic activities, like kinase activity (53) and HAT activity (188). TAF1 plays a critical role in the G1/S transition of cell cycle in yeast (275) and the HAT activity seems to be essential for this transition (54).

Yeast TAF1 is 1066 amino acids long protein. Previously, we showed that TAF1 maintains several interactions with other TAFs (292). Not only does it interact with other TAFs but also studies have shown that it maintains

interactions with several other factors and DNA thus regulating transcription (for a review see (278)). Clearly TAF1 accomplishes most of these multiple interactions by domains that are non-overlapping. We set out to delineate the TAF interaction regions within TAF1, thus our plan was to identify a smaller region of TAF1, which harbored most of its functional interactions. We hypothesized that such a region of TAF1 would be more amenable to biochemistry for furthering our understanding of TAF1. To accomplish this we designed deletions in the open reading frame of TAF1 and tethered these regions to the activation domain (AD) and DNA binding domain (DB) of a transcription activator. We utilized yeast two-hybrid analysis to identify interactions between the deletion regions of TAF1 and 12 other essential TAFs. Our study yielded several interesting and unexpected results. Studies so far on TAF1 have only delineated its interaction domains with other basal transcription factors and co-activators of transcription. This is the first study to clearly delineate the interaction surfaces on TAF1 for other TBP associated factors, which is novel and lacking from previous studies.

S.II.3 Results and Discussion

TAF 1 is an essential protein in yeast and other eukaryotes (211, 278). In spite of TAF1 being half the size of the human counter part the functional domains are mostly conserved (figure S.II.1A for comparison between yeast and human TAF1). We made deletions in the open reading frame of TAF1 (figure S.II.1B) and tethered it to the DB and AD of the GAL4 activator.

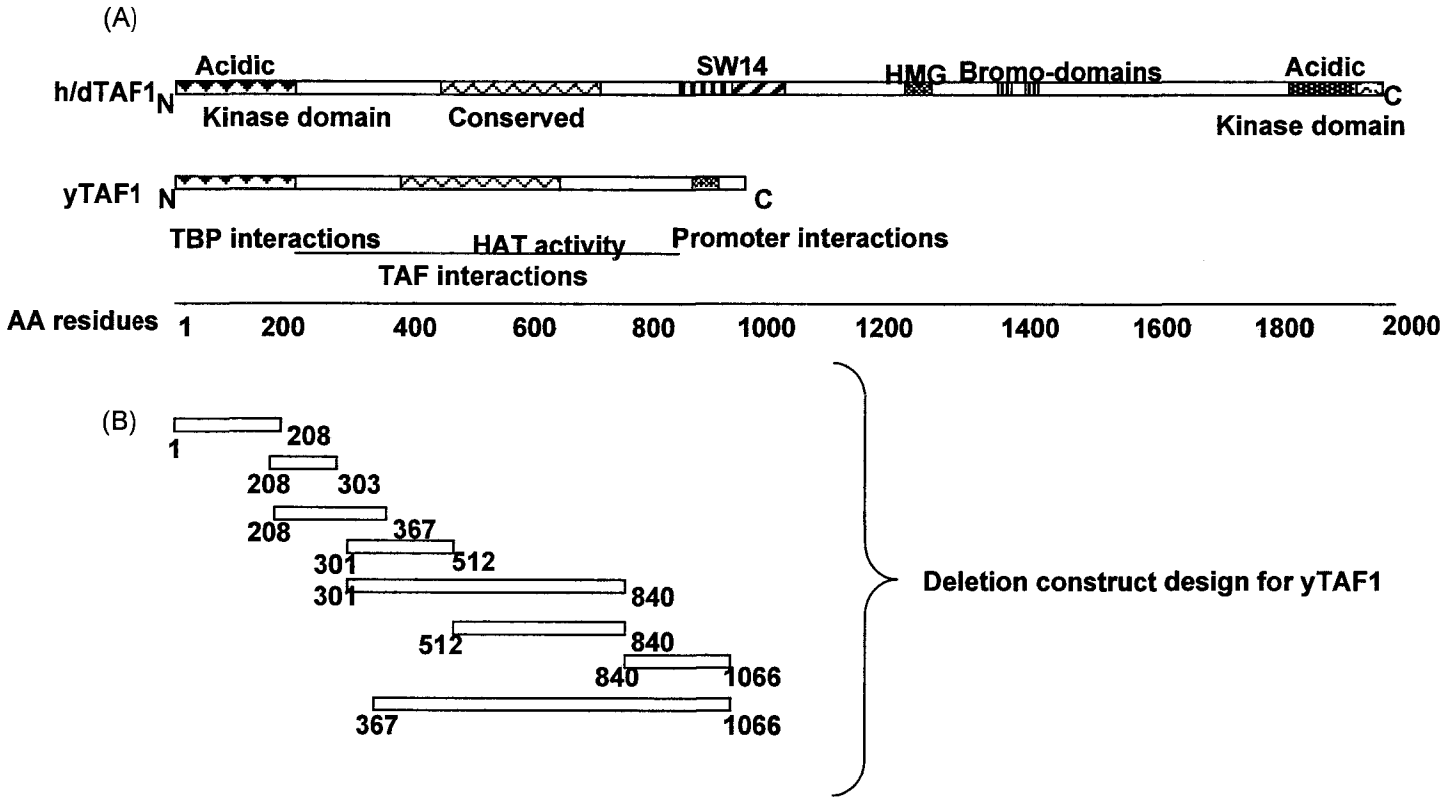

Figure S.II.1: Deletion construct design for TAF1. (A) Linear representation of human and yeast TAF1. All the conserved domains of TAF1 between human and yeast have been marked. Specific functional domains have also been represented. (B) Linear representation of TAF1 with the regions indicated used in the construction of deletion fusions.

Since the two-hybrid assays are done in presence of endogenous TAF1, these deletions would not be deleterious for cell viability. An advantage of using the two-hybrid system for identifying such kind of interactions is that they are done *in vivo*, still maintaining the wild type copy, thus the interactions are studied under normal physiological state of the cells.

We sought out to study the interaction profile of each of these deletion constructs with other TBP-associated factors and TBP. First, we transformed the AD-TAF1 deletion constructs into the CG1945 strain and assayed for interactions with itself and rest of the factors that were fused to the DNA binding domain of the GAL4 activator. Strains harboring any given AD-TAF1 deletion and individual DB-TAFs were assayed for growth on media containing 3-Aminotriazole (AT) (figure S.II.2). The interactions were scored from weak (+), to intermediate strength (++) to strong (+++) based on the robustness of the cell growth on the media containing AT (table S.II.1). We saw several interactions between TAF1 and TAF2, TAF5, TAF6, TAF9 and most strongly with TAF7 (figure S.II.2). It was interesting to note that the deletion constructs did not disclose any new interactions from the full length TAF1 (table S.II.1). This probably indicates that there were no inhibitory domains within TAF1 that would abolish certain interactions with other TAFs. However, this strategy was definitely useful to delineate the interaction domain within TAF1 for TAF7. The central conserved region in yeast that extends approximately from 440-830 amino acids (9) when used in our interaction studies was most competent for interaction with TAF7. It is interesting to note that not only is this region significantly conserved between

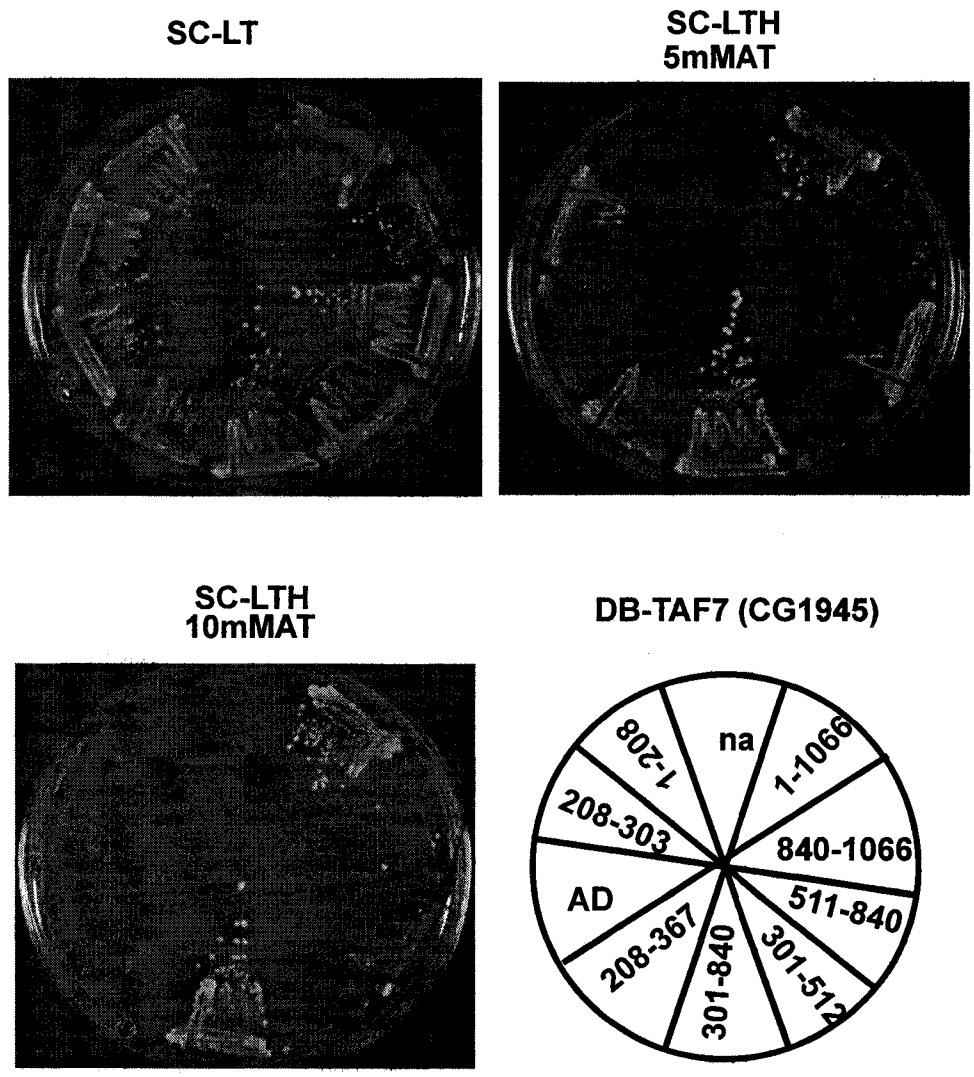

Figure S.II.2: Representative panel for the two-hybrid assays with the deletion constructs of TAF1. Yeast strains expressing DB-TAF7 in CG1945 strain background were independently transformed with different AD-TAF1 deletion constructs and their ability to interact and activate the reporter *HIS3* gene was assayed by growth on media containing amino triazole (5mM and 10mM). Yeast growth in each panel is from a single colony. Growth on control media has been indicated (SC-LT). TAF7-TAF1 (301-840) was scored as strongly positive (+++).

Table S.II.1: Interaction of AD-TAF1 deletion constructs with DB-TAFs

	Interaction phenotype[a]								
DB Fusions	AD	AD 1-208	AD 208-303	AD 208-367	AD 301-512	AD 301-840	AD 512-840	AD 840-1066	AD 1-1066
DB	-	-	-	-	-	-	-	-	-
DB TAF1[b]	+	na[c]	na	na	na	na	na	na	na
DB 1-208	+	na	na	na	na	na	na	na	na
DB 208-367	-	-	-	-	-	-	-	-	-
DB 367-1066	-	-	-	-	-	-	-	-	-
DB TAF2	-	-	-	-	-	-	-	-	+
DB TAF3[b]	+	na	na	na	na	na	na	na	na
DB 1-81	+	na	na	na	na	na	na	na	na
DB 81-353	-	-	-	-	-	-	-	-	-
DB TAF4	-	-	-	-	-	-	-	-	-
DB TAF5	-	-	+	+	-	-	-	-	+
DB TAF6	-	-	-	-	+	-	-	+	+
DB TAF7	-	-	-	-	-	+++	-	-	+++
DB TAF8	-	-	-	-	-	-	-	-	-
DB TAF9	-	-	+	-	+	-	-	-	+
DB TAF10	-	-	-	-	-	-	-	-	-
DB TAF11	-	-	-	-	-	-	-	-	-
DB TAF12[b]	+	na	na	na	na	na	na	na	na
DB 1-280	-	-	-	-	-	-	-	-	-
DB 280-539	+	na	na	na	na	na	na	na	na
DB TAF13	-	-	-	-	-	-	-	-	-
DB TBP	+	na	na	na	na	na	na	na	na

[a] Relative growth rate on aminotriazole (AT) of strains harboring the indicated AD and DB fusions. Robust growth on AT is scored as '+++' and is result of activation of *HIS3* reporter gene. Intermediate and weak growths on AT are represented as '++' and '+', respectively.
[b] Deletion constructs were used instead of the full length TAFs. Numbers in the parenthesis indicate the amino acid residues that were used in the construct for the two-hybrid analysis.
[c] The DB-TAF fusion constructs that were not appropriate for use in the two-hybrid assay since these constructs themselves activated transcription in the absence of the AD-TAF fusions. 'na' indicates not applicable.

yeast to human, but also harbors the HAT activity (188). It was previously shown in humans that TAF7 interaction with TAF1 is particularly localized to this region and inhibits its HAT activity thus affecting the transcription from a particular class of genes (79). It is exciting to note that this interaction between TAF1 and TAF7 is conserved in spite of the yeast TAF1 being almost half the size of its human counter part.

When a reciprocal experiment was performed by fusing the derivatives of TAF1 to the DNA binding domain of the GAL4 activator and transformed individually with AD-TAF constructs, we identified much stronger interactions than earlier. We show that TAF1 derivatives interact with TAF2, TAF4, TAF5, TAF6, TAF9, TAF7 and TBP (table S.II.2). The only novel interaction obtained in this orientation was with TAF4 that was absent in the previous case. The reason could be that the AD domain fusions could be less active as the AD might be interacting with other protein factors and dilute the effect that we observe with TAFs in particular. Nevertheless, we were excited that all the interactions we observed were positive when present in both the orientations.

We observed several interesting interactions of TAF1 with TAF4, TAF5, TAF6, TAF9 and TBP that can be localized within the 208-367 amino acids of TAF1 (table S.II.2). This region is particularly interesting because, in a previous study, deletion within residues 208-303 were unable to support cell viability (9). When the same study checked several sequential deletions for interaction with TBP, they noticed that the extreme N-terminus of TAF1 is required for interaction with TBP.

Table S.II.2: Interaction of DB-TAF1 constructs with AD-TAFs

	Interaction phenotype[a]							
AD Fusions	DB	DB 1-208[b]	DB 208-303	DB 208-367	DB 301-512	DB 367-1066	DB 840-1066	DB 1-1066
AD	-	+	-	-	-	-	-	+
AD TAF1	-	na[c]	-	-	-	-	-	na
AD TAF2	-	na	-	-	+	-	-	na
AD TAF3	-	na	-	-	-	-	-	na
AD TAF4	-	na	+	+++	-	+	-	na
AD TAF5	-	na	-	++	++	-	-	na
AD TAF6	-	na	-	+++	-	+++	-	na
AD TAF7	-	na	-	-	-	+++	-	na
AD TAF8	-	na	-	-	-	-	-	na
AD TAF9	-	na	-	++	-	-	-	na
AD TAF10	-	na	-	-	-	-	-	na
AD TAF11	-	na	-	+	-	-	-	na
AD TAF12	-	na	-	-	-	-	-	na
AD TAF13	-	na	-	-	-	-	-	na
AD TBP	-	na	-	++	-	-	-	na

[a] Relative growth rate on aminotriazole (AT) of strains harboring the indicated AD and DB fusions. Robust growth on AT is scored as '+++' and is result of activation of *HIS3* reporter gene. Intermediate and weak growths on AT are represented as '++' and '+', respectively.
[b] Deletion constructs were used instead of the full length TAFs. Numbers in the parenthesis indicate the amino acid residues that were used in the construct for the two-hybrid analysis.
[c] The DB-TAF fusion constructs that were not appropriate for use in the two-hybrid assay since these constructs themselves activated transcription in the absence of the AD-TAF fusions. 'na' indicates not applicable.

It is interesting to note that the extreme N-terminus deletion construct in our study harbors residues 1-208 and we saw that it was able to activate transcription on its own. This could most probably be attributed to its direct interactions with TBP. However in the same study, upon carefully scrutinizing their published results we noticed that the deletion of regions 300-367 resulted in a slight decrease (fig 4, lane 10: (9)) in binding with TBP. This observation is also consistent with our results as we show that fragment 208-367 has a weak interaction with TBP. This interaction was not identified in our previous published study. The reason could be that we performed the present assay in a different strain background MaV103 in comparison to CG1945 strain in our previous study. We identified interactions of TAF1 with TAF9 in both the orientations that can be mapped to residues 300-367. We also identified interactions of TAF1 with TAF2, which was again specific to residues 303-512. Other novel interactions mapped were of TAF4 and TAF5 to regions 208-367. By further extrapolating our information, we can assume that the region 300-367 seems to be the most active region, which harnesses most of the full-length TAF1 interactions. Previous studies have shown that residues 300-367 are most important within the N-terminus of TAF1, since deletion of this small region was unable to support cell viability (9). In light of present studies, this can be attributed to the fact that loss of the region 300-367 results in loss of its interactions with most of the TAFs thus resulting in destabilized TFIID and cell death.

In the present study we show a level of detail in understanding the functional domains of TAF1 that is novel and lacking from other studies. We

show that the most important interactions of TAF1 can be attributed to the N-terminal 300-367 amino acids, where as the functional interaction of TAF7 can be mapped to the whole central conserved region of TAF1. The smaller region of TAF1 (300-367), can be used in future studies to understand the functional role of TAF1 much better. It is interesting to note that TAF1, a 1066 amino acid long protein, also famously known as a scaffolding protein, harbors most of its TAF interactions within this small region.

Chapter III

TAF11 as a mediator of TFIID-TFIIA interaction

This chapter focuses on TAF11, which has been shown to functionally act as a mediator between TFIID and TFIIA. TFIIA interacts with TFIID via association with TBP and TAF11. This work was done as a collaborative effort with Dr. Mary Robinson, former student in the Stargell laboratory. The work done here is in conjunction with my primary interest on TBP associated factors. Some of the sections have been excised from the written manuscript. However, to keep this chapter compact, only the work performed by me is explained in detail. This work has been written and is ready for submission by Dr. Robinson as the first author. For the purpose of the manuscript as well as the thesis, I created the figures III.2, III.3, III.5, III.6 & III.7 in the thesis. Figures III.1 and III.3 were created by Drs. Mary Robinson and Ryan Ranallo. Once published the literature citation would be as follows for the paper:

TAF11 interacts with TFIIA via two distinct domains to promote stable association of TFIIA-TBP-DNA complexes: Robinson, M.M., A. Bric, G. Yatherajam, R.T. Ranallo, A. Borland, M.R. Paule and L.A. Stargell

III.1 Abstract

TFIIA interacts with TFIID via association with TATA binding protein (TBP) and TAF11. The importance of the TFIIA-TBP interaction and its contributions to transcription has been well characterized. However, TAF11 also provides an additional functional link between TFIIA and TFIID. To further understand the functional connection made by TAF11, an earlier study in our laboratory identified compensatory mutations in TAF11 that were competent for interaction with a mutant allele of TFIIA that is defective for its interaction with TAF11. Analysis of compensatory mutations revealed that the interaction with TFIIA requires two distinct regions of TAF11, the conserved histone fold domain (HFD) and the N-terminal region. In the present study we show that the N-terminus of TAF11 is specifically required for an interaction with TFIIA. Cells lacking the N-terminus of TAF11 are temperature sensitive and exhibit defects in transcription. However, deletion of the N-terminus of TAF11 does not compromise the stability of TFIID suggesting that the TAF11-TFIIA interaction is essential for normal cell growth. Here we also establish that interaction between TAF11 and TFIIA are compensatory *in vivo*, thus a functional connection is established for TAF11 as a mediator of interactions between TFIID and TFIIA. This study provides additional evidence for the molecular organization of the TAF11-TFIIA interaction.

III.2 Introduction

Transcription factor IIA (TFIIA) enhances the pre-initiation complex (PIC) assembly by increasing the affinity of TBP for DNA (17, 120, 126, 152) and stabilizes the TBP-TATA association through direct interactions with both DNA and TBP (80, 256). TFIIA influences TFIID promoter functions through associations with TAFs (91, 145, 294). Studies show that TFIID association with promoters is TFIIA dependent (231). In higher eukaryotes, addition of TFIIA alters the DNA protection pattern of TFIID (30, 31, 164). In UV crosslinking experiments, addition of TFIIA induces a rearrangement within TFIID, enhancing additional DNA contacts downstream of the TATA element (199). These changes likely contribute to the overall affinity and stability of TFIID promoter associations. In addition, TFIIA can relieve TAF mediated repression by competing with the N-terminal region of TAF1 for TBP binding (141).

Our previous studies demonstrated that TFIIA interacts directly and specifically with TFIID specific TAF11 (145). The functional significance of this interaction is demonstrated by the observations that TFIIA strains expressing mutations that disrupt interaction with TAF11 exhibit conditional growth phenotypes and defects in transcription (145). Our studies also show that TAF11 interaction with TFIIA enhances TFIIA-TBP-DNA complex formation, suggesting this protein association has important core promoter functions (145).

To better understand the molecular organization of TFIIA-TFIID associations, studies in our laboratory extended the characterization of the TAF11-TFIIA interaction to define regions of TAF11 required for this interaction.

Yeast TFIIA has two subunits, Toa1 and Toa2, which are 32 and 13.5 KDa in molecular weights respectively. Previously, we identified mutations in the small subunit of TFIIA, Toa2, that affect interaction with TAF11. Specifically, a toa2 allele containing a single lysine substitution at isoleucine 27 (toa2-I27K) is defective for interaction with TAF11 (145). The location of the I27 residue within the four-helix bundle domain of Toa2, a region that projects away from the TBP-TFIIA-DNA complex, implicates this region as important for interaction with TAF11. To further define the TAF11-TFIIA interaction surfaces, toa2-I27K allele was employed in a genetic screen to isolate mutants in TAF11 that suppress the toa2-I27K interaction defect in a two-hybrid reporter gene context. The objective of this study was to isolate TAF11 compensatory mutations that restore interaction with toa2-I27K and thus reveal the surface(s) of TAF11 required for interaction with TFIIA. A two-hybrid approach was used to exploit the loss of interaction exhibited by toa2-I27K to isolate TAF11 compensatory alleles that suppress the toa2-I27K interaction defect. In such a screen we identified several potential compensatory alleles. From this pool, one of the alleles that had a single substitution at the residue E182 was able to suppress the interaction defect of toa2-I27K most strongly in the two-hybrid context. Some times the artificial nature of the two-hybrid assay can magnify or diminish protein interactions, the use of this assay for isolation of compensatory mutant allowed direct isolation of alleles, which specifically restore the interaction with toa2-I27K. However, it is essential to establish a compensatory interaction outside the context of this artificial reporter gene system. Thus, in the present study we

employed E182 allele, and integrated it at a chromosomal locus and establish a compensatory interaction with toa2-I27K *in vivo* outside the context of the reporter gene.

Out of the 14 TAFs, 9 of them have histone fold domains and TAF11/TAF13 pair is one of the well-characterized HFD partner pairs. In mammalian system it was established that TAF11 interacts with TAF13 and this interaction is mediated through their histone fold domains (HFD) (13). A recent study proposes that TAF11 and its dimerization partner TAF13 are required for TBP recruitment, and potentially facilitate primary contacts with TBP during activator-mediated recruitment (241). In yeast it was also shown that over-expression of TAF13 could rescue temperature sensitive phenotypes of TAF11 (143). Thus, there is a strong and functional interaction between TAF11 and TAF13. In the present study we show that all the three α helices of TAF11 are essential for a functional interaction with TAF13.

It is obvious that TAF11 maintains functional contacts with TFIID either via TAF13 or other TAFs. It was pertinent to understand how the alleles isolated in the genetic screen affect the interaction capabilities of TAF11 with its dimerization partner TAF13. Thus, all the alleles of TAF11 were subjected for interaction test with TAF13 in two-hybrid assays. It was interesting to note that none of the alleles were defective for interactions with both TAF13 and Toa2. Upon further analysis of all the alleles it was suggested that perhaps TAF11 interaction with Toa2 and TAF13 is mediated by separate surfaces. Since TAF11 is highly conserved throughout evolution, the three-dimensional structure of the

human TAF13/TAF11 heterodimer (13) was used as a template to model the location of residues involved in the compensatory interaction. The residue E182 mapped to a solvent exposed surface within the α-2 helix of the histone fold domain (HFD), a surface not involved in dimerization with TAF13 (figure III.1). Thus, the region encompassing these residues within the α-2 helix of the HFD defines a distinct region of TAF11 important for interaction with Toa2.

The genetic screen clearly implicated α-2 helix of the HFD as the TAF11-TFIIA interaction surface. However, it is possible that other regions of TAF11 might also be required to facilitate the interaction. When the pool of alleles was further investigated, some mutations of TAF11 were also contained in a region at a distinct distance from the HFD in the N-terminus. Loss of the N-terminus resulted in loss of interaction with Toa2. This suggested that a second region of TAF11 might also be required for interaction with TFIIA. When the N-terminus of TAF11 was deleted, the cells resulted in temperature sensitive phenotypes and severe transcriptional defects. Thus suggesting that this region might be important for interaction with TFIIA. To attribute the phenotypes to loss of interaction with TFIIA, it is essential to establish it is not due to loss of Δ N association with TFIID. To show that a loss of functional interaction between TAF11-TFIIA results in conditional growth phenotypes and defects in transcription, in the present study we present evidence corroborating that the N-terminus is indeed essential for these contacts and removal of residues in N-terminus does not result in a destabilized TFIID complex.

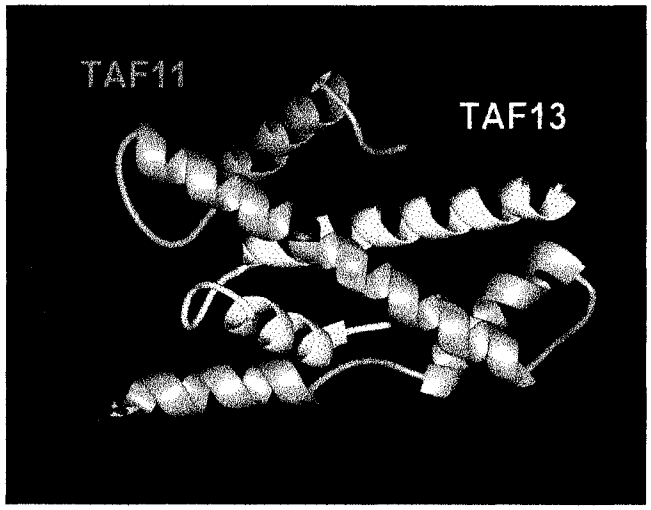

Figure III.1 Crystal structure of human TAF11/ TAF13 heterodimer. TAF11 is shown in blue ribbon and TAF13 is shown in yellow ribbon. The location of the residue homologous to the yeast E182 is shown in red.

III.3 Materials and Methods

III.3a DNA constructs: Activation domain (AD) hybrids of TAF11 mutant derivatives were constructed using primers designed to incorporate changes and subcloned using *in vivo* recombination to the 2μ *LEU2* marked vector pACT2.2-*LEU2* described previously (55, 145, 292). All PCR derived AD plasmids were completely sequenced. The DNA-binding domain (DB) hybrid constructs used in this study were constructed as previously described (145, 292) using the pPC97-*TRP1* vector (273). TAF11-YCP22 constructs containing the TAF11derivatives driven by the TAF11 native promoter and terminator were generated by PCR from genomic DNA. An *EcoR1* site was engineered at the ATG start codon and utilized for inserting three Myc epitopes (GEQKLISEEDLN), creating Myc-TAF11-YCP22.

III.3b Yeast strains: All *Saccharomyces cerevisiae* strains used in the yeast two-hybrid assays and for genetic selection of compensatory mutants and interaction studies were transformants of either MaV103 (273) or CG1945 (65). Both strains contain the *HIS3* reporter but in different promoter contexts as described previously (292). The compensatory interaction (*in vivo*) assay was performed in the *TOA2* deletion strain expressing Toa2 derivative toa2-I27K described previously (145). However, integrating the taf11-E182G derivative at the chromosomal locus of TAF11 using a PCR derived fragment containing the *URA3* gene modified the strain. Viability testing and phenotypic characterization of TAF11 mutant derivatives were conducted in YSB366, a derivative of YSB373, (relevant genotype *MAT**a**, ura3-52, leu2, his3 Δ200, taf11Δ::LEU2)* (143). Co-

immunoprecipitation experiments were performed in YSB366 that was modified by chromosomal integration of sequences encoding HA epitope at the 3' end of the coding sequence for TAF1. The HA tag was amplified from pFA6a –3HA-*TRP1* construct using corresponding primers as previously described (169).

III.3c Two-hybrid assays and phenotypic studies: Gal4 DB plasmids and Gal4 AD plasmids were transformed into yeast strains CG1945 or MaV103 using standard lithium acetate transformation. The resulting strains were spotted in 10-fold serial dilutions or streaked onto the appropriate selection media that either contained or lacked 3-aminotriazole (AT) and grown at 30^0 C for 4-7 days. For temperature sensitivity assays, yeast strains were streaked to the appropriate media and incubated at either 30^0C or 38^0C.

III.3d Protein expression of integrated TAF11 deletion strain YSB366: Extracts were prepared from strains growing on media containing no histidine essentially as described in (44). Approximately 25-30 μg of protein was loaded onto the SDS-PAGE gels. Immunoblots were performed using monoclonal antibodies directed against HA (BAbCO) at 1:1000 dilution, and the anti-mouse secondary antibody (Promega) was prepared at 1:20,000. Signals were developed using a chemiluminiscence kit (Pierce).

III.3e Co-immunopreciptiation assays: Co-immunoprecipitation experiments were essentially performed as described previously (66, 191) with a few modifications. The TAF11 deletion strain YSB366 was modified by integration of a PCR-derived HA tag to the C-terminus of the chromosomal copy of TAF1. Modified TAF11 deletion strains expressing epitope-tagged TAF11 derivatives

were grown in rich medium containing 2% glucose to optical density (600 nm) of approximately 1.0. Protein extracts were prepared immediately and precleared using 50 μl of protein A- sepharose beads (Pharmacia) for 1 hr at 4°C. Anti-TBP or anti-myc antibodies were coupled to protein A-sepharose beads, and protein extracts were incubated with 50 μl of antibody-coupled beads at room temperature for 2 hrs. Following six washes, the beads were boiled in loading buffer, and 15 μl was loaded for SDS-PAGE followed by immunoblot.

III.4 Results

III.4a Functional interaction between TAF11 and TFIIA: To establish a functional interaction between TAF11 and TFIIA, our previous study identified taf11-E182G allele that suppresses the interaction defect of toa2-I27K with TAF11 (manuscript in preparation). This allele was identified in the context of a artificial reporter gene based assay (two-hybrid assay). However, to see if the interaction between the two alleles of TAF11 and TFIIA is compensatory *in vivo*, we need to establish this in their natural context *in vivo.* In which case, taf11-E182G should suppress the phenotypes associated with toa2-I27K. To do this, we improvised a 'gene knock in' technique to replace the chromosomal copy of the wild type TAF11 with E182G mutation in a strain harboring the toa2-I27K allele. To follow the integration of the point mutant at the chromosomal locus, we also integrated a *URA3* marker gene at the C-terminus of TAF11 along with the point mutant (scheme: figure III.2A). Cells that have the integrated point mutant of TAF11 can be selected by the strains ability to grow on media containing no uracil.

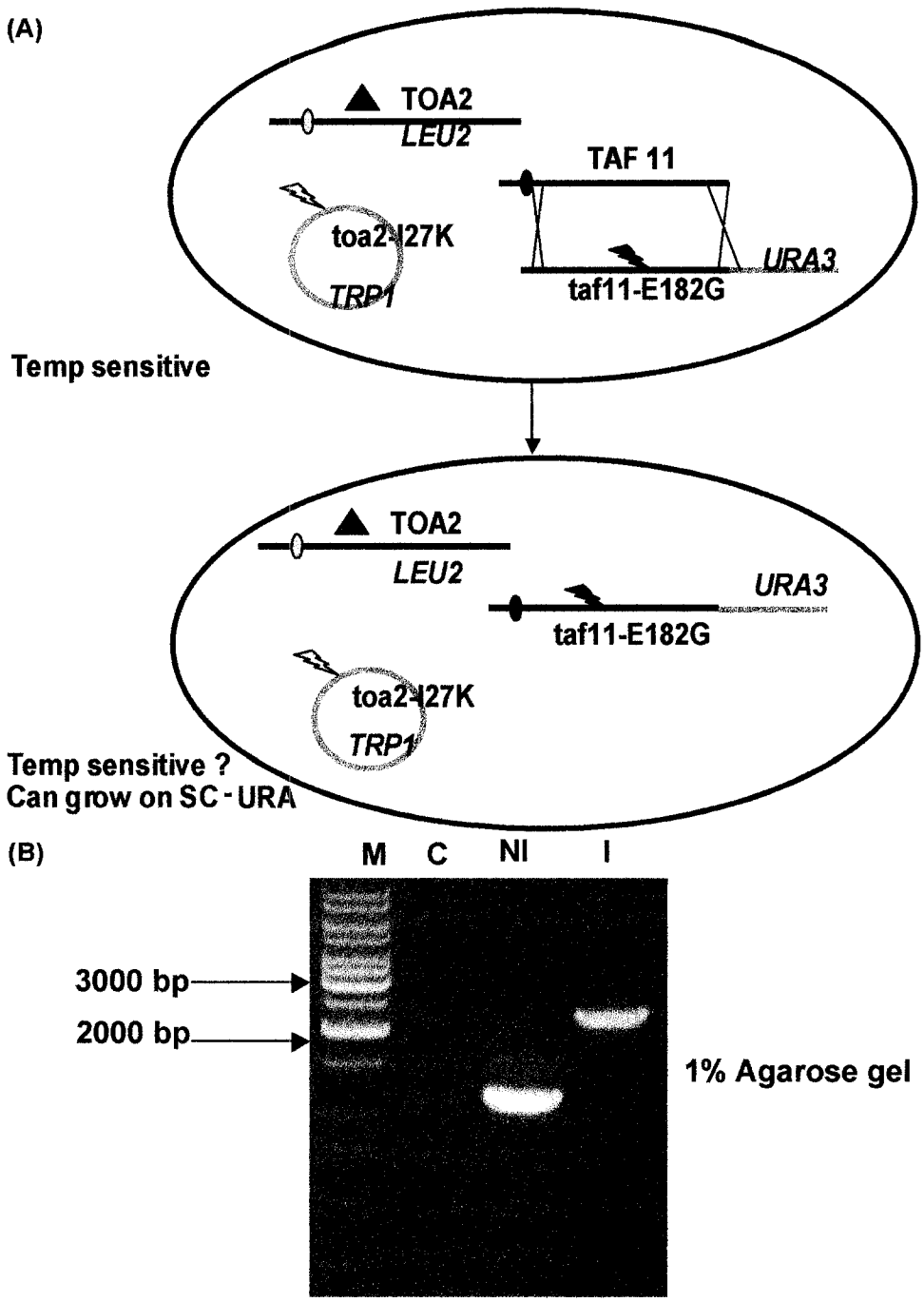

Figure III.2: Scheme for integration of the taf11-E182G at the chromosomal locus in strain harboring toa2-I27K. (A) The compensatory interaction *in vivo* assay was performed in the *TOA2* deletion strain expressing Toa2 derivative toa2-I27K. This strain was modified by integrating the taf11-E182G derivative at the chromosomal locus of TAF11 using a PCR derived fragment containing the *URA3* gene. (B) The integration of taf11-E182G at the chromosomal locus was identified by PCR amplifying the region of TAF11 from 125 aminoacids to the terminator. Lane C is a control lane with the PCR reaction performed using water as the template. Lane I is the integrated strain. Lane NI is non-integrated strain where the product size is smaller, as there is no taf11-E182G with *URA3* integration.

The integration was reconfirmed by PCR (figure III.2B). Cells from yeast strains expressing either toa2-I27K, which has a wildtype copy of the TAF11 at the chromosomal locus or toa2-I27K with taf11-E182G were spotted onto the appropriate selection media and incubated at 30^0C and 38^0C. Consistent with our previous observations, the yeast strain expressing toa2-I27K does not grow at 38^0C (figure III.3), however when taf11-E182G is co-expressed with toa2-I27K, growth is restored at 38^0C (figure III.3). Thus, we conclude that the TAF11 mutation E182G confers a functional compensatory interaction with toa2-I27K *in vivo*, resulting in the suppression of the temperature sensitive phenotype. Such a study is strong evidence for a functional interaction between TAF11 and TFIIA via this region.

III.4b The N-terminus of TAF11 is important for interaction with Toa2: To determine if interaction with TFIIA requires additional regions of TAF11 than the HFD (figure III.4A), we constructed a series of deletion derivatives encompassing TAF11 domains (figure III.4B). Using the yeast two-hybrid assay, TAF11 deletion derivatives were tested for interaction with TFIIA (Toa2) and TAF13 (figure III.4B). As expected, disrupting the HFD by deletion of the α-3 helix, (ΔC), is detrimental for TAF11 interaction with TAF13. Moreover, the ΔC derivative is also defective for interaction with TFIIA further indicating the importance of this domain for interaction with TFIIA. In contrast, the TAF11 derivative ΔN, which includes the HFD but is missing the first 73 N-terminal amino acids, can interact with TAF13 but is not sufficient for interaction with TFIIA suggesting the N-terminal region of TAF11 is also required for interaction with TFIIA.

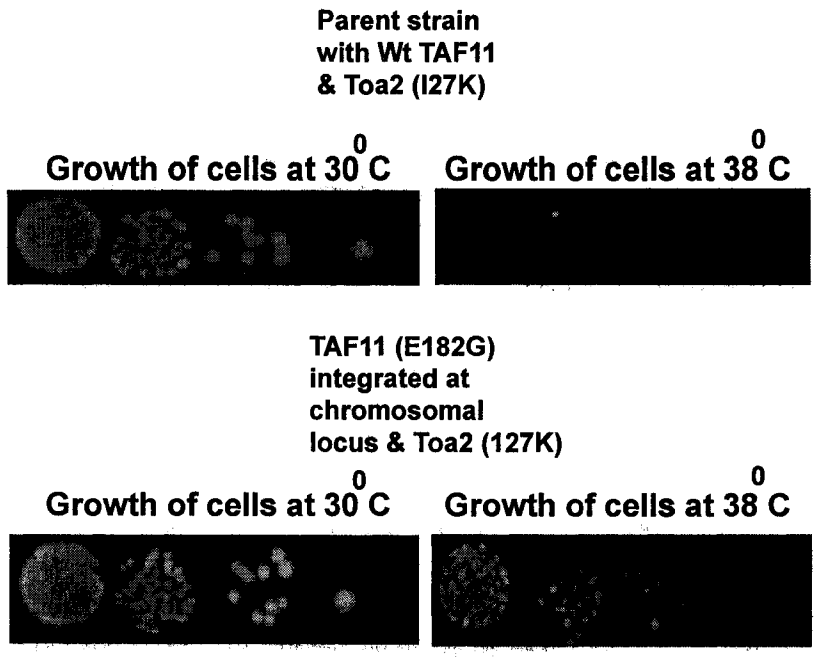

Figure III.3: Compensatory mutant taf11-E182G can suppress conditional phenotypes in cells expressing toa2-I27K. Cells from strains expressing toa2-I27K and taf11-E182G were spotted in serial dilutions on the appropriate selection media and incubated at 30^0C and 38^0C. Growth on plates incubated at 38^0C indicates suppression of the temperature sensitive phenotype.

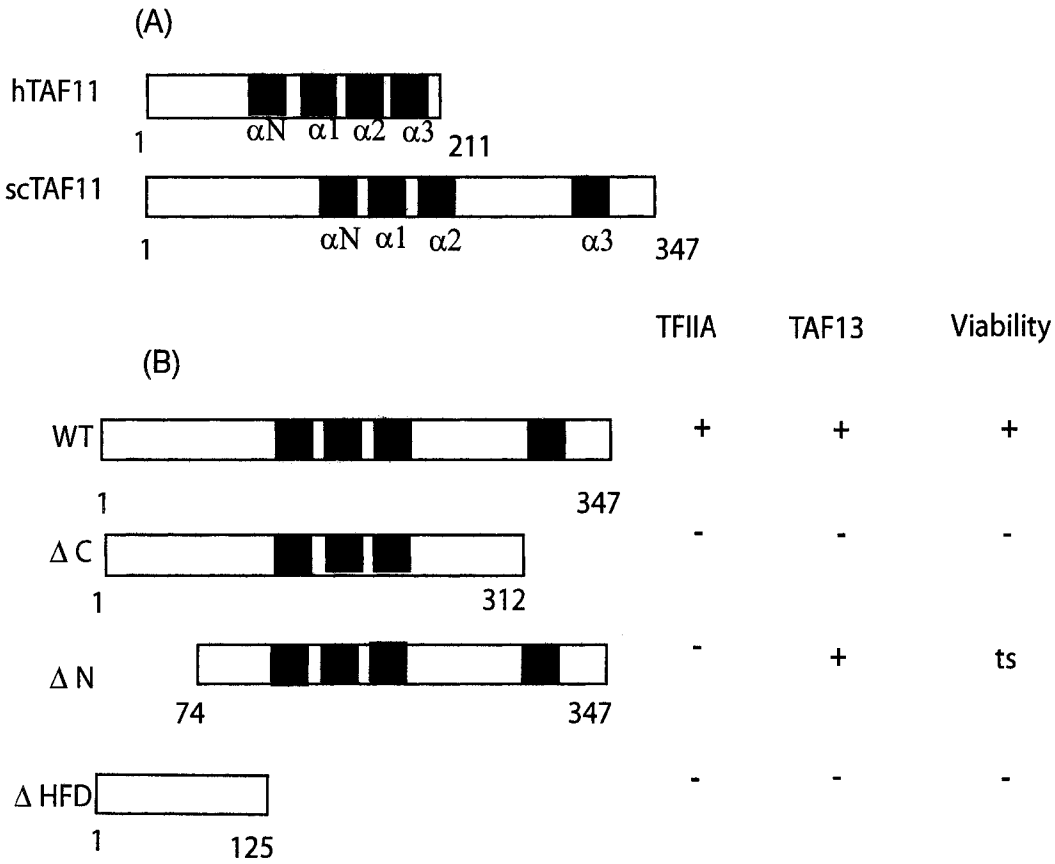

Figure III.4: Interaction with TFIIA requires both the N-terminus and HFD of TAF11. (A) Sequence alignment of human and yeast TAF11. The conserved regions, which comprise the helices of the HFD are indicated by black boxes. (B) The N and C terminal residues are numbered for each deletion construct. The ability of deletion constructs to interact with TFIIA and TAF13 are denoted with a "+" or "-". Viability denotes the growth (+) or no growth (-) phenotype of cells when indicated derivative is tested for its ability to cover a genomic deletion of TAF11 gene in a plasmid shuffle experiment.

Furthermore neither the HFD nor the N-terminus alone is sufficient for TFIIA interaction since both ΔN and ΔHFD derivatives fail to interact with TFIIA. Taken together, we conclude that the HFD is necessary and sufficient for interaction with TAF13. However, TAF11 requires both the N-terminus and the HFD for interaction with TFIIA.

In order to determine how loss of specific interactions affects TAF11 function *in vivo*, we next tested deletion derivatives for their ability to support cell viability. TAF11 deletion derivatives were expressed (under the control of an endogenous TAF11 promoter and terminator) in a strain in which the chromosomal copy of TAF11 had been deleted. There was a strong correlation between cell viability and the ability of TAF11 to interact with TAF13 and TFIIA (figure III.4B). That is, TAF11 deletions that disrupt interaction with both TAF13 and TFIIA were unable to support cell viability. Of particular interest was the TAF11 derivative, ΔN, which supported cell viability yet exhibited a temperature-sensitive phenotype. Because this derivative was specifically defective for interaction with TFIIA, the inability to grow at the elevated temperature could result from loss of this interaction.

III.4c The N-terminus of TAF11 is not required for TFIID integrity: Although deletion of the N-terminus of TAF11 does not affect interaction with TAF13 in the two hybrid assay, it is possible that the N-terminus is necessary for functional contacts within TFIID and the observed conditional phenotype is due to loss of the TFIID function. To examine if TFIID integrity is affected by this mutation, co-immunoprecipitation assays were performed in yeast strains expressing the wild

type or the ΔN TAF11 derivatives first at permissive temperatures. We used TAF1 as a marker for TFIID since it is also known as scaffolding TAF and its presence in a TFIID preparation is indicative of an intact TFIID complex. Availability of antibodies specifically recognizing the TAF1 were not handy and developing antibodies to a particular protein would have been a time consuming effort. So, we employed a more economical and versatile technique to tag the genes of our interest with markers recognized by commercially available monoclonal antibodies (169). We integrated a HA tag along with the *HIS3* marker at the C-terminus of TAF1 at the genomic locus in strains that were expressing either the full length or TAF11 deletion strains that had a Myc tag (scheme: figure III.5A). The integration of the tag is confirmed by the ability of the cells to grow on media lacking histidine and also by western blot analysis (figure III.5B). Whole cell extracts were prepared from TAF11 strains expressing HA-tagged TAF1 and Myc-tagged TAF11 derivatives. Polyclonal antibodies directed at TBP were used for immunoprecipitation, and the precipitated complexes were analyzed by immunoblot, using monoclonal antibodies against the Myc or HA epitopes. Immunoprecipitation by antibodies directed to TBP indicates that wild type TAF11 associates with both TBP and TAF1 (figure III.6 A and B). In addition, immunoprecipitates from the TAF11 strain expressing the ΔN derivative also show association with TBP and TAF1 indicating that the intactness of TFIID is not affected by loss of the N- terminus (figure III.6A and B). These results indicate that deletion of the N-terminus of TAF11 does not affect TFIID integrity.

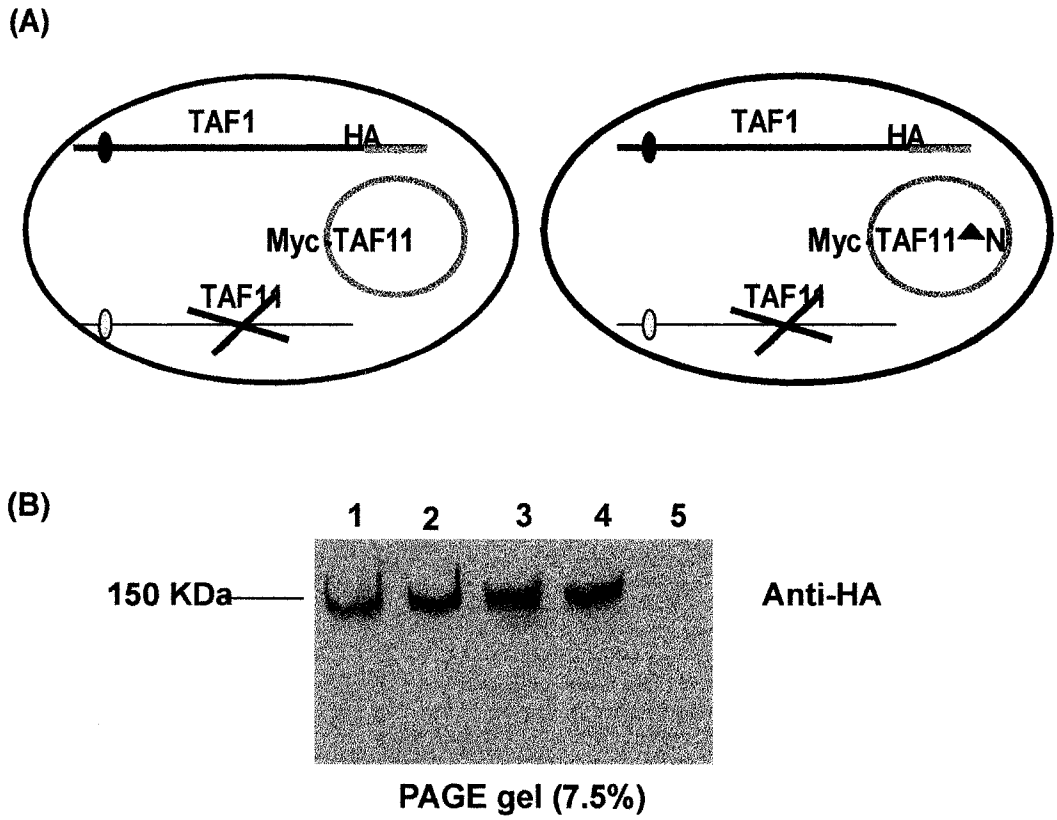

Figure III.5: Chromosomal integration of HA-tag and *HIS3* marker at the C-terminus of TAF1. (A) Representation of the strains used to tag the markers at the chromosomal location of TAF1 in strains expressing wild type or N-terminus deleted TAF11. For the purpose of co-immunoprecipitation experiments, YSB 366 strain was used, which has TAF11 deleted from the chromosomal location and was supported by either full length TAF11 (Myc) of deletion N derivative (Myc) on a plasmid copy. These strains were modified by chromosomal integration of sequences encoding the HA epitope at the 3' end of the coding sequence of TAF1. The HA tag was amplified from pFA6a-3HA-TRP1 construct using corresponding primers as described earlier. (B) The integration of the HA tag in the YSB 366 strain was confirmed by western blot analysis. Protein extracts (25 μg) from strains harboring the integrated HA tag were loaded on the gels and signals were detected using antibodies directed against the HA epitope. Lanes (1-4) show four different strains that have integrated tags. Lane 5 is a control strain showing no integration of HA.

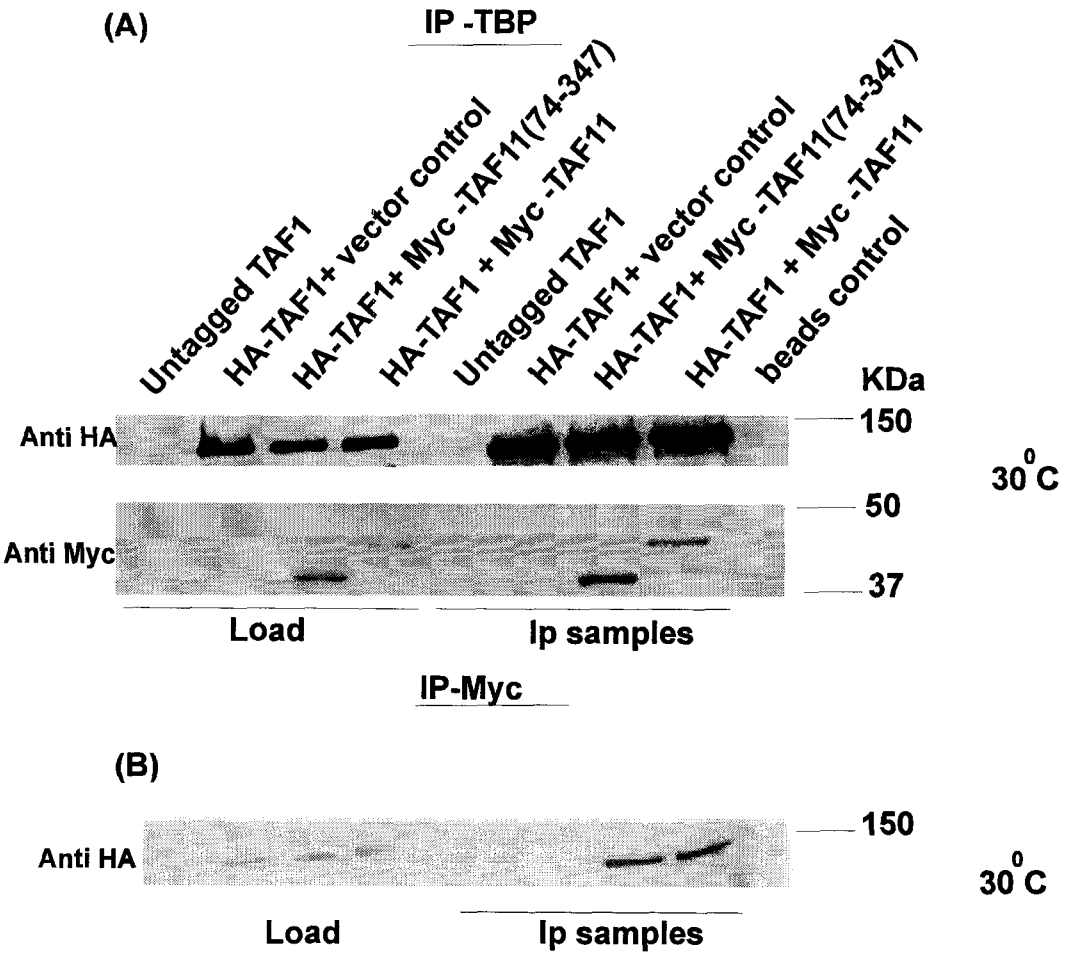

Figure III.6: TAF11 N-terminal deletion (74-347) is stably associated with TFIID *in vivo* at 30°C. (A) Co-immunoprecipitation was performed on cell extracts from strains expressing HA-tagged TAF1, Myc-tagged TAF11 derivatives or untagged vector alone. Complexes were immunoprecipitated (IP) by using polyclonal antibodies to TBP. Immunoprecipitated complexes were resolved by SDS-PAGE and probed by using antibodies to detect signals for TAF1 and TAF11. Input from each extract (Load) and sample of antibody conjugated protein-A beads (beads) are indicated. (B) Co-immunoprecipitation was performed in a similar fashion as above. Here Myc antibodies were used for immunoprecipitation. A similar immunoblot analysis detects the signals for TAF1.

Therefore, the phenotype exhibited by strains expressing ΔN-TAF11 derivative is very likely due to loss of its interaction with TFIIA.

III.4d The N-terminus of TAF11 does not affect the stability of TFIID at elevated temperatures: We observed that the ΔN derivative of TAF11 had a temperature sensitive phenotype. It was essential to see the stability of the TFIID complex at elevated temperatures (38^0C). To do this assay we collected the whole cell extracts from the TAF11 strains expressing HA-tagged TAF1 and Myc-tagged TAF11 derivatives in the same fashion as indicated above. However, one hour prior to collecting the cells for the whole cell extraction process, the strains were subjected to growth at 38^0C for 60 minutes. The immunoprecipitations were followed in similar fashion as above and further subjected to western blot analysis. At higher temperatures (38^0C) we show that the TFIID integrity is not compromised (figure III.7A and B). Thus, even at higher temperatures, it is clear that the conditional growth defect of ΔN-TAF11 can be attributed to the loss of its interaction with TFIIA and not due to a destabilized TFIID complex.

III.5 Discussion

Previous isolation of TAF11 compensatory mutants confirmed that toa2-I27K is specifically defective for interaction with TAF11 and it effectively maps the TAF11 interaction surface to the four-helix bundle domain of TFIIA, a surface not involved in interaction with TBP or DNA. In addition, TAF11 compensatory mutants identified regions of TAF11 required for the TFIIA interaction. Through the analysis of over- represented mutations, this study identified single substitutions that restore interaction with toa2-I27K and function *in vivo* by

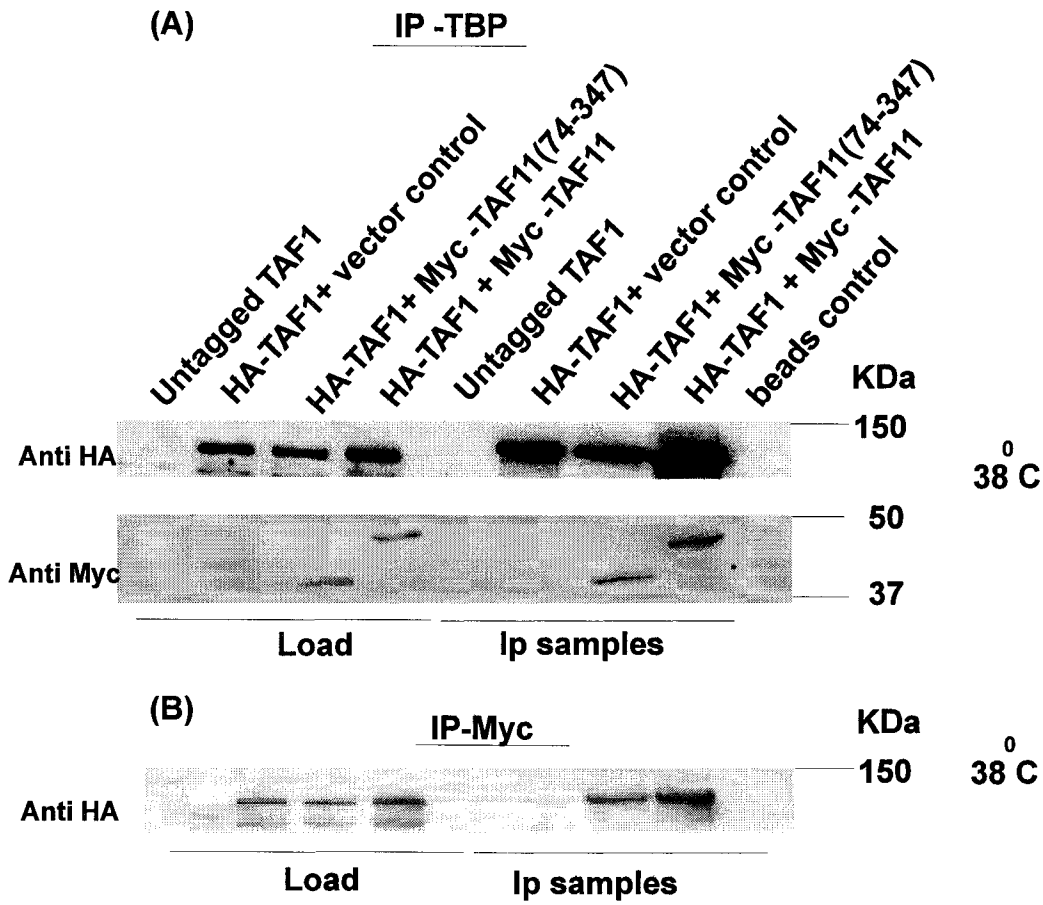

Figure III.7: TAF11 N-terminal deletion derivative (74-347) is stably associated with TFIID *in vivo* at 38^{0}C. (A) Co-immunoprecipitation was performed on cell extracts from strains expressing HA-tagged TAF1, Myc-tagged TAF11 derivatives or untagged vector alone. Complexes were immunoprecipitated (IP) by using polyclonal antibodies to TBP. Immunoprecipitated complexes were resolved by SDS-PAGE and probed by using antibodies to detect signals for TAF1 and TAF11. Input from each extract (Load) and sample of antibody conjugated protein-A beads (beads) are indicated. (B) Co-immunoprecipitation was performed in a similar fashion as above. Here Myc antibodies were used for immunoprecipitation. A similar immunoblot analysis detects the signals for TAF1.

suppressing the temperature sensitive phenotype caused by loss of interaction between TFIIA and TAF11. Moreover, mapping the location of these compensatory mutations to the surface of the structurally defined α-2 helix within the HFD implicates the involvement of this region for interaction with TFIIA.

The predominance of HFD in TAFs and the identification of TAF-TAF dimers has led to the proposal that, like histones, the HFD of TAFs dictate dimerization specificity. Recently, we, as well as others, demonstrate that this domain provides an accessible surface for other TAF-TAF interactions, which are important for transcription (74, 292). In this study, we also show that the HFD of TAF11 can mediate interactions with both TAF13 and TFIIA. Furthermore, this data suggests that the accessibility of HFDs in TFIID may extend to additional protein interactions required for efficient initiation complex assembly. These findings are further supported by studies of human TAF11, which show that mutations in residues on the solvent exposed surface of the α-2 helix abolish synergistic transcription activation by nuclear receptors (151). Interestingly, the residues implicated for this activity correspond to the same residue we identified as important for interaction with TFIIA (E182), indicating the functional importance of this interaction surface in the human system.

In addition to the HFD, TAF11 mutational analysis indicated that the N-terminus also provides an important surface for TFIIA interaction. Mutations at specific N-terminal residues and deletion of the first 73 N-terminal residues result in loss of interaction with TFIIA. Cells that expressed the TAF11 derivative ΔN (lacking the 73 N-terminal residues), were temperature sensitive and exhibited

transcription defects at a number of Pol II transcribed genes. We confirmed that deletion of these N-terminal residues does not alter TAF11 interactions within TFIID, since cells expressing this TAF11 derivative can associate with TAF1 and TBP. In addition, two-hybrid analysis determined that interaction with TAF13 was not affected by this mutation. Although we cannot rule out that other TAF11 interactions might be affected by the N-terminal deletion, loss of interaction with TFIIA could contribute to the conditional growth defects and impaired transcription observed. These findings are consistent with the defects observed when TAF11-TFIIA interaction was disrupted by mutations in Toa2 (145).

The characterization of the TAF11-TFIIA interaction presented here clearly defines regions within both proteins important for this association. Combined with structural information presented in a number of studies, this new data provides the foundation for understanding the molecular architecture and organization of TFIIA-TFIID-promoter complexes. We have demonstrated in an earlier study that TAF11 interaction with TFIIA has a mechanistic role during complex formation, and loss of interaction results in molecular defects that affect cell growth and transcription *in vivo*. In the present study we precisely show that TAF11 via two distinct regions interacts with TFIIA. We also show that there is an *in vivo* mechanistic functional correlation between TAF11 and TFIIA. Taken together, the data presented in this study provides further evidence that TAF11 can serve as a functional link between TFIIA and TFIID.

III.6 Acknowledgements: Drs. Ryan Ranallo and Mary Robinson, both performed the study presented in figure III.3. Dr. Mary Robinson performed the

compensatory interaction screen. I thank her for sharing her unpublished results and her manuscript in preparation, for the purposes of this chapter and her collaboration on this project with me.

Chapter IV

Functional characterization of MOT1 *in vivo.*

This chapter focuses on MOT1, a known regulator of transcription. Previous *in vitro* experiments on MOT1 show that it interacts with TBP and dissociates it from DNA, thus MOT1 was generally assigned a negative function with regard to transcription. However, recent gene array analysis has shown that MOT1 can affect transcription both positively as well as negatively. Chromatin immunoprecipitation analyses have shown that MOT1 is localized to genes that it both positively and negatively regulates. Thus, the role of MOT1 *in vivo* is still unclear. To understand the *in vivo* function of MOT1 more clearly, we have tethered it to a DNA binding domain and recruited it to test promoters and studied its effects. The work presented in this chapter has provided us insights into MOT1 function and furthered our understanding of its mechanistic role. This work was done in collaboration with Dr. David Auble, a pioneer in the area of MOT1 research and is a co-author on our manuscript. This work is in conjunction with my primary interest on TBP associated factors. I have performed all the experiments, created all the figures, and written the manuscript. Once published the literature citation would be as follows for the paper:

MOT1 represses transcription when recruited to a promoter *in vivo.* Yatherajam.G, Auble. DT and Stargell.LA.

IV.1 Abstract

MOT1 is an essential TBP associated factor that belongs to the Snf2/Swi2 ATPase family of proteins. *In vitro*, MOT1 utilizes its inherent ATPase activity to dissociate TBP from DNA, suggesting a role in repression *in vivo*. Studies have established that MOT1 regulates transcription both positively as well as negatively. Previously, there has been no direct *in vivo* assays that could differentiate MOT1's functional ability to repress transcription. Here we present an *in vivo* system to further investigate MOT1 function. We demonstrate that artificial recruitment of MOT1 to a test promoter results in repression of transcription. In addition, this effect is independent of the DNA-binding domain used to target MOT1, as well as the test promoter. We also show that repression by MOT1 is dependent on its inherent ATPase function and the ability to physically interact with TBP. Surprisingly, the occupancy of TBP is not substantially altered at the promoter when MOT1 is targeted and is repressing transcription. This indicates that TBP occupancy does not correlate with the transcriptional output at these test promoters in the presence of MOT1. Thus, MOT1 does not necessarily repress transcription by occluding the binding of TBP or dissociating the TBP that is already bound. Repression at these test promoters could in fact be a more direct effect of MOT1 compromising the functional ability of TBP to promote transcription. Thus our studies provide further insights into the mechanism of MOT1 action.

IV.2 Introduction

TBP is a global factor required for eukaryotic transcription. Binding of TBP to the TATA element is the first and a major rate-limiting step in transcription initiation (17, 45, 269). In addition to TBP participation at this initial stage of transcription, TBP associated factors (TAFs) also regulate transcription initiation. TAFs have both positive and negative roles in the regulation of transcription initiation. TBP associated factors present in B-TFIID and NC2-TBP complexes are known to effect transcription negatively. MOT1 (Modifier Of Transcription 1, (51)) is a TAF present as a component of an alternate form of TFIID, B-TFIID (212, 261). MOT1 was isolated in genetic screens as a mutation that led to an increase in the basal level expression of several pheromone responsive genes in the absence of a trans-activator as well as pheromones (51). In an additional genetic screen, MOT1 was also isolated as a trans-acting factor that affects DNA polymerase α and δ essential genes (209). MOT1 is an essential gene and in yeast it encodes a 210 KDa protein, which is conserved from yeast to mammals (51).

In vitro studies have shown that MOT1 forms a stable complex with TBP in solution and on promoter DNA (6). MOT1 utilizes ATP hydrolysis to dissociate TBP from DNA (7). This ability to dissociate TBP is associated with repression of basal and activated Pol II gene transcription (7). As such, the classical role of MOT1 has been as a transcription repressor, however recent microarray analyses have shown that MOT1 regulates transcription both positively and negatively (50, 82). In addition to these large scale profiling studies, others have

also shown that when used in small amounts, MOT1 activates transcription *in vitro* (193). A few genetic characterization studies on MOT1 have shown that mutations in MOT1 resulted in decreased transcription of certain genes (36, 172) thus positively effecting the Pol II mediated gene transcription.

It is puzzling that MOT1 is associated with genes that are both positively as well as negatively regulated (82). This indicates that MOT1 may participate directly in both transcription activation as well as repression (50, 82). ATPase function of MOT1 seems to be inherent for its dual functions *in vivo* (50, 92). It is very important to separate transcription activation function from repression of MOT1 *in vivo*, to better understand how it accomplishes these two independent tasks and to see if one is a more direct effect than the other.

In an effort to understand MOT1 function more precisely *in vivo*, we targeted MOT1 to a test promoter by tethering it to a DNA binding domain of a transcription factor. Using this system we establish that targeting MOT1 to DNA results in repression of transcription. This repression is independent of the context of the test promoters as well as the fusion construct designs. In addition, repression is dependent on the ATPase function of MOT1 and ability of MOT1 to interact with TBP. Strikingly, we see that TBP is occupied/present at these test promoters in the presence of MOT1 even though transcription is repressed. It is interesting to note that in the case of wild type and MOT1 alleles defective for ATPase function and ability to interact with TBP, the transcriptional output does not correlate with TBP occupancy. Based on these results we hypothesize that MOT1 compromises the functional ability of TBP. Thus resulting in repression of

transcription by affecting the TBP activity. This system provides us insights into MOT1 function and also is a technique to identify factors that might be involved in this pathway.

IV.3 Materials and methods

IV.3a DNA constructs: Full length MOT1 (cloned by homologous recombination from the genomic DNA) and the mutants of MOT1 (subcloned from PRS backbone vector) were made as fusions to the DNA binding (DB) region of GAL 4 activator (residues from 1-147) in a pPC97-*TRP1*. The vector pPC97 (CEN, *TRP1*) also contains an *ADH1* promoter and a nuclear localization sequence. Mutants of MOT1 (K1303A (7) and MOT1 allele1-260 (49)) were subcloned into the pPC97 vector backbone. LexA fusions of the full length MOT1 were subcloned into a BTM116 (2μ, *TRP1*) (122) as fusions to the E.coli protein LexA. BTM116 also has an *ADH1* promoter and terminator. For the plasmid based artificial recruitment assays, a YCP111 vector which contains a LEXA operator fused to the *HIS3* gene was used.

IV.3b Yeast Strains: MaV103 strain (273) is essentially a two-hybrid strain having two reporter genes (*HIS3 and URA3*) fused upstream with the UAS of GAL that contains the binding sites for the GAL4 activator. GAL4 and GAL80, which are the regulators of this region have been deleted from the strain. CG1945 (Clontech) is also a similar two-hybrid strain with two reporter genes a *HIS3* and a *LACZ* gene. BY4741 strain, which was used for our artificial recruitment assays that were plasmid driven for the reporter gene had a genotype of (MATa his3Δ1, leu2Δ0, met15Δ0, ura3Δ0).

IV.3c Protein expression: Extracts were prepared from strains harboring the indicated plasmid constructs essentially as described (44). Approximately 20-25 μg of the protein was loaded onto SDS-PAGE gels. Immunoblotting was performed using monoclonal antibodies directed against the HA (BabCO) epitope tag present in the vectors at 1:1000 dilution. The blot was further probed with anti-mouse secondary antibody (Promega) at a dilution of 1:20,000. Signals were further developed using a chemiluminiscence kit (Pierce).

IV.3d S1 nuclease transcription assays: S1 nuclease analyses were conducted as described (123). Cultures were grown to an optical density (600 nm) close to 1.0 and RNA was prepared by hot phenol extraction and quantitated spectrophotometrically at 260 nm. Hybridizations with excess probe were normally done with 25–30 μg of RNA overnight at 55°. S1 nuclease digestion was performed on the hybridized samples for 30–45 min at 37°. Band intensity was normalized to the intensity of the tRNA band of each panel. Quantitation of the signals was done by using Image Quant software.

IV.3e Co-Immunoprecipitation assays: Co-immunoprecipitation experiments were performed essentially as described previously (189) with a few modifications. Cultures were grown to an optical density (600 nm) of 1.0 in rich medium containing 2% dextrose. Cell extracts (300 μg) were used immediately following preparation and were precleared by incubation with 50 μl plain protein A-sepharose beads (Pharmacia) for 1 hr at 4°. A small sample was taken after the preclear step to provide a load control. Anti-TBP antibodies were coupled to protein A-sepharose beads, and the remaining extract was incubated with 50 μl

of these coupled beads for 2 hr at room temperature. After six washes, the beads were boiled in loading buffer and 15 µl was loaded for SDS-PAGE, followed by immunoblot analysis.

IV.3f Chromatin Immunoprecipitation assays: Chromatin immunoprecipitations were performed as described in (249) with few modifications. Cells (150 ml) were grown to an O.D between (0.8-1.0) at 600 nm. Cells were treated with a final concentration of 1% formaldehyde for 15 minutes with occasional swirling of the flasks at intervals of 5 minutes. Glycine was added to a final concentration of 125 mM at room temperature for 5 minutes to stop crosslinking. Cells were collected and washed twice in ice cold TBS. Cells were resuspended in lysis buffer (500 µl of Lysis buffer for a total of 50 ml of cell culture). Chromatin was sheared by sonication using a Branson W-350 model of sonifier (10 times at 10 seconds each on continuous pulse at a microtip power setting of 6). Input controls were 10% of the chromatin material used for the immunoprecipitation and were processed after reversing the cross-links and purifying the DNA. 500 µl of the chromatin material was incubated with approximately 5 µl of TBP antibodies by rotation overnight at 4^0C. Protein-A sepharose beads (Pharmacia-prepared as slurry as per the manufacturers directions) was further incubated with the chromatin material for 2 hours at 4^0C. The beads were spun down and the antigen-antibody complexes bound to the beads were recovered and further treated with TE/SDS buffer for 15 minutes at 65^0C to elute the complexes. Protein-DNA cross-links were reversed by incubation overnight at 65^0 and the DNA was purified by phenol-chloroform

extraction and used for the PCR analysis.

PCR reactions were carried out in a total volume of 50 µl. Different template amounts from the immunoprecipitated material was used to determine the linear range of the PCR reaction. The samples were run on 1% agarose gels and ethidium bromide stained. Image was captured on to the computer and quantified using Image Quant software analysis to detect the strengths of various signals. No antibody samples and strains with no plasmid transformation were used as controls. Primers for the PCR were designed at the promoter region of the reporter gene encompassing the start site of the *HIS3* gene and 250 bases away from the start site. As a control promoter of the *ADH2* gene was amplified. Since the cells were grown in presence of glucose, there should be essentially no signal for the *ADH2* promoter.

IV.4 Results

IV.4a MOT1 represses transcription when targeted to a test promoter: A system was designed to determine the effect on transcription of directly targeting MOT1 to a promoter *in vivo*. To accomplish this, MOT1 was fused to the DNA binding domain (DB) of the GAL4 transcription factor (DB-MOT1). DB-MOT1 was recruited to a test promoter, via binding sites for DB. MaV103 strain (273) has a *HIS3* reporter gene fused with the binding sites for DB (67). Thus, the *HIS3* gene is completely under the control of the plasmid driven DB or DB-MOT1. Proper expression of these proteins was confirmed by performing western blot analysis with antibodies directed against the HA epitope tag (figure IV.1).

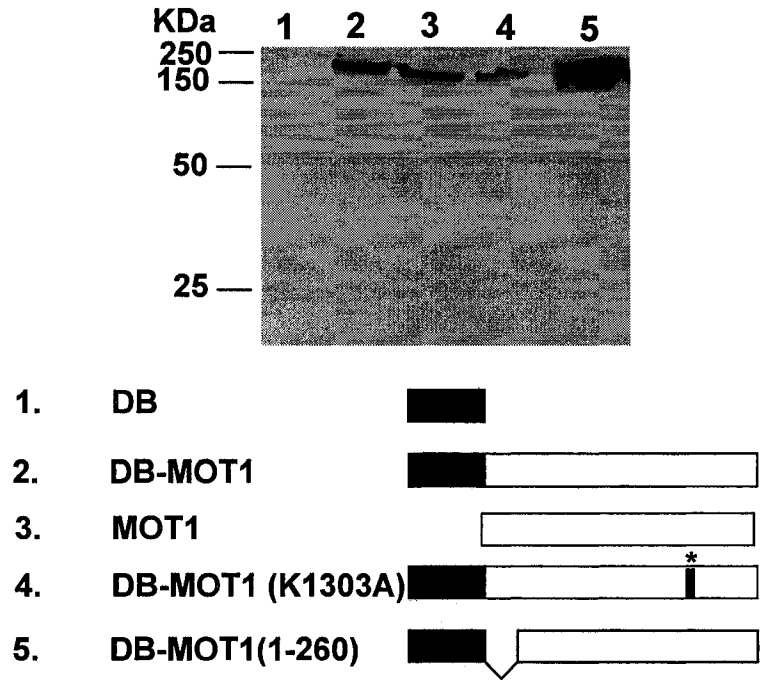

Figure IV.1: Expression of MOT1 fusion constructs *in vivo.* Protein extracts (25μg) from strains harboring the indicated derivatives were loaded onto 7.5% SDS-PAGE gels. Expressions were detected using antibodies directed against the HA epitope tag.

The ability of this fusion construct to affect the expression of *HIS3* was checked by growth on media containing the competitive inhibitor of the *HIS3* gene product, 3-Amino triazole (AT) (figure IV.2A). The construction of this reporter gene allows for a very minimal amount of *HIS3* gene expression in the presence of DB (control). In the case of DB-MOT1, this strain was unable to grow on AT containing media (figure IV.2A). This suggests that when MOT1 is targeted to this promoter it represses the expression of the gene. This is in contrast to the results obtained with the majority of the TAFs in TFIID, which when targeted to a test promoter in similar fashion were capable of positively effecting the expression of the reporter gene (292). However, in the present study a similar targeting of MOT1 that is also a TAF, albeit present in a different complex of TFIID (212, 261) affected the expression of the reporter gene in a negative fashion.

To determine if the growth on AT is mirrored at the level of transcription, S1 nuclease assays were performed and the abundance of the transcripts was measured directly. We observed two-three fold decrease in the transcription of *HIS3* in the case of DB-MOT1 in comparison to the DB alone (figure IV.2B). This clearly established that targeting MOT1 to a test promoter resulted in repression.

To determine that the repression effect was a direct result of targeting MOT1 to the *HIS3* gene and not due to a non-specific binding effect or a general effect by DB-MOT1, levels of transcription from the *PGK1* gene that did not harbor any upstream binding sites for DB was measured.

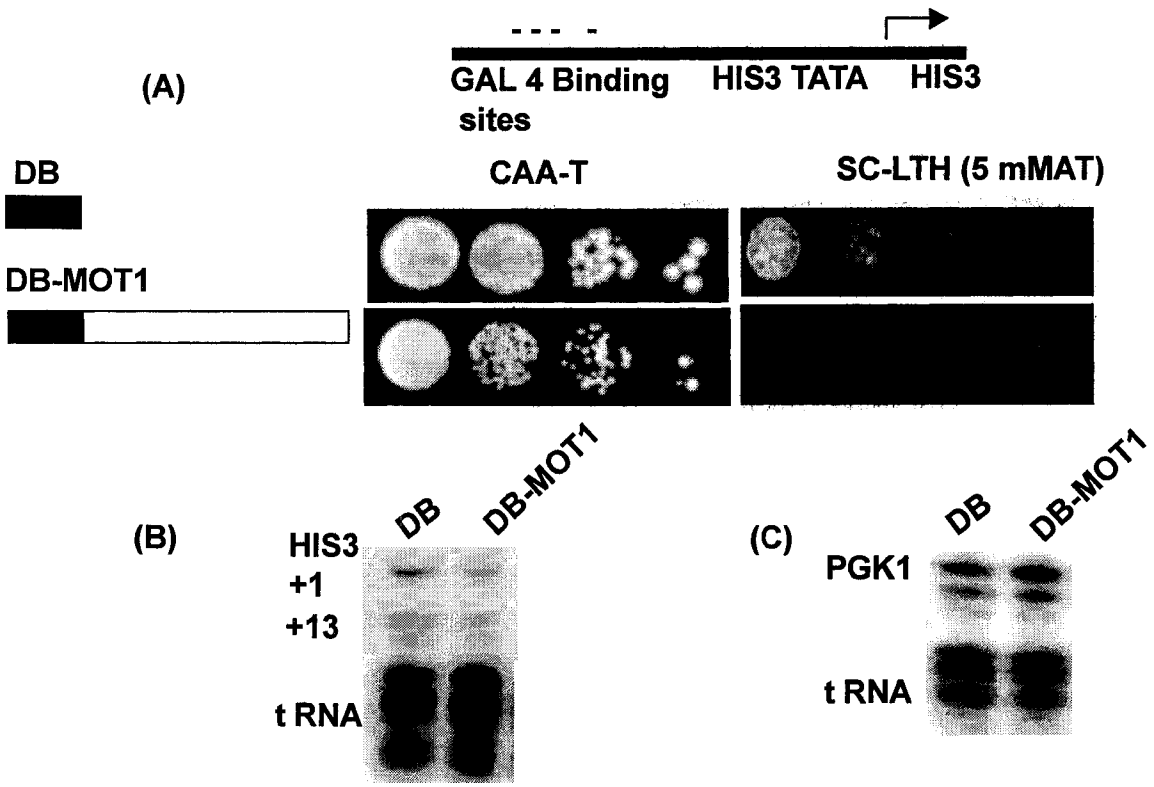

Figure IV.2: Repression by MOT1 upon targeting to the test promoters. (A) Growth phenotypes of strains bearing plasmids DB and DB-MOT1 in MaV103 strain. These strains were assayed for ability to grow on AT containing media (5 mM). Cell growth on AT is indicative of expression of the HIS3 reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown. (B) Transcription analysis of the *HIS3* reporter gene. *HIS3* mRNA levels of the strain harboring indicated plasmids was analyzed by the S1 nuclease method using 25 μg of the total RNA. The total RNA was hybridized to the ^{32}P labeled HIS3 probe and tRNA probe. This was further treated with S1 nuclease enzyme to digest the single strand nucleic acids and the undigested DNA was further resolved on denaturing PAGE gel. ^{w}tRNA was used as a load control. (C) Transcription analysis of the *PGK1* gene. Similar to panel B, transcription analysis was performed at the *PGK1* gene using the probes directed against it. The strains harboring the indicated plasmids were processed for the total RNA and using S1 nuclease assay the transcript levels of the *PGK1* gene was detected. ^{w}tRNA was used as a load control.

There was no decrease in transcript levels of PGK1 from DB-MOT1 strain in comparison to DB (figure IV.2C). Thus the repression effect we saw earlier at our test promoter was specifically due to the targeting of MOT1.

IV.4b Repression is recruitment dependent: One might argue that expressing a DNA binding domain tethered MOT1 in normal cells would result in additional copies of MOT1 overall, thus, resulting in deleterious effects on cell growth and adverse effects on transcription by sequestering TBP *in vivo*. To determine if that was the case and if over expression of MOT1 resulted in any non-specific effects on transcription, we removed the DNA binding domain from the fusion construct and expressed MOT1 in the cells. The expression level was similar to the fusion construct (figure IV.1). Such a MOT1 was checked for cell growth on AT (figure IV.3A). Growth on AT is indicative of no repressive effect on *HIS3* expression. There was no change in the transcript levels of *HIS3* and *PGK1* (figure IV.3B and 3C). It was clear that the specific targeting of the MOT1 directly affected the transcription repression and this was not due to any other indirect effects or over expression of MOT1.

IV.4c Targeted repression by MOT1 is not promoter or fusion construct specific: Previous studies have shown that effect of MOT1 on transcription was variable based on the strength of the TATA and the gene (36, 82). To see if the repression effect is test promoter or fusion construct specific, first, we targeted MOT1 to a *HIS3* gene (CG1945-Clontech), which contains the promoter element derived from the *GAL* gene instead of the *HIS3*. Similar to earlier case and as we predicted, we see that MOT1 was still able to repress transcription (figure IV.4A).

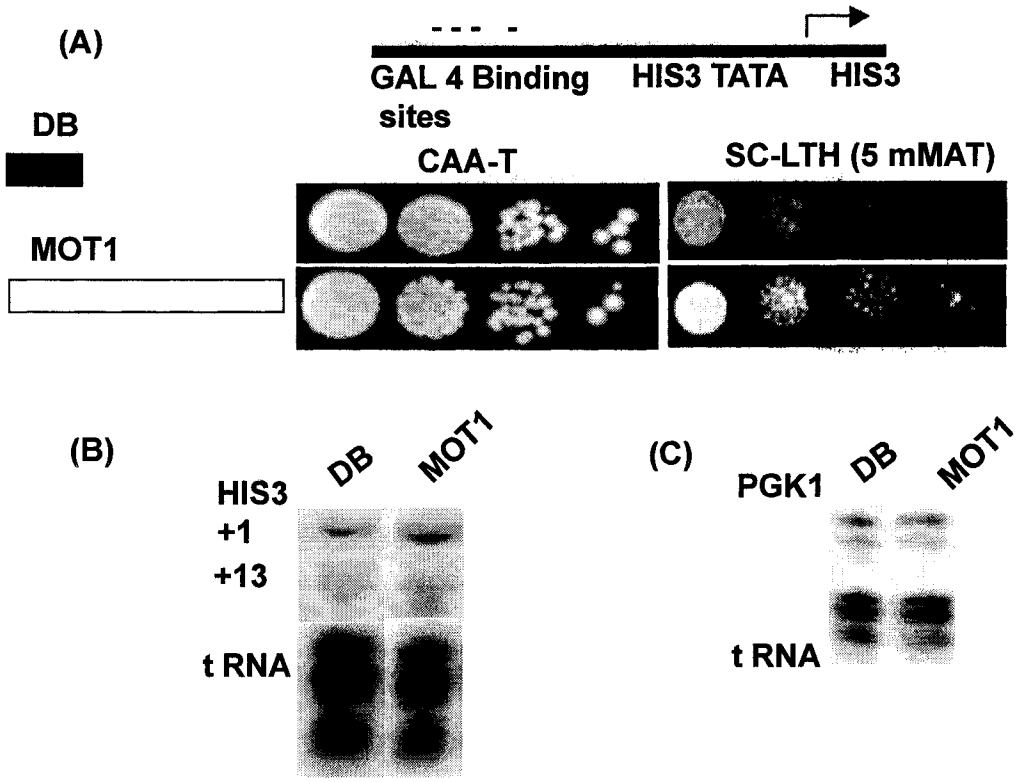

Figure IV.3: Overexpression of MOT1 does not result in repression.
(A) Growth phenotypes of strains bearing plasmids DB and MOT1 in MaV103 strain. These strains were assayed for ability to grow on AT containing media (5 mM). Cell growth on AT is indicative of expression of the HIS3 reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown. (B) Transcription analysis of the *HIS3* reporter gene. *HIS3* mRNA levels of the strain harboring indicated plasmids was analyzed by the S1 nuclease method using 25 μg of the total RNA. The total RNA was hybridized to the ^{32}P labeled HIS3 probe and tRNA probe. This was further treated with S1 nuclease enzyme to digest the single strand nucleic acids and the undigested DNA was further resolved on denaturing PAGE gel.[w]tRNA was used as a load control. (C) Transcription analysis of the *PGK1* gene. Similar to panel B, transcription analysis was performed at the *PGK1* gene using the probes directed against it. The strains harboring the indicated plasmids were processed for the total RNA and using S1 nuclease assay the transcript levels of the *PGK1* gene was detected.[w]tRNA was used as a load control.

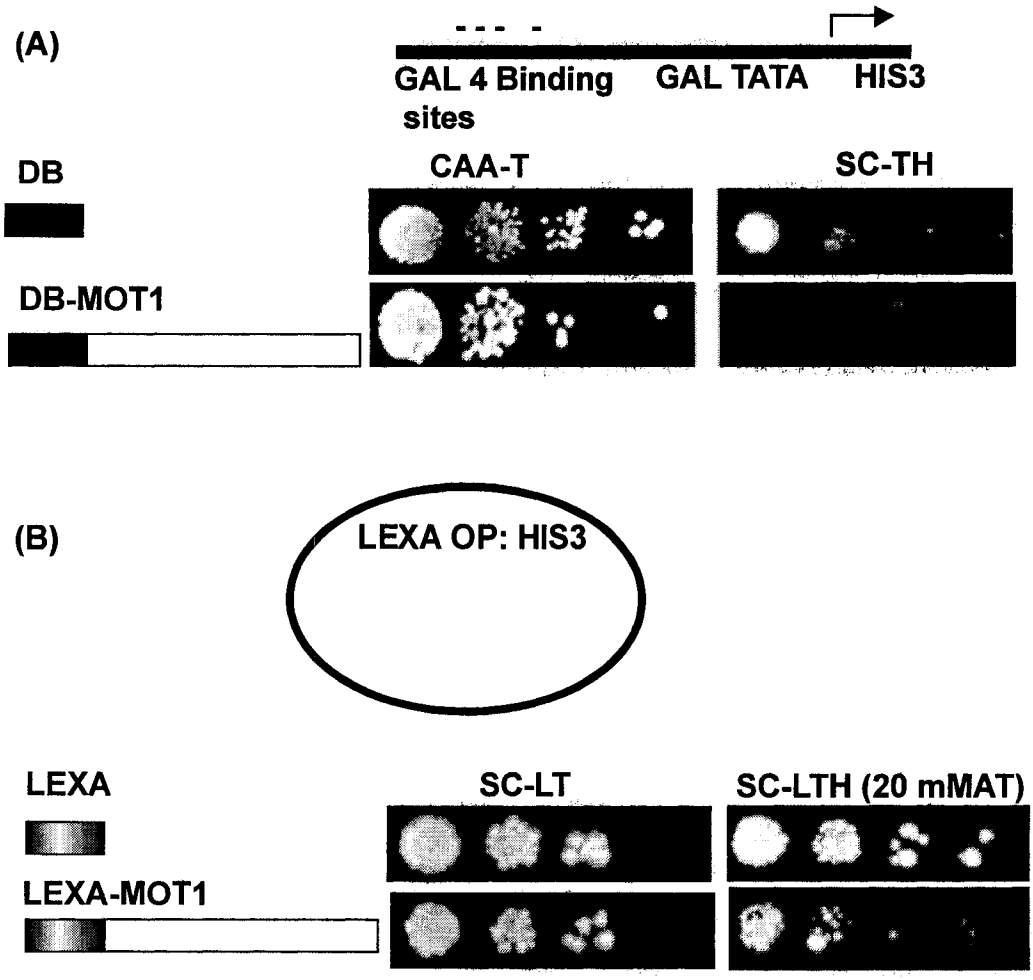

Figure IV.4: MOT1 repression is a general effect. (A) Growth phenotypes of strains bearing plasmids DB, DB-MOT1 in CG1945 strain that has a *HIS3* reporter gene with binding sites for GAL4 DNA binding domain and GAL TATA. These strains are assayed for ability to grow on media containing no histidine. Cell growth on media with no histidine is indicative of expression of the *HIS3* reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown. (B) Growth phenotypes of strains bearing plasmids LEXA and LEXA-MOT1 in strain that has plasmid driven HIS3 reporter gene with LEXA operator upstream. These strains were assayed for ability to grow on AT containing media (20mM). Cell growth on AT is indicative of expression of the *HIS3* reporter gene.

To see if the repression effect is fusion construct specific, we have also used a LexA (bacterial protein) fusion of the wild type MOT1 instead of the DB fusion and targeted it to a plasmid based reporter gene. This reporter gene, unlike the previous two reporter genes, is plasmid based and contains a LexA operator upstream of the *HIS3* gene. When LexA or LexA-MOT1 were transformed into a strain containing the LexA operator fused to the *HIS3* and were assayed for the cells ability to grow on AT containing media, it was clear that similar to previous case we see repression of transcription by DB-MOT1 (figure IV.4B). Thus, MOT1 dependent repression of transcription was independent of the test promoter context or the fusion domain.

IV.4d ATPase function of MOT1 is required for transcription repression:

MOT1 belongs to a Swi2/Snf2 family of the ATPase dependent proteins (51). Several studies since have shown that ATPase activity of MOT1 is inherent for its functional ability to dissociate TBP from DNA *in vitro*. However it is unclear if the ATPase function is required for transcription repression. To test if the ATPase function of MOT1 is required for repression function *in vivo*, we made a DB fusion of a point mutant of MOT1-K1303A (7) similar to the wild type. MOT1-K1303A was shown to be inactive for dissociating the TBP from DNA *in vitro* (7). DB-MOT1 (K1303A) when targeted to the test promoter was incompetent for repressing transcription, indicating that repression effect is ATP dependent (Figures IV.5A, B and C).

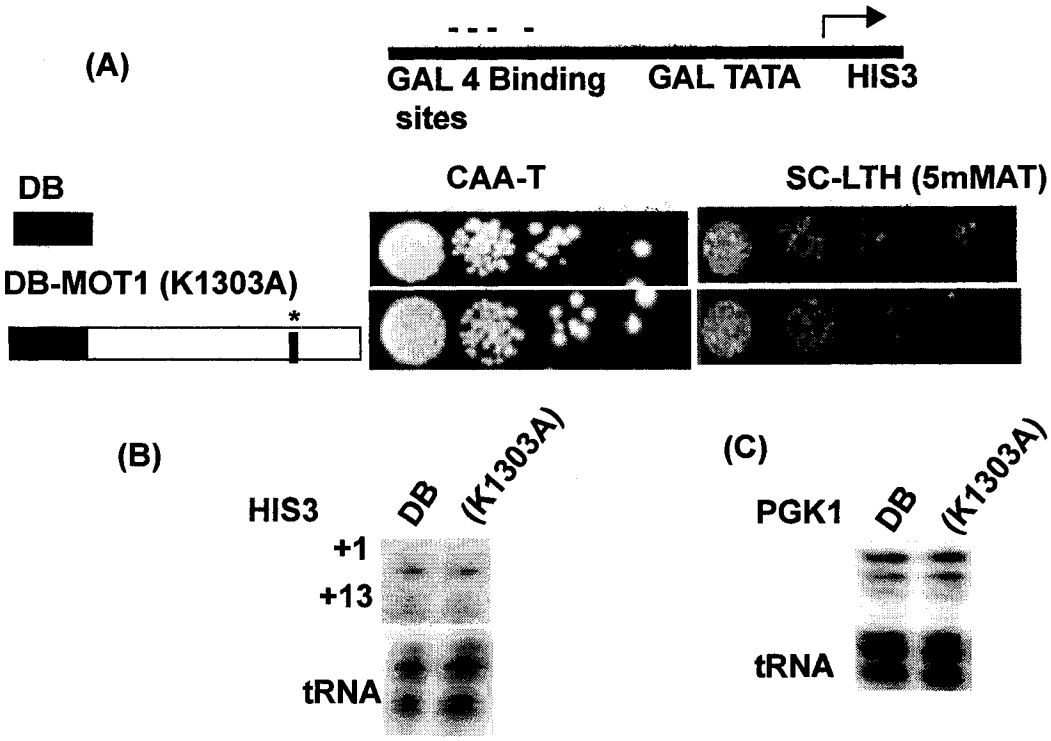

Figure IV.5: No repression of transcription by MOT1 (K1303A), upon targeting to the test promoters. (A) Growth phenotypes of strains bearing plasmids DB and DB-MOT1 (K1303A) in MaV103 strain. These strains were assayed for ability to grow on AT containing media (5mM). Cell growth on AT is indicative of expression of the HIS3 reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown. (B) Transcription analysis of the *HIS3* reporter gene. *HIS3* mRNA levels of the strain harboring indicated plasmids was analyzed by the S1 nuclease method using 25 μg of the total RNA. The total RNA was hybridized to the ^{32}P labeled HIS3 probe and tRNA probe. This was further treated with S1 nuclease enzyme to digest the single strand nucleic acids and the undigested DNA was further resolved on denaturing PAGE gel. tRNA was used as a load control. (C) Transcription analysis of the *PGK1* gene. Similar to panel B, transcription analysis was performed at the *PGK1* gene using the probes directed against it. The strains harboring the indicated plasmids were processed for the total RNA and using S1 nuclease assay the transcript levels of the *PGK1* gene was detected. tRNA was used as a load control.

We also performed co-immunoprecipitation assays with DB-MOT1 and DB-MOT1 (K1303A) to see their interaction profiles with TBP. Protein-A Sepharose beads were conjugated to TBP and incubated with the whole cell protein extracts of the strains harboring the wild type and the mutant of MOT1. It was interesting to note that both wild type and K1303A mutant were enriched in the immunoprecipitated samples for their presence in comparison to the load controls (figure IV.6). This indicates that both the wild type and the K1303A fusion constructs were competent for interactions with TBP.

To compare our results of MOT1 and MOT1 (K1303A) with known transcription factors like FOS and TAF2, we made similar fusion constructs and targeted them to the test promoter. When we compare the level of activation we get from these factors (figure IV.7) it was clear that the expression of *HIS3* by DB-MOT1 (K1303A) is reversed for the repression of transcription but does not activate transcription as DB-FOS or DB-TAF2. DB-MOT1 (K1303A) behaves essentially like DB, thus, was dead for any effects on transcription in our system.

IV.4e Repression is dependent on the ability of MOT1 to interact with TBP:

It has been well characterized that the ability of MOT1 to dissociate TBP from DNA is dependent on its interaction with TBP (1, 8, 207). We used a mutant of MOT1 that was deleted for 94 amino acids towards the N-terminus, which was shown to be incompetent for supporting the cell viability (49). The same study shows that this allele was defective for interaction with TBP and also the dissociation of the TBP-DNA-MOT1 ternary complex *in vitro*.

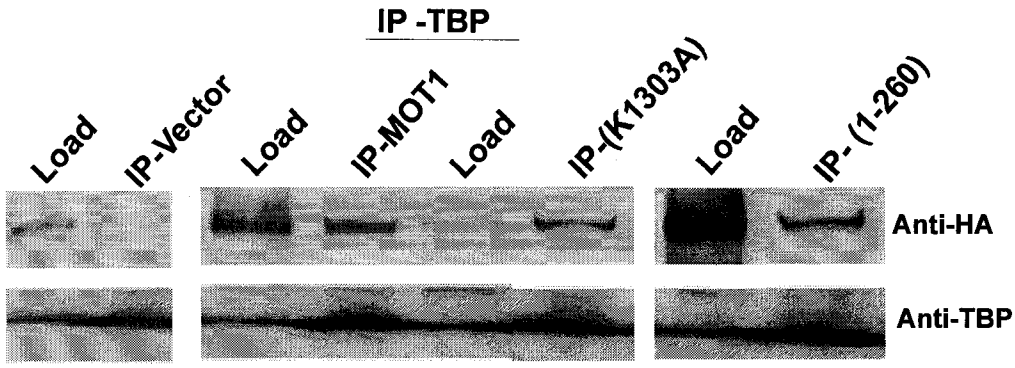

Figure IV.6: interaction of MOT1, MOT1(K1303A) and MOT1(1-260) alieles with TBP. Co-immunoprecipitation assay was performed on cell extracts from strains expressing DB (vector), DB-MOT1, DB-MOT1 (K1303A) and DB-MOT1 (1-260) by using antibodies directed against TBP. Immuno-precipitated complexes (IP) were resolved by SDS-PAGE and probed by immunoblot analysis using moclonal antibodies specific to HA. Input from each extract (Load) is indicated. Immunoblot analysis for the presence of TBP was performed as a control.

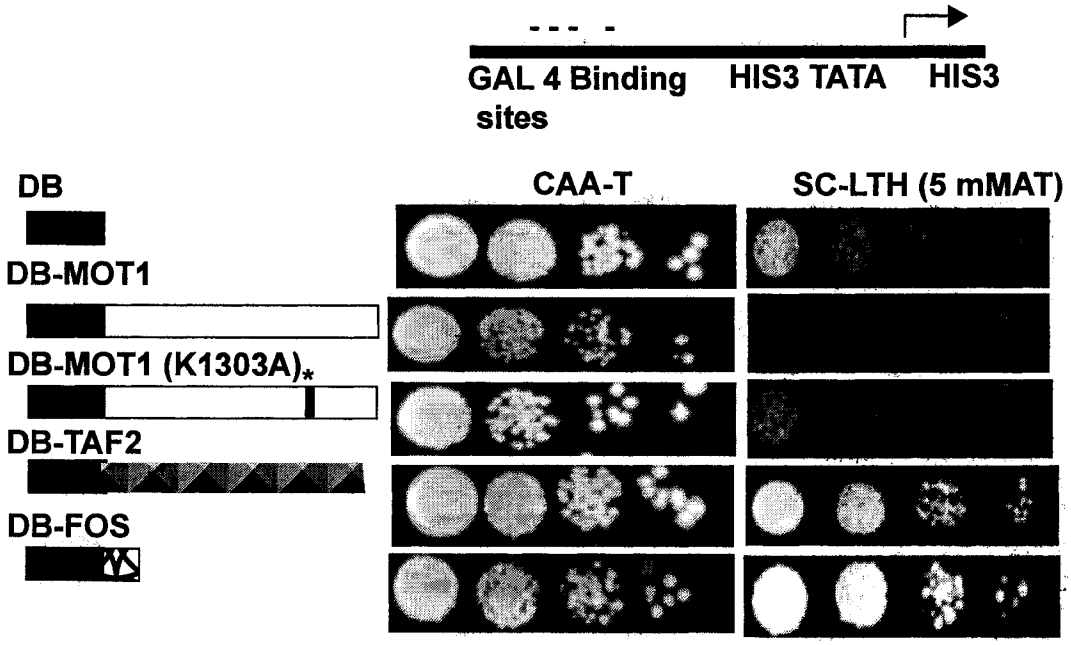

Figure IV.7: Comparison of fusion constructs of MOT1 and fusion constructs of other known transcription activators. Growth phenotypes of MaV103 strains bearing plasmids DB, DB-MOT1, DB-MOT1 (K1303A), DB-TAF2 and DB-FOS. These strains were assayed for ability to grow on AT containing media (5mM). Cell growth on AT is indicative of expression of the *HIS3* reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown.

We tethered this particular mutant to the DB and targeted it to DNA to check its effect at the test promoter. The advantage of our system is that, we can still use this allele in our *in vivo* assay and essentially not disturb the normal physiology of the cell. Expression of this fusion construct was also confirmed by performing western blot analysis (figure IV.1).

We assayed for cell growth on media containing AT (figure IV.8A). We show that this allele is defective for the repression of transcription. We also measured the transcription levels of the *HIS3* gene to make sure that the repression is at the transcription level and specificity (figure IV.8B and IV.8C). We see that similar to the case of MOT1 (K1303A) mutant, the MOT1 (1-260) was unable to repress transcription. This indicates that a physical association between MOT1 with TBP is essential for repression of transcription function.

When co-immunoprecipitation assays were performed with DB-MOT1 (1-260), there was no enrichment of the signals in the IP samples (figure IV.6). In comparison to DB-MOT1 and DB-MOT1 (K1303A), DB-MOT1 (1-260) does not show an enrichment of signals when immunoprecipitated with TBP antibody bound beads. An enrichment of signal in the IP sample compared to the load is indicative of an association between TBP and the MOT1 constructs. Previous studies have shown that three regions in the N-terminus of MOT1 are involved in interaction with TBP (207). The small interaction signal that we see may be due to some weak interactions still maintained by other regions of MOT1 (figure IV.6).

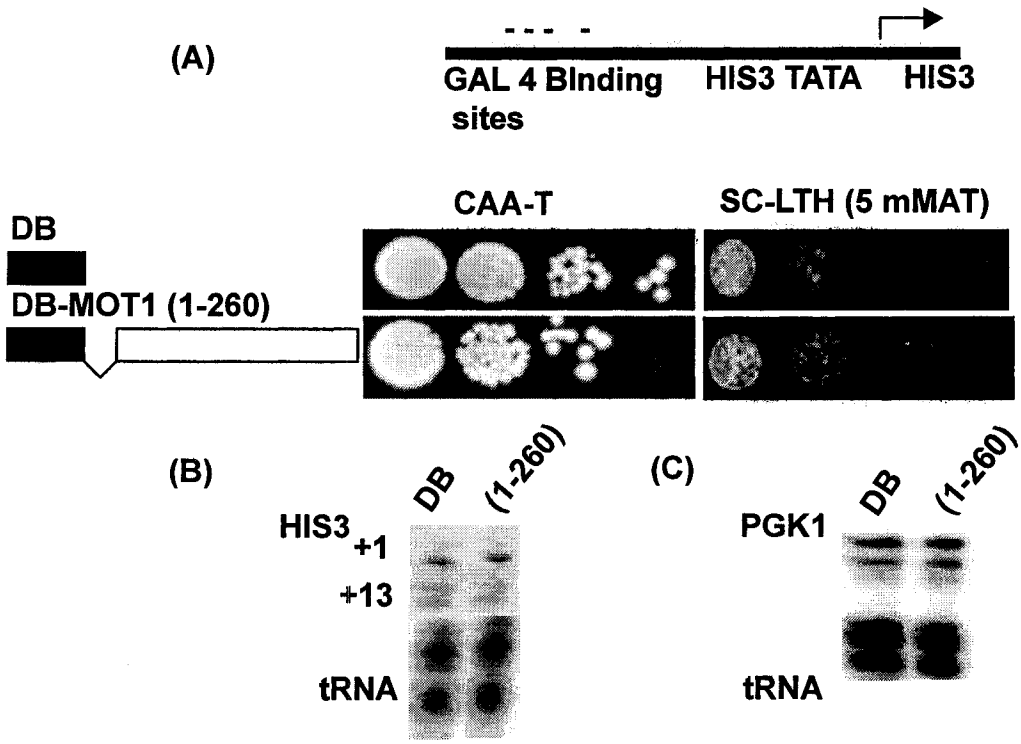

Figure IV.8: No repression of transcription by MOT1 (1-260) allele on targeting to the test promoter. (A) Growth phenotypes of strains bearing plasmids DB and DB-MOT1 (1-260) in MaV103 strain. These strains were assayed for ability to grow on AT containing media (5mM). Cell growth on AT is indicative of expression of the HIS3 reporter gene. Yeast growth in each panel is a serial dilution of same amount of cells. Growth on regular media for selection of plasmids is also shown. (B) Transcription analysis of the *HIS3* reporter gene. *HIS3* mRNA levels of the strain harboring indicated plasmids was analyzed by the S1 nuclease method using 25 μg of the total RNA. The total RNA was hybridized to the ^{32}P labeled HIS3 probe and tRNA probe. This was further treated with S1 nuclease enzyme to digest the single strand nucleic acids and the undigested DNA was further resolved on denaturing PAGE gel. ^{w}tRNA was used as a load control. (C) Transcription analysis of the *PGK1* gene. Similar to panel B. transcription analysis was performed at the *PGK1* gene using the probes directed against it. The strains harboring indicated plasmids were processed for the total RNA and using S1 nuclease assay the transcript levels of the *PGK1* gene was detected. ^{w}tRNA was used as a load control.

IV.4f TBP occupancy at genes targeted by MOT1: To further shed light on mechanism of MOT1 action at these test promoters, chromatin immunoprecipitation assays were performed in strains harboring the DB and DB fusion constructs of MOT1. Upon immunoprecipitation using TBP antibodies, the DNA protein cross-links were reversed and the reporter gene was amplified. Efforts to amplify the binding sites of GAL4 to the start site of *HIS3* failed. These attempts resulted in amplification of several random and non-specific products. Thus, we used primers to amplify the reporter gene from *HIS3* start site to 250 bases downstream of the start site. Since, we were in the window of 500 bp from the GAL4 binding site, the quantitation should provide us an approximate measure of TBP binding within this region.

In strains harboring DB alone, the TBP occupancy at the reporter gene was minimal, reminiscent of low levels of transcription. Based on the *in vitro* studies one would hypothesize that DB-MOT1 would cause lower occupancy of TBP compared to DB, since MOT1 would dissociate TBP from DNA. However, quite contrary to this hypothesis, we see that strains harboring DB-MOT1 did not have diminished TBP occupancy compared to DB alone (figure IV.9). This result indicates that the tethered MOT1, when targeted to DNA was repressing transcription in a way that did not correlate directly with TBP dissociation from DNA. Owing to the fact that DB-MOT1 exhibited 2-3 fold decreased transcription compared to DB alone and no decrease in TBP occupancy we reached at a probable conclusion for the mechanism of MOT1 action at these test promoters. We propose that MOT1 at these test promoters compromises the activity of TBP

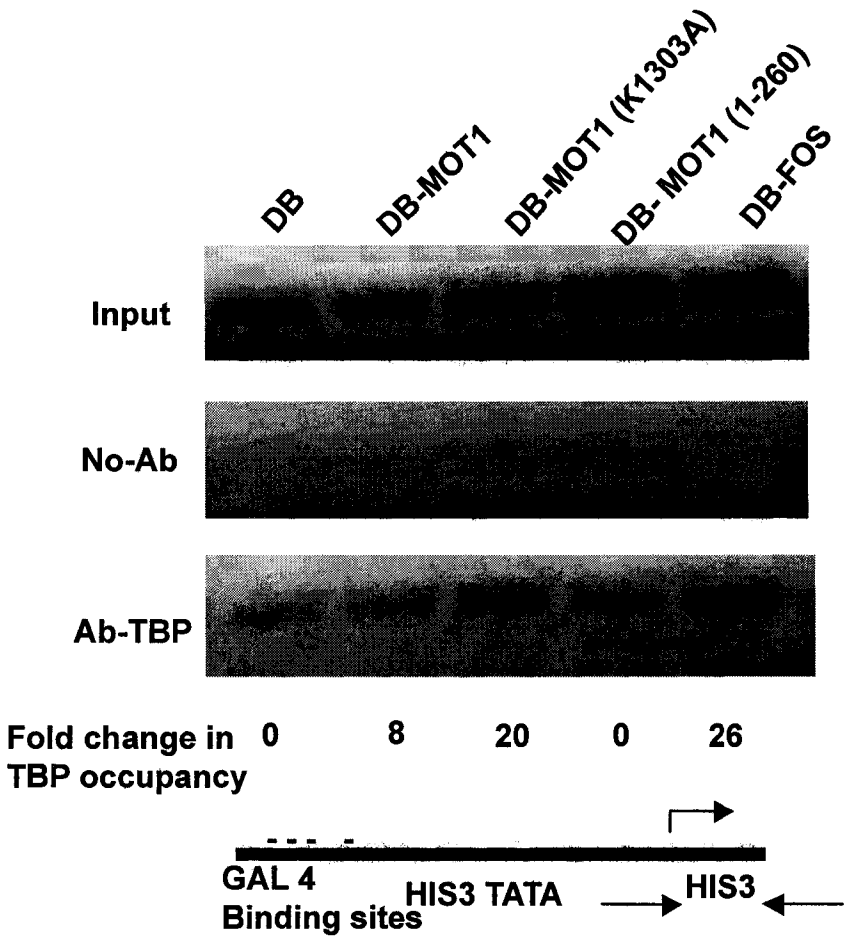

Figure IV.9: Occupancy of TBP at the reporter gene . Chromatin immunoprecipitation from strains bearing the indicated plasmids was performed using polyclonal antibodies directed against TBP. Top panel indicates the amplification of the reporter gene product of the total input control, which was 10% of the chromatin used for immunoprecipitation. Middle panel is amplification of the reporter gene using no antibodies and the lower panel is for the IP material. Aprroximately a 250 base pair product is visualized by running a small amount of the total PCR reaction mixture on 1% agarose gel and further stained with ethidium bromide dye. Fold change in occupancy was calculated from the values obtained by IP/Input ratios in each category. The value obtained for the vector alone (DB) was subtracted from the value obtained by IP/Input ratios in each category to show the fold change in TBP occupancy.

such that now there is repression of transcription. This may be one way by which MOT1 represses transcription and falls in line with the studies which show that TBP is still occupied both at promoters that are positively and negatively regulated by MOT1 (82).

In case of the DB-MOT1 (K1303A), we see a slightly increased TBP occupancy (figure IV.9). However, In spite of a difference in TBP occupancy levels between the strains harboring DB and DB-MOT1 (K1303A), their transcriptional outputs are similar. This further strengthens our prediction that probably MOT1 has an ability to inactivate TBP in a fashion so as to affect the transcriptional output. In case of DB-MOT1 (1-260), we see very less TBP occupancy similar to the DB control (figure IV.9). This would indicate that infact the N-terminus is essential for loading of TBP onto the DNA. We also checked the TBP occupancy of a known transcription activator, FOS. We saw that there is a high TBP occupancy (figure IV.9), and directly correlates with the transcriptional output (148, 161).

In an entirely different study it was shown that inactivation of MOT1 increased the TBP binding at a set of genes. However, at all these genes, TBP occupancy did not match with the transcriptional output (161). These studies are in line with the observations that we note here. By recruiting either the wild type or different alleles of MOT1 we see differential TBP occupancy, which does not exactly match with their transcriptional outputs. Chromatin immunoprecipitation assays use formaldehyde to crosslink the protein with DNA, which not only necessarily crosslink the protein-protein and protein-DNA but also those proteins

that are in the proximity of DNA. The occupancy of TBP we see in the case of MOT1 might be an indirect indication of TBP dissociated from the DNA but still present in the proximity. Thus chromatin immunoprecipitation results need to be interpreted with caution. However, measurements of transcript levels from the reporter genes are true indication of the final outcome of the effects by wild type and alleles of MOT1.

IV.5 Discussion

There are several transcription repressors that either form complexes with TBP or interact with TBP and repress activated as well as basal transcription (121, 180, 184). MOT1 also belongs to this category of repressors which modulate TBP to effect transcription. MOT1 seems to be more complicated than many repressors as it has both transcription activation and repressor functions. In the present study, we have observed interesting facets of MOT1's mechanism of action in transcription regulation. Our study utilizes test promoters *in vivo* instead of *in vitro* techniques, hoping that we maintain the normal physiology of the cell to gain insights into MOT1 function. MOT1 relaxes the requirement for the UAS (51) indicating in our case that targeting of MOT1 to a test promoter should be indifferent to how an activator might modify transcription at these test promoters. By tethering MOT1 to DNA binding domains of transcription activators and targeting it to test promoters we show repression of transcription at these test promoters. Inherent ATPase function of MOT1 is essential for its repression function, as well as the ability of MOT1 to interact with TBP is required. We also

show that repression function is independent of the test promoters or the fusion construct used.

We observed that TBP occupancy was not decreased in the case of MOT1 at these test promoters, thus in the present setup, TBP occupancy does not parallel the effects on transcription. This indicates that when targeted to a gene, MOT1 represses transcription by forming an inactive complex with TBP that is now unable to activate transcription. Significant repression of transcription, when MOT1 is targeted to DNA irrespective of fusion design and test promoter tells us that transcription repression is a more direct effect of MOT1. However, further experimentation along these lines would be required.

One would assume, based on *in vitro* assays, that ATPase function of MOT1 is required for the removal of TBP from DNA at our test promoters. Once MOT1 defective for dissociating TBP is targeted to the DNA, we would predict that it should no longer repress transcription, since higher TBP directly correlates with transcription activation (148, 161). However, we see that this is not the case.

Here, we present a novel *in vivo* technique that can be further used to look for factors that influence the way MOT1 effects transcription. By using this *in vivo* technique, we can study constructs of MOT1 that do not cover a knock out. By this technique we can specifically target the repression function of MOT1 *in vivo*. There is a large possibility for MOT1 to maintain a multitude of interactions with other transcription regulatory factors. It is also interesting to note that, not all MOT1 present in the yeast extracts is in association with TBP (212) indicating that there could be several other factors that MOT1 could associate with that

potentially regulate MOT1 function. Our system allows us to conduct genetic screens to identify any such novel interacting factors.

Since its original discovery in genetic screens, MOT1 has been suggested to perform any of the several roles that would explain the requirement for such a factor in the cell. It was suggested to either properly position the TBP, render the TBP/TFIID binding as the rate limiting step in transcription initiation, remove the TBP/TFIID once the polymerase kicks off into elongation stage, or in a more positive sense remove the TBP from the strong TATA and help the limiting pool of the TBP to initiate transcription from weak or TATA less promoters (36). We would also like to suggest that MOT1 could inactivate TBP directly by either masking its interaction surfaces for other basal transcription machinery or activators or by forming an inactive/non-functional complex with TBP or importantly change the conformation of TBP at the DNA, as one of the mechanisms by which MOT1 can regulate transcription. We feel that our study provides an *in vivo* perspective to mechanistically gain insights into the MOT1 mechanism of action, which is novel and lacking from previous studies.

Chapter V

Perspectives and Future directions

The work presented in this dissertation is focused on understanding various facets of TAF function. Initially, I utilized an *in vivo* approach to identify the interactions between all of the TBP associated factors and TBP. This information was used to develop an interaction map and model the structure of transcription factor IID in yeast. Many of the interactions have been shown previously via several genetic and biochemical methods, but over 50% of the interactions were novel (292). It was interesting to note that each TAF had a unique interaction profile in our study.

Every TAF has a unique functional role in transcription. It was shown in a gene array analysis that mutation of each TAF seems to differentially effect gene expression (241). Combined with our identification of novel interactions between TAFs and their significant participation in gene expression, the study of each TAF individually will provide us with more clues for their functional roles at different genes. Each TAF-TAF interaction profile might provide us with important clues to their involvement at different genes. By defining these TAF-TAF interactions, I feel that I have laid the groundwork for future studies in this area.

As an extension of the TAF-TAF interaction mapping studies, I looked at the interactions of TAF1 in further detail. TAF1 is known as the scaffolding TAF and aptly we found that it interacts with several other TAFs in our two-hybrid analysis. I delineated a smaller region within this TAF, which is functional and encompasses most of its TAF interactions. Isolating a small region (approx 100 residues), within this large 1066 amino acids protein should allow future biochemical characterization of TAF1 function.

I have also made special contributions in understanding the functional role of TAF11 in mediating interactions between TFIID and TFIIA. Here, we specifically show that deletion of the first 73 N-terminal amino acids of TAF11 (taf11-ΔN) abolishes interaction with TFIIA, but does not effect the TAF11-TAF13 interaction. This particular allele of TAF11 was fully functional for TFIID integrity. This suggests that the conditional phenotypes and transcriptional defects previously observed could be attributed to the loss of its interaction with TFIIA and not due to instability of TFIID. Identifying and establishing a compensatory interaction between two different alleles of TAF11 and TFIIA was a significant finding that further establishes a functional connection between TAF11 and TFIIA. The taf11-ΔN allele can be further exploited in a genetic screen for suppressors of its phenotypes. We hypothesize that analysis of such suppressor strains would potentially identify factors that might be important for TAF11 functions like chromatin remodeling factors and other transcription regulators.

I feel that I have established a sound base for MOT1 function *in vivo*. This is a very valuable tool, as many researchers so far could only study its function *in*

vitro, in isolation from other factors that might have an impact on MOT1 function. In our studies, we show that tethering MOT1 to a DNA binding domain of a transcription factor and recruiting it to the DNA represses transcription from a test promoter. This effect was independent of the fusion design or the test promoter context. We also show that the inherent ATPase activity of MOT1 and its ability to interact physically with TBP are essential features for its repression function *in vivo*. Thus, our fusion construct is a fully functional MOT1 *in vivo*. This system can be utilized to look for other factors that might interact with MOT1 and regulate its function.

First we plan to use a collection of mutant strains that have been deleted for a single non-essential factor. We intend to transform the MOT1 fusion construct along with the reporter gene into a set of these deletion strains. By doing so we hope that in the absence of a factor required for MOT1 to repress transcription, repression will be lost. This strategy should identify several factors like chromatin remodeling factors, other transcription regulators or general transcription factors that might affect the MOT1 function *in vivo.* Such a study will provide us valuable insights not only into MOT1 function but also will establish a functional connection between transcription regulators and other co-regulators that modulate transcription.

Finally, I see great potential in *SPN1* research. *SPN1* is a novel factor identified in a screen looking for factors that genetically interact with TBP. *SPN1* was also shown to have post-recruitment functions in transcription. I applied the chromatin immunoprecipitation technique to understand the occupancy of *SPN1*

and other factors that were found to interact with *SPN1* (in a genetic screen) at the post recruitment regulated gene, *CYC1*. In this study I show that, the *CYC1* promoter is occupied by Spt6 and Snf2. This study has provided new functional insights into *SPN1* mechanism of action in conjunction with known chromatin remodelers like Spt6 and Snf2.

Summary

The work presented in this dissertation provides a solid foundation for advancing our understanding of several TBP associated factors that regulate TBP function both positively and negatively. The experimental ideas proposed above will extend the analysis of these TAFs and will further our understanding of RNA Pol II mediated regulation of transcription.

Appendix

SPN1, a TBP interacting factor

It is clearly established that recruitment of TBP at the promoters can be the rate- limiting step for transcription initiation (28, 35, 133, 258, 290), and TBP occupancy at a promoter is known to correlate strongly with transcriptional output (148, 161). As such, many factors may either directly or indirectly interact with TBP to influence its activity.

Previous studies done by several groups show that by tethering TBP to LexA and recruiting it artificially to a reporter gene, overcomes the requirement for a transcription activator (28, 133, 247, 290). However, stages like promoter clearance and recruitment of Pol II, cannot be bypassed by tethered TBP. Previously, the TBP allele F237D was isolated, which was unable to initiate transcription upon recruitment to a reporter gene (66).

To identify factors that genetically interact with TBP, our laboratory conducted a spontaneous suppressor screen looking for factors that allowed lexA-F237D to transcribe a reporter gene containing a LexA operator. In this screen we identified a gene called 'suppressor for post-recruitment defective gene number 1' (*SPN1*) (66). Further functional characterization of *SPN1* shows that a mutation (K192N) alters transcription by Pol II and confers SPT

phenotypes. Thus *SPN1* is an spt gene along with TBP, which is *SPT*15. *SPN1* is essential and conserved throughout evolution (66). This initial report from our group shows that *SPN1* has functional interactions with TBP and affects the Pol II mediated transcription (66). *SPN1* was also identified in other studies and was called *IWS1* (interacts with Spt6).

Many activators affect transcription by enhancing the PIC formation and stability by direct interactions with TBP, TAFs, GTFs, co-activators, mediators and chromatin remodeling complexes (147, 148, 161, 165, 260). In addition to affecting transcription at this initial step, activators also influence subsequent steps in transcription process. They play important roles in promoter clearance, release of Pol II pausing and elongation rates of Pol II (16, 146). Thus, activators affect both recruitment and post-recruitment steps in transcription.

SPN1 clearly has roles in post-recruitment stages of transcription, since it was identified in the screen for factors suppressing a post-recruitment defective TBP allele. *CYC1* is a post-recruitment regulated gene that encodes isoform 1 of cytochrome C1 (29, 174). *CYC1* is unusual since it defies the general rule that recruitment of transcription machinery is rate-limiting step (148, 161). It was previously shown that both TBP and Pol II are occupied at the *CYC1* promoter, under transcription inactivating states (29, 174). Thus activation at this gene is regulated in a post-recruitment step. At such a gene it was shown that its transcription is facilitated by a mutation in *SPN1* under both partially activating and repressing conditions, thus providing further evidence for the involvement of *SPN1* in post-recruitment functions. Recent studies in our laboratory have

established a functional connection between Spn1 & Spt6 and Spn1 & Snf2. Here, we look at the factor occupancy at the *CYC1* gene under partially repressing and activating conditions. I hope that by determining the occupancy of these factors, we can establish a correlation between their known mechanistic functions.

Materials and Methods:

Yeast strains: Spn1-Myc tagged strain is in the background of BY4741. Ycp22 plasmid vectors containing Wt *SPN1*-Myc or *SPN1* (K192N)-Myc were in the background of SK1 strain, which was deleted for SPN1 from its chromosomal location. In the same strain an HA epitope was integrated at the chromosomal location of the *SPT6* gene. SPN1 was also deleted in the background of BY4741 and supported by Ycp22 plasmid vectors containing Wt *SPN1*-Myc or *SPN1* (K192N)-Myc where the *SNF2* gene is tagged with a HA epitope at the chromosomal location. LZ and JF created all the strains.

Cell growth conditions: Cell growth conditions to induce partially repressed and activated conditions to perform chromatin immunoprecipitation analysis are as follows. In case of partially repressed conditions, cells were grown in YPD media i.e. in the presence of glucose. For activated conditions, after growing overnight a small batch of culture in YPD, the cells were washed thrice in media containing no glucose and inoculated further into media containing 3% ethanol without any glucose and were grown for 6 hours. All the cells were cultivated at an OD of 1.0 (600 nm).

Chromatin immunoprecipitation assays: Chromatin immunoprecipitations were performed as described (249) with few modifications. Cells (150 ml) were grown to an OD of 0.8-1.0 (600 nm). Cells were treated with a final concentration of 1% formaldehyde for 15 minutes with occasional swirling of the flasks at intervals of 5 minutes. Glycine was added to a final concentration of 125 mM at room temperature for 5 minutes to stop the crosslinks. Cells were collected and washed twice in ice cold TBS. Cells were resuspended in FA-lysis buffer (500 µl of FA-Lysis buffer for a total of 50 ml of cell culture). Chromatin was sheared by sonication using a Branson W-350 model of sonifier (10 times at 10 seconds each on continuous pulse at a microtip power setting of 6). Input was 10% of the chromatin material used for the immunoprecipitation and was processed as the after reversing the cross-links and purifying the DNA. 500 µl of the chromatin material was incubated with approximately 5 µl of either TBP, Spn1, Myc or HA antibodies by rotation overnight at 4^0C. 50 µl of protein-A sepharose beads (Pharmacia-prepared as slurry as per the manufacturers directions) was further incubated with the chromatin material for 2 hours at 4^0C. The beads were spun down and the antigen-antibody complexes bound to the beads were recovered and further treated with TE/SDS buffer for 15 minutes at 65^0C to elute the complexes. Protein-DNA cross-links were reversed by incubation overnight at 65^0 C and the DNA was purified by phenol-chloroform extraction and used for PCR analysis.

PCR reactions were carried out in a total volume of 50 µl. Different template amounts from the immunoprecipitated material were used to determine the linear

range of the PCR reaction. Primers for the PCR were designed at the promoter region of the *CYC1* gene and amplified a product of 312 bp (174). DNA was run on 1% agarose gels, and scanned into the computer. The intensity of the bands was quantified by using Image Quant software. The signal strength ratio between the IP sample and the input is an indication of the occupancy of the protein.

Results and Discussion

Spn1 was present at *CYC1* gene under both partially repressing and activated conditions similar to TBP (figure A.1). The Spn1 mutant (K192N) has a diminished Spn1 occupancy at this gene under both partially repressing and activated conditions (figure A.2). Several previous studies have shown that Spn1 interacts with Spt6 (Venugopal Pujari: personal communications). Here, we show that Spt6 is present at the *CYC1* in the presence of wild type Spn1 and absent in the presence of mutant Spn1 (figure A.2).

A genetic interaction between Spn1 and Snf2 has been established previously (Lei Zhang: unpublished results). Here, we show that Snf2 has a diminished occupancy in Spn1 wild type cells under partially repressing conditions and normal occupancy under activating conditions (figure A.3). However, in the Spn1 mutant, Snf2 was present under both partially repressing and activated conditions (figure A.3). This observation probably suggests a negative regulation of Snf2 by Spn1. However, further experimentation and additional studies by other methods are required to develop a concrete model of Spn1 mode of action at the post-recruitment regulated *CYC1* gene.

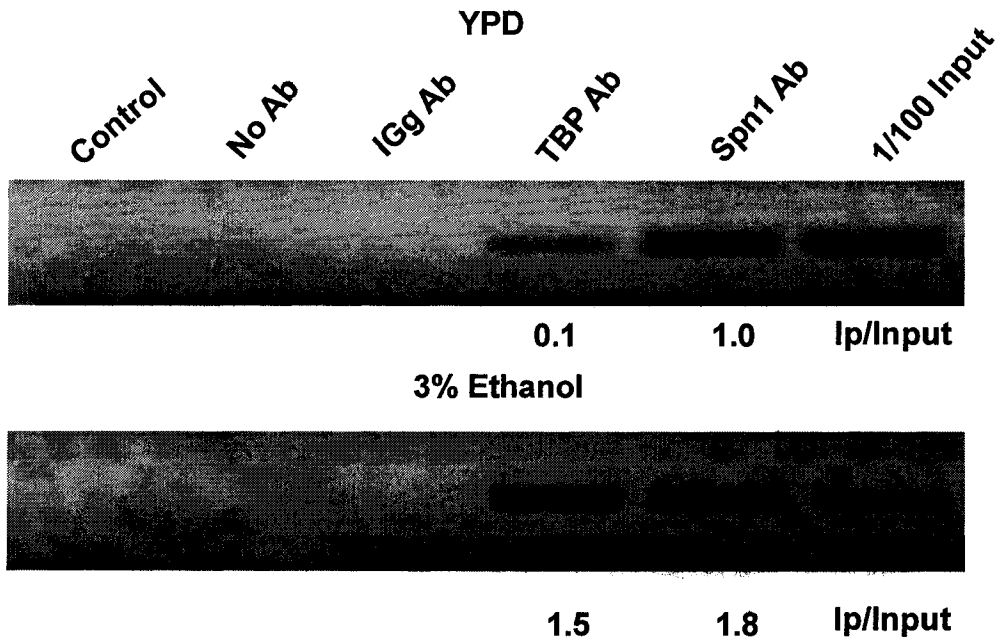

Figure A.1: Spn1 occupies *CYC1* under both partialiy repressing and activated conditions. Chromatin immunoprecipitation was performed using no antibody (Ab) as control, an irrelevant IgG Ab, Polyclonal TBP Ab and Spn1 antibodies. Quantitative PCR was performed on the DNA material once the protein-DNA cross-links were reversed and DNA was extracted from the cell that were grown both in presence of YPD and 3% ethanol. 1/100 amount of the input DNA was amplified along with the IP samples. After ethidium bromide staining, images were captured and signals were quantified by using the image quant software. The signal strength ratio between the IP samples and corresponding input samples indicate the occupancy of the protein.

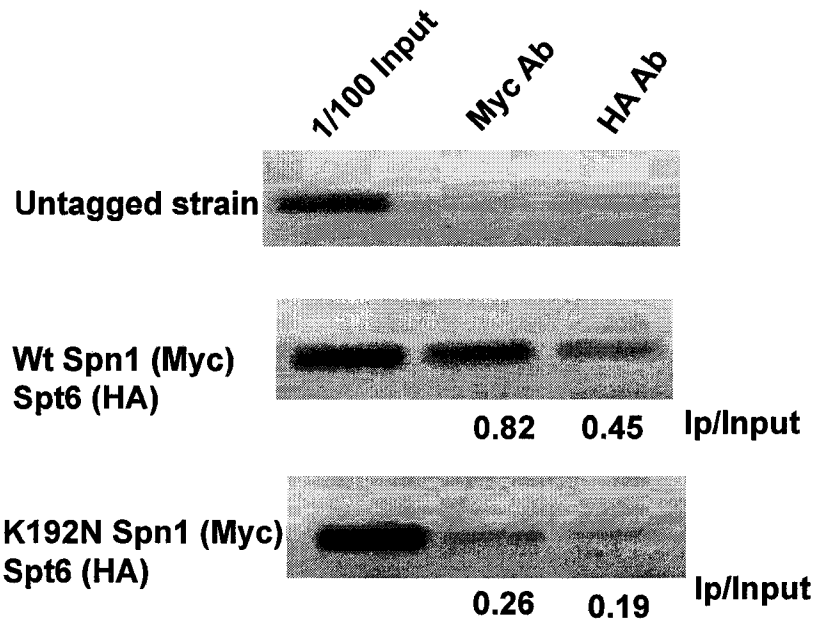

Figure A.2: Diminished occupancy of Spn1 (K192N) and Spt6 at the *CYC1* under partialiy repressing conditions. Chromatin immunoprecipitation was performed using no antibody (Ab) as control, and monoclonal Myc and HA antibodies. Quantitative PCR was performed on the DNA material once the protein-DNA cross-links were reversed and DNA was extracted from the cells that were grown in YPD media. 1/100 amount of the input DNA was amplified along with the IP samples. After ethidium bromide staining, images were captured and signals were quantified by using image quant software. The signal strength ratio between the IP samples and corresponding input indicates the occupancy of that protein. Top panel shows a control strain, which has no tags, thus resulting in no signal. The two strains used have either Wt Spn1 (Myc) or K192N Spn1-(Myc) with Spt6-HA.

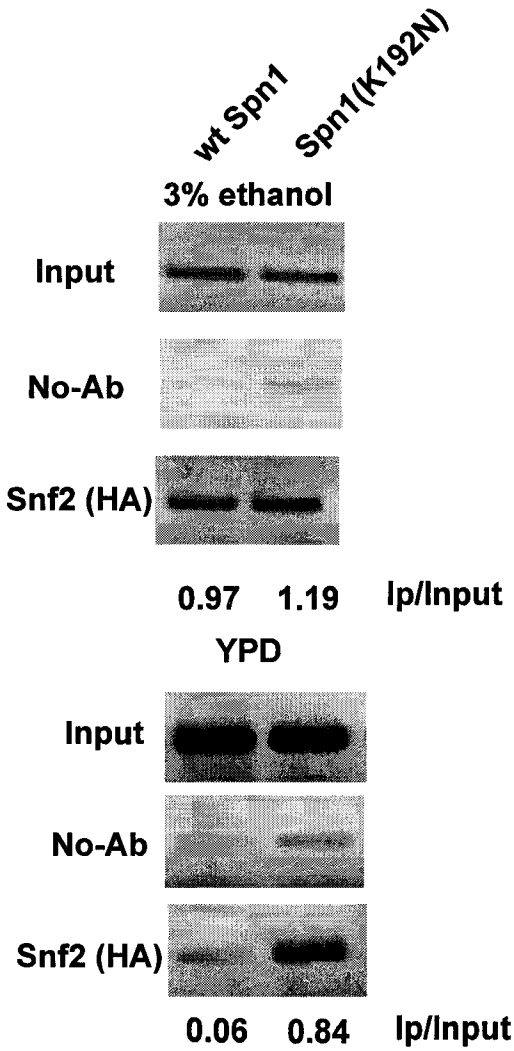

Figure A.3: Variable occupancy of Snf2 at the *CYC1* under partially repressing and activating conditions. Chromatin immunoprecipitation was performed using no antibody (Ab) as control, and monoclonal Myc and HA antibodies. Quantitative PCR was performed on the DNA material once the protein-DNA cross-links were reversed and DNA was extracted from the cells that were grown in 3% ethanol and YPD media. 1/100 amount of the input DNA was amplified along with the IP samples. After ethidium bromide staining, images were captured and signals were quantified by using image quant software. The signal strength ratio between the IP samples and corresponding input indicates the occupancy of that protein. The two strains used have either Wt Spn1 (Myc) or K192N Spn1 (Myc) with Snf2-HA.

References

1. **Adamkewicz, J. I., K. E. Hansen, W. A. Prud'homme, J. L. Davis, and J. Thorner .** 2001. High affinity interaction of yeast transcriptional regulator, Mot1, with TATA box-binding protein (TBP). J. Biol. Chem. **276:**11883-94.

2. **Andell III, F., A. G. Ladurner, C. Inouye, R. Tjian, and E. Nogales.** 1999. Three-dimensional structures of the human TFIID-IIA-IIB complex. Science **286:**2153-2156.

3. **Apone, L. M., and M. R. Green.** 1998. Transcription sans TBP. Nature **393:**114-115.

4. **Apone, L. M., C. A. Virbasius, J. C. Reese, and M. R. Green.** 1996. Yeast $TAF_{II}90$ is required for cell-cycle progression through G_2/M but not for general transcription activation. Genes Dev. **10:**2368-2380.

5. **Apone, L. M., C.-A. Virbasius, F. C. Holstege, J. Wang, R. A. Young, and M. R. Green.** 1998. Broad, but not universal, transcriptional requirement for $yTAF_{II}17$, a histone H3-like TAF_{II} present in TFIID and SAGA. Mol. Cell **2:**653-661.

6. **Auble, D. T., and S. Hahn.** 1993. An ATP-dependent inhibitor of TBP binding to DNA. Genes Dev. **7:**844-856.

7. **Auble, D. T., K. E. Hansen, C. G. F. Mueller, W. S. Lane, J. Thorner, and S. Hahn.** 1994. Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev. **8:**1920-1934.

8. **Auble, D. T., D. Wang, K. W. Post, and S. Hahn.** 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:**4842-51.

9. **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:**3081-3093.

10. **Banik, U., J. M. Beechem, E. Klebanow, S. Schroeder, and P. Weil, 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.

11. **Barberis, A., and L. Gaudreau.** 1998. Recruitment of the RNA polymerase II holoenzyme and its implications in gene regulation. Biol. Chem. **379:**1397-1405.

12. **Bengal, E., O. Flores, A. Krauskopf, D. Reinberg, and Y. Aloni .** 1991. Role of the mammalian transcription factors IIF, IIS, and IIX during elongation by RNA polymerase II. Mol. Cell. Biol. **11:**1195-1206.

13. **Birck, C., O. Poch, C. Romier, M. Ruff, G. Mengus, A. Lavigne, I. Davidson, and D. Moras.** 1998. Human TAF(II)28 and TAF(II)18 interact through a histone fold encoded by atypical evolutionary conserved motifs also found in the SPT3 family. Cell **94:**239-249.

14. **Bjorklund, S., and Y. J. Kim.** 1996. Mediator of transcriptional regulation. Trends Biochem. Sci. **21:**335-337.

15. **Brand, M., C. Leurent, V. Mallouh, L. Tora, and P. Schultz.** 1999. Three-dimensional structures of the TAF_{II}-containing complexes TFIID and TFTC. Science **286:**2151-2153.

16. **Brown, S. A., A. N. Imbalzano, and R. E. Kingston.** 1996. Activator-dependent regulation of transcriptional pausing on nucleosomal templates. Genes Dev. **10:**1479-1490.

17. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56:**549-561.

18. **Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente.** 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature **334:**37-42.

19. **Buratowski, S., and P. A. Sharp.** 1992. Initiation of transcription by RNA polymerase II, p. 227-246. *In* S. L. McKnight, and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Press, Cold Spring Harbor, NY.

20. **Burke, T., and J. Kadonaga.** 1997. The downstream core promoter element, DPE, is conserved from Drosophila to humans and is recognized by $TAF_{II}60$ of Drosophila. Genes Dev. **11:**3020-3031.

21. **Burley, S. K., and R. G. Roeder.** 1996. Biochemistry and structural biology of transcription factor IID (TFIID). Annu. Rev. Biochem. **65:**769-799.

22. **Cairns, B. R., Y. J. Kim, M. H. Sayre, B. C. Laurent, and R. D. Kornberg.** 1994. A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc. Natl. Acad. Sci. U.S.A. **91:**1950-1954.

23. **Carrozza, M. J., and N. A. DeLuca.** 1996. Interaction of the viral activator protein ICP4 with TFIID through TAF250. Mol. Cell. Biol. **16:**3085-3093.

24. **Cavallini, B., I. Faus, H. Matthes, J.-M. Chipoulet, B. Winsor, J. M. Egly, and P. Chambon.** 1989. Cloning of the gene encoding the yeast protein BTF1, which can substitute for the human TATA box-binding factor. Proc. Natl. Acad. Sci. U.S.A. **89:**9803-9807.

25. **Chalkley, G. E., and C. P. Verrijzer.** 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.

26. **Chang, M., and J. Jaehning.** 1997. A multiplicity of mediators: alternative forms of transcription complexes communicate with transcriptional regulators. Nucleic Acids Research **25:**4861-4865.

27. **Chao, D. M., E. L. Gadbois, P. J. Murray, S. F. Anderson, M. S. Sonu, J. D. Parvin, and R. A. Young.** 1996. A mammalian SRB protein associated with an RNA polymerase II holoenzyme. Nature **380:**82-84.

28. **Chatterjee, S., and K. Struhl.** 1995. Connecting a promoter-bound protein to the TATA-binding protein overrides the need for a transcriptional activation region. Nature **374:**820-822.

29. **Chen, J., M. Ding, and D. S. Pederson.** 1994. Binding of TFIID to the CYC1 TATA boxes in yeast occurs independently of upstream activating sequences. Proc Natl Acad Sci U S A. **91:**11909-13.

30. **Chi, T., and M. Carey.** 1996. Assembly of the isomerized TFIIA-TFIID-TATA ternary complex is necessary and sufficient for gene activation. Genes Dev. **10:**2540-2550.

31. **Chi, T., P. Lieberman, K. Ellwood, and M. Carey.** 1995. A general mechanism for transcriptional synergy by eukaryotic activators. Nature **377:**254-257.

32. **Chiang, C. M., and R. G. Roeder.** 1995. Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators. Science **267:**531-536.

33. **Cho, E. J., T. Takagi, C. R. Moore, and S. Buratowski.** 1997. mRNA capping enzyme is recruited to the transcription complex by phosphorylation of the RNA polymerase II carboxy-terminal domain. Genes Dev. **11:**3319-3326.

34. **Cho, H., E. Maldonado, and D. Reinberg.** 1997. Affinity purification of a human RNA polymerase II complex using monoclonal antibodies against transcription factor IIF. J Biol Chem. **272:**11495-11502.

35. **Colgan, J., and J. L. Manley.** 1992. TFIID can be rate limiting in vivo for TATA-containing, but not TATA-lacking, RNA polymerase II promoters. Genes Dev. **6:**304-315.

36. **Collart, M. A.** 1996. The NOT, SPT3, and MOT1 genes functionally interact to regulate transcription at core promoters. Mol. Cell. Biol. **16:**6668-6676.

37. **Collart, M. A., and K. Struhl.** 1993. CDC39, an essential nuclear protein that negatively regulates transcription and differentially affects the constitutive and inducible *his3* promoters. EMBO J. **12:**177-186.

38. **Collart, M. A., and K. Struhl.** 1994. *NOT1(CDC39)*, *NOT2(CDC36)*, *NOT3*, and *NOT4* encode a global negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. **8:**525-537.

39. **Comai, L., N. Tanese, and R. Tjian.** 1992. The TATA-binding protein and associated factors are integral components of the RNA polymerase I transcription factor, SL1. Cell **68:**965-976.

40. **Conaway, J. W., and R. C. Conaway.** 1989. A multisubunit transcription factor essential for accurate initiation by RNA polymerase II. J. Biol. Chem. **264:**2357-2362.

41. **Conaway, R. C., J. N. Bradsher, and J. W. Conaway.** 1992. Mechanism of assembly of the RNA polymerase II preinitiation complex. Evidence for a functional interaction between the carboxyl-terminal domain of the largest subunit of RNA polymerase II and a high molecular mass form of the TATA factor. J. Biol. Chem. **267:**8464-8467.

42. **Conaway, R. C., and J. W. Conaway.** 1993. General initiation factors for RNA polymerase II. Ann. Rev. Biochem. **62:**161-190.

43. **Conaway, R. C., and J. W. Conaway.** 1989. An RNA polymerase II transcription factor has an associated DNA-dependent ATPase (dATPase) activity strongly stimulated by the TATA region of promoters. Proc. Natl. Acad. Sci. U S A **86:**7356-7360.

44. **Cormack, B. P., and K. Struhl.** 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69:**685-696.

45. **Cortes, P., O. Flores, and D. Reinberg.** 1992. Factors involved in specific transcription by mammalian RNA polymerase II: purification and analysis of transcription factor IIA and identification of transcription factor IIJ. Mol. Cell. Biol. **12:**413-421.

46. **Crowley, T. E., T. Hoey, J. K. Liu, Y. N. Jan, L. Y. Jan, and R. Tjian.** 1993. A new factor related to TATA-binding protein has highly restricted expression patterns in Drosophila. Nature **361:**557-61.

47. **Dahmus, M. E.** 1994. The role of multisite phosphorylation in the regulation of RNA polymerase II activity. Prog. Nucleic Acid Res. Mol. Biol. **48:**143-179.

48. **Damania, B., P. Lieberman, and J. C. Alwine.** 1998. Simian virus 40 large T antigen stabilizes the TATA-binding protein-TFIIA complex on the TATA element. Mol. Cell. Biol. **18:**3926-3935.

49. **Darst, R. P., A. Dasgupta, C. Zhu, J. Y. Hsu, ., A. Vroom, T. Muldrow , and D. T. Auble.** 2003. Mot1 regulates the DNA binding activity of free TATA-binding protein in an ATP-dependent manner. J. Biol. Chem. **278:**13216-13226.

50. **Dasgupta, A., R. P. Darst, K. J. Martin, C. A. Afshari, and D. T. Auble.** 2002. Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. Proc. Natl. Acad. Sci. U S A. **99:**2666-71.

51. **Davis, J. L., R. Kunisawa, and J. Thorner.** 1992. A presumptive helicase (*MOT1* gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**1879-1892.

52. **DeJong, J., R. Bernstein, and R. Roeder.** 1995. Human general transcription factor TFIIA: characterization of a cDNA encoding the small subunit and requirement for basal and activated transcription. Proc. Natl. Acad. Sci. U S A **92:**3313-3317.

53. **Dikstein, R., S. Ruppert, and R. Tjian.** 1996. $TAF_{II}250$ is a bipartite protein kinase that phosphorylates the basal transcription factor RAP74. Cell **84:**781-790.

54. **Dunphy, E. L., T. Johnson, ., S. S. Auerbach, and E. H. Wang.** 2000. Requirement for TAF(II)250 acetyltransferase activity in cell cycle progression. Mol. Cell. Biol. **20:**1134-1139.

55. **Durfee, T., K. Becherer, P.-L. Chen, S.-H. Yeh, Y. Yang, A. E. Kilburn, W.-H. Lee, and S. J. Elledge.** 1993. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev. **7:**555-569.

56. **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:**7331-7344.

57. **Dvir, A., R. C. Conaway, and J. W. Conaway.** 1997. A role for TFIIH in controlling the activity of early RNA polymerase II elongation complexes. Proc. Natl. Acad. Sci. U S A **94:**9006-90010.

58. **Dvir, A., K. P. Garrett, C. Chalut, J. Egly, J. W. Conaway, and R. C. Conaway.** 1996. A Role for ATP and TFIIH in Activation of the RNA Polymerase II Preinitiation Complex Prior to Transcription Initiation. J. Biol. Chem. **271:**7245-7248.

59. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66:**563-576.

60. **Eichbaum, Q. G., R. Iyer, D. P. Raveh, C. Mathieu, and R. A. Ezekowitz.** 1994. Restriction of interferon gamma responsiveness and basal expression of the myeloid human Fc gamma R1b gene is mediated by a functional PU.1 site and a transcription initiator consensus. J. Exp. Med. **179:**1985-1996.

61. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58:**1183-1191.

62. **Fang, S. M., and Z. F. Burton.** 1996. RNA polymerase II-associated protein (RAP) 74 binds transcription factor (TF) IIB and blocks TFIIB-RAP30 binding. J. Biol.Chem. **271:**11703-11709.

63. **Feaver, W. J., N. L. Henry, D. A. Bushnell, M. H. Sayre, J. H. Brickner, O. Gileadi, and R. D. Kornberg.** 1994. Yeast TFIIE. Cloning, expression, and homology to vertebrate proteins. J Biol Chem **269:**27549-27553.

64. **Feaver, W. J., N. L. Henry, Z. Wang, X. Wu, J. Q. Svejstrup, D. A. Bushnell, E. C. Friedberg, and R. D. Kornberg.** 1997. Genes for Tfb2, Tfb3, and Tfb4 subunits of yeast transcription/repair factor IIH. Homology to human cyclin-dependent kinase activating kinase and IIH subunits. J. Biol. Chem. **272:**19319-19327.

65. **Feilotter, H., G. Hannon, C. Ruddell, and D. Beach.** 1994. Construction of an improved host strain for two hybrid screening. Nucleic Acids Res **22:**1502.

66. **Fischbeck, J. A., S. M. Kraemer, and L. A. Stargell.** 2002. SPN1, a conserved yeast gene identified by suppression of a post-recruitment defective yeast TATA-binding protein mutant. Genetics **162:**1605-16.

67. **Flick, J. S., and M. Johnston.** 1990. Two systems of glucose repression of the *GAL1* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**4757-4769.

68. **Flores, A., J. Briand, O. Gadal, J. Andrau, L. Rubbi, V. Van Mullem, C. Boschiero, M. Goussot, C. Marck, C. Carles, P. Thuriaux, A. Sentenac, and M. Werner.** 1999. A protein-protein interaction map of yeast RNA polymerase III. Proc. Natl. Acad. Sci. USA **96:**7815-7820.

69. **Flores, O., I. Ha, and D. Reinberg.** 1990. Factors involved in specific transcription by mammalian RNA polymerase II. Purification and subunit composition of transcription factor IIF. J. Biol. Chem. **265:**5629-5634.

70. **Flores, O., H. Lu, and D. Reinberg.** 1992. Factors involved in specific transcription by mammalian RNA polymerase II. Identification and characterization of factor IIH. J. Biol. Chem. **267:**2786-2793.

71. **Flores, O., E. Maldonado, and D. Reinberg.** 1989. Factors involved in specific transcription by mammalian RNA polymerase II. Factors IIE and IIF independently interact with RNA polymerase II. J. Biol.Chem. **264:**8913-8921.

72. **Fujigasaki, H., J. J. Martin, P. P. De Deyn, A. Camuzat, D. Deffond, G. Stevanin , B. Dermaut, C. Van Broeckhoven, A. Durr, and A. Brice.** 2001. CAG repeat expansion in the TATA box-binding protein gene causes autosomal dominant cerebellar ataxia. Brain **124:**1939-1947.

73. **Furukawa, T., and N. Tanese.** 2000. Assembly of partial TFIID complexes in mammalian cells reveals distinct activitites assoicated with individual TATA box-binding protein-associated factors. J. Biol. Chem. **275:**29847-29856.

74. **Gangloff, Y. G., S. L. Sanders, C. Romier, D. Kirschner, P. A. Weil, L. Tora, and I. Davidson.** 2001. Histone folds mediate selective heterodimerization of yeast $TAF_{II}25$ with TFIID components $yTAF_{II}47$ and $yTAF_{II}65$ and with SAGA component ySPT7. Mol. Cell. Biol. **21:**1841-1853.

75. **Gangloff, Y. G., S. Werten, C. Romier, L. Carre, O. Poch, D. Moras, and I. Davidson.** 2000. The human TFIID components TAF(II)135 and TAF(II)20 and the yeast SAGA components ADA1 and TAF(II)68 heterodimerize to form histone-like pairs. Mol. Cell. Biol. **20:**340-351.

76. **Garraway, I. P., K. Semple, and S. T. Smale.** 1996. Transcription of the lymphocyte-specific terminal deoxynucleotidyltransferase gene requires a specific core promoter structure. Proc. Natl. Acad. Sci. U S A. **93:**4336-4341.

77. **Gaudreau, L., M. Keaveney, J. Nevado, Z. Zaman, G. Bryant, K. Struhl, and M. Ptashne.** 1999. Transcriptional activation by artificial recruitment in yeast is influenced by promoter architecture and downstream sequences. Proc. Natl. Acad. Sci. USA **96:**2668-2673.

78. **Ge, H., and R. G. Roeder.** 1994. Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. Cell **78:**513-523.

79. **Gegonne, A., J. D. Weissman, and D. S. Singer.** 2001. TAFII55 binding to TAFII250 inhibits its acetyltransferase activity. Proc. Natl. Acad. Sci. U S A **98:**12432-12437.

80. **Geiger, J. H., S. Hahn, S. Lee, and P. B. Sigler.** 1996. Crystal structure of the yeast TFIIA/TBP/DNA complex. Science **272:**830-836.

81. **Geisberg, J. V., J. L. Chen, and R. P. Ricciardi.** 1995. Subregions of the adenovirus E1A transactivation domain target multiple components of the TFIID complex. Mol. Cell. Biol. **15:**6283-6290.

82. **Geisberg, J. V., Z. Moqtaderl, L. Kuras, and K. Struhl.** 2002. Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in Saccharomyces cerevisiae. Mol. Cell. Biol. **22:**8122-8134.

83. **Giieadi, O., W. Feaver, and R. Kornberg.** 1992. Cloning of a subunit of yeast RNA polymerase II transcription factor b and CTD kinase. Science **257:**1389-1392.

84. **Gill, G.** 2001. Regulation of the initiation of eukaryotic transcription. Essays Biochem. **37:**33-43.

85. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tjian.** 1993. Drosophila $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519-530.

86. **Goodrich, J. A., and R. Tijan.** 1994. TBP-TAF complexes: selectivity factors for eukaryotic transcription. Current Opinion in Cell Biology **6:**403-409.

87. **Goppelt, A., G. Stelzer, F. Lottseich, and M. Meisterernst.** 1996. A mechanism for the repression of class II gene transcription through specific binding of NC2 to TBP-promoter complexes via heteodimeric histone fold domains. EMBO J. **15:**3105-3116.

88. **Grant, P. A., D. Schieltz, M. G. Pray-Grant, D. Steger, J. C. Reese, J. R. Yates III, 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:**45-53.

89. **Green, M. R.** 2000. TBP-associated factors (TAFIIs): multiple, selective transcriptional mediators in common complexes. Trends Biochem. Sci. **25:**59-63.

90. **Greenblatt, J.** 1991. RNA polymerase-associated transcription factors. Trends Biochem. Sci. **16:**408-411.

91. **Guermah, M., Y. Tao, and R. G. Roeder.** 2001. Positive and negative TAF(II) functions that suggest a dynamic TFIID structure and elicit synergy with traps in activator-induced transcription. Mol. Cell.Biol. **21:**6882-6894.

92. **Gumbs, O. H., A. M. Campbell, and P. A. Weil.** 2003. High-affinity DNA binding by a Mot1p-TBP complex: implications for TAF-independent transcription. EMBO J. **22:**3131-3141.

93. **Ha, I., S. Roberts, E. Maldonado, X. Sun, L. U. Kim, M. Green, and D. Reinberg.** 1993. Multiple functional domains of human transcription factor IIB: distinct interactions with two general transcription factors and RNA polymerase II. Genes Dev **7:**1021-1032.

94. **Hahn, S.** 1998. Activation and the role of reinitiation in the control of transcription by RNA polymerase II. Cold Spring Harb. Symp. Quant .Biol. **63:**181-188.

95. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 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.

96. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Yeast TATA-binding protein TFIID binds to TATA elements with both consensus

and nonconsensus DNA sequences. Proc. Natl. Acad. Sci. U.S.A. **86**:5718-5722.

97. **Hampsey, M.** 1998. Molecular genetics of the RNA polymerase II genreal transcriptional machinery. Microbiology and Molecular Biology Reviews **62**:465-503.

98. **Hampsey, M., and D. Reinberg.** 1999. RNA polymerase II as a control panel for multiple coactivator complexes. Curr. Opin. Genet. Dev. **9**:132-139.

99. **Hansen, S., and R. Tjian.** 1995. TAFs and TFIIA mediate differential utilization of the tandem Adh promoters. Cell **82**:565-75.

100. **Happel, A. M., M. S. Swanson, and F. Winston.** 1991. The *SNF2*, *SNF5*, and *SNF6* genes are required for Ty transcription in *Saccharomyces cerevisiae*. Genetics **128**:69-77.

101. **Hekmatpanah, D. S., and R. A. Young.** 1991. Mutations in a conserved region of RNA polymerase II influence the accuracy of mRNA start site selection. Mol. Cell. Biol **11**:5781-5791.

102. **Henry, N. L., A. M. Campbell, W. J. Feaver, D. Poon, P. A. Weil, and R. D. Kornberg.** 1994. TFIIF-TAF-RNA polymerase II connection. Genes Dev. **8**:2868-2878.

103. **Henry, N. L., M. H. Sayre, and R. D. Kornberg.** 1992. Purification and characterization of yeast RNA polymerase II general initiation factor g. J. Biol. Chem. **267**:23388-92.

104. **Hisatake, K., T. Ohta, R. Takada, M. Guermah, M. Horikoshi, Y. Nakatani, and R. G. Roeder.** 1995. Evolutionary conservation of human TATA-binding-polypeptide-associated factors $TAF_{II}31$ and $TAF_{II}80$ and interactions of $TAF_{II}80$ with other TAFs and with general transcription factors. Proc. Natl. Acad. Sci. USA **92**:8195-8199.

105. **Hobbs, N., A. Bondareva, S. Barnett, M. Capecchi, and E. Schmidt.** 2002. Removing the Vertebrate-Specific TBP N-terminus Disrupts Placental Beta2M-Dependent Interactions with the Maternal Immune System. Cell **110**:43-54.

106. **Hochheimer, A., S. Zhou, S. Zheng, M. C. Holmes, and R. Tjian.** 2002. TRF2 associates with DREF and directs promoter-selective gene expression in Drosophila. Nature **420**:439-445.

107. **Hoey, T., B. D. Dynlacht, M. G. Peterson, B. F. Pugh, and R. Tjian.** 1990. Isolation and characterization of the Drosophila gene encoding the TATA box binding protein, TFIID. Cell **61:**1179-1186.

108. **Hoey, T., R. O. J. Weinzierl, G. Gill, J.-L. Chen, B. D. Dynlacht, and R. Tjian.** 1993. Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. Cell **72:**247-260.

109. **Hoffmann, A., C. Chiang, T. Oelgeschlager, X. Xie, S. K. Burley, Y. Nakatani, and R. G. Roeder.** 1996. A histone octamer-like structure within TFIID. Nature **380:**356-359.

110. **Hoffmann, A., M. Horikoshi, C. K. Wang, S. Schroeder, P. A. Weil, and R. G. Roeder.** 1990. Cloning of *Schizosaccharomyces pombe* TFIID gene reveals a strong conservation of functional domains present in *Saccharomyces cerevisiae* TFIID. Genes Dev. **4:**1141-1148.

111. **Hoffmann, A., T. Oelgeschlager, and R. Roeder, G.** 1997. Considerations of transcriptional control mechanisms: do TFIID-core promoter complexes recapitulate nucleosome-like functions? Proc. Natl. Acad. Sci. USA **94:**8928-8935.

112. **Hoffmann, A., E. Sinn, Y. T. J. Wang, A. Roy, M. Horikoshi, and R. G. Roeder.** 1990. Highly conserved core domain and unique N-terminus with presumptive regulatory motifs in a human TATA factor (TFIID). Nature **346:**387-390.

113. **Holmes, M. C., and R. Tjian.** 2000. Promoter-selective properties of the TBP-related factor TRF1. Science. **288:**867-870.

114. **Holstege, F. C., v. d. V. .P.C., and H. T. Timmers.** 1996. Opening of an RNA polymerase II promoter occurs in two distinct steps and requires the basal transcription factors IIE and IIH. EMBO J. **15:**1666-1677.

115. **Holstege, F. C. P., E. G. Jennings, C. 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.

116. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Weil, and R. G. Roeder.** 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature **341:**299-303.

117. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Weil, and R. G. Roeder.** 1989. Purification of a yeast TATA box-binding protein that

exhibits human transcription factor IID activity. Proc. Natl. Acad. Sci. U S A **86:**4843-7.

118. **Horikoshi, M., T. Yamamoto, Y. Ohkuma, P. A. Weil, and R. G. Roeder.** 1990. Analysis of structure-function relationships of yeast TATA box binding factor TFIID. Cell **61:**1171-1178.

119. **Huet, J., and A. Sentenac.** 1992. The TATA-binding protein participates in TFIIIB assembly on tRNA genes. Nucleic Acids Res. **20:**6451-6454.

120. **Imbalzano, A. N., K. S. Zaret, and R. E. Kingston.** 1994. Transcription factor (TF) IIB and TFIIA can independently increase the affinity of the TATA-binding protein for DNA. J. Biol. Chem. **269:**8280-8286.

121. **Inostroza, J. A., F. H. Mermelstein, I. Ha, W. S. Lane, and D. Reinberg.** 1992. Dr1, a TATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. Cell **70:**477-489.

122. **Iwabuchi, K., B. Li, P. Bartel, and S. Fields.** 1993. Use of the two-hybrid system to identify the domain of p53 involved in oligomerization. Oncogene **8:**1693-1696.

123. **Iyer, V., and K. Struhl.** 1996. Absolute mRNA levels and transcriptional initiation rates in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. U S A **93:**5208-5212.

124. **Izban, M. G., and D. S. Luse.** 1992. Factor-stimulated RNA polymerase II transcribes at physiological elongation rates on naked DNA but very poorly on chromatin templates. J. Biol. Chem. **267:**13647-55.

125. **Jiang, Y., S. J. Triezenberg, and J. D. Gralla.** 1994. Defective transcriptional activation by diverse VP16 mutants associated with a common inability to form open promoter complexes. J. Biol. Chem. **269:**5505-5508.

126. **Kang, J. J., D. T. Auble, J. A. Ranish, and S. Hahn.** 1995. Analysis of yeast transcription factor TFIIA: Distinct functional regions and a Polymerase II-specific role in basal and activated transcription. Mol. Cell. Biol. **15:**1234-1243.

127. **Kao, C. C., P. M. Lieberman, M. C. Schmidt, Q. Zhou, R. Pei, and A. J. Berk.** 1990. Cloning of a transcriptionally active human TATA binding factor. Science **248:**1646-1650.

128. **Kassavetis, G. A., C. A. P. Joazeiro, M. Pisano, E. P. Geiduschek, T. Colbert, S. Hahn, and J. A. Blanco.** 1992. The role of the TATA-binding

protein in the assembly and function of the multisubunit yeast RNA polymerase transcription factor, TFIIIB. Cell **71:**1055-1064.

129. **Kaufmann, J., and S. T. Smale.** 1994. Direct recognition of initiator elements by a component of the transcription factor IID complex. Genes Dev. **8:**821-829.

130. **Killeen, M., B. Coulombe, and J. Greenblatt.** 1992. Recombinant TBP, transcription factor IIB, and RAP30 are sufficient for promoter recognition by mammalian RNA polymerase II. J. Biol. Chem. **267:**9463-6.

131. **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:**3178-3193.

132. **Kitajima, S., Y. Tanaka, T. Kawaguchi, T. Nagaoka, S. M. Weissman, and Y. Yasukochi .** 1990. A heteromeric transcription factor required for mammalian RNA polymerase II. Nucleic Acids Res. **18:**4843-4849.

133. **Klages, N., and M. Strubin.** 1995. Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature **374:**822-823.

134. **Klemm, R. D., J. A. Goodrich, S. Zhou, and R. Tjian.** 1995. Molecular cloning and expression of the 32-kDa subunit of human TFIID reveals interactions with VP16 and TFIIB that mediate transcriptional activation. Proc. Natl. Acad. Sci. U S A **92:**5788-92.

135. **Knezetic, J. A., and D. S. Luse.** 1986. The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II in vitro. Cell **45:**95-104.

136. **Kobayashi, A., T. Miyake, Y. Ohyama, M. Kawaichi, and T. Kokubo.** 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.

137. **Kobayashi, N., T. G. Boyer, and A. J. Berk.** 1995. A class of activation domains interacts directly with TFIIA and stimulates TFIIA-TFIID-promoter complex assembly. Mol. Cell. Biol. **15:**6465-6473.

138. **Koh, S. S., A. Z. Ansari, M. Ptashne, and R. A. Young.** 1998. An activator target in the RNA polymerase II holoenzyme. Mol. Cell **1:**895-904.

139. **Kokubo, T., D. W. Gong, R. G. Roeder, M. Horikoshi, and Y. Nakatani.** 1993. 1993. Proc. Natl. Acad. Sci. U S A. **90:**5896-5900.

140. **Kokubo, T., D. W. Gong, S. Yamashita, M. Horikoshi, R. G. Roeder, and Y. Nakatani.** 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 Dev. **7:**1033-1046.

141. **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:**1003-1012.

142. **Koleske, A. J., and R. A. Young.** 1995. The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. **20:**113-116.

143. **Komarnitsky, P. B., B. Michel, and S. Buratowski.** 1999. TFIID-specific yeast TAF40 is essential for the majority of RNA polymerase II-mediated transcription in vivo. Genes Dev. **13:**2484-2489.

144. **Kotani, T., T. Miyake, Y. Tsukihashi, A. Hinnebusch, Y. Nakatani, M. Kawaichi, and T. Kokubo.** 1998. Identification of highly conserved amino-terminal segments of $dTAF_{II}230$ and $yTAF_{II}145$ 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.

145. **Kraemer, S. M., R. T. Ranallo, R. C. Ogg, and L. A. Stargell.** 2001. TFIIA interacts with TFIID via association with TATA-binding protein and TAF40. Mol. Cell. Biol. **21:**1737-46.

146. **Kumar, K. P., S. Akoulitchev, and D. Reinberg.** 1998. Promoter-proximal stalling results from the inability to recruit transcription factor IIH to the transcription complex and is a regulated event. Proc. Natl. Acad. Sci. USA **95:**9767-9772.

147. **Kuras, L., P. Kosa, M. Mencia, and K. Struhl.** 2000. TAF-Containing and TAF-independent forms of transcriptionally active TBP in vivo. Science **288:**1244-1248.

148. **Kuras, L., and K. Struhl.** 1999. Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. Nature **399:**609-613.

149. **Lagrange, T., T.-K. Kim, G. Orphanides, Y. W. Ebright, R. H. Ebright, and D. Reinberg.** 1996. High-resolution mapping of nucleoprotein complexes by site-specific protein-DNA photocrosslinking: organization of the TBP-TFIIA-TFIIB-DNA quaternary complex. Proc. Natl. Acad. Sci. U.S.A. **93:**10620-10625.

150. **Lavigne, A., G. Mengus, M. May, V. Dubrovskaya, L. Tora, P. Chambon, and I. Davidson.** 1996. Multiple interactions between $hTAF_{II}55$ and other TFIID subunits. Requirements for the formation of stable ternary complexes between $hTAF_{II}55$ and the TATA-binding protein. J. Biol. Chem. **271:**19774-19780.

151. **Lavigne, A. C., Y.-G. Gangloff, L. Carre, G. Mengus, C. Birck, O. Poch, C. Romier, D. Moras, and I. Davidson.** 1999. Synergistic transcriptional activation by TATA-binding protein and $hTAF_{II}28$ requires specific amino acids of $hTAF_{II}28$ histone fold. Mol. Cell. Biol. **19:**5050-5060.

152. **Lee, D. K., J. Dejong, S. Hashimoto, M. Horikoshi, and R. G. Roeder.** 1992. TFIIA induces conformational changes in TFIID via interactions with the basic repeat. Mol. Cell. Biol. **12:**5189-5196.

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

154. **Lee, T. I., and R. A. Young.** 1998. Regulation of gene expression by TBP-associated proteins. Genes Dev. **12:**1398-1408.

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

156. **Leuther, K. K., D. A. Bushneli, and R. D. Kornberg.** 1996. Two-dimensional crystallography of TFIIB- and IIE-RNA polymerase II complexes: implications for start site selection and initiation complex formation. Cell **85:**773-779.

157. **Li, B., and J. C. Reese.** 2000. Derepression of DNA damage-regulated genes requires yeast TAF(II)s. EMBO J. **19:**4091-4100.

158. **Li, W.-Z., and F. Sherman.** 1991. Two types of TATA elements for the *CYC1* gene of the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**666-676.

159. **Li, X. Y., S. R. Bhaumik, and M. R. Green.** 2000. Distinct classes of yeast promoters revealed by differential TAF recruitment. Science **288:**1242-1244.

160. **Li, X. Y., S. R. Bhaumik, X. Zhu, L. Li, W. C. Shen, B. L. Dixit, and M. R. Green.** 2002. Selective recruitment of TAFs by yeast upstream activating sequences. Implications for eukaryotic promoter structure. Curr. Biol. **12:**1240-1244.

161. **Li, X. Y., A. Virbasius, X. Zhu, and M. R. Green.** 1999. Enhancement of TBP binding by activators and general transcription factors. Nature **399:**605-609.

162. **Li, Y., S. Bjorklund, Y. W. Jiang, Y. J. Kim, W. S. Lane, D. J. Stillman, and R. D. Kornberg.** 1995. Yeast global transcriptional regulators SIN4 and RGR1 are components of mediator complex RNA polymerase II holoenzyme. Proc. Natl. Acad. Sci. U.S.A. **92:**10864-10868.

163. **Li, Y., P. M. Flanagan, H. Tschochner, and R. D. Kornberg.** 1994. RNA polymerase II initiation factor interactions and transcription start site selection. Science **263:**805-807.

164. **Lieberman, P. M., and A. J. Berk.** 1994. A mechanism for TAFs in transcriptional activation: activation domain enhancement of TFIID-TFIIA-promoter DNA complex formation. Genes Dev. **8:**995-1006.

165. **Lieberman, P. M., J. Ozer, and D. Gursel.** 1997. Requirement for transcription factor IIA (TFIIA)-TFIID recruitment by an activator depends on promoter structure and template competition. Mol. Cell. Biol. **17:**6624-32.

166. **Liu, D., R. Ishima, K. I. Tong, S. Bagby, T. Kokubo, D. R. Muhandiram, L. E. Kay, Y. Nakatani, and M. Ikura.** 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.

167. **Liu, Q., S. E. Gabriel, K. L. Roinick, R. D. Ward, and K. M. Arndt.** 1999. Analysis of TFIIA function in vivo: Evidence for a role in TATA-binding Protein recruitment and gene-specific activation. Mol. Cell. Biol. **19:**8673-8685.

168. **Lobo, S. M., J. Lister, M. L. Sullivan, and N. Hernandez.** 1991. The cloned RNA Polymerase II transcription factor IID selects RNA polymerase III to transcribe the U6 gene in vitro. Genes Dev. **5:**1477-1489.

169. **Longtine, M. S., A. r. McKenzie, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Phllippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast **14:**953-961.

170. **Lorch, Y., J. W. LaPointe, and R. D. Kornberg.** 1987. Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell **49:**203-210.

171. **Lu, H., L. Zawel, L. Fisher, J.-M. Egly, and D. Reinberg.** 1992. Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. Nature **358:**641-645.

172. **Madison, J. M., and F. Winston.** 1997. Evidence that Spt3 Functionally Interacts with Mot1, TFIIA, and TATA-Binding Protein To Confer Promotor-Specific Transcriptional Control in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:**287-295.

173. **Maldonado, E., I. Ha, P. Cortes, L. Weiss, and D. Reinberg.** 1990. Factors involved in specific transcription by mammalian RNA polymerase II: role of transcription factors IIA, IID, and IIB during formation of a transcription-competent complex. Mol. Cell. Biol. **10:**6335-6347.

174. **Martens, C., B. Krett, and P. J. Laybourn.** 2001. RNA polymerase II and TBP occupy the repressed CYC1 promoter. Mol. Microb. **40:**1009-1019.

175. **Martinez, E., T. K. Kundu, J. Fu, and R. G. Roeder.** 1998. A human SPT3-TAFII31-GCN5-L acetylase complex distinct from transcription factor IID. J. Biol. Chem. **273:**23781-23785.

176. **Matsui, T., J. Segall, P. A. Weil, and R. G. Roeder.** 1980. Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. J. Biol. Chem. **255:**11992-11996.

177. **Maxon, M. E., and R. Tjian.** 1994. Transcriptional activity of transcription factor IIE is dependent on zinc binding. Proc. Natl. Acad. Sci. U S A. **91:**9529-9533.

178. **McCracken, S., N. Fong, E. Rosonina, K. Yankulov, G. Brothers, D. Siderovski, A. Hessel, S. Foster, S. Shuman, and D. L. Bentley.** 1997. 5'-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. Genes Dev. **11:**3306-3318.

179. **McKune, K., P. A. Moore, M. W. Hull, and N. A. Woychik.** 1995. Six human RNA polymerase subunits functionally substitute for their yeast counterparts. Mol. Cell. Biol. **15:**6895-6900.

180. **Meisterernst, M., and R. G. Roeder.** 1991. Family of proteins that interact with TFIID and regulate promoter activity. Cell **67:**557-567.

181. **Melcher, K., and S. A. Johnston.** 1995. GAL4 interacts with TATA-binding protein and coactivators. Mol. Cell. Biol. **15:**2839-2848.

182. **Mencia, M., Z. Moqtaderi, J. V. Geisberg, L. Kuras, and K. Struhl.** 2002. Activator-Specific Recruitment of TFIID and Regulation of Ribosomal Protein Genes in Yeast. Mol. Cell **9:**823-833.

183. **Mencia, M., and K. Struhl.** 2001. Region of yeast TAF 130 required for TFIID to associate with promoters. Mol. Cell. Biol. **21:**1145-1154.

184. **Merino, A., K. R. Madden, W. S. Lane, J. J. Champoux, and D. Reinberg.** 1993. DNA topoisomerase I is involved in both repression and activation of transcription. Nature **365:**227-232.

185. **Mermelstein, F., K. Yeung, J. Cao, J. A. Inostroza, H. Erdjument-Bromage, K. Eagelson, D. Landsman, P. Levitt, P. Tempst, and D. Reinberg.** 1996. Requirement of a corepressor for Dr1-mediated repression of transcription. Genes Dev **10:**1033-48.

186. **Michel, B., P. Komarnitsky, and S. Buratowski.** 1998. Histone-like TAFs are essential for transcription in vivo. Mol. Cell **2:**663-672.

187. **Mitsiou, D. J., and H. G. Stunnenberg.** 2000. TAC, a TBP-sans-TAFs complex containing the unprocessed TFIIA alphabeta precursor and the TFIIA gamma subunit. Mol. Cell **6:**527-537.

188. **Mizzen, C. A., X. 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:**1261-1270.

189. **Moqtaderi, Z., Y. Bai, D. Poon, P. A. Weil, and K. Struhl.** 1996. TBP-associated factors are not generally required for transcriptional activation in yeast. Nature **383:**188-191.

190. **Moqtaderi, Z., M. Keaveney, and K. Struhl.** 1998. The histone H3-like TAF is broadly required for transcription in yeast. Mol. Cell **2:**675-682.

191. **Moqtaderi, Z., J. D. Yale, K. Struhl, and S. Buratowski.** 1996. Yeast homologues of higher eukaryotic TFIID subunits. Proc. Natl. Acad. Sci. USA **93:**14654-14658.

192. **Muhich, M. L., C. T. Iida, M. Horikoshi, R. G. Roeder, and C. S. Parker.** 1990. cDNA clone encoding *Drosophila* transcription factor TFIID. Proc. Natl. Acad. Sci. U.S.A. **87:**9148-9152.

193. **Muldrow, T. A., A. M. Campbell, P. A. Weil, and D. T. Auble.** 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:**2834-2845.

194. **Nakamura, K., S. Y. Jeong , T. Uchihara , M. Anno, K. Nagashima, T. Nagashima, S. Ikeda, S. Tsuji, and I. Kanazawa.** 2001. SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. Hum. Mol. Genet. **10:**1441-1448.

195. **Natarajan, K., B. Jackson, E. Rhee, and A. Hinnebusch.** 1998. $yTAF_{II}61$ has a general role in RNA polymerase II transcription and is required by Gcn4p to recruit the SAGA coactivator complex. Mol. Cell **2:**683-692.

196. **Nevado, J., L. Gaudreau, M. Adam, and M. Ptashne.** 1999. Transcriptional activation by artificial recruitment in mammalian cells. Proc. Natl. Acad. Sci. USA **96:**2674-2677.

197. **Nikolov, D. B., H. Chen, E. D. Halay, A. A. Usheva, K. Hisatake, D. K. Lee, R. G. Roeder, and S. K. Burley.** 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature **377:**119-128.

198. **Nikolov, D. B., S.-H. Hu, J. Lin, A. Gasch, A. Hoffman, M. Horikoshi, N.-H. Chua, R. G. Roeder, and S. K. Burley.** 1992. Crystal structure of TFIID TATA-box binding protein. Nature **360:**40-46.

199. **Oelgeschlager, T., C. Chiang, and R. Roeder.** 1996. Topology and reorganization of a human TFIID-promoter complex. Nature **382:**735-738.

200. **Ogryzko, V. V., T. Kotani, X. Zhang, R. L. Schiltz, T. Howard, X.-J. Yang, B. H. Howard, J. Qin, and Y. Nakatani.** 1998. Histone-like TAFs within the PCAF histone acetylase complex. Cell **94:**35-44.

201. **Ohbayashi, T., T. Kishimoto, Y. Makino, M. Shimada, T. Nakadai, T. Aoki, T. Kawata, S. Niwa, and T. Tamura.** 1999. Isolation of cDNA, chromosome mapping, and expression of the human TBP-like protein. Biochem. Biophys. Res. Commun. **255:**137-142.

202. **Ohkuma, Y., S. Hashimoto, C. K. Wang, M. Horikoshi, and R. G. Roeder.** 1995. Analysis of the role of TFIIE in basal transcription and TFIIH-mediated carboxy-terminal domain phosphorylation through structure-function studies of TFIIE-alpha. Mol. Cell. Biol. **15:**4856-4866.

203. **Ohkuma, Y., and R. G. Roeder.** 1994. Regulation of TFIIH ATPase and kinase activities by TFIIE during active initiation complex formation. Nature **368:**160-163.

204. **Orphanides, G., T. Lagrange, and D. Reinberg.** 1996. The general transcription factors of RNA polymerase II. Genes Dev. **10:**2657-2683.

205. **Ozer, J., K. Mitsouras, D. Zerby, M. Carey, and P. Lieberman.** 1998. Transcription factor IIA derepresses TATA-binding protein (TBP)-associated factor inhibition of TBP-DNA binding. J. Biol. Chem. **273:**14293-300.

206. **Ozer, J., P. A. Moore, A. H. Bolden, A. Lee, C. A. Rosen, and P. M. Lieberman.** 1994. Molecular cloning of the small (g) subunit of human TFIIA reveals functions critical for activated transcription. Genes Dev. **8:**2324-2335.

207. **Pereira, L. A., J. A. van der Knaap, V. van den Boom, F. A. van den Heuvel, and H. T. Timmers.** 2001. TAF(II)170 interacts with the concave surface of TATA-binding protein to inhibit its DNA binding activity. Mol. Cell. Biol. **21:**7523-34.

208. **Peterson, M. G., N. Tanese, F. Pugh, and R. Tjian.** 1990. Functional domains and upstream activation properties of cloned human TATA binding proteins. Science **248:**1625-1630.

209. **Piatti, S., R. Tazzi, A. Pizzagalli, P. Plevani , and G. Lucchini .** 1992. Control of DNA synthesis genes in budding yeast: involvement of the transcriptional modulator MOT1 in the expression of the DNA polymerase alpha gene. Chromosoma. **102:**S107-113.

210. **Pinto, I., D. E. Ware, and M. Hampsey.** 1992. The yeast *SUA7* gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection in vivo. Cell **68:**977-988.

211. **Poon, D., Y. Bai, A. M. Campbell, S. Bjorklund, Y. J. Kim, S. Zhou, R. D. Kornberg, and P. A. Weil.** 1995. Identification and characterization of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **92:**8224-8228.

212. **Poon, D., A. M. Campbell, Y. Bai, and P. A. Weil.** 1994. Yeast Taf170 is encoded by MOT1 and exists in a TATA box-binding protein (TBP)-TBP-associated factor complex distinct from transcription factor IID. J Biol Chem **269:**23135-40.

213. **Price, D. H., A. E. Sluder, and A. L. Greenleaf.** 1989. Dynamic interaction between a Drosophila transcription factor and RNA polymerase II. Mol. Cell. Biol. **9:**1465-1475.

214. **Ptashne, M., and A. Gann.** 1997. Transcriptional activation by recruitment. Nature **386:**569-577.

215. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell **61:**1187-1197.

216. **Purnell, B. A., P. A. Emanuel, and D. S. Gilmour.** 1994. TFIID sequence recognition of the initiator and sequences farther downstream in *Drosophila* class II genes. Genes Dev. **8:**830-842.

217. **Rabenstein, M. D., S. Zhou, J. T. Lis, and R. Tjian.** 1999. TATA box-binding protein (TBP)-related factor 2 (TRF2), a third member of the TBP family. Proc. Natl. Acad. Sci. U S A. **96:**4791-4796.

218. **Ramakrishnana, V.** 1997. Histone structure and the organization of the nucleosome. Annu Rev Biophys Biomol Struct. **226:**83-112.

219. **Reese, J. C., L. Apone, S. S. Walker, L. A. Griffin, and M. R. Green.** 1994. Yeast TAF_{II}s in a multisubunit complex required for activated transcription. Nature **371:**523-527.

220. **Reinberg, D., M. Horikoshi, and R. G. Roeder.** 1987. Factors involved in specific transcription by mammalian RNA polymerase II: Functional analysis of initiation factors IIA and IID, and identification of a new factor operating at sequences downstream of the initiation site. J. Biol. Chem. **262:**3322-3330.

221. **Reinberg, D., G. Orphanides, R. Ebright, S. Akoulitchev, J. Carcamo, H. Cho, P. Cortes, R. Drapkin, O. Flores, I. Ha, J. A. Inostroza, S. Kim, T. K. Kim, P. Kumar, T. Lagrange, G. LeRoy, H. Lu, D. M. Ma, E. Maldonado, A. Merino, F. Mermelstein, I. Olave, M. Sheldon, R. Shiekhattar, and L. Zawel.** 1998. The RNA polymerase II general transcription factors: past, present, and future, vol. 63.

222. **Roebuck, K. A., and M. Saifuddin.** 1999. Regulation of HIV-1 transcription. Gene Expr. **8:**67-84.

223. **Roeder, R. G.** 1996. The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. **21:**327-335.

224. **Roeder, R. G., L. B. Schwartz, and V. E. Sklar.** 1976. Function, structure, and regulation of eukaryotic nuclear RNA polymerases. Symp. Soc. Dev. Biol. **34:**29-52.

225. **Roy, A. L., H. Du, P. D. Gregor, C. D. Novina, E. Martinez, and R. G. Roeder.** 1997. Cloning of an inr- and E-box-binding protein, TFII-I, that interacts physically and functionally with USF1. EMBO J. **16:**7091-7104.

226. **Roy, R., L. Schaeffer, S. Humbert , W. Vermeulen, G. Weeda, and J. M. Egly.** 1994. The DNA-dependent ATPase activity associated with the class II basic transcription factor BTF2/TFIIH. J. Biol.Chem. **269:**9826-9832.

227. **Ruppert, S., and R. Tjian.** 1995. $TAF_{II}250$ interactsw with RAP74: implications for RNA polymerase II initiation. Genes & Dev. **9:**2747-2755.

228. **Ruppert, S., E. Wang, and R. Tjian.** 1993. Cloning and expression of human TAF250: a TBP associated factor implicated in cell cycle regulation. Nature **362:**175-179.

229. **Ryan, M. P., G. A. Stafford, L. Yu, and R. H. Morse.** 2000. Artificially recruited TATA-binding protein fails to remodel chromatin and does not activate three promoters that require chromatin remodeling. Mol. Cell. Biol. **20:**5847-5857.

230. **Samuels, M., A. Fire, and P. A. Sharp.** 1982. Separation and characterization of factors mediating accurate transcription by RNA polymerase II. J. Biol. Chem. **257:**14419-14427.

231. **Sanders, S. L., K. A. Garbett, and P. A. Weil.** 2002. Molecular Characterization of Saccharomyces cerevisiae TFIID. Mol. Cell. Biol. **22:**6000-6013.

232. **Sanders, S. L., J. Jennings, A. Canutescu, A. J. Link, and P. A. Weil.** 2002. 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.

233. **Sanders, S. L., and P. A. Weil.** 2000. Identification of two novel TAF subunits of the yeast Saccharomyces cerevisiae TFIID complex. J. Biol. Chem. **275:**13895-13900.

234. **Sawadogo, M., and A. Sentenac.** 1990. RNA polymerase B (II) and general transcription factors. Ann. Rev. Biochem. **59:**711-754.

235. **Schultz, M. C., R. H. Reeder, and S. Hahn.** 1992. Variants of the TATA-binding protein can distinguish subsets of RNA polymerase I, II, and III promoters. Cell **69:**697-702.

236. **Schwyter, D. H., J. D. Huang, T. Dubnlcoff, and A. J. Courey.** 1995. The decapentaplegic core promoter region plays an integral role in the spatial control of transcription. Mol. Cell. Biol. **15:**3960-3968.

237. **Selleck, W., R. Howley, Q. Fang, V. Podolny, M. Fried, G., S. Buratowski, and S. Tan.** 2001. A histone fold TAF octamer within the yeast TFIID transcriptional coactivator. Nat. Struct. Biol. **8:**695-700.

238. **Serizawa, H., R. C. Conaway, and J. W. Conaway.** 1992. A carboxyl-terminal-domain kinase associated with RNA polymerase I transcription factor d from rat liver. Proc. Natl. Acad. Sci. U S A **89:**7476-7480.

239. **Shao, Z., S. Ruppert, and P. D. Robbins.** 1995. The retinoblastoma-susceptibility gene product binds directly to the human TATA-binding protein-associated factor TAFII250. Proc. Natl. Acad. Sci. U S A. **92:**3115-3119.

240. **Shaw, S. P., J. Wingfield, M. J. Dorsey, and J. Ma.** 1996. Identifying a species-specific region of yeast TF11B in vivo. Mol. Cell. Biol. **16:**3651-3657.

241. **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:**3395-3402.

242. **Simmen, K. A., J. Bernues, H. D. Parry, H. G. Stunnenberg, A. Berkenstam, B. Cavallini, J.-M. Egly, and I. W. Mattaj.** 1991. TFIID is required for *in vitro* transcription of the human U6 gene by RNA polymerase III. EMBO J. **10:**1853-1862.

243. **Smale, S. T., and D. Baltimore.** 1989. The "initiator" as a transcription control element. Cell **57:**103-113.

244. **Smale, S. T., A. Jain, J. Kaufmann, K. H. Emami, K. Lo, and I. P. Garraway.** 1998. The initiator element: a paradigm for core promoter heterogeneity within metazoan protein-coding genes. Cold Spring Harb Symp Quant Biol. **63:**21-31.

245. **Smale, S. T., M. C. Schmidt, A. J. Berk, and D. Baltimore.** 1990. Transcriptional activation by Sp1 as directed through TATA or initiator: a specific requirement for mammalian TFIID. Proc. Natl. Acad. Sci. U S A. **87:**4509-4513.

246. **Stargell, L. A., R. C. Ogg, J. N. Adkins, M. M. Robinson, and K. J. Lumb.** 2001. Transcriptional activity of the TFIIA four-helix bundle in vivo. Proteins **43:**227-232.

247. **Stargell, L. A., and K. Struhl.** 1996. A new class of activation-defective TATA-binding protein mutants: Evidence for two steps of transcriptional activation *in vivo*. Mol. Cell. Biol. **16:**4456-4464.

248. **Starr, D. B., and D. K. Hawley.** 1991. TFIID binds in the minor groove of the TATA box. Cell **67:**1231-1240.

249. **Strahl-Bolsinger, S., A. Hecht, K. Luo, and M. Grunstein.** 1997. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. Genes Dev. **11:**83-93.

250. **Struhl, K.** 1999. Fundamentally different logic of gene regulation in eukaryotes and prokaryotes. Cell. **98:**1-4.

251. **Sun, X., D. Ma, M. Sheldon, K. Yeung, and D. Reinberg.** 1994. Reconstitution of human TFIIA activity from recombinant polypeptides: a role in TFIID-mediated transcription. Genes Dev. **8:**2336-2348.

252. **Sun, Z., A. Tessmer, and M. Hampsey.** 1996. Functional interaction between TFIIB and the Rpb9 (Ssu73) subunit of RNA polymerase II in *Saccharomyces cerevisiae*. Nucleic Acids Res. **24:**2560-2566.

253. **Sypes, M. A., and D. S. Gilmour.** 1994. Protein/DNA crosslinking of a TFIID complex reveals novel interactions downstream of the transcription start. Nucleic Acids Res. **22:**807-814.

254. **Taggart, A. K. P., T. S. Fisher, and B. F. Pugh.** 1992. The TATA-binding protein and associated factors are components of Pol III transcription factor TFIIIB. Cell **71:**1015-1028.

255. **Takada, R., Y. Nakatani, A. Hoffman, T. Kokubo, S. Hasegawa, R. G. Roeder, and M. Horikoshi.** 1992. Identification of human TFIID components and direct interaction between a 250 kDa polypeptide and the TATA-box binding protein (TFIID tau). Proc. Natl. Acad. Sci. U S A **89:**11809-11813.

256. **Tan, S., Y. Hunziker, D. F. Sargent, and T. J. Richmond.** 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature **381:**127-134.

257. **Tanese, N., B. F. Pugh, and R. Tjian.** 1991. Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. Genes Dev. **5:**2212-2224.

258. **Tansey, W. P., S. Ruppert, R. Tjian, and W. Herr.** 1994. Multiple regions of TBP participate in the response to transcriptional activators in vivo. Genes Dev. **8:**2756-2769.

259. **Tao, Y., M. Guermah, E. Martinez, T. Oelgeschlager, S. Hasegawa, R. Takada, T. Yamamoto, M. Horikoshi, and R. G. Roeder.** 1997. Specific interactions and potential functions of human $TAF_{II}100$. J. Biol. Chem. **272:**6714-6721.

260. **Thompson, J. S., A. Hecht, and M. Grunstein.** 1993. Histones and the regulation of heterochromatin in yeast. Cold Spring Harbor Symp. Quant. Biol. **58:**247-256.

261. **Timmers, H. T., R. E. Meyers, and P. A. Sharp.** 1992. Composition of transcription factor B-TFIID. Proc. Natl. Acad. Sci. U S A **89:**8140-8144.

262. **Timmers, H. T. M., and P. A. Sharp.** 1991. The mammalian TFIID protein is present in two functionally distinct complexes. Genes Dev. **5:**1946-1956.

263. **Tora, L.** 2002. A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. Genes Dev. **16:**673-675.

264. **Tsukiyama, T., C. Daniel, J. Tamkun, and C. Wu.** 1995. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell **83:**1021-1026.

265. **Uetz, P., L. Giot, G. Cagney, T. Mansfield, R. Judson, J. R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover, T. Kalbfleisch, G. Vijayadamodar, M. Yang, M. Johnston, S. Fields, and J. M. Rothberg.** 2000. A comprehenisive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature **403:**623-627.

266. **Um, M., J. Yamauchi, S. Kato, and J. L. Manley.** 2001. Heterozygous disruption of the TATA-binding protein gene in DT40 cells causes reduced cdc25B phosphatase expression and delayed mitosis. Mol. Cell. Biol. **21:**2435-2448.

267. **Usheva, A., E. Maldonado, A. Goldring, H. Lu, C. Houbavi, D. Reinberg, and Y. Aloni.** 1992. Specific interaction between the nonphosphorylated form of RNA polymerase II and the TATA-binding protein. Cell **69:**871-881.

268. **van der Knaap, J. A., J. W. Borst, P. C. van der Vliet, R. Gentz, and H. T. Timmers.** 1997. Cloning of the cDNA for the TATA-binding protein-associated factorII170 subunit of transcription factor B-TFIID reveals homology to global transcription regulators in yeast and Drosophila. Proc. Natl. Acad. Sci. U S A. **94:**11827-11832.

269. **Van Dyke, M. W., R. G. Roeder, and M. Sawadogo.** 1988. Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. Science **241:**1335-1338.

270. **Verrijzer, C. P., J.-L. Chen, K. Yokomori, and R. Tjian.** 1995. Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. Cell **81:**1115-1125.

271. **Verrijzer, C. P., and R. Tijan.** 1996. TAFs mediate transcriptional activation and promoter selectivity. Trends Biochem. Sci. **21:**338-342.

272. **Verrijzer, C. P., K. Yokomori, J.-L. Chen, and R. Tjian.** 1994. *Drosophila* $TAF_{II}150$: Similarity to yeast gene TSM-1 and specific binding to core promoter DNA. Science **264:**933-941.

273. **Vidal, M., R. K. Brachmann, A. Fattaey, E. Harlow, and J. D. Boeke.** 1996. Reverse two-hybrid and one-hybrid systems to detect dissociation of protein-protein interactions. Proc. Natl. Acad. Sci. USA **93:**10315-10320.

274. **Walker, S. S., J. C. Reese, L. M. Apone, and M. R. Green.** 1996. Transcription activation in cells lacking $TAF_{II}s$. Nature **383:**185-188.

275. **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:**607-614.

276. **Wang, W., M. Carey, and J. D. Gralla.** 1992. Polymerase II promoter activation: closed complex formation and ATP-driven start site opening. Science **255:**450-453.

277. **Wang, Z., S. Buratowski, ., J. Q. Svejstrup, W. J. Feaver , X. Wu, R. D. Kornberg, T. F. Donahue, and E. C. Friedberg.** 1995. The yeast TFB1 and SSL1 genes, which encode subunits of transcription factor IIH, are

required for nucleotide excision repair and RNA polymerase II transcription. Mol. Cell. Biol. **15:**2288-2293.

278. **Wassarman, D. A., and F. Sauer.** 2001. $TAF_{II}250$: a transcription toolbox. J. Cell. Sci. **114:**2895-2902.

279. **Weideman, C. A., R. C. Netter, L. R. Benjamin, J. J. McAllister, L. A. Schmiedekamp, R. A. Coleman, and B. F. Pugh.** 1997. Dynamic interplay of TFIIA, TBP and TATA DNA. J. Mol. Biol. **271:**61-75.

280. **Weil, P. A., D. S. Luse, J. Segall, and R. G. Roeder.** 1979. Selective and accurate initiation of transcription at the Ad2 major late promotor in a soluble system dependent on purified RNA polymerase II and DNA. Cell **18:**469-484.

281. **Weinzerl, R. O. J., B. D. Dynlacht, and R. Tjian.** 1993. Largest subunit of *Drosophila* transcription factor IID directs assembly of a complex containing TBP and a coactivator. Nature **362:**511-517.

282. **Weinzierl, R. O. J., S. Ruppert, B. D. Dynlacht, N. Tanese, and R. Tjian.** 1993. Cloning and expression of *Drosophila* $TAF_{II}60$ and human $TAF_{II}70$ reveal conserved interactions with other subunits of TFIID. EMBO J **12:**5303-5309.

283. **Werten, S., A. Mitschler, C. Romier, Y. Gangloff, G., S. Thuault, I. Davidson, and D. Moras.** 2002. Crystal structure of a subcomplex of human TFIID formed by TBP-associated factors hTAF4 (hTAFII135) and hTAF12 (hTAFII20). J. Biol. Chem. **277:**45502-45509.

284. **White, R. J., and S. P. Jackson.** 1992. Mechanism of TATA-biding protein recruitment to a TATA-less class III promoter. Cell **71:**1041-1053.

285. **Wieczorek, E., M. Brand, X. Jacq, and L. Tora.** 1998. Function of TAF(II)-containing complex without TBP intranscription by RNA polymerase II. Nature **393:**187-191.

286. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387-391.

287. **Workman, J., and R. Kingston.** 1998. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. **67:**545-79.

288. **Wu, S. Y., and C. M. Chiang.** 2001. TATA-binding protein-associated factors enhance the recruitment of RNA polymerase II by transcriptional activators. J. Biol. Chem. **276:**34235-24243.

289. **Wu, Y., R. J. Reece, and M. Ptashne.** 1996. Quantitation of putative activator-target affinities predicts transcriptional activating potentials. EMBO J. **15:**3951-3963.

290. **Xiao, H., J. D. Friesen, and J. T. Lis.** 1995. Recruiting TATA-binding protein to a promoter: transcriptional activation without an upstream activator. Mol. Cell. Biol. **15:**5757-5761.

291. **Yankulov, K., and D. Bentley.** 1998. Transcriptional control: Tat cofactors and transcriptional elongation. Curr. Biol. **8:**R447-449.

292. **Yatherajam, G., L. Zhang, S. M. Kraemer, and L. A. Stargell.** 2003. Protein-protein interaction map for yeast TFIID. Nucleic Acids Res. **31:**1252-1260.

293. **Yokomori, K., A. Admon, J. A. Goodrich, J. L. Chen, and R. Tjian.** 1993. Drosophila TFIIA-L is processed into two subunits that are associated with the TBP/TAF complex. Genes Dev **7:**2235-45.

294. **Yokomori, K., M. P. Zeidler, J.-L. Chen, C. P. Verrijzer, M. Mlodzik, and R. Tjian.** 1994. *Drosophila* TFIIA directs cooperative DNA binding with TBP and mediates transcriptional activation. Genes Dev. **8:**2313-2323.

295. **Yoon, H., S. P. Miller, E. K. Pabich, and T. F. Donahue.** 1992. SSL1, a suppressor of a HIS4 5'-UTR stem-loop mutation, is essential for translation initiation and affects UV resistance in yeast. Genes Dev. **6:**2463-2477.

296. **Yue, Z., E. Maldonado, R. Pillutla , H. Cho, D. Reinberg, and A. J. Shatkin.** 1997. Mammalian capping enzyme complements mutant Saccharomyces cerevisiae lacking mRNA guanylyltransferase and selectively binds the elongating form of RNA polymerase II. Proc. Natl. Acad. Sci. U S A **94:**12898-12903.

297. **Zawel, L., K. P. Kumar, and D. Reinberg.** 1995. Recycling of the general transcription factors during RNA polymerase II transcription. Genes Dev **9:**1479-90.

298. **Zawel, L., and D. Reinberg.** 1993. Initiation of transcription by RNA polymerase II: a multi-step process. Prog. Nucleic Acid Res. Mol. Biol. **44:**67-108.

299. **Zhao, X., and W. Herr.** 2002. A Regulated Two-Step Mechanism of TBP Binding to DNA: A Solvent-Exposed Surface of TBP Inhibits TATA Box Recognition. Cell **108:**615-627.

300. **Zhou, Q., T. Boyer, and A. Berk.** 1993. Factors (TAFs) required for activated transcription interact withthe TATA box -binding protein conserved core domain. Genes Dev. **7:**180-187.

301. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964-1974.