THE ROLE OF yFACT IN TRANSCRIPTIONAL INITIATION IN SACCHAROMYCES CEREVISIAE

by

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A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Experimental Pathology

Department of Pathology

The University of Utah

December 2006

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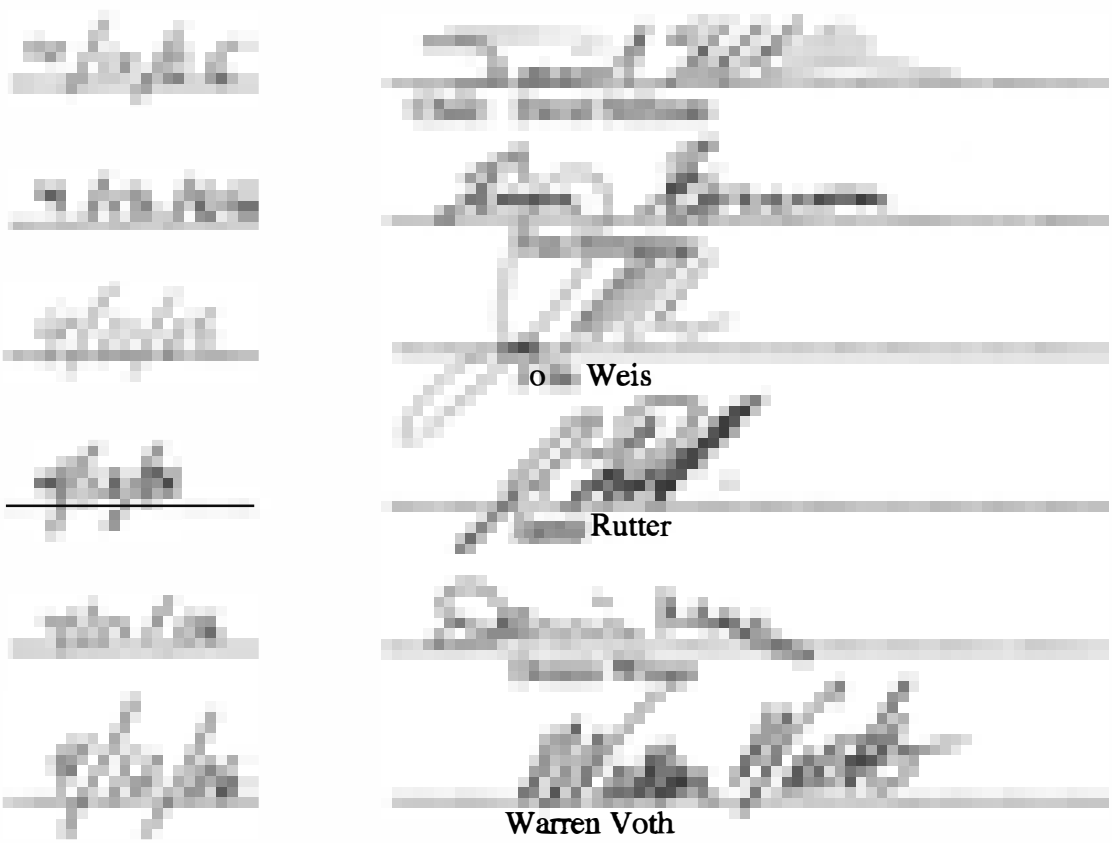

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ABSTRACT

The yeast Nhp6 protein is an architectural transcription factor in the budding yeast *Saccharomyces cerevisiae*. Genetic experiments suggest that Nhp6 can affect gene expression both positively and negatively, depending on the gene. In this dissertation, I have explored the functions of Nhp6 in relation to the yFACT chromatin reorganizing complex. The results show that the transcriptional regulation by Nhp6 in regulating binding of the TATA-binding protein (TBP) is mediated through the yFACT complex.

A genetic screen conducted in yeast identified TBP mutations that are lethal in the absence of Nhp6, but viable in a *NHP6+* strain. Further analysis of these TBP mutations showed a functional interaction among Spt3, TBP and Nhp6 in regulating essential functions *in vivo*, including formation of the complex of TBP and TFIIA with DNA. Using both genetic and biochemical assays, we have shown that Nhp6, histone acetylation by Gcn5, and chromatin remodeling by the Swi/Snf chromatin remodeling complex, have a role in TBP-TFIIA complex formation.

We have also explored functional relationships between Nhp6 and Gcn5 with negative regulators of TBP binding such as Mot1 and the Ccr4/Not complex. We show that Mot1 and Ccr4/Not also have a positive role in formation of the TBP-TFIIA complex *in vivo*.

Previous work suggested that posttranslational histone methylation at H3-K36 regulates transcription at the elongation step. Our results show that this histone

modification also negatively regulates yFACT mediated TBP and RNA polymerase II binding at the promoter regions of some genes. The ATP-dependent chromatin remodeler Chd1 physically and genetically interacts with components of the yFACT complex. *In vivo* Chd1 has been implicated in negative regulation of transcription. We provide evidence that shows an opposite role of Chd1 in the yFACT mediated stimulation of TBP and RNA polymerase II binding at promoter regions. The role of components of the yFACT complex in regulating transcription at the promoter region is discussed.

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ACKNOWLEDGMENTS

I would like to thank my supervisor Prof. David J. Stillman for his excellent guidance and encouragement. I would also like to thank my thesis committee members for their ideas and suggestions during my graduate study. This work was carried out in the friendly and constructive environment of Stillman lab. I thank all the members of the Stillman Lab for their help and suggestions. Special thanks go to my dear wife Rinku Dutta-Biswas and my family members especially to my Ma, Baba, Dada, Boudibhai, Didi, Jamaibabu, Babai, Bon, Swapan Da, Mimi, our family members at Palta and members of Jathamasai's family.

CHAPTER 1

INTRODUCTION

An overview of eukaryotic transcription

Eukaryotic transcription involves three different steps: initiation, elongation and termination. Research from the last several years has identified a plethora of transcription factors that are involved in regulation of specific steps of transcription. Eukaryotic DNA is packaged into a highly compacted structure known as chromatin. Because of this compaction, the underlying DNA sequences are not accessible for binding by most DNA-binding transcription factors. Because of the nature of chromatin, the default state of most of protein coding genes is "off". However, in response to different stimuli, specific sets of genes are expressed through recruitment of gene-specific transcription factors. The process of turning on a particular gene starts with the recruitment of sequence-specific transcriptional activator proteins to the promoter region. Binding of the activator protein complex may in turn recruit several transcriptional co-activator complexes. These co-activators may change the structure of chromatin and thereby help in the recruitment of several other transcription factors, ultimately resulting in RNA polymerase recruitment to initiate transcription.

Once RNA polymerase starts transcribing the DNA sequences from the promoter region, progression of the elongating RNA polymerase is regulated by several factors. These include: i) accessibility of the DNA sequences to the elongating RNA polymerase for transcription, ii) restoration of transcription after RNA polymerase experiences a pause during elongation, iii) co-transcriptional recruitment of RNA processing machinery, and iv) reassembly or restoration of chromatin structure after RNA polymerase passes through the chromatin. Several transcription factors have been identified that have specific roles in one or more of these events *in vivo*. These factors are

recruited sequentially in a co-transcriptional manner during the entire elongation process (Orphanides and Reinberg, 2000; Sims et al., 2004). Elongation by RNA polymerase starts with a specific set of transcription factors at the 5' end of a transcription unit. These are exchanged with a different set of transcription factors during progression to the 3' end (Kim et al., 2004; Pokholok et al., 2002). However, some factors remain bound to elongating RNA polymerase throughout the ORF. The process of transcription termination starts once the elongating RNA polymerase encounters the poly-adenylation (poly-A) sequence of the transcribed region. Recruitment of the cleavage poly-adenylation factors at the poly-A site in turn recruits the transcription termination factors that ultimately cause pausing and dissociation of the elongating RNA polymerase from the coding region (Buratowski, 2005).

Although several transcription factors have been identified that have specific roles during specific step of transcription, there are instances in which a factor may have roles in regulating two different steps of transcription. It is not known whether these factors generally act within two different complexes or their effect at a particular step in transcription has an indirect role in regulating other step.

Overview of transcriptional regulation by different factors in *Saccharomyces cerevisiae*

The budding yeast, *Saccharomyces cerevisiae* (*S. cerevisiae*), has been used as a great model system for studying eukaryotic transcriptional regulation. The power of yeast genetics in combination with biochemical studies has promoted understanding of various modes of regulating transcription in eukaryotes. Most the essential genes are conserved from yeast to human during evolution, and thus most findings on transcriptional

regulation in yeast also hold true for other higher organisms. In all eukaryotic organisms, the DNA sequence is wrapped around the histone octamer to form the nucleosome. Nucleosomes are the building blocks of the highly compacted structures known as chromosomes. One of the important tasks of the eukaryotic transcription machinery is to make the underlying DNA sequence available to sequence-specific DNA-binding transcription factors. The ultimate goal of all of the transcription factors involved in the transcription initiation is to recruit RNA polymerase and initiate transcription by the RNA polymerase. There are three major mechanisms by which the transcriptional elongation factors regulate transcription by the RNA polymerase: i) by changing the structure of chromatin so as to facilitate passage for RNA polymerase through the inhibitory chromatin, ii) by resuming transcription by RNA polymerase once it pauses or arrests during transcription, iii) by reassembling the normal repressive chromatin structure to prevent inappropriate transcriptional initiation from TATA elements within coding regions. The factors responsible for the transcription termination are co-transcriptionally recruited during the transcription elongation process (Problem of redundancy). Studies from the last few years have implicated a growing number of transcription factors that are involved in regulating all the steps of transcription. In this introduction, I will discuss the factors and mechanisms by which different steps of transcription are regulated, with emphasis to transcriptional regulation in *S. cerevisiae.*

Transcriptional regulation by general transcription factors

The regulation of expression of specific sets of genes starts with recruitment of transcription factors in a promoter specific manner. Recruitment of general transcription factors (GTFs) at the promoter region helps in promoter melting and DNA unwinding

for transcriptional initiation (Naar et al., 2001; Reinberg et al., 1998). These factors include TFIIA, B, D, E, F and H. Recruitment of TFIID to a TATA sequence forms a scaffold for binding of the other GTFs. The sequence specific binding of TFIID to a TATA-box containing DNA is achieved through sequence specific binding of TATA-binding protein (TBP), a subunit of the TFIID complex. *In vitro* binding experiments have shown that TBP binding is followed by binding of TFIIA and TFIIB to form the TBP-TFIIA-TFIIB ternary complex with DNA. Binding of the TFIIA and TFIIB to the TBP-DNA complex stabilizes TBP binding to the DNA. Formation of the TBP-TFIIA-TFIIB complex is one of the critical steps in transcriptional initiation in eukaryotes. DNA-binding by TBP is required for transcription by all three RNA polymerases. TBP is associated with a variety of complexes, such as SL1 (Comai et al., 1992), TFIID and TFIIIB (Pugh, 2000). SL1 functions at RNA polymerase I promoters, the TFIID is specific for some promoters of mRNA genes transcribed by RNA polymerase II, and the TFIIIB complex targets RNA polymerase III genes. Several transcription factors have been identified that regulate transcription by regulating TBP binding. The positive regulators of TBP binding include several transcriptional coactivators containing histone acetyl transferases, chromatin remodelers, etc. (Pugh, 2000).

The most studied negative regulators of the TBP binding are Mot1 (Auble et al., 1994; Auble et al., 1997; Darst et al., 2003; Dasgupta et al., 2002), NC2 (Cang et al., 1999; Goppelt and Meisterernst, 1996; Mermelstein et al., 1996) and the Ccr4/Not complex (Badarinarayana et al., 2000; Oberholzer and Collart, 1999). Mot1 is a Snf2/Swi2-related ATPase factor that can drive dissociation of the TBP-DNA complex *in vitro* (Auble et al., 1997). The negative regulation by Mot1 is attributed to the ability to

dissociate the TBP-DNA complex. Evidence suggests that Mot1 also positively regulates transcription (Dasgupta et al., 2002; Geisberg et al., 2002). It has been hypothesized that the ability of Mot1 to dissociate abortive TBP-DNA complexes is also responsible for Mot1 positively regulating transcription of some genes. The two subunits of NC2 are encoded by *BUR6* and *YDR1* in *S. cerevisiae*. NC2 associates with the promoter-bound TBP, thereby preventing the recruitment of TFIIA and TFIIB to the TBP-DNA complex *in vitro* (Kim et al., 1995). Studies in yeast have identified a mutation in the largest subunit of TFIIA that acts as a suppressor of the essential role for NC2, providing *in vivo* support for the results of the *in vitro* studies (Xie et al., 2000). A crystal structure of NC2 recognizing the TBP-DNA complex has been described that shows that NC2 binding precludes recruitment of TFIIA and TFIIB to the preformed TBP-DNA complex (Kamada et al., 2001). There are two Ccr4-Not complexes, each a large multiprotein complex of either 1.2- or 2-MDa molecular weight (Liu et al., 1998). Five Not proteins are associated with Ccr4 to form the Ccr4/Not complex. This complex has a role in various cellular processes including mRNA deadenylation and thereby regulates mRNA degradation (Denis and Chen, 2003). However, evidence also suggested that the Ccr4/Not complex regulates TBP binding to DNA. For example, i) components of the Ccr4/Not complex show genetic interaction with TBP (Badarinarayana et al., 2000), and ii) Not1 protein physically interacts with TBP and components of the TFIID complex (Deluen et al., 2002; Huisinga and Pugh, 2004). The mechanism of negative regulation of the Ccr4/Not complex in the TBP binding is unknown. There is substantial evidence suggesting that DNA-binding by TBP can also by negatively regulated by the ability of TBP to form a homodimer (Alexander et al., 2004; Coleman et al., 1995; Weideman et

al., 1997). Transcription factors such as TFIIA and Brf1 can inhibit TBP homodimer formation and thereby positively regulate transcription (Alexander et al., 2004; Weideman et al., 1997).

Promoter specific recruitment of TBP has been studied in relation to SAGA and the TFIID complex. Genome-wide analysis shows that for 90% of yeast genes TBP binds as part of the TFIIA complex, and at the remaining 10% of yeast genes TBP binding requires the SAGA co-activator complex (Huisinga and Pugh, 2004). SAGA-dependent TBP binding occurs at promoters with canonical TATA boxes, while TFIID binds to promoters with imperfect TATA sequences (Basehoar et al., 2004). Therefore the promoter specific recruitment of TBP may actually be dependent on other proteins that are associated with both the SAGA and TFIID complexes. The multiprotein complexes TFIID and SAGA share some common subunits, known as TAFs (TBP-associated factors). The actual roles of all these TAFs in recruiting SAGA and TFIID to specific promoters and their role in facilitating TBP binding are still unclear.

Work from the last several years has shown that while GTFs are absolutely required to promote transcription, regulation of chromatin structure is also important. Chromatin regulation of transcription includes histone modifications, chromatin remodeling by the ATP-dependent chromatin remodeling complexes, assembly of multiprotein complexes by architectural transcription factors and chromatin reorganization by chromatin binding factors. I will discuss the roles of these factors in brief in the subsequent sections of this introduction.

Transcriptional regulation by histone modification

The nucleosome is a highly compacted structure composed of 147 bp of DNA and a histone octamer with two copies each of four different histone proteins: H2A, H2B, H3 and H4 (Luger et al., 1997). Each nucleosome is a highly structured complex, although the tails of histone proteins that protrude out of the nucleosome are unstructured. These histone tails are subject to post-translational modifications that play an important role in regulating eukaryotic transcription (Strahl and Allis, 2000). During the last few years the field of eukaryotic transcription has witnessed the identification of several transcription factors that regulate transcription through post translational modification of histones (Strahl and Allis, 2000). These post translational histone modifications include the acetylation of lysine residues, methylation of lysine and arginine residues, phosphorylation of serine and threonine residues, ubiquitylation of lysine residues, sumoylation of lysine residues, and the poly-ADP-ribosylation of glutamic acid residues (de la Cruz et al., 2005). Several transcription factors have also been described that recognize specific histone modifications, with this recognition leading to physical interaction. Significant progress has been made in understanding the roles played by histone acetylation, histone methylation and histone mono-ubiquitylation in regulating eukaryotic transcription as described below.

Transcriptional regulation by histone acetylation

The importance of histone acetylation in regulating the eukaryotic transcription has been known for a long period of time. Hyperacetylation of histones at genes is correlated with transcriptional activity (Hebbes et al., 1988). Because acetylation of lysine residues neutralizes the positive charge of this residue, initially it was assumed that a decrease in

the electrostatic interaction between DNA and the histone proteins is the major acetylation-dependent mechanism that regulates gene expression. The first histone acetyltransferase (HAT) protein identified was a 55 kDa *Tetrahymena* protein with HAT activity (Brownell and Allis, 1995). It was subsequently shown that the sequence of this HAT protein strikingly matches the yeast Gcn5 protein (Brownell et al., 1996). HAT enzymes form a trimeric complex with acetyl-CoA and the lysine residue, enabling the direct transfer of the acetyl group from acetyl CoA to the lysine residue (Roth et al., 2001). A number of budding yeast proteins have been identified that show HAT activity (Table 1.1). These HAT proteins are mostly found in large complexes with other proteins. Some HAT proteins specifically modify only certain lysine residues, while others show broad specificity. Although the mechanism behind this residue specificity of HAT proteins is not clear, it is thought that the associated proteins in a HAT complex determine the specificity of the HAT proteins for their substrates.

In *Saccharomyces cerevisiae*, the HATs are targeted to promoters by specific DNA-binding proteins. For example, the transcriptional activator Gcn4 recurits the HAT Gcn5, which is a subunit of the SAGA complex, to the promoter of the *HIS3* gene (Kuo et al., 2000; Utley et al., 1998). This causes histone acetylation by Gcn5 primarily at histone H3 and H2B. The histone acetyltransferase Esa1, which is the catalytic component of the NuA4 (nucleosome acetyltransferase of histone H4) HAT complex, acetylates histones H4 and H2A in yeast (Allard et al., 1999; Doyon and Cote, 2004). Although the HAT activities of most of the HATs have not been described in relation to specific promoters and coding regions, some HATs acetylate only within coding regions of genes. For example, Elp3, which acetylates the Lys residues of the histones H3 and H4 tails *in vitro*,

Table 1.1. The HAT complexes in *S. cerevisiae*

HAT	Organism	Complex	Functions in transcription
Gcn5	Yeast	SAGA, SLIK, SALSA, ADA, HAT-A2	Transcriptional activation
Esa1	Yeast	NuA4 complex	Transcriptional activation
Spt10	Yeast	Spt10/Spt21	Transcriptional activation
Sas3	Yeast	NuA3 complex	Transcriptional activation
Sas2	Yeast	SAS-I complex	Anti-silencing
Hat1	Yeast	Hat1/2 complex	Histone deposition
TAF1	Mammal/fly/yeast	TFIID	Transcriptional initiation by RNA polymerase II
Elp3	Yeast	Elongator	Transcription elongation
Hpa2	Yeast		Unknown
Med5	Yeast	Mediator	Transcriptional initiation by RNA polymerase II

has been shown to associate with the elongating form of RNA polymerase II as part of the elongator complex and to acetylate the coding region of genes (Winkler et al., 2002).

Genome-wide analysis shows that histone acetylation is primarily present at the 5'end of genes (Kurdistani et al., 2004; Pokholok et al., 2005). In a striking finding, it was found that acetylation of only certain lysine residues correlates with transcriptional activity of a promoter. For example, acetylation at histone H3-K18, K27 and K9 is

correlated with high transcriptional activity (Kurdistani et al., 2004). In contrast, acetylation of histone H4-K8, K12, and K16 is seen at transcriptionally inactive genes (Kurdistani et al., 2004). Based on these genome-wide analyses of histone acetylation patterns, acetylation clusters have been described for some gene groups and transcription factors that are required for specific biological functions (Kurdistani et al., 2004).Recent work also shows that acetylation within the globular domain of histone H3 at K56 is also important in regulating transcription of some genes, and that Spt10 is responsible for this modification (Xu et al., 2005).

The dynamics of histone acetylation *in vivo* is maintained by deacetylation of acetylated histone by histone deacetylases (HDACs). There are five HDACs in *S. cerevisiae*, including Rpd3, Hda1, Hos1, Hos2 and Hos3 (Kurdistani and Grunstein, 2003). All of these HDACs function in multiprotein complexes. The Rpd3 HDAC complex is the best characterized histone deacetylase complex in yeast. There are two different forms of Rpd3 HDAC complexes in yeast, Rpd3 large (Rpd3(L)) and Rpd3 small (Rpd3(S)) (Kasten et al., 1997). The Ume6 DNA-binding protein, which is part of the Rpd3(L) complex, recruits Rpd3(L) through Sin3, to a specific DNA element (*URS1*) in the *INO1* promoter (Carrozza et al., 2005; Kadosh and Struhl, 1997; Rundlett et al., 1998). Chromatin immunoprecipitation (ChIP) studies show that the Rpd3(L) complex is enriched at the *INO1* promoter where it deacetylates almost all sites of acetylation on histones H4, H3, H2A and H2B (Kadosh and Struhl, 1998; Suka et al., 2001). The region of deacetylation is highly localized, and limited to a region of one or two nucleosomes immediately adjacent to the *URS1* element of the *INO1* promoter (Kadosh and Struhl, 1998). This repressive chromatin structure formed by deacetylation may block the

recruitment of other transcription factors, resulting in negative regulation of transcription. The Rpd3(S) complex has been shown to have a role during transcription elongation at the 3' end of some genes. This aspect of Rpd3(S) in regulating transcription is discussed later.

The Hda1 HDAC complex of yeast can be recruited to its target promoters through the Ssn6/Tup1 corepressor complex (Wu et al., 2001) and deacetylates histones H3 and H2B. Like Rpd3 deacetylation, recruitment of the Ssn/Tup1 complex also results in local deacetylation of a region that spans one to two nucleosomes adjacent to the recruitment site (Wu et al., 2001). The Hos2 HDAC complex associates physically with coding regions of genes when they are transcribed and specifically deacetylates histones H3 and H4 *in vivo* (Wang et al., 2002). Hos2 has been described in regulating transcription both positively and negatively.

The bromodomain and recognition of histone acetylation modification

The bromodomain containing proteins recognize the acetylated histone residues, and helps in recruiting other transcription co-activators such as the nucleosome remodeling and HAT-containing complexes (de la Cruz et al., 2005). The yeast bromodomain factors and their roles in transcription are described in Table 1.2. Interaction between acetylated histones and bromodomain containing complexes is thought to stabilize the interaction of these complexes with nucleosomes and promote nucleosome remodeling (Hassan et al., 2002), histone acetylation (Syntichaki et al., 2000), and TFIID recruitment (Martinez-Campa et al., 2004; Matangkasombut et al., 2000). The recognition of the acetylated histone tails by the bromodomain containing factors can also promote exchange of

Table 1.2. Bromodomain containing proteins in yeast (*S. cerevisiae*)

Protein activity	Gene	Complex	Protein	Molecular function
Histone interaction	BDF1	SWR1/ TFIID	Bdf1	Histone exchange Transcriptional coactivation
Chromatin remodeling	*SWI2/SNF2*	Swi/Snf	Swi2	Transcriptional coactivation
	RSC4	RSC	Rsc4	Transcriptional coactivation
	STH1	RSC	Sth1	Transcriptional coactivation
Histone acetylation	GCN5	SAGA	Gcn5	Transcriptional coactivation

histones. For example, Bdf1, a component of the SWR1 complex, is able to exchange the conventional histone H2A in nucleosomes for a histone variant H2A.Z (Htz1) (Krogan et al., 2003b; Raisner et al., 2005; Zhang et al., 2005a).

Although acetylation of histone tails by HAT proteins and their roles in regulating transcription have been studied in great detail, acetylation of other non-histone proteins by the p300/CBP complex has also been shown to stimulate transcription in higher eukaryotes (Kimura and Horikoshi, 2004). Acetylation substrates of the mammalian p300/CBP complex include Sp1, KLF1, FOXO1, MEF2C, SRY, GATA-4, HNF6, and Stat3 (Kimura and Horikoshi, 2004). Acetylation of these factors enhances transcriptional activity either by stimulating their DNA-binding activity or by promoting interaction with other transcription factors

Significant progress has been achieved in understanding the molecular mechanisms by which histone acetylation regulates eukaryotic transcription. However, the fundamental question of how multiple histone modifications specifically affect transcriptional processes remains unknown. A detailed analysis of both how these HAT-containing complexes are recruited to chromatin and the effects of complexes that specifically recognize acetylated histones may provide insights into the underlying mechanisms.

Regulation of transcription by histone methylation

Unlike the histone acetylation, which is involved mainly in transcriptional activation, histone methylation has been implicated in both the transcriptional activation and transcriptional repression. Lysines of histone H3 at 4, 9, 14, 27, 36, 79 and histone H4 at 20 and 59 are methylated by residue specific histone methyltransferases (Lee et al., 2005; Margueron et al., 2005). In general, lysine methylation of histone H3 at 4, 36 and 79 are associated with transcriptional activation and lysine methylation of histone H3 at 9, 27 and H4 at 20 are associated with heterochromatin formation and transcriptional repression. Histone methyl transferases (HMTs) that are specific for specific lysine residues have been characterized. Most of these histone methyl transferases contain a SET [Su(var), Enhancer of zeste, trithorax] domain structure that is responsible for catalysis and binding of cofactor S-adenosyl-L-methionine (AdoMet) (Bottomley., 2004; Cheng et al., 2005; Marmorstein, 2003; Xiao et al., 2003a). HMTs then transfer one or more methyl groups to the ε-amino group of the specific lysine residues, resulting in mono-, di-, or trimethylated lysine (Martin and Zhang, 2005; Zhang and Reinberg, 2001). Unlike acetylation, methylation of lysine residues does not change the net positive charge

on the nucleosome, rather it increases the bulkiness and hydrophobicity, which may disrupt the nucleosomal structure or create new sites for proteins that preferentially bind to the methylated histone proteins. How these histone modifications regulate transcription is an intense area of study. The known roles for some of these histone modifications in regulating transcription in *S. cerevisiae* are discussed below.

Histone H3 methylation at lysine-4

Set1 histone methyltransferase methylates histone H3 at lysine-4 (H3-K4) (Briggs et al., 2001). This histone modification is generally associated with transcriptional activation (Bernstein et al., 2002; Santos-Rosa et al., 2002). By microarray analysis it has been shown that the deletion of *SET1* results in reduced expression of ~ 80% of genes in *S. cerevisiae* (Boa et al., 2003). Genome-wide analysis of the presence of different forms of the methylated histone H3-K4 revealed that the trimethylated form of the histone H3-K4 is predominant at the 5' end of the genes whereas the di- and monomethylated forms of histone H3-K4 is predominantly present at the coding region and 3' end of the genes, respectively (Pokholok et al., 2005). However, the functional relationship between the presence of these modifications and transcriptional regulation is not known. In *S. cerevisiae*, Set1 is present as a member of a large multiprotein complex called COMPASS (Complex Proteins Associated with Set1) (Miller et al., 2001). It has been shown that Cps40 and Cps60 subunits of the COMPASS complex are required for the trimethylation of the histone H3-K4 but not for the di- and mono-methylation (Schneider et al., 2005).

Although H3-K4 methylation is associated with transcriptional activation (Bernstein et al., 2002; Noma and Grewal, 2002; Santos-Rosa et al., 2002), some evidence suggests

a role in gene repression. In fact, Set1 was originally identified as a protein important in gene silencing in *S. cerevisiae* (Nislow et al., 1997). Deletion of the *SET1* gene led to disruption of silencing of reporter genes integrated near telomeres, at the mating type loci, and at the rDNA locus (Briggs et al., 2001; Bryk et al., 2002). Therefore, it appears that Set1 plays a complex role in both gene activation and repression in budding yeast and more studies will be required to understand how it participates in both positive and negative regulation of transcription.

In *S. cerevisiae*, recruitment of Set1 to genes is dependent on several events, including phosphorylation of the C-terminal domain (CTD) of RNA polymerase II (Ng et al., 2003b), the presence of the Paf1 elongation complex (Krogan et al., 2003a), and monoubiquitylation of histone H2B-K123 by the Rad6 enzyme (Ng et al., 2003a; Shahbazian et al., 2005; Wood et al., 2003b). The CTD of the RNA polymerase II consists of a long series of heptapeptide repeats, Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The phosphorylation status of the CTD correlates with the stages of RNA polymerase II in the transcription process (Ng et al., 2003b). Ser-5 phosphorylation of the CTD is important for facilitating the transition from transcription initiation to elongation and correlates with the initiation and the early phase of the transcriptional elongation process, whereas Ser-2 phosphorylation of the CTD is associated with the late phase of transcriptional elongation (Palancade and Bensaude, 2003). In *S. cerevisiae*, Ser-5 is phosphorylated by the TFIIH-associated Kin28 kinase, whereas Ser-2 is phosphorylated by Ctk1 kinase (Kobor and Greenblatt, 2002). The recruitment of the Set1 containing COMPASS complex at the 5' end of the genes is regulated by several cooperative mechanisms. First, Set1 associates with RNA polymerase II when the CTD is phosphorylated at Ser-5 but not at Ser-2 (Ng et

al., 2003b). A *kin28* mutation results in decreased recruitment of Set1 to the 5' coding region (Ng et al., 2003b). This indicates that the CTD phosphorylation of the newly initiated RNA polymerase II recruits the Set1 containing COMPASS complex, resulting in trimethylation of histone H3-K4 at the 5' end of genes. However, the relationship between the Set1 recruitment and predominant existence of the di- and monomethylated form of the histone H3-K4 is still unclear. Second, components of the Paf1 transcription elongation complex interact with Set1 and are also required for recruitment of Set1 (Krogan et al., 2003a). Third, as discussed later, methylation of the histone H3-K4 is also dependent on monoubiquitylation of the histone H2B-K123 residue. Interestingly, the di- and tri-methylation of histone H3-K4 are dependent on monoubiquitylation of histone H2B-K123 residue, but mono-methylation does not required this monoubiquitylation (Dehe et al., 2005; Shahbazian et al., 2005). The Bur2 kinase selectively phosphorylates the CTD of RNA polymerase II and has been shown to regulate the tri-methylation by Set1 (Laribee et al., 2005). A *bur2* mutation also regulates monoubiquitylation of histone H2B-K123 and recruitment of the Paf1 elongation complex, thereby regulating the tri-methylation by the Set1 containing COMPASS complex.

Histone H3 methylation at lysine-36

Lysine-36 residue of the histone H3 (H3-K36) is methylated by another SET domain containing protein, Set2. The K36 residue of histone H3 lies at the junction between the histone tail and core domains. Because of this unique location, methylation of this residue may directly alter the nucleosome structure and thus affect binding of different transcription factors that recognize either the modification or the altered chromatin structure.

Similar to histone H3-K4 methylation, histone H3-K36 methylation is also associated with active genes. Set2 preferentially binds to the Ser-2-phosphorylated form of CTD as compared with the unphosphorylated CTD of RNA polymerase II. This suggests that Set2 is recruited to the coding regions of the transcribed genes. Deletion of approximately 10 heptapeptide repeats of the RNA polymerase II CTD resulted in a significant loss of histone H3-K36 methylation globally, while having no effect on histone H3-K4 or H3-K79 methylation. Deletion of individual components of the Ctk kinase complex also results in complete loss of H3-K36 methylation. This provides strong evidence that Ser-2 phosphorylation of the RNA polymerase II CTD by the Ctk kinase complex controls H3-K36 methylation. The mechanism by which CTD phosphorylation at Ser-2 recruits Set2 to the 3' regions of genes is not known. The Paf1 complex also plays an important role in the recruitment of Set2, as was also observed for Set1. Thus, the Paf1 complex and the Ser-2 phosphorylation of the RNA polymerase II CTD may work together to target Set2 and Set1 to the coding regions of actively transcribed genes. In fact, genome wide analysis shows that the di- and trimethylated forms of histone H3-K36 are located predominantly present at the 3' end of the genes. However, the presence of the monomethylated histone H3-K36 in a genome wide manner has not been investigated.

Similar to Set1, Set2 was also originally implicated in transcriptional repression in *S. cerevisiae*. Tethering of Set2 to the promoter of a reporter gene by fusing Set2 to a LexA DNA-binding domain results in repression of reporter gene expression, and this repression was partially relieved by mutations in the SET domain. A *GAL4* promoter lacking its UAS element is expressed poorly, but a deletion of *SET2* or a mutation at H3-K36 allows higher expression from this mutant promoter. Recent reports show a

functional link between histone H3-K36 methylation and the Rpd3(S) complex. These studies have shown that the Eaf3 chromodomain-containing protein in the Rpd3(S) complex recognizes the methylated histone H3-K36 mark at the 3' end of some genes. Recruitment of this Rpd3(S) complex results in deacetylation of histones, allowing nucleosomes to return to the normal state following passage of RNA polymerase II. Reassembling the perturbed nucleosome to a pretranscribed state may be important to inhibit the usage of the cryptic TATA sequence present in the coding sequences of some genes. One study reported dynamic changes in methylation at H3-K4 and H3-K36 at the *MET16* gene (Morillon et al., 2005). It has been proposed that the histone H3-K4 trimethylation and the H3-K36 di- and trimethylation regulate the post-initiation step of transcription, while the H3-K4 dimethylation and the H3-K79 trimethylation mark the onset of transcription elongation (Morillon et al., 2005).

Histone H3 methylation at lysine-79

Lys-79 of histone H3 (H3-K79) is methylated by Dot1. Dot1 is a unique HMT, because it does not contain a SET domain and methylates a histone residue that is in the core of the nucleosome structure rather than a histone tail. In *S. cerevisiae*, histone H3-K79 dimethylation is present in the heterochromatic regions, including the rDNA, telomere, and silent mating type regions. Methylation at H3-K79 inhibits Sir2/3 binding and thereby disrupts silencing in *S. cerevisiae*. Histone methylation by Dot1 is also regulated by the Paf1 complex and by histone monoubiquitylation by the Rad6/Bre1 complex. However, only the di- and tri-methylation at H3-K79, not the monomethylation, are regulated by histone monoubiquitylation at H3-K123 residue.

The chromodomain and recognition of histone methylation modification

The protein motif that recognizes methylated lysine residues is called a chromodomain. In *S. cerevisiae*, several chromodomain containing transcription factors have been described (Table 1.3). Chromodomain containing proteins are involved in both transcriptional activation and transcriptional silencing (heterochromatin formation) *in vivo*.

The HP1 protein in human and Swi6, Clr3 and Clr4 proteins in fission yeast (*Schizosaccharomyces pombe*) are involved in formation of heterochromatin structures (Grewal and Moazed, 2003; Hiragami and Festenstein, 2005). As described earlier, the chromodomain of the Eaf3 protein in *S. cerevisiae* is involved in transcriptional regulation at the 3' end of the genes. The chromodomain of Esa1 is required for targeting the NuA4 HAT complex to the *PHO5* promoter, and this acetylation of H4 recruits the Pho4 activator (Nourani et al., 2004). The chromodomain of Chd1 has been shown to be important for recognizing dimethylated H3-K4 *in vitro* (Pray-Grant et al., 2005). Chd1 is associated with the SAGA/SLIK HAT complex, and thus K4 methylation results in binding of this coactivator complex (Pray-Grant et al., 2005).

Dynamics of lysine methyl modification of histone tails

It was long believed that histone methylation was an irreversible post translational modification, with histone methylation changed only by turnover of nucleosomes. However the existence of lysine demethylases and arginine deiminases have recently been demonstrated. The only known lysine demethylase that selectively demethylates the

Table 1.3. Chromodomain containing proteins in yeast (*S. cerevisiae*)

Protein activity	Gene	Complex	Protein	Molecular function
Chromatin reassembly	EAF3	Rpd3(S)/NuA4	Eaf3	Transcriptional repression
Histone acetylation	ESA1	NuA4	Esa1	Transcriptional coactivation
Chromatin remodeling	CHD1	SAGA/SLIK	Chd1	Transcriptional coactivation

mono- and dimethylated form of the histone H3-K4 is LSD1 (Lysine Specific Demethylase 1) (Shi et al., 2004; Shi et al., 2005). However, since most active genes are tri-methylated at H3-K4, it is tempting to speculate the existence of a histone demethylase specific for the trimethylated form of histone H3. No homolog of LSD1 is present in *S. cerevisiae*. The only known arginine deiminase described in mammals is PADI4 (Protein Arginine Deiminase) (Cuthbert et al., 2004; Wang et al., 2004). This enzyme converts the arginine residue of histone to citrulline by a deimination reaction. The fate of this citrulline in nucleosomal structure is unknown. So far no arginine deiminase has been described in *S. cerevisiae* (Bannister and Kouzarides, 2005).

Histone monoubiquitylation and regulation of transcription

Unlike protein polyubiquitylation that signals for protein degradation via the proteasome pathway, monoubiquitylation of a protein is a stable postranslational

modification. An E2 ubiquitin conjugating enzyme, Rad6 is responsible for the monoubiquitylation of histone H2B at residue K123 (Kao et al., 2004). Deletion of *RAD6* results in the elimination of the global H2B monoubiquitylation, as well as loss of dimethylation at K4 and K79 of histone H3 (Kao et al., 2004). A *rad6* mutation does not affect K36 methylation. Bre1, an E3 ubiquitin ligase, associates with Rad6 and is required for targeting of Rad6 to chromatin (Wood et al., 2003a). Deletion of *BRE1* also results in loss of monoubiquitylated H2B and a loss of histone H3-K4 and K79 dimethylation. The components of the Paf1 complex are also required for H2B K123 monoubiquitylation and thus, H3 K4 and K79 methylation as well (Wood et al., 2003b). Like Set1, Rad6/Bre1 also associates with the elongating RNA polymerase II via the Paf1 complex (Xiao et al., 2005).

Transcription regulation by chromatin remodeling complexes

The histone-DNA interactions in chromatin can be changed by chromatin remodeling complexes, such that the underlying DNA becomes more accessible to DNA-binding proteins. This remodeling of the nucleosome may lead to displacement of histone octamers exposing a particular DNA sequence to DNA-binding proteins. One of the hallmarks of chromatin remodeling complexes is their dependence on ATP hydrolysis for their functions (Becker and Horz, 2002).

The chromatin remodeling complexes are multiprotein in nature. All the chromatin remodelers have an ATPase subunit of the Swi2/Snf2 family of ATPases. The enzymes in this family can be grouped into several subfamilies based on the sequence features outside of their ATPase domains. Direct evidence of chromatin remodeling activity has been demonstrated for some of these enzymes, including the Swi2/Snf2-related enzymes,

the ISWI/SNF2L-type ATPases, the CHD1 family member Mi-2 and the INO80 complex (Cairns, 2005; Wang, 2003).

Biochemical characterization of the SWI/SNF chromatin remodeling complex has identified 11 different subunits (Vignali et al., 2000). The motor of this complex is the nucleosome remodeling ATPase Swi2/Snf2. The functions of many of the other subunits in this complex are less well understood. This complex has been shown to increase the accessibility of the nucleosomal DNA in an ATP-dependent manner. The proteins present in the SWI/SNF complex were originally discovered and characterized as transcriptional activators, which fits nicely with the observation that they remodel the chromatin to enable transcription. However, genome-wide analyses showed some unexpected results. Only a small fraction of the yeast genes require the SWI/SNF complex for their activation, and the SWI/SNF complex also seems to be involved in repression of almost the same number of genes (Holstege et al., 1998; Sudarsanam et al., 2000).

Another chromatin remodeling complex called RSC (Remodels Structure of Chromatin) has been studied in *S. cerevisiae*. The RSC complex subunits have strong homology with the Swi/Snf complex subunits (Mohrmann and Verrijzer, 2005). Two proteins, Arp7 and Arp9, are common to Swi/Snf and RSC. In contrast to all currently known genes that encode the protein subunits of the SWI/SNF complex, most of the genes coding for subunits of the RSC complex are essential in nature. RSC is much more abundant than SWI/SNF. So far, evidence suggests that RSC is involved in cell cycle progression, repair of double strand breaks, association of cohesin with centromeres and chromosome arms, and regulation of expression of genes encoding ribosomal proteins, cell wall proteins, and sporulation specific genes. The ISWI chromatin remodeling

complexes are characterized by presence of two SANT-like domains in the C-terminal regions of the enzymes. The *in vivo* functions of the ISWI complexes include transcriptional activation and repression, chromatin assembly, nucleosome spacing or sliding and maintenance of higher order chromatin structure (Mellor and Morillon, 2004). The compositions of the ISWI complexes in yeast are described in Table 1.4.

In yeast, the Isw2 complex is recruited by the general transcription repressor Ume6. This recruitment leads to repression of a variety of genes (Goldmark et al., 2000). Whole genome expression analysis suggests that the Isw1 complex primarily plays a role in transcriptional repression in association with the Sin3/Rpd3 HDAC (Fazzio et al., 2001). Some studies suggest a role for the Isw1 complex in both the transcription elongation and termination processes. The Isw1 complex has been proposed to sequentially regulate each stage of transcription by coordinating the events occurring at the 5' and 3' end during a transcription cycle, and by controlling the amount of RNA polymerase II entering into a productive elongation step (Morillon et al., 2003). The functions of the two different forms of ISW1 complexes, Isw1a and Isw1b, in regulating transcription have been examined in this study. The Isw1a complex acts as a repressor and prevents transcriptional initiation whereas the Isw1b complex controls the transcriptional elongation by coordinating CTD phosphorylation, RNA 3' end formation, and release of the RNA polymerase II during termination. The ability of the ISWI complexes to regulate transcription has been shown to be dependent on histone methylation at H3-K4 and the Isw1 protein has been shown to recognize histone methylation at H3-K4 *in vitro* (Santos-Rosa et al., 2003). *In vivo*, Isw1 displaces TBP in a promoter specific manner in association with Cbf1 (Moreau et al., 2003).

Table 1.4. ISWI protein complexes in yeast (*S. cerevisiae*)

Complex	Subunits	*In vivo* functions
Isw1a	Isw1 and Ioc3	Transcription repression
Isw1b	Isw1, Ioc2 and Ioc4	Transcription elongation and termination
Isw2	Isw2 and Itc1	Transcription repression and nucleosome sliding
Isw2/yCHRAC	Isw2, Itc1, Dpb4 and D1s1	Telomere position effect and heterochromatin structure

Chromatin remodeling by ATPases of the CHD type are characterized by the presence of a pair of chromodomains (Woodage et al., 1997). Several members of the CHD family of proteins have been described and these are listed in Table 1.5. The most studied CHD family protein in *S. cerevisiae* is Chd1. Initial observations implicated Chd1 as a negative regulator of transcription (Woodage et al., 1997). The uracil analog, 6-azauracil (6-AU) causes an imbalance in the pools of ribonucleotide tri-phosphate (Exinger and Lacroute, 1992). Strains defective in transcriptional elongation are sensitive to grow on 6-AU containing media. Deletion of *CHD1* results in increased resistance to growth on media containing 6-AU than wild type. This observation suggests that Chd1 may have a negative role in transcriptional elongation *in vivo*. The synthetic lethal interaction between *chd1*Δ and *isw1*Δ *isw2*Δ indicates a role of Chd1 in chromatin remodeling *in vivo* (Tsukiyama et al., 1999). An *in vitro* biochemical study showed that Chd1 has an ATP-dependent chromatin remodeling activity which is different from that of Swi/Snf (Tran et al., 2000). Several genetic interactions have been documented

Table 1.5. The members of CHD family proteins

Complex	Catalytic ATPase subunit (organism)	Biochemical function	*In vivo* function
Chd1	Chd1 (*S. cerevisiae*)	Disruption of nucleosome, recognition of methylated H3-K4 tail peptide	Transcription elongation, formation of repressive chromatin structure
HRP1	HRP1 (*S. pombe*)	DNA-stimulated ATPase activity	Chromosome separation
Mi2	Chd4 (Drosophila)	ATPase activity stimulated by nucleosome	Methylation induced gene silencing
Mi2	Chd4 but also Chd3 (Mouse)	ATPase activity stimulated by nucleosome	Involved in T-cell development
NuRD/NURD	Chd3 and/or Chd4 (human)	ATPase activity stimulated by mononucleosome	Methylation induced gene silencing, heterochromatin, DNA repair
CHD1	Chd1 (Drosophila/Mouse)	ATP-dependent chromatin assembly	Associated with active transcription

between *chd1* and other transcriptional elongation factors (Simic et al., 2003; Zhang et al., 2005b). Physical interactions between Chd1 and transcription elongation factors (e.g., components of the yFACT complex, the Spt4-Spt5 complex, and the Rtf1 component of the Paf1 complex) indicates a role for Chd1 in regulating transcriptional elongation (Krogan et al., 2002; Lindstrom et al., 2003; Simic et al., 2003). Chromatin isolated from a *chd1*Δ strain of *S. cerevisiae* is hypersensitive to digestion by micrococcal nuclease. This signifies that Chd1 has a role in producing a repressive chromatin structure *in vivo* (Robinson and Schultz, 2003). Recently, a physical association between Chd1 and the SAGA/SLIK co-activator complex has been reported (Pray-Grant et al., 2005). Association of Chd1 with the SAGA/SLIK complex has been proposed to be important for histone acetylation that is dependent on histone H3-K4 methylation. However, another study failed to detect binding of Chd1 to methylated K4 (Sims et al., 2005).

Transcriptional regulation by architectural transcription factors

The architectural transcription factors are group of proteins that bend linear DNA or bind preferentially to bent or distorted DNA *in vitro*, and have a role *in vivo* in the assembly of the multiprotein complexes. These factors are relatively abundant in nature (about 1 molecule per 10–15 nucleosomes on average). Like histones, they bind to DNA without sequence specificity and were initially regarded as probable chromatin structural components. High mobility group (HMG) proteins are the best studied architectural transcription factors in vertebrates. There are two groups of HMG proteins in vertebrates, HMGA and HMGB. HMGAs are directly involved in the transcriptional control of

specific genes. They are key regulators of enhanceosome formation with the help of several other transcription factors (Bianchi and Agresti, 2005).

There are several mechanisms by which the HMGBs promote transcription of several genes. The HMGBs interact directly with nucleosomes and thereby may loosen the wrapped DNA in the nucleosome structure enhancing the accessibility of DNA sequences to the chromatin-remodeling complexes as well as to the other transcription factors. The interactions between HMGBs with TBP and other general transcription factors may also regulate expression of several genes *in vivo* (Bianchi and Agresti, 2005).

In *S. cerevisiae*, Nhp6a and Nhp6b are two architectural transcription factors of the HMGB family. Nhp6a and Nhp6b have 87% sequence identity (Kolodrubetz and Burgum, 1990), and are redundant in nature. Deletion of one of the copies of *NHP6* genes does not cause any phenotype. However, deletion of both copies of *NHP6* results in temperature sensitivity, and sensitivity to grow on media containing 6-AU (Formosa et al., 2001b; Kruppa et al., 2001; Yu et al., 2000). The temperature sensitive phenotype of the *nhp6a nhp6b* (*nhp6ab*) double mutants arises from a defect in transcription of the *SNR6* gene (coding for U6 small nuclear RNA) by RNA polymerase III (Kruppa et al., 2001). Nhp6 proteins directly facilitate the binding of TFIIIC and TBP to the *SNR6* promoter. Overexpression of Brf1, a subunit of the RNA polymerase III-specific general transcription factor TFIIIB, and an activating mutation in TFIIIC, were each found to restore *SNR6* transcription and to suppress the *nhp6ab* growth defect (Kruppa et al., 2001). Nhp6 also interacts with the RSC chromatin remodeling complex (Szerlong et al., 2003), and with the Ssn6/Tup 1 complex (Fragiadakis et al., 2004). Nhp6 also acts as a

fidelity factor for transcriptional initiation by RNA polymerase III (Kassavetis and Steiner, 2006b).

Nhp6 proteins also facilitate or repress the transcription of many RNA polymerase II-dependent genes by various mechanisms. Studies from our laboratory have shown that Nhp6 stimulates preinitiation complex formation both *in vitro* and *in vivo*. Nhp6 also combines with Spt16-Pob3 heterodimers in yeast to form the yFACT complex that binds to nucleosome *in vitro* (Formosa et al., 2001b). The nucleosomes with bound Nhp6 or the Spt16–Pob3–Nhp6 complex have an altered electrophoretic mobility and a distinct pattern of enhanced sensitivity to digestion by DNase I.

The yFACT complex and its role in transcription *in vivo*

The mammalian FACT complex was first identified as a factor that assisted RNA polymerase II transcription through a chromatin template in an *in vitro* transcription assay (Orphanides et al., 1998). The mammalian FACT complex is a heterodimer comprising the HMG-box containing protein SSRP1 and p140/hSpt16, a human homologue of the yeast Spt16 protein. The genetic and biochemical evidence suggest that the FACT complex is not just an elongation specific transcription factor, rather it is a general chromatin specific factor. For example, the *Xenopus laevis* homologue of the mammalian FACT complex termed DUF (DNA unwinding factor) was purified as an activity from oocyte extracts that is required for DNA replication (Okuhara et al., 1999). Also Pob3, the small subunit of the yeast FACT (yFACT) complex, was originally identified through its ability to bind to DNA polymerase α, which is involved in the initiation of DNA replication. Genetic studies demonstrated that mutations in *SPT16* and *POB3* genes show phenotypes that are relevant to both replication and transcription. The

yeast homologue of SSRP1, Pob3, lacks an HMG domain. Nhp6 protein serves the DNA binding function of the yeast Spt16-Pob3 complex. It is interesting to note that multiple Nhp6 molecules are required for yFACT recruitment to chromatin *in vitro*. Thus it is possible that Nhp6 acts as a loading factor for the Spt16-Pob3 complex onto nucleosomes, and that Nhp6 is not a stable subunit of yFACT. Nucleosome reorganization by yFACT is proposed to occur in two steps: first, by binding the Nhp6 proteins to nucleosomes and second, by targeting the Spt16-Pob3 complex to the nucleosome.

Several observations suggest a role for the yFACT complex in regulating the elongation step of transcription. Chromatin immunoprecipitation (ChIP) studies performed in yeast and *Drosophila* and immunostaining of *Drosophila* polytene chromosomes demonstrate that FACT subunits are present at actively transcribed genes, along with other transcription elongation factors such as Spt5 and Spt6. High-resolution ChIP analyses show that the Spt6 and FACT complex are recruited to the *Drosophila HSP* genes upon transcriptional induction and travel across the genes with elongating RNA polymerase II. ChIP experiments in yeast show that yFACT also travels with elongating RNA polymerase II upon transcriptional induction. In addition to genetics, ChIP, and immunolocalization analyses, proteomic studies suggest a connection between FACT and transcription elongation. Subunits of the yFACT complex interact physically with transcription elongation factors, such as the Spt4–Spt5 complex, Spt6, Chd1 and the Paf1 complex. However several lines of evidence suggest that the FACT complex may also have a role in transcriptional initiation. The SPT16 gene was initially identified as a factor which shows the Spt$^-$ phenotype upon over-expression or mutation. The Spt$^-$

phenotype results from aberrant TATA site utilization at the promoter region. The Drosophila FACT complex helps in GAGA factor recruitment at the promoter region of *HOX* genes (Shimojima et al., 2003). So, it is possible that the yFACT complex have a role in both transcriptional initiation and elongation steps.

Rationale for thesis research

This thesis research has been focused on studying the roles played by the yFACT complex in regulating transcriptional initiation. One of the critical steps of eukaryotic transcriptional initiation is formation of the TBP-TFIIA-TFIIB complex with DNA. Initially we began studying the role of the Nhp6 protein in regulating the TBP-TFIIA-TFIIB complex formation. Our initial observations suggested that several transcription factors that change the structure of chromatin also have a role in regulating *HO* transcription (McBride et al., 1997). These factors are encoded by the genes *SWI2*, and *NHP6A* and *NHP6B*. *SWI2* encodes the catalytic subunit of the Swi/Snf chromatin remodeling factor. We have shown that Nhp6 and Gcn5 activate transcription in parallel pathways (Yu et al., 2000). The yeast strains with deletion of either *NHP6* genes or *GCN5* are defective in *HO* expression. This defect in *HO* expression can be partially suppressed by overexpression of TBP (Yu et al., 2003), suggesting that Nhp6 and histone acetylation by Gcn5 promote TBP binding at the *HO* promoter.

There were a number of unanswered questions regarding the roles played by Nhp6. These questions were

1. What are the mechanisms of action of Nhp6, Gcn5 and the Swi/Snf chromatin remodeling complex, all of which have been shown to positively regulate *HO* expression *in vivo*?

2. How does Nhp6 regulate TBP binding in the context of chromatin? Earlier it was shown that a TATA site that is embedded into a nucleosome is refractory to TBP binding. Nhp6 may have a role in TBP binding as a part of the yFACT complex that reorganizes the nucleosome structure after binding.
3. What is the correlation between histone methylation and yFACT activity in regulating transcription? Our earlier genetic evidence suggested a positive correlation between the histone acetylation and the yFACT activity.

We were therefore interested in examining the functional correlation between the histone methylation and the yFACT activity.

Chapter 2 in this thesis describes a genetic screen for TBP mutants that are synthetically lethal with the absence of Nhp6 proteins. This chapter also shows that *SNR6* is the limiting component in an *nhp6ab* strain. Over-expression of *SNR6* from a multicopy plasmid suppresses several synthetic lethalities between TBP mutations and *nhp6ab*. Chapter 3 demonstrates that Nhp6 and two other transcriptional co-activators, Gcn5 in the SAGA complex and the Swi/Snf chromatin remodeling complex, work in the same pathway to promote formation of the TBP-TFIIA-DNA complex *in vivo*. In Chapter 4, we have explored the genetic relationship between Nhp6 and Gcn5 with Mot1 and the Ccr4/Not complex that were earlier shown to have negative roles in TBP binding. Our study suggested that in addition to having negative roles with TBP, Mot1 and the Ccr4/Not complex also have a positive role in regulating TBP binding at some promoters *in vivo*. In Chapter 5, using biochemical and genetic analysis, we show that yFACT has a role in formation of the TBP-TFIIA complex both *in vivo* and *in vitro*. In Chapter 6, the functional relationship between the histone methylation and the yFACT complex has

been described. We focused mainly on the functional relationship between the yFACT complex and the histone methylation at H3-K36 by Set2. Although regulation of transcription by histone methylation at H3-K4 has been studied for a long time, the role played by histone methylation at H3-K36 is poorly known. In Chapter 7, I have explored the functional relationship between yFACT and another ATP-dependent chromatin remodeler Chd1, in regulating transcriptional initiation. Chapter 8 summarizes all of these results on how the yFACT complex regulates transcriptional initiation in *S. cerevisiae*.

References

Alexander, D.E., Kaczorowski, D.J., Jackson-Fisher, A.J., Lowery, D.M., Zanton, S.J. and Pugh, B.F. (2004) Inhibition of TATA binding protein dimerization by RNA polymerase III transcription initiation factor Brf1. *J Biol Chem*, **279**, 32401-32406.

Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L. and Cote, J. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *Embo Journal*, **18**, 5108-5119.

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

Auble, D.T., Wang, D., Post, K.W. and Hahn, S. (1997) Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP-driven enzyme that dissociates TATA-binding protein from DNA. *Mol Cell Biol*, **17**, 4842-4851.

Badarinarayana, V., Chiang, Y.C. and Denis, C.L. (2000) Functional interaction of CCR4-NOT proteins with TATAA-binding protein (TBP) and its associated factors in yeast. *Genetics*, **155**, 1045-1054.

Bannister, A.J. and Kouzarides, T. (2005) Reversing histone methylation. *Nature*, **436**, 1103-1106.

Bannister, A.J., Schneider, R., Myers, F.A., Thorne, A.W., Crane-Robinson, C. and Kouzarides, T. (2005) Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. *J Biol Chem*, **280**, 17732-17736.

Basehoar, A.D., Zanton, S.J. and Pugh, B.F. (2004) Identification and distinct regulation of yeast TATA box-containing genes. *Cell*, **116**, 699-709.

Becker, P.B. and Horz, W. (2002) ATP-dependent nucleosome remodeling. *Annu Rev Biochem*, **71**, 247-273.

Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T. and Schreiber, S.L. (2002) Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc Natl Acad Sci U S A*, **99**, 8695-8700.

Bianchi, M.E. and Agresti, A. (2005) HMG proteins: dynamic players in gene regulation and differentiation. *Curr Opin Genet Dev*, **15**, 496-506.

Boa, S., Coert, C. and Patterton, H.G. (2003) *Saccharomyces cerevisiae* Set1p is a methyltransferase specific for lysine 4 of histone H3 and is required for efficient gene expression. *Yeast*, **20**, 827-835.

Bottomley, M.J. (2004) Structures of protein domains that create or recognize histone modifications. *EMBO Rep*, **5**, 464-469.

Brewster, N.K., Johnston, G.C. and Singer, R.A. (2001) A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. *Mol Cell Biol*, **21**, 3491-3502.

Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y., Winston, F. and Allis, C.D. (2001) Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes Dev*, **15**, 3286-3295.

Brownell, J.E. and Allis, C.D. (1995) An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in Tetrahymena macronuclei. *Proc Natl Acad Sci U S A*, **92**, 6364-6368.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y. and Allis, C.D. (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843-851.

Bryk, M., Briggs, S.D., Strahl, B.D., Curcio, M.J., Allis, C.D. and Winston, F. (2002) Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in S. cerevisiae by a Sir2-independent mechanism. *Curr Biol*, **12**, 165-170.

Buratowski, S. (2005) Connections between mRNA 3' end processing and transcription termination. *Curr Opin Cell Biol*, **17**, 257-261.

Cairns, B.R. (2005) Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr Opin Genet Dev*, **15**, 185-190.

Cang, Y., Auble, D.T. and Prelich, G. (1999) A new regulatory domain on the TATA-binding protein. *Embo Journal*, **18**, 6662-6671.

Carrozza, M.J., Florens, L., Swanson, S.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005a) Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochim Biophys Acta*, **1731**, 77-87; discussion 75-76.

Carrozza, M.J., Li, B., Florens, L., Suganuma, T., Swanson, S.K., Lee, K.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005b) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell*, **123**, 581-592.

Cheng, X., Collins, R.E. and Zhang, X. (2005) Structural and sequence motifs of protein (histone) methylation enzymes. *Annu Rev Biophys Biomol Struct*, **34**, 267-294.

Coleman, R.A., Taggart, A.K., Benjamin, L.R. and Pugh, B.F. (1995) Dimerization of the TATA binding protein. *J Biol Chem*, **270**, 13842-13849.

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

Costa, P.J. and Arndt, K.M. (2000) Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. *Genetics*, **156**, 535-547.

Cuthbert, G.L., Daujat, S., Snowden, A.W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P.D., Tempst, P., Bannister, A.J. and Kouzarides, T. (2004) Histone deimination antagonizes arginine methylation. *Cell*, **118**, 545-553.

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

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

de la Cruz, X., Lois, S., Sanchez-Molina, S. and Martinez-Balbas, M.A. (2005) Do protein motifs read the histone code? *Bioessays*, **27**, 164-175.

Dehe, P.M., Pamblanco, M., Luciano, P., Lebrun, R., Moinier, D., Sendra, R., Verreault, A., Tordera, V. and Geli, V. (2005) Histone H3 lysine 4 mono-methylation does not require ubiquitination of histone H2B. *J Mol Biol*, **353**, 477-484.

Deluen, C., James, N., Maillet, L., Molinete, M., Theiler, G., Lemaire, M., Paquet, N. and Collart, M.A. (2002) The Ccr4-not complex and yTAF1 (yTaf(II)130p/yTaf(II)145p) show physical and functional interactions. *Mol Cell Biol*, **22**, 6735-6749.

Denis, C.L. and Chen, J. (2003) The CCR4-NOT complex plays diverse roles in mRNA metabolism. *Prog Nucleic Acid Res Mol Biol*, **73**, 221-250.

Doyon, Y. and Cote, J. (2004) The highly conserved and multifunctional NuA4 HAT complex. *Curr Opin Genet Dev*, **14**, 147-154.

Exinger, F., and Lacroute, F. (1992). 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr Genet *22*, 9-11.

Fazzio, T.G., Kooperberg, C., Goldmark, J.P., Neal, C., Basom, R., Delrow, J. and Tsukiyama, T. (2001) Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol*, **21**, 6450-6460.

Feng, Q., Wang, H., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and Zhang, Y. (2002) Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr Biol*, **12**, 1052-1058.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y. and Stillman, D.J. (2001) Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *Embo Journal*, **20**, 3506-3517.

Formosa, T., Ruone, S., Adams, M.D., Olsen, A.E., Eriksson, P., Yu, Y., Rhoades, A.R., Kaufman, P.D. and Stillman, D.J. (2002) Defects in SPT16 or POB3 (yFACT) in *Saccharomyces cerevisiae* cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. *Genetics*, **162**, 1557-1571.

Fragiadakis, G.S., Tzamarias, D. and Alexandraki, D. (2004) Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for FRE2 transcriptional activation. *Embo Journal*, **23**, 333-342.

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

Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, G.M. and Tsukiyama, T. (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell*, **103**, 423-433.

Goppelt, A. and Meisterernst, M. (1996) Characterization of the basal inhibitor of class II transcription NC2 from *Saccharomyces cerevisiae*. *Nucleic Acids Res*, **24**, 4450-4455.

Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science*, **301**, 798-802.

Hassan, A.H., Prochasson, P., Neely, K.E., Galasinski, S.C., Chandy, M., Carrozza, M.J. and Workman, J.L. (2002) Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell*, **111**, 369-379.

Havas, K., Whitehouse, I. and Owen-Hughes, T. (2001) ATP-dependent chromatin remodeling activities. *Cell Mol Life Sci*, **58**, 673-682.

Hebbes, T.R., Thorne, A.W. and Crane-Robinson, C. (1988) A direct link between core histone acetylation and transcriptionally active chromatin. *Embo Journal*, **7**, 1395-1402.

Hiragami, K. and Festenstein, R. (2005) Heterochromatin protein 1: a pervasive controlling influence. *Cell Mol Life Sci*, **62**, 2711-2726.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S. and Young, R.A. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell*, **95**, 717-728.

Huisinga, K.L. and Pugh, B.F. (2004) A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell*, **13**, 573-585.

Joshi, A.A. and Struhl, K. (2005) Eaf3 Chromodomain Interaction with Methylated H3-K36 Links Histone Deacetylation to Pol II Elongation. *Mol Cell*, **20**, 971-978.

Kadosh, D. and Struhl, K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell*, **89**, 365-371.

Kadosh, D. and Struhl, K. (1998) Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. *Mol Cell Biol*, **18**, 5121-5127.

Kamada, K., Shu, F., Chen, H., Malik, S., Stelzer, G., Roeder, R.G., Meisterernst, M. and Burley, S.K. (2001) Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex. *Cell*, **106**, 71-81.

Kao, C.F., Hillyer, C., Tsukuda, T., Henry, K., Berger, S. and Osley, M.A. (2004) Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. *Genes Dev*, **18**, 184-195.

Kassavetis, G.A. and Steiner, D.F. (2006) Nhp6 Is a Transcriptional Initiation Fidelity Factor for RNA Polymerase III Transcription in Vitro and in Vivo. *J Biol Chem*, **281**, 7445-7451.

Kasten, M.M., Dorland, S. and Stillman, D.J. (1997) A large protein complex containing the yeast Sin3p and Rpd3p transcriptional regulators. *Mol Cell Biol*, **17**, 4852-4858.

Keogh, M.C., Kurdistani, S.K., Morris, S.A., Ahn, S.H., Podolny, V., Collins, S.R., Schuldiner, M., Chin, K., Punna, T., Thompson, N.J., Boone, C., Emili, A., Weissman, J.S., Hughes, T.R., Strahl, B.D., Grunstein, M., Greenblatt, J.F., Buratowski, S. and Krogan, N.J. (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell*, **123**, 593-605.

Kim, M., Ahn, S.H., Krogan, N.J., Greenblatt, J.F. and Buratowski, S. (2004) Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. *Embo Journal*, **23**, 354-364.

Kim, T.K., Zhao, Y., Ge, H., Bernstein, R. and Roeder, R.G. (1995) TATA-binding protein residues implicated in a functional interplay between negative cofactor NC2 (Dr1) and general factors TFIIA and TFIIB. *J Biol Chem*, **270**, 10976-10981.

Kimura, A. and Horikoshi, M. (2004) Partition of distinct chromosomal regions: negotiable border and fixed border. *Genes Cells*, **9**, 499-508.

Kimura, A., Matsubara, K. and Horikoshi, M. (2005) A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. *J Biochem (Tokyo)*, **138**, 647-662.

Kobor, M.S. and Greenblatt, J. (2002) Regulation of transcription elongation by phosphorylation. *Biochim Biophys Acta*, **1577**, 261-275.

Kolodrubetz, D. and Burgum, A. (1990) Duplicated NHP6 genes of *Saccharomyces cerevisiae* encode proteins homologous to bovine high mobility group protein 1. *J Biol Chem*, **265**, 3234-3239.

Krogan, N.J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Ryan, O.W., Golshani, A., Johnston, M., Greenblatt, J.F. and Shilatifard, A. (2003a) The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol Cell*, **11**, 721-729.

Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R.A., Pootoolal, J., Tong, A., Canadien, V., Richards, D.P., Wu, X., Emili, A., Hughes, T.R., Buratowski, S. and Greenblatt, J.F. (2003b) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell*, **12**, 1565-1576.

Krogan, N.J., Kim, M., Ahn, S.H., Zhong, G., Kobor, M.S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S. and Greenblatt, J.F. (2002) RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. *Mol Cell Biol*, **22**, 6979-6992.

Krogan, N.J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D.P., Beattie, B.K., Emili, A., Boone, C., Shilatifard, A., Buratowski, S. and Greenblatt, J. (2003c) Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol*, **23**, 4207-4218.

Kruppa, M., Moir, R.D., Kolodrubetz, D. and Willis, I.M. (2001) Nhp6, an HMG1 protein, functions in SNR6 transcription by RNA polymerase III in S. cerevisiae. *Mol Cell*, **7**, 309-318.

Kuo, M.H., vom Baur, E., Struhl, K. and Allis, C.D. (2000) Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol Cell*, **6**, 1309-1320.

Kurdistani, S.K. and Grunstein, M. (2003) Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol*, **4**, 276-284.

Kurdistani, S.K., Tavazoie, S. and Grunstein, M. (2004) Mapping global histone acetylation patterns to gene expression. *Cell*, **117**, 721-733.

Landry, J., Sutton, A., Hesman, T., Min, J., Xu, R.M., Johnston, M. and Sternglanz, R. (2003) Set2-catalyzed methylation of histone H3 represses basal expression of GAL4 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **23**, 5972-5978.

Laribee, R.N., Krogan, N.J., Xiao, T., Shibata, Y., Hughes, T.R., Greenblatt, J.F. and Strahl, B.D. (2005) BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. *Curr Biol*, **15**, 1487-1493.

Lee, D.Y., Teyssier, C., Strahl, B.D. and Stallcup, M.R. (2005) Role of protein methylation in regulation of transcription. *Endocr Rev*, **26**, 147-170.

Li, B., Howe, L., Anderson, S., Yates, J.R., 3rd and Workman, J.L. (2003) The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J Biol Chem*, **278**, 8897-8903.

Lindstrom, D.L., Squazzo, S.L., Muster, N., Burckin, T.A., Wachter, K.C., Emigh, C.A., McCleery, J.A., Yates, J.R., 3rd and Hartzog, G.A. (2003) Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. *Mol Cell Biol*, **23**, 1368-1378.

Liu, H.Y., Badarinarayana, V., Audino, D.C., Rappsilber, J., Mann, M. and Denis, C.L. (1998) The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. *Embo Journal*, **17**, 1096-1106.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389**, 251-260.

Margueron, R., Trojer, P. and Reinberg, D. (2005) The key to development: interpreting the histone code? *Curr Opin Genet Dev*, **15**, 163-176.

Marmorstein, R. (2003) Structure of SET domain proteins: a new twist on histone methylation. *Trends Biochem Sci*, **28**, 59-62.

Martin, C. and Zhang, Y. (2005) The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol*, **6**, 838-849.

Martinez-Campa, C., Politis, P., Moreau, J.L., Kent, N., Goodall, J., Mellor, J. and Goding, C.R. (2004) Precise nucleosome positioning and the TATA box dictate

requirements for the histone H4 tail and the bromodomain factor Bdf1. *Mol Cell*, **15**, 69-81.

Mason, P.B. and Struhl, K. (2003) The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol*, **23**, 8323-8333.

Matangkasombut, O., Buratowski, R.M., Swilling, N.W. and Buratowski, S. (2000) Bromodomain factor 1 corresponds to a missing piece of yeast TFIID. *Genes Dev*, **14**, 951-962.

McBride, H.J., Brazas, R.M., Yu, Y., Nasmyth, K. and Stillman, D.J. (1997) Long-range interactions at the HO promoter. *Mol Cell Biol*, **17**, 2669-2678.

Mellor, J. and Morillon, A. (2004) ISWI complexes in *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, **1677**, 100-112.

Mermelstein, F., Yeung, K., Cao, J., Inostroza, J.A., Erdjument-Bromage, H., Eagelson, K., Landsman, D., Levitt, P., Tempst, P. and Reinberg, D. (1996) Requirement of a corepressor for Dr1-mediated repression of transcription. *Genes Dev*, **10**, 1033-1048.

Miller, T., Krogan, N.J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., Greenblatt, J.F. and Shilatifard, A. (2001) COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. *Proc Natl Acad Sci U S A*, **98**, 12902-12907.

Mohrmann, L. and Verrijzer, C.P. (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim Biophys Acta*, **1681**, 59-73.

Moreau, J.L., Lee, M., Mahachi, N., Vary, J., Mellor, J., Tsukiyama, T. and Goding, C.R. (2003) Regulated displacement of TBP from the PHO8 promoter in vivo requires Cbf1 and the Isw1 chromatin remodeling complex. *Mol Cell*, **11**, 1609-1620.

Morillon, A., Karabetsou, N., Nair, A. and Mellor, J. (2005) Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. *Mol Cell*, **18**, 723-734.

Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N. and Mellor, J. (2003) Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell*, **115**, 425-435.

Naar, A.M., Lemon, B.D. and Tjian, R. (2001) Transcriptional coactivator complexes. *Annu Rev Biochem*, **70**, 475-501.

Ng, H.H., Dole, S. and Struhl, K. (2003a) The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J Biol Chem*, **278**, 33625-33628.

Ng, H.H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y. and Struhl, K. (2002) Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev*, **16**, 1518-1527.

Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2003b) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell*, **11**, 709-719.

Nislow, C., Ray, E. and Pillus, L. (1997) SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. *Mol Biol Cell*, **8**, 2421-2436.

Noma, K. and Grewal, S.I. (2002) Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. *Proc Natl Acad Sci U S A*, **99 Suppl 4**, 16438-16445.

Nourani, A., Utley, R.T., Allard, S. and Cote, J. (2004) Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *Embo Journal*, **23**, 2597-2607.

Oberholzer, U. and Collart, M.A. (1999) In vitro transcription of a TATA-less promoter: negative regulation by the Not1 protein. *Biol Chem*, **380**, 1365-1370.

Okuhara, K., Ohta, K., Seo, H., Shioda, M., Yamada, T., Tanaka, Y., Dohmae, N., Seyama, Y., Shibata, T. and Murofushi, H. (1999) A DNA unwinding factor involved in DNA replication in cell-free extracts of Xenopus eggs. *Curr Biol*, **9**, 341-350.

Orphanides, G., LeRoy, G., Chang, C.H., Luse, D.S. and Reinberg, D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell*, **92**, 105-116.

Orphanides, G. and Reinberg, D. (2000) RNA polymerase II elongation through chromatin. *Nature*, **407**, 471-475.

Orphanides, G., Wu, W.H., Lane, W.S., Hampsey, M. and Reinberg, D. (1999) The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature*, **400**, 284-288.

Palancade, B. and Bensaude, O. (2003) Investigating RNA polymerase II carboxyl-terminal domain (CTD) phosphorylation. *Eur J Biochem*, **270**, 3859-3870.

Pokholok, D.K., Hannett, N.M. and Young, R.A. (2002) Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. *Mol Cell*, **9**, 799-809.

Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E., Zeitlinger, J., Lewitter, F., Gifford,

D.K. and Young, R.A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell*, **122**, 517-527.

Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, **433**, 434-438.

Pugh, B.F. (2000) Control of gene expression through regulation of the TATA-binding protein. *Gene*, **255**, 1-14.

Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J. and Madhani, H.D. (2005) Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell*, **123**, 233-248.

Rao, B., Shibata, Y., Strahl, B.D. and Lieb, J.D. (2005) Dimethylation of histone H3 at lysine 36 demarcates regulatory and nonregulatory chromatin genome-wide. *Mol Cell Biol*, **25**, 9447-9459.

Reinberg, D., Orphanides, G., Ebright, R., Akoulitchev, S., Carcamo, J., Cho, H., Cortes, P., Drapkin, R., Flores, O., Ha, I., Inostroza, J.A., Kim, S., Kim, T.K., Kumar, P., Lagrange, T., LeRoy, G., Lu, H., Ma, D.M., Maldonado, E., Merino, A., Mermelstein, F., Olave, I., Sheldon, M., Shiekhattar, R., Zawel, L. and et al. (1998) The RNA polymerase II general transcription factors: past, present, and future. *Cold Spring Harb Symp Quant Biol*, **63**, 83-103.

Robinson, K.M. and Schultz, M.C. (2003) Replication-independent assembly of nucleosome arrays in a novel yeast chromatin reconstitution system involves antisilencing factor Asf1p and chromodomain protein Chd1p. *Mol Cell Biol*, **23**, 7937-7946.

Roth, S.Y., Denu, J.M. and Allis, C.D. (2001) Histone acetyltransferases. *Annu Rev Biochem*, **70**, 81-120.

Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M. and Grunstein, M. (1998) Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. *Nature*, **392**, 831-835.

Ruone, S., Rhoades, A.R. and Formosa, T. (2003) Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. *J Biol Chem*, **278**, 45288-45295.

Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J. and Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature*, **419**, 407-411.

Santos-Rosa, H., Schneider, R., Bernstein, B.E., Karabetsou, N., Morillon, A., Weise, C., Schreiber, S.L., Mellor, J. and Kouzarides, T. (2003) Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. *Mol Cell*, **12**, 1325-1332.

Saunders, A., Werner, J., Andrulis, E.D., Nakayama, T., Hirose, S., Reinberg, D. and Lis, J.T. (2003) Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. *Science*, **301**, 1094-1096.

Schlesinger, M.B. and Formosa, T. (2000) POB3 is required for both transcription and replication in the yeast *Saccharomyces cerevisiae*. *Genetics*, **155**, 1593-1606.

Schneider, J., Wood, A., Lee, J.S., Schuster, R., Dueker, J., Maguire, C., Swanson, S.K., Florens, L., Washburn, M.P. and Shilatifard, A. (2005) Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. *Mol Cell*, **19**, 849-856.

Shahbazian, M.D., Zhang, K. and Grunstein, M. (2005) Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Mol Cell*, **19**, 271-277.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A. and Casero, R.A. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, **119**, 941-953.

Shi, Y.J., Matson, C., Lan, F., Iwase, S., Baba, T. and Shi, Y. (2005) Regulation of LSD1 histone demethylase activity by its associated factors. *Mol Cell*, **19**, 857-864.

Simic, R., Lindstrom, D.L., Tran, H.G., Roinick, K.L., Costa, P.J., Johnson, A.D., Hartzog, G.A. and Arndt, K.M. (2003) Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo Journal*, **22**, 1846-1856.

Sims, R.J., 3rd, Belotserkovskaya, R. and Reinberg, D. (2004) Elongation by RNA polymerase II: the short and long of it. *Genes Dev*, **18**, 2437-2468.

Sims, R.J., 3rd, Chen, C.F., Santos-Rosa, H., Kouzarides, T., Patel, S.S. and Reinberg, D. (2005) Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *J Biol Chem*, **280**, 41789-41792.

Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41-45.

Strahl, B.D., Grant, P.A., Briggs, S.D., Sun, Z.W., Bone, J.R., Caldwell, J.A., Mollah, S., Cook, R.G., Shabanowitz, J., Hunt, D.F. and Allis, C.D. (2002) Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol Cell Biol*, **22**, 1298-1306.

Sudarsanam, P., Iyer, V.R., Brown, P.O. and Winston, F. (2000) Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*, **97**, 3364-3369.

Suka, N., Suka, Y., Carmen, A.A., Wu, J. and Grunstein, M. (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. *Mol Cell*, **8**, 473-479.

Sun, Z.W. and Allis, C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature*, **418**, 104-108.

Syntichaki, P., Topalidou, I. and Thireos, G. (2000) The Gcn5 bromodomain co-ordinates nucleosome remodelling. *Nature*, **404**, 414-417.

Szerlong, H., Saha, A. and Cairns, B.R. (2003) The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *Embo Journal*, **22**, 3175-3187.

Tran, H.G., Steger, D.J., Iyer, V.R. and Johnson, A.D. (2000) The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *Embo Journal*, **19**, 2323-2331.

Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J. and Wu, C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev*, **13**, 686-697.

Utley, R.T., Ikeda, K., Grant, P.A., Cote, J., Steger, D.J., Eberharter, A., John, S. and Workman, J.L. (1998) Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature*, **394**, 498-502.

van Leeuwen, F., Gafken, P.R. and Gottschling, D.E. (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell*, **109**, 745-756.

Vignali, M., Hassan, A.H., Neely, K.E. and Workman, J.L. (2000) ATP-dependent chromatin-remodeling complexes. *Mol Cell Biol*, **20**, 1899-1910.

Wang, A., Kurdistani, S.K. and Grunstein, M. (2002) Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science*, **298**, 1412-1414.

Wang, W. (2003) The SWI/SNF family of ATP-dependent chromatin remodelers: similar mechanisms for diverse functions. *Curr Top Microbiol Immunol*, **274**, 143-169.

Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.H., Perlin, J.R., Leonelli, L., Sonbuchner, L.S., McDonald, C.H., Cook, R.G., Dou, Y., Roeder, R.G., Clarke, S., Stallcup, M.R.,

Allis, C.D. and Coonrod, S.A. (2004) Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science*, **306**, 279-283.

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

Winkler, G.S., Kristjuhan, A., Erdjument-Bromage, H., Tempst, P. and Svejstrup, J.Q. (2002) Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo. *Proc Natl Acad Sci U S A*, **99**, 3517-3522.

Wittmeyer, J., Joss, L. and Formosa, T. (1999) Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. *Biochemistry*, **38**, 8961-8971.

Wood, A., Krogan, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F., Johnston, M. and Shilatifard, A. (2003a) Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol Cell*, **11**, 267-274.

Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2003b) The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J Biol Chem*, **278**, 34739-34742.

Woodage, T., Basrai, M.A., Baxevanis, A.D., Hieter, P. and Collins, F.S. (1997) Characterization of the CHD family of proteins. *Proc Natl Acad Sci U S A*, **94**, 11472-11477.

Wu, J., Suka, N., Carlson, M. and Grunstein, M. (2001) TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. *Mol Cell*, **7**, 117-126.

Xiao, B., Wilson, J.R. and Gamblin, S.J. (2003a) SET domains and histone methylation. *Curr Opin Struct Biol*, **13**, 699-705.

Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H. and Strahl, B.D. (2003b) Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes Dev*, **17**, 654-663.

Xiao, T., Kao, C.F., Krogan, N.J., Sun, Z.W., Greenblatt, J.F., Osley, M.A. and Strahl, B.D. (2005) Histone H2B ubiquitylation is associated with elongating RNA polymerase II. *Mol Cell Biol*, **25**, 637-651.

Xie, J., Collart, M., Lemaire, M., Stelzer, G. and Meisterernst, M. (2000) A single point mutation in TFIIA suppresses NC2 requirement in vivo. *Embo Journal*, **19**, 672-682.

Xu, F., Zhang, K. and Grunstein, M. (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell*, **121**, 375-385.

Yu, Y., Eriksson, P., Bhoite, L.T. and Stillman, D.J. (2003) Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. *Mol Cell Biol*, **23**, 1910-1921.

Yu, Y., Eriksson, P. and Stillman, D.J. (2000) Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. *Mol Cell Biol*, **20**, 2350-2357.

Zhang, H., Roberts, D.N. and Cairns, B.R. (2005a) Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell*, **123**, 219-231.

Zhang, L., Schroeder, S., Fong, N. and Bentley, D.L. (2005b) Altered nucleosome occupancy and histone H3K4 methylation in response to 'transcriptional stress'. *Embo Journal*, **24**, 2379-2390.

Zhang, Y. and Reinberg, D. (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev*, **15**, 2343-2360.

CHAPTER 2

TATA-BINDING PROTEIN MUTANTS THAT ARE LETHAL IN THE ABSENCE OF Nhp6 HIGH MOBILITY GROUP PROTEIN

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Molecular and Cellular Biology 2004 Jul;24(14):6419-29.

MOLECULAR AND CELLULAR BIOLOGY, July 2004, p. 6419–6429 Vol. 24, No. 14
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.14.6419–6429.2004
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TATA-Binding Protein Mutants That Are Lethal in the Absence of the Nhp6 High-Mobility-Group Protein

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Received 26 November 2003/Returned for modification 9 January 2004/Accepted 20 April 2004

The *Saccharomyces cerevisiae* Nhp6 protein is related to the high-mobility-group B family of architectural DNA-binding proteins that bind DNA nonspecifically but bend DNA sharply. Nhp6 is involved in transcriptional activation by both RNA polymerase II (Pol II) and Pol III. Our previous genetic studies have implicated Nhp6 in facilitating TATA-binding protein (TBP) binding to some Pol II promoters in vivo, and we have used a novel genetic screen to isolate 32 new mutations in TBP that are viable in wild-type cells but lethal in the absence of Nhp6. The TBP mutations that are lethal in the absence of Nhp6 cluster in three regions: on the upper surface of TBP that may have a regulatory role, near residues that contact Spt3, or near residues known to contact either TFIIA or Brf1 (in TFIIIB). The latter set of mutations suggests that Nhp6 becomes essential when a TBP mutant compromises its ability to interact with either TFIIA or Brf1. Importantly, the synthetic lethality for some of the TBP mutations is suppressed by a multicopy plasmid with *SNR6* or by an *spt3* mutation. It has been previously shown that *nhp6ab* mutants are defective in expressing *SNR6*, a Pol III-transcribed gene encoding the U6 splicing RNA. Chromatin immunoprecipitation experiments show that TBP binding to *SNR6* is reduced in an *nhp6ab* mutant. Nhp6 interacts with Spt16/Pob3, the yeast equivalent of the FACT elongation complex, consistent with *nhp6ab* cells being extremely sensitive to 6-azauracil (6-AU). However, this 6-AU sensitivity can be suppressed by multicopy *SNR6* or *BRF1*. Additionally, strains with *SNR6* promoter mutations are sensitive to 6-AU, suggesting that decreased *SNR6* RNA levels contribute to 6-AU sensitivity. These results challenge the widely held belief that 6-AU sensitivity results from a defect in transcriptional elongation.

The TATA-binding protein (TBP) is required for all eukaryotic transcription, whether the genes are transcribed by RNA polymerase I (Pol I), Pol II, or Pol III (28). TBP binding to promoters of genes transcribed by Pol II correlates with transcriptional activity; therefore, regulation of TBP binding may be the critical event in determining transcriptional activation (36, 40). Full-length TBP binds DNA slowly in a multistep process (29, 69), and crystallographic studies demonstrate that TBP bends DNA sharply upon binding (46, 47). Transcriptional activation by Pol II requires the assembly of a complex of general transcription factors at a promoter (18, 27). It is believed that transcriptional coactivators function by stimulating DNA binding by TBP and facilitating formation of a complex between TBP and the general transcription factors TFIIA and TFIIB.

The Spt3 factor can regulate TBP binding to promoters. Spt3, which is part of the SAGA histone acetyltransferase complex (63), physically interacts with TBP and plays an important role in transcriptional start site selection for RNA Pol II (19). Spt3 can act as a positive or negative transcriptional regulator, depending on the promoter (5, 17, 67). Although Spt3 acts to promote TBP binding at the *GAL1* promoter (17), we have shown that Spt3 inhibits TBP binding to *HO* (67). Genetic interactions have been seen between *SPT3* and other regulators of TBP binding (4, 13, 43).

The *Saccharomyces cerevisiae* Nhp6 protein is similar to the high-mobility-group B class of architectural DNA-binding proteins (1). Nhp6 is an abundant protein (50), and it has multiple roles in transcription, including transcriptional initiation and elongation by Pol II and promoting transcription by Pol III. For its role in elongation, Nhp6 interacts genetically and biochemically with Spt16/Pob3 (10, 22), the yeast equivalent of FACT that promotes elongation through chromatin templates (49). Nhp6 is required for Spt16/Pob3 to bind to nucleosomes (22, 54).

Nhp6 is encoded by two genes, *NHP6A* and *NHP6B*, that are functionally redundant, as only the *nhp6ab* double mutants show any observable phenotype. *nhp6ab* mutants are unable to grow at 37°C, but this growth defect can be suppressed by a multicopy plasmid with either *SNR6* or *BRF1* (35). *SNR6* encodes the U6 RNA required for mRNA splicing, and it is suggested that a deficiency in *SNR6* RNA contributes to the temperature-sensitive growth defect seen in *nhp6ab* mutants. Brf1 is the limiting component in TFIIIB, a factor required for Pol III transcription (56); therefore, *BRF1* overexpression could increase *SNR6* expression and facilitate growth of the *nhp6ab* mutant at 37°C. Overexpression of TBP also suppresses the temperature-sensitive growth defect of an *nhp6ab* mutant (67). In addition to its well-documented role in Pol II transcription, TBP is also a component of the RNA Pol III factor TFIIIB and is required for Pol III transcription (33). Thus, TBP overexpression could suppress the *nhp6ab* growth defect by affecting either Pol II or Pol III transcription. Data

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from Paull et al. (50) suggest that Nhp6 stimulates transcription by promoting formation of preinitiation complexes.

Our genetic studies suggest that Nhp6 and the Gcn5 histone acetyltransferase function in parallel to activate expression of the yeast *HO* gene (67, 68). A *gcn5 nhp6a nhp6b* triple mutant is extremely sick, but this growth defect can be suppressed by mutations in *SIN4*, *SPT3*, or *SPT8*. The data suggested that TBP was the critical target, as overexpression of TBP suppresses the temperature-sensitive growth defect of *nhp6ab* mutants and partially restores *HO* expression in the absence of either Nhp6 or Gcn5. In this study, we continue the genetic analysis examining the relationship between Nhp6 and TBP. We performed a novel synthetic lethal screen to identify strains with TBP mutations that are unable to grow in the absence of Nhp6. We also find that a multicopy plasmid with *SNR6* can suppress some of the TBP *nhp6ab* synthetic lethalities. Our results suggest that Nhp6 and TBP work together to facilitate transcriptional activation by both Pol II and Pol III.

MATERIALS AND METHODS

Strains and media. All *S. cerevisiae* strains used are listed in Table 1 and are isogenic in the W303 background (65). Standard genetic methods were used for strain construction (53, 59). W303 strains with disruptions in *gcn5*, *nhp6a*, *nhp6b*, and *swi2* have been described previously (67, 68). The TBP-Myc-tagged allele (51) was provided by Rick Young and crossed to generate the strains used here. Cells were grown in yeast extract-peptone-dextrose (YEPD) medium (59) at 30°C, except where the use of other temperatures is noted, or where synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids, as appropriate, but lacking essential components was used to select for plasmids. Medium containing 5-fluoroorotic acid (5-FOA) was prepared as described previously (8).

Plasmids. All plasmids used are listed in Table 2, except for the TBP mutant plasmids, which were all YCp-*TRP1* plasmids. A 1.2-kb XhoI-PstI fragment with *SNR6* from pRS314-*SNR6*, provided by Dave Brow, was cloned into pRS425 and pRS422, constructing M4479 and M4488, respectively. A 3.8-kb BamHI fragment with *ADE3* from plasmid pTF96, provided by Tim Formosa, was cloned into BamHI-cleaved plasmid pDE28-6 (19), generating M4373.

Screen for TBP mutants. Strain DY7244 was transformed with a library of mutagenized TBP genes on YCp-*TRP1* plasmids (2), generously provided by Karen Arndt, and we screened for solid red (nonsectoring) colonies that could not lose the wild-type TBP gene (the YCp-*URA3-ADE3-SPT15* plasmid). The solid red colonies were plated onto fresh plates to verify the nonsectoring phenotype, and growth on medium containing FOA confirmed the inability of these strains to lose the YCp-*ADE3-URA3*-TBP plasmid. The majority of these nonsectoring colonies contained lethal TBP mutations, and these mutations were eliminated by mating the strain to strain DY7472. Plasmids which produced a nonsectoring and 5-FOA-sensitive phenotype in the *NHP6/nhp6ab* diploid were discarded, while plasmids with a sectored, 5-FOA-resistant phenotype were retained. Plasmids were purified after passage through *Escherichia coli*, retransformed into *nhp6ab* and *NHP6*$^+$ strains to verify the phenotypes, and then sequenced.

ChIP. Chromatin immunoprecipitation (ChIP) was performed as described previously (7) using either 9E11 monoclonal antibody to the Myc epitope or polyclonal anti-TBP serum provided by Laurie Stargell. Multiplex PCR was performed with oligonucleotides specific to the *HO* and *PGK1* promoter and the *TRA1* open reading frame, and products were visualized on ethidium bromide-stained 2.6% MetaPhor agarose (BioWhittaker) gels. Quantitative ChIP was performed by real-time PCR with a LightCycler (Roche) and primers specific to *SNR6* or *TRA1*. The amounts of specific target DNA regions amplified in immunoprecipitated samples were determined with LightCycler software (version 3.5; Roche) by comparing the PCR logarithmic amplification threshold (crossing point) values for ChIP DNAs versus a standard dilution series of input samples prior to IP. Each PCR was performed in triplicate, and the normalized mean and standard deviation of the ratio of *SNR6* to *TRA1* values was calculated according to equation 7 of van Kempen and van Vliet (66) to determine the relative enrichment of the specific target versus the nontarget control. Results of at least two independent ChIP reactions are reported here. The sequences of the PCR primers are as follows: for *SNR6*, CTGGCATGAACAGTGGTAAA and GGGGAACTGCTGATCATCTC; for *TRA1*, CTGAGTATGCACTATGGGAA and CTTGATCTCCTTTTCTGCTT; and for *PGK1*, CCAGAGCAAAGTTCGTTCGA and GCTTGTCCTTCAAGTCCAAA.

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Relevant genotype
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY151	*MAT*α *ade2 can1 his3 leu2 trp1 ura3*
DY2381	*MAT*α *nhp6a*::*URA3 nhp6b*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY3398	*MAT***a** *ade2 can1 his3 leu2 trp1*
DY6439	*MAT*α *nhp6a*::*URA3 nhp6b*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY7242	*MAT***a** *spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 ade3 can1 his3 leu2 trp1 ura3*
DY7244	*MAT*α *nhp6a*::*KanMX nhp6b*::*HIS3 spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 ade3 can1 his3 leu2 lys2 trp1 ura3*
DY7472	*MAT***a** *spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7474	*MAT***a** *spt3*::*ADE2 spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7588	*MAT***a** *nhp6a*::*URA3 nhp6b*::*ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7723	*MAT***a** *nhp6a*::*KanMX nhp6b*::*HIS3 spt3*::*ADE2 spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8162	*MAT*α *nhp6a*::*KanMX nhp6b*::*HIS3 spt15*::*ADE2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 ade3 can1 his3 leu2 lys2 trp1 ura3*
DY8356	*MAT***a** *nhp6a*::*KanMX nhp6b*::*HIS3 spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8360	*MAT***a** *SPT15*-MYC(18)::*TRP1 nhp6a*::*KanMX nhp6b*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY8408	*MAT***a** *SPT15*-MYC(18)::*TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8409	*MAT*α *SPT15*-MYC(18)::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY8855	*MAT***a** *nhp6a*::*KanMX nhp6b*::*ADE2 spt3*::*TRP1 ade2 can1 his3 leu2 trp1*
DY8858	*MAT*α *snr6*::*LEU2* + *SNR6*-wild type(YCp-*TRP1*) *ade2 can1 his3 leu2 lys2 met2 trp1*
DY8859	*MAT*α *snr6*::*LEU2* + *SNR6*-Δ42(YCp-*TRP1*) *ade2 can1 his3 leu2 lys2 met2 trp1*
DY8860	*MAT*α *snr6*::*LEU2* + *SNR6*-T14A(YCp-*TRP1*) *ade2 can1 his3 leu2 lys2 met2 trp1*
DY8861	*MAT*α *snr6*::*LEU2* + *SNR6*-T5-flip(YCp-*TRP1*) *ade2 can1 his3 leu2 lys2 met2 trp1*
DY8980	*MAT***a** *spt3*::*TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*

Other methods. For the *SNR6* Northern blot, RNA was separated on an 8% polyacrylamide gel containing 8 M urea, transferred to a Biodyne B nylon membrane (Pall), and probed with labeled oligonucleotides specific for *SNR6* (CCTTATGCAGGGGAACTGCTGATC) and *SNR128* (CCGAGAGTACTAACGATGGGTTCGTAAGCGTACTCC) RNAs. The Northern blots were quantitated using ImageQuant software and a Molecular Dynamics PhosphorImager. The Hi Lithium method (31) was used for yeast transformations. For dilution

TABLE 2. Plasmids used in this study[a]

Plasmid	Description	Reference or source
pRS422	YEp-*ADE2* vector	9
pRS423	YEp-*HIS3* vector	60
pRS425	YEp-*LEU2* vector	60
pRS314	YCp-*TRP1* vector	60
YEplac195	YEp-*URA3* vector	25
pRS426-*SNR6*	*SNR6* in YEp-*URA3* plasmid	Dave Brow
M4479	*SNR6* in YEp-*LEU2* plasmid	This work
M4488	*SNR6* in YEp-*ADE2* plasmid	This work
pRS423/B70	*BRF1* in YEp-*HIS3* plasmid	56
pDE28-6	TBP (*SPT15*) in YCp-*URA3* plasmid	19
pTM8	TBP (*SPT15*) in YCp-*TRP1* plasmid	34
M4373	TBP (*SPT15*) in YCp-*URA3-ADE3* plasmid	This work
pSH346	TFIIA (*TOA1* and *TOA2*) in YEp-*LEU2* plasmid	Steve Hahn

[a] All plasmids used in this study except for the TBP mutant plasmids which were all YCp-*TRP1*.

plating assays, cells were grown to saturation in either rich or selective medium (depending on the plasmid) and washed with water, and then aliquots of diluted samples (10-fold dilutions) were plated on appropriate medium.

RESULTS

Reduced TBP binding to the *SNR6* promoter in *nhp6ab* mutant cells. To investigate whether Nhp6 affects TBP binding to promoters in vivo, we performed ChIP assays to compare TBP binding in *NHP6* and *nhp6ab* cells. We used PCR to examine the amount of DNA from a number of promoters transcribed by Pol II, including *HO*, *HIS3*, *TRP3*, *URA1*, *ELP3*, *STE3*, *ADH2*, *MET14*, *MFA2*, *SER3*, *VID28*, *VPS38*, and *XRS2*. However, none of these promoters showed a strong difference in TBP binding when immunoprecipitated material from wild-type and *nhp6ab* cells were compared (data not shown). It is possible that the *nhp6ab* mutation affects TBP binding but only modestly or that TBP binding to other Pol II promoters that we have not tested is affected.

We next examined TBP binding to the *SNR6* promoter in the *nhp6ab* mutant. Reduced expression of *SNR6*, transcribed by RNA Pol III, in an *nhp6ab* mutant is at least partially responsible for the 37°C growth defect, as a multicopy plasmid with *SNR6* can at least partially suppress this growth defect (35, 42). We constructed isogenic strains with a TBP-Myc-tagged allele, either *NHP6* or *nhp6ab*, and performed ChIP experiments. The experiment was done in duplicate, using cells grown at 30°C. Real-time PCR was used to quantitate the amount of *SNR6* DNA in the immunoprecipitated chromatin. As shown in Fig. 1A, TBP-Myc shows strong binding to *SNR6*, compared to the untagged control strains. Importantly, binding of TBP-Myc to *SNR6* was significantly reduced in the *nhp6ab* mutant.

The TBP-Myc-tagged *nhp6ab* strain shows a marked growth defect, and we have previously reported synthetic lethality between *nhp6ab* and hemagglutinin-tagged TBP alleles (67). Therefore, we wanted to confirm that TBP binding to *SNR6* is reduced in the *nhp6ab* mutant, but using a polyclonal TBP antibody instead of an epitope-tagged strain. *nhp6ab* mutants do not grow at 37°C, and Kruppa et al. (35) showed that shifting an *nhp6ab* strain to 37°C results in reduced *SNR6* RNA levels. Thus, we grew wild-type and *nhp6ab* cells at 30°C and then shifted them to 37°C for 60 min. More than 85% of *nhp6ab* cells retain viability after 60 min at 37°C. Samples were taken for ChIP analysis both before and after the temperature shift. In addition to testing for TBP binding to *SNR6*, TBP binding to the *PGK1* promoter and *TRA1* open reading frame were assessed as positive and negative controls, respectively. The ChIP material was analyzed by multiplex PCR, with products separated on gels. The results show clearly that the occupancy of the *SNR6* TATA element by TBP is Nhp6 dependent (Fig. 1B). Quantitation of the *SNR6* ChIP signal, normalized to the *PGK1* control, shows TBP binding to *SNR6* of 3 to 5.5 binding units (arbitrary units) of that in wild-type cells, while TBP binding to *SNR6* is reduced in the *nhp6ab* mutant to

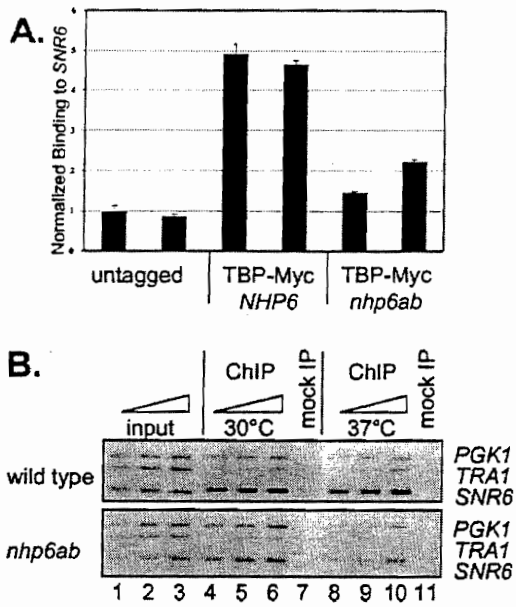

FIG. 1. TBP binding to *SNR6* is reduced in *nhp6ab* mutant. (A) ChIP was performed with untagged strains, TBP-Myc-tagged *NHP6* strains, and TBP-Myc-tagged *nhp6ab* strains. Real-time PCR quantitation of TBP-Myc binding to the *SNR6* gene shows reduced binding in the *nhp6ab* mutant. The units are arbitrary units after normalization to a *TRA1* internal control. Strains DY150, DY151, DY8408, DY8409, DY8360, and DY8361 were used. (B) After an aliquot of *NHP6* and *nhp6ab* strains grown at 30°C were harvested, the remaining cells were shifted to 37°C for 60 min and then harvested. Chromatin samples were prepared and immunoprecipitated with polyclonal anti-TBP antibody. Serial twofold dilutions were used to assess binding of native TBP to *PGK1* (positive control), *TRA1* (negative control), and *SNR6* by multiplex PCR. Lanes 1 to 3, input controls; lanes 4 to 6, ChIP from 30°C samples; lane 7, mock IP from 30°C sample; lanes 8 to 10, ChIP from 37°C samples; lane 11, mock IP from 37°C sample. Strains DY151 and DY2381 were used.

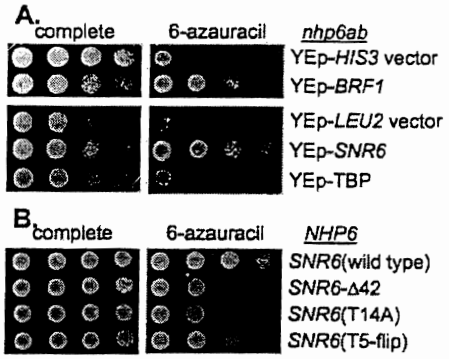

FIG. 2. Multicopy *BRF1* or *SNR6* suppresses the 6-AU sensitivity of *nhp6ab* mutants. (A) Dilutions of strain DY6439 transformed with the indicated plasmid were plated on plates containing complete medium or medium containing 6-AU (25 μg/ml) and incubated at 30°C for 2 days (complete medium) or 6 days (medium containing 6-AU). Plasmids pRS423 (YEp-*HIS3* vector), pRS423/B70 (YEp-*BRF1*), pRS425 (YEp-*LEU2* vector), M4479 (YEp-*SNR6*), and M4480 (YEp-TBP) were used. (B) Dilutions of strains DY8858 (*SNR6* wild type), DY8859 (*SNR6*-Δ42), DY8860 (*SNR6*-T14A), and DY8861 (*SNR6*-T5-flip), were plated on plates containing complete medium or medium containing 6-AU (75 μg/ml) and incubated at 30°C for 2 days (complete medium) or 6 days (medium containing 6-AU).

about 1 binding unit, the *SNR6*/*PGK1* ratio seen in the input DNA. We conclude that TBP binding to *SNR6* is reduced in an *nhp6ab* mutant.

Multicopy *SNR6* and *BRF1* suppress the 6-AU sensitivity of *nhp6ab* mutants. The uracil analog 6-azauracil (6-AU) inhibits transcriptional elongation by causing imbalances in the pools of ribonucleotide triphosphates (20, 57), and some believe that mutants sensitive to 6-AU are defective in elongation. An *nhp6ab* mutant is very sensitive to 6-AU (10, 22), and a concentration of 25 μg of 6-AU per ml is sufficient to effectively inhibit growth (Fig. 2A). We wanted to know whether specific genes on multicopy plasmids will suppress this 6-AU sensitivity.

Nhp6 is required for efficient transcription of the *SNR6* gene by RNA Pol III (35, 42, 44). *SNR6*, the gene for U6 RNA, and *BRF1*, encoding a Pol III transcription factor, act as multicopy suppressors of the temperature-sensitive phenotype of *nhp6ab* mutants (35). A multicopy plasmid with TBP also suppresses the temperature-sensitive growth defect of an *nhp6ab* mutant (67). We determined whether *SNR6*, *BRF1*, or TBP, when present on a multicopy plasmid, could suppress 6-AU sensitivity of the *nhp6ab* mutant (Fig. 2). The results show strong suppression from either overexpression of the *BRF1* Pol III factor or increased copies of the *SNR6* structural gene. This is a surprising result, suggesting that decreased *SNR6* expression contributes to the 6-AU sensitivity. Interestingly, overexpression of TBP does not suppress the 6-AU sensitivity.

It is possible that reduced *SNR6* RNA levels contribute to the sensitivity to 6-AU, as the steady-state levels of *SNR6* RNA are lower in an *nhp6ab* mutant than in wild-type cells (42). To test this idea, we constructed strains with mutations in the *SNR6* promoter that reduce expression, and these mutations result in a 6-AU-sensitive phenotype (Fig. 2B). Interestingly, all three of these *SNR6* promoter mutations are lethal in the absence of Nhp6 (44). It is possible that the decrease in *SNR6* RNA affects mRNA splicing, and this results in 6-AU sensitivity (see Discussion). Nonetheless, these experiments clearly show that sensitivity to 6-AU can result from a mutation in a gene not having a direct role in transcriptional elongation.

Screen for TBP mutations that are lethal in the absence of Nhp6. Cells containing TBP with a hemagglutinin epitope tag, whether the tag is at the N or C terminus, are viable and healthy, but the tagged versions of TBP are lethal in an *nhp6ab* mutant (67). Similarly, TBP(K138T Y139A), TBP(G174E), and TBP(F237D) mutants are viable in an *NHP6*$^+$ strain but lethal in an *nhp6ab* mutant (67). We wondered what other types of TBP mutations would not be tolerated in the absence of Nhp6, and we have taken two approaches. First, we set up a screen to look for TBP point mutations that are specifically lethal in an *nhp6ab* strain. Second, we tested a variety of TBP mutations that had been characterized in other labs.

We used a red and white sectoring assay (6) to identify specific TBP mutations. We constructed an *ade2 ade3 spt15::LEU2* strain containing a YCp plasmid with three genes, the wild-type TBP gene and two nutritional markers, *URA3* and *ADE3*. The *SPT15* gene, which encodes TBP, is essential for viability, so this strain cannot lose the YCp-*ADE3-URA3-SPT15* plasmid, and the strains are red in color and sensitive to 5-FOA. This strain was transformed with a PCR-mutagenized TBP library on a YCp-*TRP1* vector (2), and we screened for solid red (nonsectoring) colonies (Fig. 3). The majority of these nonsectoring colonies contain lethal TBP mutations, and these mutants were eliminated by mating to an *NHP6*$^+$ strain and testing the resulting *NHP6*$^+$/*nhp6ab* diploids for sectoring and 5-FOA sensitivity. Finally, all of the candidate *TRP1* plasmids with TBP mutations that were synthetic lethal with *nhp6ab* were purified and retransformed into *nhp6ab spt15* and *NHP6*$^+$ *spt15* strains to verify that the mutations are specifically lethal with *nhp6ab*.

We screened 8,500 colonies transformed with mutagenized TBP plasmids and recovered 32 TBP mutations that are lethal in the absence of Nhp6 (Fig. 4). These mutants were sequenced, and all but five had a single amino acid substitution (Table 3). We also screened 33 TBP mutants previously characterized by other investigators and identified 11 additional TBP mutations that are lethal in the absence of TBP (Table 3; examples in Fig. 5A). As described below in the Discussion, these mutations cluster in three regions, near where TBP interacts with TFIIA (clusters 1, 2, and 3), where TBP interacts with Spt3 (clusters 5), and on the upper surface where the TBP core may interact with TBP-associated factors or the N-terminal region of TBP (cluster 4 [Fig. 4]). The TBP mutations that are tolerated in the *nhp6ab* mutant are interesting, and they include substitutions that eliminate interaction with TFIIB (E186L, E186 M, E188A, and L189A [38; S. Buratowski, unpublished data]) or TFIIA (K133L and K133L/K138L [11]), substitutions that cause defective DNA binding (V71A, S118L, F148H, N159D, N159L, and V161A [2, 38, 61]), substitutions that cause defective transcriptional activation (V71A, S118L, F148H, T153I, N159D, N159L, V161A, and E236P [2, 37]), and substitutions that are lethal in the presence of a Taf1 truncation (S118L, N159D, N159L, V161A, and E336P [34]).

Interestingly, two of this group of TBP point mutations that

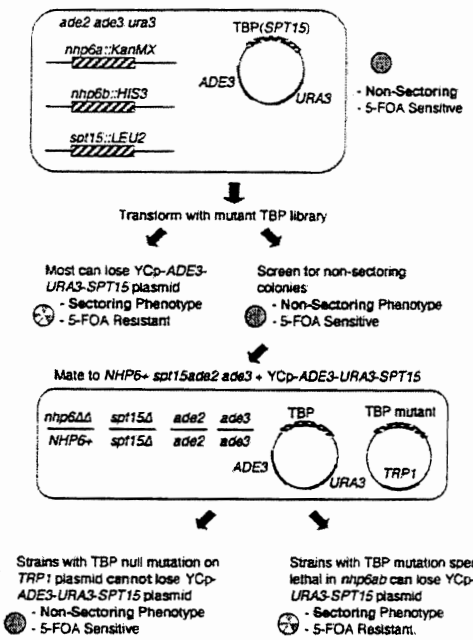

FIG. 3. Screen for TBP mutants specifically lethal in an *nhp6ab* mutant. Strain DY7244 (*MATα nhp6a nhp6b spt15 ade2 ade3 ura3* with a YCp-*URA3-ADE3-SPT15* plasmid) was transformed with a YCp-*TRP1* library of TBP mutants. Nonsectoring colonies were identified, and then mated to strain DY7244 (*MATa NHP6A NHP6B spt15 ade2 ade3 ura3* with a YCp-*URA3-ADE3-SPT15* plasmid), and the resulting diploids were tested for sectoring and 5-FOA growth phenotypes. Diploid strains giving sectoring and 5-FOA-resistant phenotypes contain a TBP plasmid specifically lethal in an *nhp6ab* mutant.

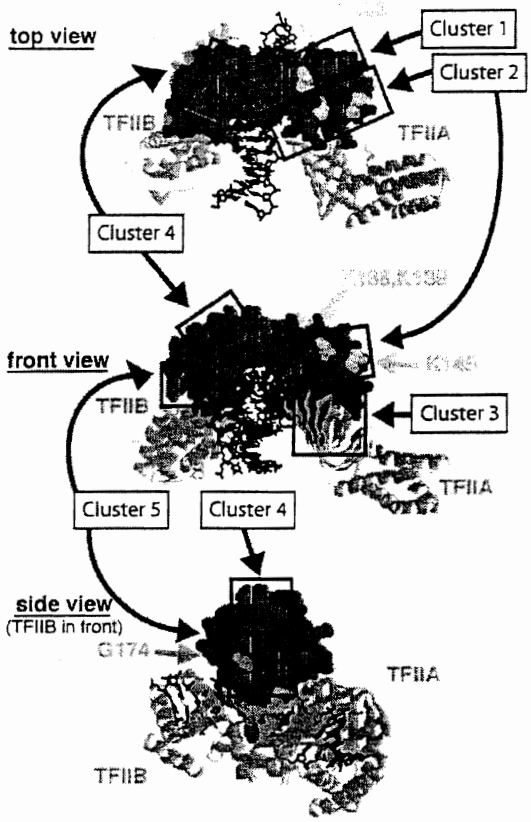

FIG. 4. TBP mutations on the TBP core structure. The structures of TBP (purple), TFIIB (light blue), TFIIA (green), and DNA (black) are shown. Mutations in TBP residues shown in red, yellow, and green are lethal in an *nhp6ab* mutant. The red residues have been previously shown to affect TBP interaction with TFIIA, and the G174 residue that contacts Spt3 is shown in green. Site-directed mutations created on the TBP surface that eliminate interaction with TFIIB are shown in orange, and these mutations are viable in an *nhp6ab* mutant. The clusters of mutations are described in the text. SwissPDB Viewer version 3.7 (26) was used to merge the TFIIB-TBP-DNA 1VOL (48) and TFIIA-TBP-DNA 1YTF (64) crystal structures into a single PDB file, and the positions of mutations on the resulting structure were visualized with RasMac version 2.6 (55).

are tolerated in the absence of Nhp6 affect interaction with TFIIA, while other TBP mutations affecting TFIIA binding are lethal in the *nhp6ab* mutant (Table 3) (67). For example, the K133L K145L double mutation is lethal in the *nhp6ab* mutant, but cells with the K133L single mutation in TBP are viable. The K133L K145L double mutation causes defective interaction with TFIIA in vitro, and this mutant shows a much stronger growth defect than the K133L single mutant (11). We suggest that cells with the K133L mutation have only a modest defect in interaction with TFIIA and thus tolerate the lack of Nhp6. Interestingly, while cells with the K133L mutation are viable in the absence of Nhp6, the K133R mutation recovered in the screen is lethal in the *nhp6ab* strain. It may be that replacement of lysine with the larger arginine residue interferes with TFIIA interaction.

Suppression by multicopy *SNR6*. TBP interacts with TFIIA and TFIIB, and transcriptional activation requires formation of a TBP-TFIIA-TFIIB-DNA complex. We therefore asked whether a multicopy plasmid with either *SUA7*, encoding TFIIB, or the two genes encoding the TFIIA subunits, *TOA1* and *TOA2*, could suppress the synthetic lethality of TBP alleles in the *nhp6ab* mutants. We tested more than half of the TBP mutants for multicopy suppression, but none of them were suppressed by either YEp-TFIIA or YEp-TFIIB. Since the 6-AU sensitivity of *nhp6ab* mutants could be suppressed by multicopy *SNR6* (Fig. 2), we determined whether YEp-*SNR6* could suppress the synthetic lethality from combining *nhp6ab* with TBP point mutations. We tested 12 TBP alleles and found that for 8 alleles a multicopy plasmid with the *SNR6* gene allowed cells to grow on 5-FOA (Table 3; examples in Fig. 5B). There is no obvious correlation between the location of the TBP substitution on the structure (Fig. 4) and the ability to be suppressed by YEp-*SNR6*.

Suppression by *spt3* mutation. Spt3 physically interacts with TBP, and Spt3 acts to promote or inhibit TBP binding, de-

TABLE 3. TBP mutations lethal in the absence of Nhp6

Substitution(s)	Cluster location(s) of substitution	Lethality in *nhp6ab* mutant suppressed by:		Source of mutant
		*spt3*Δ	YEp-*SNR6*	
Single mutations				
P65S	Cluster 4	No effect	ND	34
L67Q	Cluster 1	ND[a]	ND	This work
K83E	Cluster 3	ND	ND	This work
E93G	Cluster 3	No effect	No effect	This work
Y94C	Cluster 3	ND	ND	This work
P109A	Cluster 2	ND	ND	2
L114F	Cluster 5	Suppression	ND	3
E129G	Cluster 1	ND	Suppression	This work
E129V	Cluster 1	ND	ND	This work
K133R	Cluster 1	ND	Suppression	This work
K138L	Cluster 2	ND	ND	11
I142N	Cluster 2	ND	ND	This work
Q144K	Cluster 2	ND	ND	This work
K145L	Cluster 2	ND	ND	11
G147W	Cluster 2	Suppression	No effect	This work
K151E	Cluster 1	ND	ND	This work
F152S	Cluster 1	ND	ND	This work
F155K	Cluster 1	ND	ND	This work
I157T	Cluster 1	ND	ND	This work
C164W	Cluster 4	No effect	No effect	This work
C164Y	Cluster 4	ND	ND	This work
L172P	Cluster 5	Suppression	Suppression	This work
R173G	Cluster 5	No effect	ND	This work
G174E	Cluster 5	Partial suppression	Partial suppression	19
K211E[b]		Suppression	ND	This work
R220H	Cluster 1	No effect	ND	34
Q225P	Cluster 4	ND	ND	This work
F227L	Cluster 4	Suppression	ND	This work
F227S	Cluster 4	ND	ND	This work
E228K	Cluster 4	ND	ND	This work
Y231A	Cluster 4	No effect	ND	34
F237D	Cluster 5	No effect	ND	34
F237L	Cluster 5	Suppression	ND	This work
K239E	Cluster 5	No effect	ND	This work
K239T	Cluster 5	ND	Suppression	This work
K239Stop	Cluster 5	ND	ND	This work
Double mutations[c]				
F18L K110K[d]	Cluster 2	ND	ND	This work
K97R L193S	Cluster 3, 5	Suppression	Suppression	This work
I103T K239Stop	Cluster 3, 5	ND	Partial suppression	This work
E108G Y231H	Cluster 2, 5	ND	ND	This work
K120I C164Y	Cluster 4, 4	No effect	ND	This work
K133L K145L	Cluster 1, 2	Suppression	Partial suppression	11
K138T Y139A	Cluster 2, 2	Partial suppression	No effect	62

[a] ND, not determined.
[b] K211 does not fall within any of the clusters.
[c] For the double mutations, the cluster locations of the substitutions are shown in the same order as the substitutions.
[d] F18 is not present in the TBP crystal structure.

pending on the promoter (17, 19, 67). Additionally, an *spt3* mutation can suppress growth defects in both *nhp6a nhp6b* and *gcn5 nhp6a nhp6b* strains (67). On the basis of these results, we examined whether an *spt3* gene disruption can suppress the synthetic lethality observed between *nhp6* and mutant TBP alleles, using an *spt15 nhp6a nhp6b spt3* strain with wild-type TBP on a YCp-*URA3* plasmid. This strain was transformed with the *TRP1* plasmids carrying various mutant versions of TBP, and the transformed strains were then plated on medium containing 5-FOA to determine whether the cells were viable after loss of the YCp-*URA3* plasmid with wild-type TBP. Although we did not test all of the TBP alleles that are lethal in the *nhp6ab* mutant, we determined that for 10 of the 19 TBP alleles tested, the *spt3* mutation suppressed the synthetic lethality with *nhp6ab* (Table 3). As shown in Fig. 5C, the synthetic lethality of the F227L mutant with *nhp6ab* is strongly suppressed by *spt3*, and the F237L mutant is suppressed to a lesser extent. Again, there is no correlation between suppression by *spt3* and the position of the TBP substitution on the structure (Fig. 4).

A number of the TBP mutants show temperature-sensitive growth in an otherwise wild-type (e.g., *NHP6*$^+$) strain. We decided to determine whether an *spt3* mutation would affect the growth characteristics of these TBP mutants. Two isogenic

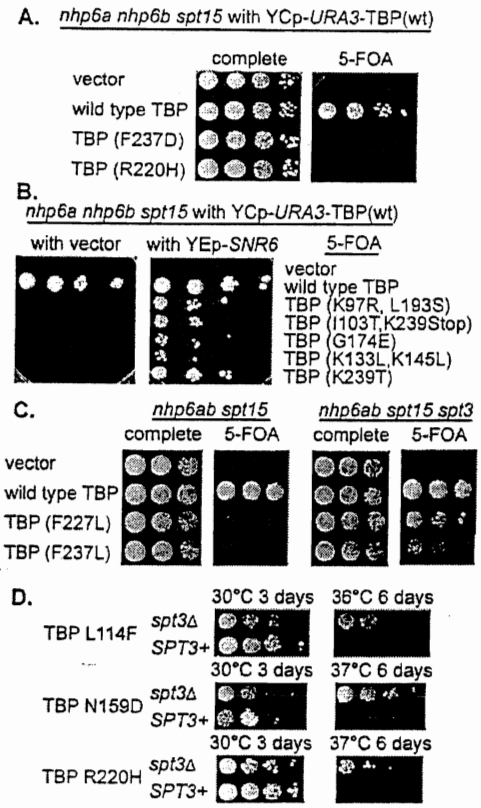

FIG. 5. TBP mutants synthetic lethal with *nhp6ab* can be suppressed by YEp-*SNR6* or by *spt3*. (A) Examples of synthetic lethality between TBP mutants and *nhp6ab*. Dilutions of strain DY7244 (*nhp6ab spt15* with a YCp-*URA3-TBP* plasmid [with the wild-type {wt} TBP gene]) transformed with the indicated TBP mutant plasmids were plated on complete medium or medium containing 5-FOA and incubated at 30°C for 3 days. Plasmids pRS314 (vector), pTM8 (with the wild-type TBP gene), YCp-TBP(F237D), and YCp-TBP(R220H) were used. (B) Strain DY7244 (*nhp6ab spt15*) was transformed with two plasmids, a *TRP1* plasmid with the indicated TBP mutant and either pRS422 (YEp-*HIS3* vector) or M4488 (YEp-*SNR6*), and dilutions of transformed strains were plated on medium containing 5-FOA and incubated at 30°C for 5 days. (C) An *SPT3* gene disruption suppresses the TBP *nhp6ab* synthetic lethality for TBP(F227L) and TBP(F237L). Strain DY7723 (*nhp6ab spt3 spt15*) was transformed with the indicated plasmids, and dilutions were plated on medium containing 5-FOA and incubated at 30°C for 2 days (complete) or 4 days (5-FOA). (D) An *SPT3* gene disruption suppresses the temperature sensitivity of TBP mutants. Dilutions of strains DY7474 (*NHP6 spt3 spt15*) and DY7472 (*NHP6 spt15*) transformed with plasmids YCp-TBP(L114F), YCp-TBP(N159D), or YCp-TBP(R220H) were plated on medium containing 5-FOA and incubated at 30°C for 3 days or at 36 or 37°C for 6 days.

*spt15*Δ strains containing the YCp-*ADE3-URA3-SPT15* plasmid were constructed, one *SPT3*$^+$ and the other *spt3*$^-$. The two strains were transformed with 15 plasmids with TBP alleles and plated at various temperatures on medium containing 5-FOA to assess the ability of these strains to grow in the absence of the plasmid with wild-type TBP. As shown in Fig. 5D, the L114F, N159D, and R220H mutants grow on 5-FOA at 30°C in both *SPT3* and *spt3* strains, although colony size is reduced in the *spt3* mutants. All three of these TBP mutants are unable to grow on 5-FOA at an elevated temperature when the *SPT3* gene is present. Interestingly, an *spt3* mutation allows these TBP mutants to grow at the higher temperature. Similar suppression of temperature-sensitive growth was also seen for L172P and R173G mutants and the K97R L193S and K120I C164Y double mutants (data not shown). These suppression results are consistent with the suggestion that Nhp6 and Spt3 affect TBP function in opposing ways (67).

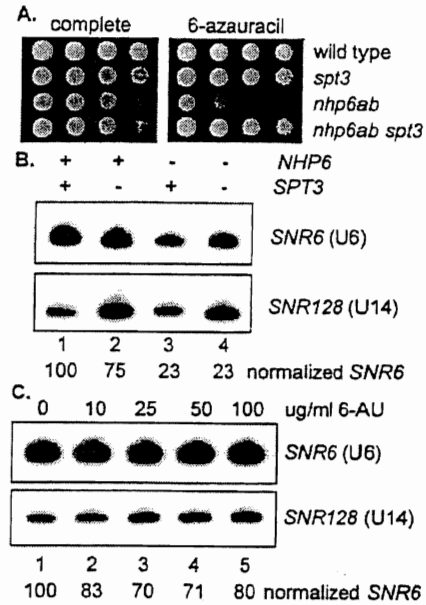

FIG. 6. The 6-azauracil-sensitive phenotype in *nhp6ab* strains can be suppressed by *spt3*. (A) Dilutions of strains DY3398 (wild type), DY8980 (*spt3*), DY7588 (*nhp6ab*), and DY8855 (*nhp6ab spt3*) were plated on complete medium or medium containing 6-AU (25 μg/ml) and incubated at 30°C for 3 days (complete medium) or 4 days (medium containing 6-AU). (B) *SPT3* does not regulate *SNR6* expression. RNA levels for *SNR6* and *SNR128* (internal control) were determined by Northern blot hybridization. RNA was isolated from strains DY3398 (wild type), DY7588 (*nhp6ab*), DY8980 (*spt3*), and DY8855 (*nhp6ab spt3*). (C) 6-AU causes a modest reduction in *SNR6* levels. RNA levels for *SNR6* and *SNR128* (internal control) were determined by Northern blot hybridization. RNA was isolated from strain DY3398 (wild type) grown for 3 h at 30°C in synthetic complete medium lacking uracil containing the indicated amount of 6-AU (in micrograms per milliliter).

Since an *spt3* gene disruption can suppress many *nhp6ab* phenotypes, we also asked whether this suppression extends to the 6-AU sensitivity of *nhp6ab* mutants. As shown in Fig. 6A, the *nhp6ab* strain is very sensitive to 6-AU, but this effect is largely suppressed in the *nhp6ab spt3* strain. This result is surprising, because the data in Fig. 2 suggest that the 6-AU sensitivity in the *nhp6ab* strain is largely due to the decreased expression of *SNR6*, a Pol III-transcribed gene. However, there is no known role of *SPT3* in Pol III transcription, although the

function of *SPT3* in Pol II transcription is well documented. To examine this question, we measured *SNR6* RNA levels by Northern hybridization in isogenic strains (Fig. 6B). *SNR6* RNA levels are reduced to 23% in the *nhp6ab* mutant, and this value is unchanged in the *nhp6ab spt3* strain. Additionally, *SNR6* RNA levels are not increased in the *spt3* single mutant, compared to the wild type. In wild-type strains, growth in the presence of 6-AU results in a modest decrease in *SNR6* RNA levels (Fig. 6C). In summary, the decrease in *SNR6* RNA levels in the *nhp6ab* strain may contribute to the strong 6-AU sensitivity. However, while the *spt3* mutation restores growth of the *nhp6ab* mutant on 6-AU, *spt3* does not suppress the *nhp6ab* defect in *SNR6* RNA levels.

DISCUSSION

Nhp6 is an abundant protein of the high-mobility-group B family of transcription factors that has been implicated in transcriptional activation by both RNA Pol II and Pol III. TBP is required for transcription by all three eukaryotic RNA polymerases. In this study, we used a novel genetic screen to identify substitutions in TBP that are viable, but lethal in the absence of Nhp6. The TBP *nhp6ab* synthetic lethality for a number of the TBP mutants can be suppressed by deletion of the *SPT3* gene. Spt3 is known to interact directly with TBP, and *spt3* mutations affect Pol II transcription start site selection (19), suggesting that Nhp6 contributes to the activation of Pol II transcription by TBP. Nhp6 also plays an important role in transcription of the *SNR6* gene by RNA Pol III, and TBP binding to *SNR6* is reduced in an *nhp6ab* mutant. A multicopy plasmid with *SNR6* suppresses the 6-AU sensitivity seen in *nhp6ab* mutants, as well as the lethality of some TBP substitutions in *nhp6ab* strains. Promoter mutations that reduce *SNR6* expression also cause 6-AU sensitivity, suggesting that decreased levels of the *SNR6* gene product contribute to sensitivity to this postulated inhibitor of transcriptional elongation. Our results suggest that Nhp6 and TBP work together to promote both Pol II and Pol III transcription.

TBP substitution mutants. The 43 TBP mutations that are synthetic and lethal in combination with *nhp6ab* cluster in interesting regions of TBP. Figure 4 shows the structure of the core of TBP, along with TFIIB, TFIIA, and DNA, merged from two separate structure determinations (48, 64) as a top view, a front view, and a 90° rotated view. New substitutions isolated in our screen that are lethal in the absence of Nhp6 are shown in red. The residues that have been previously shown to affect TBP interaction with TFIIA (K133, K138, Y139, and K145) are shown in yellow. In the TBP-TFIIA-DNA crystallographic structures, none of these four TBP residues make direct contact with TFIIA (24, 64). Only a small part of TFIIA was successfully crystallized with TBP and DNA, and it is assumed that full-length TFIIA does indeed touch these contact residues in TBP. We show that all of these previously characterized TBP double mutants (K133L K138L, K133L K145L, and K138T Y139A) that affect interaction with TFIIA are lethal in an *nhp6ab* mutant but viable in an *NHP6* strain. Residue K133 in TBP makes important contacts with TFIIA, as binding to TFIIA is lost in either the K133L K138L or K143 K145L double mutant (11, 62). We also recovered a single substitution at this position, K133R, that is lethal in the absence of Nhp6. On the top surface of TBP, K133 is part of a continuous line of substitutions, E129, K151, F152,F155, I157, and R220, that are synthetic and lethal with *nhp6ab*, and we describe these mutations as cluster 1 (top view in Fig. 4). Cluster 2 is a group of substitutions surrounding the other residues required for TFIIA interaction. K145 at the right edge of TBP, and slightly to the left are adjacent residues K138 and K139 (front view in Fig. 4). New substitutions at Q144 and G147 are just above K145, and the E108, P109, K110, and I142 mutations surround K138 and K139. The cluster 3 group of mutations, K83, E93, Y94, K97, and I103, are at the interface between TBP and TFIIA defined by the crystallographic studies. Clusters 1, 2, and 3 represent regions that probably interact with TFIIA, and some interesting patterns emerge for substitutions here. An *spt3* deletion suppresses the *nhp6ab* TBP synthetic lethality for two of four mutants tested, and three of five mutants tested can be suppressed by YEp-*SNR6*. These results show the important role of *SPT3* and *SNR6* in Nhp6 and TBP function, as discussed below.

Cluster 4 is a group of substitutions lethal in *nhp6ab*, including Q225, F227, E228, and Y231, that are on the upper left surface of TBP (top view in Fig. 4). Cluster 4 substitutions are adjacent to the P232 and V233 mutations that were identified in a screen for TBP mutations that affect NC2 binding (12) and adjacent to A226 where three mutations that specifically affect Pol II transcription were identified (14). It is not clear what this region of TBP interacts with, but one possibility is this region interacts with factors that regulate TBP binding, such as NC2, Mot1, the Not-Ccr4 complex, and TBP-associated factors (39, 52).

Cluster 5 is a collection of substitutions near G174, a residue that contacts Spt3 (shown in green in Fig. 4). Surrounding G174, we recovered clustered mutations at L172, R173, F237, and K239. Interestingly, we recovered three independent substitutions at K239. There are some interesting phenotypes common to most of the mutations in cluster 5. The synthetic lethality with *nhp6ab* for most of these TBP mutations can be suppressed by a *spt3* deletion. Interestingly, F237L is suppressed by *spt3*, but not the F237D substitution at the same position. L172P and G174E can be suppressed by *spt3*, but not the R173G mutant at the intervening position. YEp-*SNR6* suppresses the *nhp6ab* TBP synthetic lethality for all of the cluster 5 substitutions tested. Below cluster 5 are three residues shown in orange. These are positions where site-directed mutations were created on the TBP surface that eliminate interaction with TFIIB (38), based on the TFIIB-TBP-DNA cocrystal. These substitutions had no additional growth defect in an *nhp6ab* strain.

Nhp6 and Pol III transcription. Experiments suggest that Nhp6 is required for efficient transcription of the *SNR6* gene by Pol III (35, 42, 44). *SNR6* encodes the U6 RNA required for mRNA splicing, and it is suggested that a deficiency in *SNR6* RNA contributes to the temperature-sensitive growth defect seen in *nhp6ab* mutants. Brf1 is the limiting component in TFIIIB, a factor required for Pol III transcription (56); therefore, *BRF1* overexpression could increase *SNR6* expression and facilitate growth of the *nhp6ab* mutant at 37°C. Overexpression of TBP also suppresses the temperature-sensitive growth defect of an *nhp6ab* mutant (67). Additionally, TBP overexpression suppresses the defect in *HO* transcription by

RNA Pol II in an *nhp6ab* strain (67). In addition to its well-documented role in Pol II transcription, TBP is also a component of the RNA Pol III factor TFIIIB and is required for Pol III transcription (33). Thus, TBP overexpression could suppress the *nhp6ab* growth defect by affecting Pol II or Pol III transcription.

Several studies have defined how TBP interacts with Pol III transcription factors. Cormack and Struhl (14) mutagenized TBP, isolated substitutions with a temperature-sensitive phenotype, and identified 65 TBP mutants specifically defective in Pol III transcription. Although some of these residues also showed up in our screen for TBP *nhp6ab* synthetic lethality, including P65, E129, E133, Q144, F152, F155, I157, R220, Y231, and F237, not all substitutions confer the same phenotypes. For example, while F237P and F237L substitutions specifically affected Pol III transcription, F237R specifically affected Pol II, and the defects of the F237D substitution were not specific to any polymerase. Additional TBP residues important for Pol III transcription were identified through studies examining the interaction of TBP mutants with the Brf1 Pol III transcription factor (58) by the TBP-Brf1 cocrystal (32). Interestingly, all of the residues in TFIIA that are required for TFIIA-TBP interaction (K133, K138, Y139, and K145) are also required for TBP interaction with Brf1. In summary, the regions defined by clusters 1, 2, and 3 are involved in TBP binding to both TFIIA and Brf1. The fact that these substitutions are lethal in an *nhp6ab* mutant suggests that Nhp6 becomes essential when a substitution reduces that ability of TBP to interact with TFIIA or Brf1.

Several of our experiments reinforce the role for Nhp6 in Pol III transcription. ChIP experiments show strong binding of TBP to the *SNR6* gene, transcribed by Pol III, and TBP binding to *SNR6* is markedly reduced in the absence of Nhp6. *nhp6ab* mutants are extremely sensitive to 6-AU, and this could be suppressed by multicopy plasmids with either the Pol III factor *BRF1* or the Pol III target gene *SNR6*. Finally, multicopy plasmids with *SNR6* suppress the *nhp6ab* TBP synthetic lethality for several mutants, suggesting that a defect in Pol III transcription is partially responsible for the *nhp6ab* TBP synthetic lethality.

The suppression of the 6-AU sensitivity in *nhp6ab* mutants is particularly interesting. The 6-AU inhibitor affects the ribonucleotide triphosphate pools, and many transcriptional elongation mutants are sensitive to 6-AU (20, 57). Nhp6 interacts with the transcriptional elongation factor yFACT (Spt16/Pob3); therefore, the 6-AU sensitivity of *nhp6ab* mutants was attributed to a defect in transcriptional elongation (10, 22). We find that this 6-AU sensitivity can be suppressed by either *SNR6* or *BRF1* on a multicopy plasmid (Fig. 2A) or by disruption of the *SPT3* gene (Fig. 6A). Although an *spt3* mutation suppresses the 6-AU sensitivity caused by *nhp6ab*, *spt3* does not suppress the *nhp6ab* defect in *SNR6* expression (Fig. 6B).

It is not clear why an *spt3* gene deletion can suppress *nhp6ab*, in terms of sensitivity to 6-AU, but it does not suppress the defect in *SNR6* expression. An *spt3* mutation does not cause an increase in *SNR6* RNA levels, and an *spt3* strain is resistant to 6-AU (Fig. 6). However, an *spt3* mutation, by itself, has been reported to cause sensitivity to 6-AU in a different strain background (16). Previously, *SPT3* has never been shown to have a role in Pol III transcription, and *spt3* mutations have been observed to affect only RNA Pol II transcription. Thus, it is possible that the *spt3* suppression of the *nhp6ab* 6-AU sensitivity is an indirect effect of altered gene expression by RNA Pol II.

Nonetheless, our data show clearly that 6-AU sensitivity results from decreased *SNR6* expression, whether because of a *SNR6* promoter mutation or an *nhp6ab* mutation. This challenges the widely held belief that mutations affecting transcriptional elongation result in sensitivity to 6-AU. One speculative explanation is that the reduced levels of the U6 splicing factor, produced from the *SNR6* gene, affect the efficiency of mRNA splicing, and this in turn has an effect on transcriptional elongation. Several reports have recently suggested a strong link between splicing and elongation. Fong and Zhou (21) report that spliceosomal small nuclear ribonucleoproteins interact with the transcription elongation factor TAT-SF1 and stimulate Pol II elongation. A mutation in the largest subunit of RNA Pol II that slows elongation by RNA Pol II has effects on splicing, in both human and *Drosophila* cells (15). Additionally, mutations in the *SPT4* and *SPT5* yeast genes that encode elongation factors result in splicing defects (41), and growth in the presence of 6-AU also affects splicing (30). Additionally, growth of wild-type cells in the presence of 6-AU causes a slight reduction in *SNR6* RNA levels. We suggest that the *nhp6ab* mutation reduces the levels of the U6 splicing factor, produced from the *SNR6* gene, and that the reduced levels of splicing factors affect transcriptional elongation, resulting in 6-AU sensitivity. In any case, our studies show that one cannot assume that 6-AU sensitivity results from a defect in transcriptional elongation.

Nhp6 and Pol II transcription. While the lethality of TBP substitution mutants in an *nhp6ab* strain could be because Nhp6 is required for efficient *SNR6* expression by RNA Pol III, we believe Nhp6 plays a role in TBP binding at genes transcribed by RNA Pol II. In vitro binding experiments show that Nhp6 stimulates the formation of a TBP-TFIIA-TFIIB-DNA complex, and in vivo experiments with chimeric promoter constructs suggest that Nhp6 acts at core promoters (50). Nhp6 acts positively to promote activation of the *HO* gene (68) and negatively to repress *FRE2* transcription (23). Nhp6 acts both positively and negatively at the *CHA1* gene, because induced levels are reduced and basal levels are increased in an *nhp6ab* mutant (45). In support of the idea that Nhp6 promotes DNA binding by TBP at genes transcribed by RNA Pol II, in vitro binding experiments show that Nhp6 stimulates formation of a TBP-TFIIA-DNA complex (D. Biswas, A. N. Imbalzano, P. Eriksson, Y. Yu, and D. J. Stillman, submitted for publication). TFIIA is encoded by the *TOA1* and *TOA2* genes, and a *toa2* mutation that eliminates TFIIA interaction with TBP is viable in wild-type cells but lethal in an *nhp6ab* mutant strain (Biswas et al., submitted). These results suggest that Nhp6 plays a role in formation of the TBP-TFIIA-DNA complex. Additionally, we have found genetic interactions between Nhp6 and factors that regulate TBP binding (D. Biswas and D. J. Stillman, unpublished data). These strong genetic interactions between *NHP6* and basal factors exclusively used for Pol II transcription provide strong support for the idea that Nhp6 has an important role in RNA Pol II transcription. Thus, Nhp6 facilitates expression of genes transcribed by both Pol II and Pol III.

ACKNOWLEDGMENTS

Special thanks go to Karen Arndt, who provided the plasmid library with TBP mutations. We thank Karen Arndt, David Auble, David Brow, Steve Buratowski, Martine Collart, Clyde Denis, Steve Hahn, Mike Hampsey, Tetsuro Kokubo, Laurie Stargell, Ian Willis, Fred Winston, and Rick Young who provided plasmids, strains, or antisera. We thank the anonymous reviewers who made important suggestions to improve this work. We also thank Rich Holubkov of the University of Utah Biostatistical Resource Facility for statistical advice; Frank Whitby for help with the SwissPDB Viewer software; and Tim Formosa, Chris Guthrie, Maggie Kasten, and Warren Voth for helpful discussions.

This work was supported in part by a grant from the National Institutes of Health awarded to D.J.S.

REFERENCES

1. **Agresti, A., and M. E. Bianchi.** 2003. HMGB proteins and gene expression. Curr. Opin. Genet. Dev. **13**:170–178.
2. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription in vivo. EMBO J. **14**:1490–1497.
3. **Arndt, K. M., C. R. Wobbe, S. Ricupero-Hovasse, K. Struhl, and F. Winston.** 1994. Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. Mol. Cell. Biol. **14**:3719–3728.
4. **Badarinarayana, V., Y. C. Chiang, and C. L. Denis.** 2000. Functional interaction of CCR4-NOT proteins with TATAA-binding protein (TBP) and its associated factors in yeast. Genetics **155**:1045–1054.
5. **Belotserkovskaya, R., D. E. Sterner, M. Deng, M. H. Sayre, P. M. Lieberman, and S. L. Berger.** 2000. Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol. Cell. Biol. **20**:634–647.
6. **Bender, A., and J. R. Pringle.** 1991. Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:1295–1305.
7. **Bhoite, L. T., Y. Yu, and D. J. Stillman.** 2001. The Swi5 activator recruits the Mediator complex to the *HO* promoter without RNA polymerase II. Genes Dev. **15**:2457–2469.
8. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
9. **Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter, and J. D. Boeke.** 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14**:115–132.
10. **Brewster, N. K., G. C. Johnston, and R. A. Singer.** 2001. A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol. Cell. Biol. **21**:3491–3502.
11. **Buratowski, S., and H. Zhou.** 1992. Transcription factor IID mutants defective for interaction with transcription factor IIA. Science **255**:1130–1132.
12. **Cang, Y., D. T. Auble, and G. Prelich.** 1999. A new regulatory domain on the TATA-binding protein. EMBO J. **18**:6662–6671.
13. **Collart, M. A.** 1996. The *NOT, SPT3*, and *MOT1* genes functionally interact to regulate transcription at core promoters. Mol. Cell. Biol. **16**:6668–6676.
14. **Cormack, B. P., and K. Struhl.** 1993. Regional codon randomization: defining a TATA-binding protein surface required for RNA polymerase III transcription. Science **262**:244–248.
15. **de la Mata, M., C. R. Alonso, S. Kadener, J. P. Fededa, M. Blaustein, F. Pelisch, P. Cramer, D. Bentley, and A. R. Kornblihtt.** 2003. A slow RNA polymerase II affects alternative splicing in vivo. Mol. Cell **12**:525–532.
16. **Desmoucelles, C., B. Pinson, C. Saint-Marc, and B. Daignan-Fornier.** 2002. Screening the yeast "disruptome" for mutants affecting resistance to the immunosuppressive drug, mycophenolic acid. J. Biol. Chem. **277**:27036–27044.
17. **Dudley, A. M., C. Rougeulle, and F. Winston.** 1999. The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. **13**:2940–2945.
18. **Dvir, A., J. W. Conaway, and R. C. Conaway.** 2001. Mechanism of transcription initiation and promoter escape by RNA polymerase II. Curr. Opin. Genet. Dev. **11**:209–214.
19. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. **6**:1319–1331.
20. **Exinger, F., and F. Lacroute.** 1992. 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr. Genet. **22**:9–11.
21. **Fong, Y. W., and Q. Zhou.** 2001. Stimulatory effect of splicing factors on transcriptional elongation. Nature **414**:929–933.
22. **Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman.** 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. **20**:3506–3517.
23. **Fragiadakis, G. S., D. Tzamarias, and D. Alexandraki.** 2004. Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for FRE2 transcriptional activation. EMBO J. **23**:333–342.
24. **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.
25. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74**:527–534.
26. **Guex, N., and M. C. Peitsch.** 1997. SWISS-MODEL and the Swiss-Pdb-Viewer: an environment for comparative protein modeling. Electrophoresis **18**:2714–2723.
27. **Hampsey, M.** 1998. Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. **62**:465–503.
28. **Hernandez, N.** 1993. TBP, a universal eukaryotic transcription factor? Genes Dev. **7**:1291–1308.
29. **Hoopes, B. C., J. F. LeBlanc, and D. K. Hawley.** 1992. Kinetic analysis of yeast TFIID-TATA box complex formation suggests a multistep pathway. J. Biol. Chem. **267**:11539–11547.
30. **Howe, K. J., C. M. Kane, and M. Ares, Jr.** 2003. Perturbation of transcription elongation influences the fidelity of internal exon inclusion in *Saccharomyces cerevisiae*. RNA **9**:993–1006.
31. **Jiang, Y. W., and D. J. Stillman.** 1995. Regulation of *HIS4* expression by the *Saccharomyces cerevisiae SIN4* transcriptional regulator. Genetics **140**:103–114.
32. **Juo, Z. S., G. A. Kassavetis, J. Wang, E. P. Geiduschek, and P. B. Sigler.** 2003. Crystal structure of a transcription factor IIIB core interface ternary complex. Nature **422**:534–539.
33. **Kassavetis, G. A., C. A. 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 III transcription factor, TFIIIB. Cell **71**:1055–1064.
34. **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.
35. **Kruppa, M., R. D. Moir, D. Kolodrubetz, and I. M. Willis.** 2001. Nhp6, an HMG1 protein, functions in *SNR6* transcription by RNA polymerase III in *S. cerevisiae*. Mol. Cell **7**:309–318.
36. **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.
37. **Lee, M., and K. Struhl.** 1995. Mutations on the DNA-binding surface of TATA-binding protein can specifically impair the response to acidic activators in vivo. Mol. Cell. Biol. **15**:5461–5469.
38. **Lee, M., and K. Struhl.** 1997. A severely defective TATA-binding protein-TFIIB interaction does not preclude transcriptional activation in vivo. Mol. Cell. Biol. **17**:1336–1345.
39. **Lee, T. I., and R. A. Young.** 1998. Regulation of gene expression by TBP-associated proteins. Genes Dev. **12**:1398–1408.
40. **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.
41. **Lindstrom, D. L., S. L. Squazzo, N. Muster, T. A. Burckin, K. C. Wachter, C. A. Emigh, J. A. McCleery, J. R. Yates III, and G. A. Hartzog.** 2003. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol. Cell. Biol. **23**:1368–1378.
42. **Lopez, S., M. Livingstone-Zatchej, S. Jourdain, F. Thoma, A. Sentenac, and M. C. Marsolier.** 2001. High-mobility-group proteins NHP6A and NHP6B participate in activation of the RNA polymerase III *SNR6* gene. Mol. Cell. Biol. **21**:3096–3104.
43. **Madison, J. M., and F. Winston.** 1997. Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TATA-binding protein to confer promoter-specific transcriptional control in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17**:287–295.
44. **Martin, M. P., V. L. Gerlach, and D. A. Brow.** 2001. A novel upstream RNA polymerase III promoter element becomes essential when the chromatin structure of the yeast U6 RNA gene is altered. Mol. Cell. Biol. **21**:6429–6439.
45. **Moreira, J. M., and S. Holmberg.** 2000. Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6A and NHP6B. EMBO J. **19**:6804–6813.
46. **Nikolov, D. B., and S. K. Burley.** 1997. RNA polymerase II transcription initiation: a structural view. Proc. Natl. Acad. Sci. USA **94**:15–22.
47. **Nikolov, D. B., H. Chen, E. D. Halay, A. Hoffman, R. G. Roeder, and S. K. Burley.** 1996. Crystal structure of a human TATA box-binding protein/TATA element complex. Proc. Natl. Acad. Sci. USA **93**:4862–4867.
48. **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.
49. **Orphanides, G., G. LeRoy, C. H. Chang, D. S. Luse, and D. Reinberg.** 1998. FACT, a factor that facilitates transcript elongation through nucleosomes. Cell **92**:105–116.
50. **Paull, T. T., M. Carey, and R. C. Johnson.** 1996. Yeast HMG proteins

NHP6A/B potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev. **10**:2769–2781.

51. **Pokholok, D. K., N. M. Hannett, and R. A. Young.** 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell **9**:799–809.
52. **Pugh, B. F.** 2000. Control of gene expression through regulation of the TATA-binding protein. Gene **255**:1–14.
53. **Rothstein, R.** 1991. Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194**:281–302.
54. **Ruone, S., A. R. Rhoades, and T. Formosa.** 2003. Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. J. Biol. Chem. **278**:45288–45295.
55. **Sayle, R. A., and E. J. Milner-White.** 1995. RASMOL: biomolecular graphics for all. Trends Biochem. Sci. **20**:374–376.
56. **Sethy-Coraci, I., R. D. Moir, A. Lopez-de-Leon, and I. M. Willis.** 1998. A differential response of wild type and mutant promoters to TFIIB70 overexpression in vivo and in vitro. Nucleic Acids Res. **26**:2344–2352.
57. **Shaw, R. J., and D. Reines.** 2000. *Saccharomyces cerevisiae* transcription elongation mutants are defective in PUR5 induction in response to nucleotide depletion. Mol. Cell. Biol. **20**:7427–7437.
58. **Shen, Y., G. A. Kassavetis, G. O. Bryant, and A. J. Berk.** 1998. Polymerase (Pol) III TATA box-binding protein (TBP)-associated factor Brf binds to a surface on TBP also required for activated Pol II transcription. Mol. Cell. Biol. **18**:1692–1700.
59. **Sherman, F.** 1991. Getting started with yeast. Methods Enzymol. **194**:1–21.
60. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.
61. **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.
62. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators in vivo. Science **269**:75–78.
63. **Sterner, D. E., P. A. Grant, S. M. Roberts, L. J. Duggan, R. Belotserkovskaya, L. A. Pacella, F. Winston, J. L. Workman, and S. L. Berger.** 1999. Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell. Biol. **19**:86–98.
64. **Tan, S., Y. Hunziker, D. F. Sargent, and T. J. Richmond.** 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature **381**:127–151.
65. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56**:619–630.
66. **van Kempen, G. M., and L. J. van Vliet.** 2000. Mean and variance of ratio estimators used in fluorescence ratio imaging. Cytometry **39**:300–305.
67. **Yu, Y., P. Eriksson, L. T. Bhoite, and D. J. Stillman.** 2003. Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility-group protein. Mol. Cell. Biol. **23**:1910–1921.
68. **Yu, Y., P. Eriksson, and D. J. Stillman.** 2000. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol. Cell. Biol. **20**:2350–2357.
69. **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.

CHAPTER 3

ROLE FOR Nhp6, Gcn5 AND THE Swi/Snf COMPLEX IN STIMULATING FORMATION OF THE TATA-BINDING PROTEIN-TFIIA-DNA COMPLEX

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Molecular and Cellular Biology 2004 Sep;24(18):8312-21.

MOLECULAR AND CELLULAR BIOLOGY, Sept. 2004, p. 8312–8321 Vol. 24, No. 18
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.18.8312–8321.2004
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Role for Nhp6, Gcn5, and the Swi/Snf Complex in Stimulating Formation of the TATA-Binding Protein–TFIIA–DNA Complex

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Received 15 March 2004/Returned for modification 26 April 2004/Accepted 15 June 2004

The TATA-binding protein (TBP), TFIIA, and TFIIB interact with promoter DNA to form a complex required for transcriptional initiation, and many transcriptional regulators function by either stimulating or inhibiting formation of this complex. We have recently identified TBP mutants that are viable in wild-type cells but lethal in the absence of the Nhp6 architectural transcription factor. Here we show that many of these TBP mutants were also lethal in strains with disruptions of either *GCN5*, encoding the histone acetyltransferase in the SAGA complex, or *SWI2*, encoding the catalytic subunit of the Swi/Snf chromatin remodeling complex. These synthetic lethalities could be suppressed by overexpression of *TOA1* and *TOA2*, the genes encoding TFIIA. We also used TFIIA mutants that eliminated in vitro interactions with TBP. These viable TFIIA mutants were lethal in strains lacking Gcn5, Swi2, or Nhp6. These lethalities could be suppressed by overexpression of TBP or Nhp6, suggesting that these coactivators stimulate formation of the TBP-TFIIA-DNA complex. In vitro studies have previously shown that TBP binds very poorly to a TATA sequence within a nucleosome but that Swi/Snf stimulates binding of TBP and TFIIA. In vitro binding experiments presented here show that histone acetylation facilitates TBP binding to a nucleosomal binding site and that Nhp6 stimulates formation of a TBP-TFIIA-DNA complex. Consistent with the idea that Nhp6, Gcn5, and Swi/Snf have overlapping functions in vivo, *nhp6a nhp6b gcn5* mutants had a severe growth defect, and mutations in both *nhp6a nhp6b swi2* and *gcn5 swi2* strains were lethal.

The critical step in transcriptional activation by RNA polymerase II is formation of the preinitiation complex (12, 50). In vitro experiments have shown that the general transcription factors TFIIA, TFIIB, and the TATA-binding protein (TBP) are recruited onto TATA sequence-containing promoter DNA in a sequential and cooperative manner to form a TBP-TFIIA-TFIIB-DNA complex. This complex then recruits RNA polymerase II and other general transcription factors required for transcriptional initiation. In vivo experiments have shown that transcriptional activators facilitate DNA binding by TBP, and TBP binding correlates with transcriptional activity (30, 38). DNA binding by TBP may be the limiting event in transcriptional activation, and thus regulation of TBP binding is thought to be the critical step in transcription initiation. Many DNA-binding transcriptional activators recruit coactivators, such as chromatin remodeling complexes or histone acetyltransferase complexes, to promoters (12, 36). There are many ideas as to how these coactivators facilitate transcriptional activation, and many believe that they function by promoting either DNA binding by TBP or formation of the TBP-TFIIA-TFIIB-DNA complex.

The most widely studied coactivators are chromatin remodeling factors and histone acetyltransferases (48). *SWI2* encodes the catalytic subunit of the Swi/Snf chromatin remodeling complex, and an *swi2* mutation affects expression of many *Saccharomyces cerevisiae* genes (45). In support of the idea that coactivators stimulate DNA binding by basal transcription factors, Imbalzano et al. (24) reported that although TBP binds very poorly to a TATA site within a nucleosome, DNA binding of TBP and TFIIA can be stimulated by the Swi/Snf chromatin remodeler. *GCN5* encodes a histone acetyltransferase that is part of the yeast SAGA complex, and histone acetylation by Gcn5 is required for expression of many yeast genes (63). Previous studies of the regulation of the yeast *HO* gene have shown that Gcn5 functions in the same pathway as the Nhp6 architectural transcription factor (72). Nhp6 is related to the high-mobility group B (HMGB) family of small, abundant chromatin proteins that bend DNA sharply and modulate gene expression (67). Nhp6 also functions with Spt16 and Pob3, as part of the yeast FACT complex, to promote transcriptional elongation (15), and Nhp6 is important for expression of the *SNR6* gene, transcribed by RNA polymerase III (28, 43, 46).

Nhp6 is encoded by two redundant genes, as *nhp6a* and *nhp6b* single mutants are without any discernibly abnormal phenotype but the *nhp6a nhp6b* double mutant (which we describe hereafter as the *nhp6ab* mutant) is temperature sensitive for growth (7). The *gcn5 nhp6ab* triple mutant displays a strong synthetic growth defect, but this phenotype can be suppressed by mutations in the *SPT3* gene that regulates TBP binding (71). Additionally, the temperature-sensitive growth defect of *nhp6ab* strains can be suppressed either by an *spt3*

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TABLE 1. Strains used in this study

Strain	Description
DY7472	*MAT***a** *spt15::LEU2 + SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7514	*MAT***a** *gcn5::HIS3 spt15::LEU2 + SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7515	*MAT*α *gcn5::HIS3 spt15::LEU2 + SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8118	*MAT***a** *gcn5::HIS3 spt3::ADE2 spt15::LEU2 + SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8158	*MAT***a** *gcn5::HIS3 spt15::KanMX + SPT15*(YCp-*URA3-ADE3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8510	*MAT*α *nhp6a::KanMX nhp6b::ADE2 toa2::His3MX + TOA2*(YCp-*URA3*) *ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8541	*MAT***a** *toa2::His3MX + TOA2*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8660	*MAT***a** *nhp6a::KanMX nhp6b::HIS3 swi2::LEU2 + SWI2*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8688	*MAT***a** *swi2::LEU2 + SWI2*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8688	*MAT***a** *swi2::LEU2 nhp6a::KanMX nhp6b::HIS3 + NHP6A*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8709	*MAT*α *gcn5::TRP1 toa2::His3MX + TOA2*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8712	*MAT***a** *swi2::LEU2 spt15::ADE2 + SPT15*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8783	*MAT***a** *swi2::LEU2 spt15::ADE2 + SPT15*(YCp-*URA3*) *ade2 can1 leu2 lys2 trp1 ura3*
DY8811	*MAT***a** *swi2::ADE2 toa2::His3MX + TOA2*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8827	*MAT***a** *swi2::LEU2 gcn5::TRP1 + SWI2*(YCp-*URA3*) *ade2 can1 his3 leu2 lys2 trp1 ura3*

mutation or by overexpression of TBP. An *spt3* mutation or TBP overexpression also suppresses certain transcriptional defects of either *nhp6ab* or *gcn5* mutants. Spt3 interacts directly with TBP (10), and Spt3 regulates TBP binding in vivo, inhibiting TBP binding to the *HO* promoter while stimulating TBP binding to *GAL1* (32, 71). Taken together, the results of these experiments suggest that one function of the Gcn5 and Nhp6 activators, at some promoters, is to counteract the effects of inhibitors of TBP binding such as Spt3.

The genes encoding the TBP and TFIIA basal transcription factors are essential for viability. TBP is encoded by the *SPT15* gene (11, 20), and the two subunits of TFIIA are encoded by *TOA1* and *TOA2* (56). Although gene disruptions are lethal, viable mutants with point mutations have been recovered (21). Of particular interest here, viable mutants with point mutations in TBP that reduce interaction with TFIIA have been isolated (6, 62). Additionally, using the TBP-TFIIA-DNA cocrystal as a guide (17, 65), Ozer et al. (51) created site-directed mutations in the Toa2 subunit of TFIIA that eliminate interaction with TBP in vitro.

Recently, a genetic screen was conducted to identify TBP mutants that are viable in wild-type yeast strains but lethal in an *nhp6ab* strain (13). In the present study, we examined the effects of many of these TBP mutants in yeast strains with either *SWI2* or *GCN5* gene disruptions. Many of the TBP substitutions were lethal in *swi2* or *gcn5* mutants, and in some instances the synthetic lethality could be suppressed by overexpression of TFIIA. We also show genetic interactions between *TOA2*, encoding a TFIIA subunit, and *NHP6*, *GCN5*, and *SWI2*. Importantly, some of the synthetic lethalities could be suppressed by overexpression of TBP or Nhp6, indicating a possible role of these factors in formation of the TBP-TFIIA-DNA complex. Finally, in vitro DNA-binding experiments showed that Nhp6 promotes assembly of the TBP-TFIIA complex on DNA and that histone acetylation facilitates TBP binding to a nucleosomal binding site.

MATERIALS AND METHODS

Strains and media. All yeast strains used are listed in Table 1 and were isogenic in the W303 background (66). Standard genetic methods were used for strain construction (60). W303 strains with disruptions in *gcn5*, *nhp6a*, *nhp6b*, and *swi2* have been described previously (71, 72). The *toa2* gene disruption cassette was made by PCR using plasmid pFA6a:His3MX6 (42) as a template and was confirmed by Southern blotting. Cells were grown in yeast extract-peptone-dextrose medium (60) at 30°C, except when the use of other higher temperatures is noted or when synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids, as appropriate, but lacking essential components was used to select for plasmids. 5-Fluoroorotic acid (5-FOA) medium was prepared as described previously (4).

Plasmids. The multicopy plasmids used are listed in Table 2. A 2.3-kb BamHI-PstI fragment with *SPT15* from pSH223, provided by Steve Hahn, was cloned into pRS327 (14) and YEplac112 (18), generating M4533 and M4827, respectively. Plasmid M4793 was constructed by moving a 4.2-kb SalI fragment with *TOA1* and *TOA2* from pSH346 into pRS327 (14). A 937-bp BamHI-SacI fragment with *NHP6A* generated by PCR with oligonucleotides F822 (TCATGGA TCCTGGCAAAAATCGTCCTCTGT) and F833 (CTCAGAGCTCAAGAGC TGCACTCGGTCTAC) and restriction enzyme cleavage was cloned into YEplac195 (18) to create M4221; a PstI-SacI fragment with *NHP6A* from M4221 was then cloned into pRS327 (14), generating M4797. Descriptions of the YCp-*LEU2* plasmids with mutations in the Toa2 subunit of TFIIA have been published previously (51), except for that of the plasmid with the Y10G R11Δ allele, and this plasmid was generously provided by Paul Lieberman. The references for the TBP mutations on YCp-*TRP1* plasmids are given in Table 3. Descriptions of the E186L and E186M TBP mutants are unpublished, and these mutants were generously provided by Steve Buratowski.

In vitro binding experiments. Mononucleosome particles were assembled by salt dilution exactly as described previously (24) by using the PH MLT (+3) and PH MLT (+3)-Mu templates. Histones were purified as described previously (70) from logarithmically growing HeLa cells or from growing HeLa cells treated with 10 mM sodium butyrate, pH 7.0, for 16 h prior to harvest. Triton-acid-urea (TAU) gel electrophoresis was performed as described previously (75). Binding reaction mixtures contained 0.3 ng of labeled naked DNA or labeled nucleosome (in 3 ng of total nucleosomes), 12 mM HEPES, pH 7.9, 60 mM KCl, 7 mM $MgCl_2$, 15% glycerol, 0.6 mM dithiothreitol, 0.06 mM EDTA, 500 ng of bovine serum albumin, and 1.5 uM (nucleosome reaction mixtures) or 20 nM (naked DNA reaction mixtures) recombinant yeast TBP. Reaction mixtures with naked DNA also contained 100 ng of poly(dG:dC). Samples were incubated at 30°C for

TABLE 2. Multicopy plasmids

Plasmid	Description	Source or reference
pRS327	YEp-*LYS2* vector	14
M4533	TBP (*SPT15*) on YEp-*LYS2* plasmid	This work
M4793	TFIIA (*TOA1* and *TOA2*) on YEp-*LYS2* plasmid	This work
M4797	*NHP6A* on YEp-*LYS2* plasmid	This work
YEp351	YEp-*LEU2* vector	22
pSH346	TFIIA (*TOA1* and *TOA2*) on YEp-*LEU2* plasmid	Steve Hahn
YEplac223	YEp-*TRP1* vector	18
M4827	TBP (*SPT15*) on YEp-*TRP1* plasmid	This work

TABLE 3. TBP mutations with synthetic phenotypes with *gcn5* or *swi2* mutations[a]

Substitution(s) (reference)	Synthetic phenotype with *swi2*	Effect of YEp-*NHP6A* on lethality in *swi2* strain	Effect of YEp-TFIIA on lethality in *swi2* strain	Synthetic phenotype with *gcn5*	Effect of *spt3* Δ on lethality in *gcn5* strain	Effect of YEp-TFIIA on lethality in *gcn5* strain
L67Q (13)	Viable			Lethal	ND[c]	ND
K83E (13)	Viable			Lethal at 37°C	ND	None
E93G (13)	Lethal	None	Suppression	Lethal	None	Suppression
Y94C (13)	Lethal at 33°C	Partial suppression	None	Lethal	ND	None
P109A (2)	Very sickly	Partial suppression	None	Very sickly	None	Suppression
L114F (3)	Lethal	None	None	Viable		
E129G (13)	Viable			Lethal at 37°C	ND	None
E129V (13)	Lethal at 33°C	None	Partial suppression	Lethal at 37°C	ND	None
G147W (13)	Lethal at 33°C	None	Suppression	Lethal	None	Suppression
N159D (2)	Lethal	None	Suppression	Lethal	None	Suppression
L172P (13)	Very sickly at 33°C	Suppression	None	Lethal	None	Suppression
G174E (10)	Very sickly	None	Partial suppression	Very sickly	Partial suppression	Suppression
E186L[b]	Lethal at 33°C	Suppression	None	Viable		
K211E (13)	Lethal	Partial suppression	None	Very sickly	None	Suppression
R220H (27)	Very sickly	Suppression	None	Viable		
F227S (13)	Viable			Lethal at 37°C	ND	None
F237D (61)	Lethal	Partial suppression	None	Viable		
K97R, L193S (13)	Very sickly at 33°C	None	None	Very sickly	None	Suppression
I103T, K239Stop (13)	Very sickly	Partial suppression	Partial suppression	Lethal at 37°C	ND	None
K133L, K145L (6)	Lethal at 33°C	Partial suppression	None	Viable		
K138T, Y139A (62)	Lethal	None	None	Lethal	None	Suppression

[a] The following TBP mutants were viable in both the *swi2* and *gcn5* strains: V71A, S118L, K133L, K133R, F148H, C164W, E188A, L189A, F227L, Y231A, F237L, K239T, and K239Stop and the K133L K138L double mutant.
[b] Source: Steve Buratowski.
[c] ND, not determined.

25 min, treated with 0.2 U (nucleosome reaction mixtures) or 0.02 U (naked DNA reaction mixtures) of DNase I (Promega) for 2 min at room temperature, and prepared for electrophoresis as described previously (25).

For the in vitro binding experiments involving Nhp6, the two subunits of recombinant TFIIA were expressed separately in bacteria by using plasmids pLH44 and pLH41, provided by Steve Hahn, expressing Toa1 and Toa2, respectively. After induction of protein expression, the insoluble material was denatured in 7 M urea, the solubilized Toa1 and Toa2 extracts were mixed and renatured by slow dialysis, and TFIIA was purified by MonoQ chromatography. A 1.15-kb NdeI-BamHI fragment with the *SPT15* open reading frame was cloned into a modified pGEX2T vector (WISP1-69) (69), and the bacterially expressed glutathione-*S*-tranferase–TBP fusion protein was purified by glutathione affinity chromatography followed by thrombin cleavage to remove glutathione-*S*-transferase, as described previously (74). Nhp6 (untagged) purified from bacteria was generously provided by Tim Formosa (15). The DNA template for binding studies was prepared by annealing two oligonucleotides, GGACCTGGGGCTA TAAAAGGGGCCATGGGC and GCCCATGGCCCCTTTTATAGCCCCAG GTCC, followed by end labeling with polynucleotide kinase and [γ-^{32}P]ATP. The 20-μl binding reaction mixtures contained TBP, Nhp6, and TFIIA (amounts are indicated in the legend to Fig. 6) and were incubated for 30 min at 25°C by using a buffer described previously (74) and then separated at room temperature on a 6% polyacrylamide gel (ratio of acrylamide to bisacrylamide, 39:1) in 1× Tris-borate-EDTA running buffer run at room temperature. The gel was dried and autoradiographed.

RESULTS

Genetic interactions of Swi/Snf with Nhp6 and TBP. Genetic interactions occur between Nhp6 and the Swi/Snf chromatin remodeling complex. *SNF5* encodes a subunit of Swi/Snf, and Brewster et al. (5) reported that an *nhp6ab snf5* triple mutant is unable to grow at 32.5°C. *SWI2* encodes the catalytic subunit of the Swi/Snf complex, and we decided to determine whether *swi2* is synthetically lethal with *nhp6ab*. We constructed an *nhp6a*Δ/+ *nhp6b*Δ/+ *swi2*Δ/+ triply heterozygous diploid strain and transformed it with either a YCp-*URA3-NHP6A* plasmid or a YCp-*URA3-SWI2* plasmid. The diploids were induced to undergo meiosis, tetrads were dissected, and we isolated haploid strains with the *nhp6ab swi2* genotype containing either the YCp-*URA3-NHP6A* or the YCp-*URA3-SWI2* plasmid. These strains were unable to grow on medium containing 5-FOA at 25 or 30°C, and we conclude that *swi2* is synthetically lethal with *nhp6ab* (Fig. 1A).

Based on this genetic interaction, we next decided to determine whether any of the TBP mutants with point mutations that are lethal in the absence of Nhp6 showed genetic effects in a strain lacking the Swi/Snf complex. We constructed an *swi2 spt15* double deletion mutant, kept alive by the wild-type *SPT15* (TBP) gene on a YCp-*URA3* plasmid. This strain was transformed with YCp-*TRP1* plasmids with various TBP mutations, and we used plasmid shuffling to assess the viability of the *swi2 spt15* strains on 5-FOA medium on which the YCp-*URA3-SPT15* (wild type) plasmid must be lost for cells to grow. We tested 35 TBP mutants, and 17 showed a synthetic phenotype in the absence of Swi2 (Table 3; examples in Fig. 1B). Interestingly, two TBP mutants (K133L K145L and K138T Y139A) affecting interaction with TFIIA (6, 62) and two TBP mutants (E186L and E186M) affecting interaction with TFIIB (34) were lethal in the *swi2* mutant, either at all temperatures or at 33°C. For the TBP substitutions that were lethal in the absence of Swi2, there was not an obvious correlation in terms of locations on the TBP structure.

Multicopy plasmids with either TFIIA or *NHP6A* suppressed the TBP-*swi2* synthetic lethalities for selected alleles (Table 3; examples in Fig. 1C). The *swi2-nhp6ab* synthetic lethality and the partial suppression of the TBP-*swi2* synthetic lethality by YEp-*NHP6A* suggest that Swi/Snf and Nhp6 func-

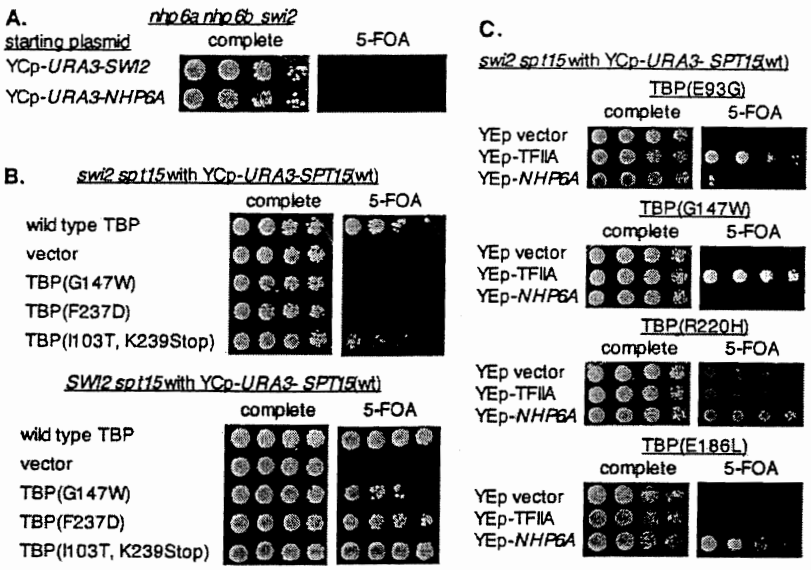

FIG. 1. Genetic interactions among *SWI2*, *NHP6*, TBP, and TFIIA. (A) *nhp6ab* is synthetically lethal with *swi2*. Dilutions of strains DY8660 (*nhp6ab swi2* strain with a YCp-*URA3-SWI2* plasmid) and DY8668 (*nhp6ab swi2* strain with a YCp-*URA3-NHP6A* plasmid) were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 30°C for 3 days. (B) Examples of synthetic lethality of TBP mutants and *swi2*. Dilutions of strains DY8712 (*swi2 spt15*) and DY7472 (*spt15*) transformed with the indicated TBP mutation plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 30°C for 3 days. *SPT15*(wt), wild-type *SPT15*. (C) Multicopy TFIIA suppresses the TBP mutant E93G-*swi2* and TBP mutant G147W-*swi2* synthetic lethalities, and multicopy *NHP6A* suppresses the TBP mutant R220H-*swi2* and TBP mutant E186L-*swi2* synthetic lethalities. Strain DY8783 (*swi2 spt15*) was transformed with two plasmids, a *TRP1* plasmid corresponding to the indicated TBP mutant and either pRS327 (YEp-*LYS2* vector), M4793 (YEp-TFIIA), or M4797 (YEp-*NHP6A*), dilutions were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for either 2 (complete medium) or 3 (5-FOA) days.

tion in the same pathway of transcriptional activation. Suppression of the TBP-*swi2* synthetic lethality by YEp-TFIIA, combined with the fact that the TBP mutants that affect interaction with TFIIB were lethal in the *swi2* mutant, suggests that Swi/Snf facilitates formation of the TBP-TFIIA-TFIIB-DNA complex.

TBP mutants lethal in the absence of Gcn5. It has previously been shown that Nhp6 and the Gcn5 histone acetyltransferase function in similar pathways in the transcriptional activation of specific genes (71, 72). Additionally, the TBP K138T Y139A double mutant that was lethal in an *nhp6ab* strain is also lethal in a *gcn5* mutant (71). With this in mind, we asked whether the new TBP mutants isolated as lethal in the *nhp6ab* mutant were also lethal in the absence of Gcn5. YCp-*TRP1* plasmids carrying the TBP mutants were used to transform a *gcn5 spt15* strain carrying a YCp-*URA3-SPT15* (wild type) plasmid, and these transformants were plated onto 5-FOA. We found that 16 TBP mutants that were synthetic lethal with *nhp6ab*, out of 35 tested, were either lethal or very sickly in the absence of Gcn5 (Table 3; examples in Fig. 2A). Additionally, we found that N159D and E186M TBP mutants, which were viable in an *nhp6ab* strain, were lethal in the *gcn5* mutant. As noted with the *swi2* mutants, the TBP mutants that were lethal in the absence of Gcn5 did not define a unique surface of TBP. This synthetic lethality of *gcn5* and TBP mutants suggests that the

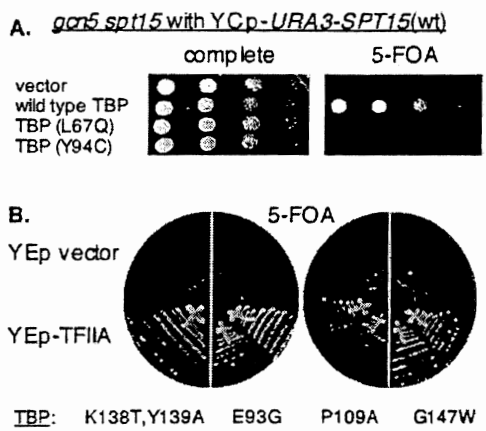

FIG. 2. Genetic interactions among *GCN5*, TBP, and TFIIA. (A) Examples of synthetic lethality of TBP mutants and *gcn5*. Dilutions of strain DY7514 (*gcn5 spt15*) transformed with the indicated TBP mutation plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 25°C for 4 days. *SPT15*(wt), wild-type *SPT15*. (B) Multicopy TFIIA suppresses the TBP-*gcn5* synthetic lethality for certain TBP mutants. Strain DY8158 (*gcn5 spt15*) was transformed with two plasmids, a *TRP1* plasmid corresponding to the indicated TBP mutant and either YEp351 (YEp-*LEU2* vector) or pSH346 (YEp-TFIIA), and was plated onto 5-FOA-containing plates, and the plates were incubated at 30°C for 4 days.

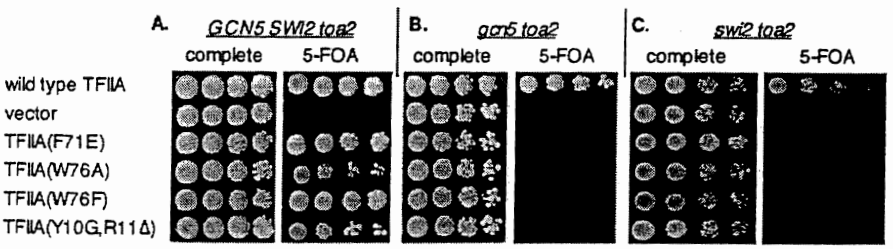

FIG. 3. TFIIA mutants are lethal in *gcn5* or *swi2* mutant strains. (A) Dilutions of strain DY8541 (*toa2*) transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for 3 days. (B) Dilutions of strain DY8709 (*gcn5 toa2*) transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for 3 days. (C) Dilutions of strain DY8811 (*swi2 toa2*) transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for 2 days. Note that the TFIIA mutants designated in the figure correspond to substitutions in the Toa2 subunit of TFIIA.

Gcn5 histone acetyltransferase assists TBP in its role of promoting transcriptional activation.

We next determined whether these TBP alleles that either were lethal or resulted in a marked growth defect in the *gcn5* mutant could be suppressed, either by an *spt3* mutation or by multicopy plasmids with TFIIA or TFIIB (Table 3). An *spt3* mutation improved growth for only one of the eight TBP mutants tested, G174E. Interestingly, the G174 residue interacts with Spt3 (10). The synthetic lethality with *gcn5* could be suppressed by overexpression of TFIIA for half of the alleles tested (Table 3; examples in Fig. 2B). The K138T Y139A double mutation affects in vitro binding to TFIIA (6), and structural studies show that E93, K97, and G147 residues are positioned nearby so that they may interact with TFIIA. In contrast, while YEp-TFIIA was an effective multicopy suppressor, overexpression of TFIIB did not suppress the synthetic lethality with *gcn5* for any of the TBP mutants tested.

Synthetic lethality of *gcn5* and *swi2* with TFIIA. Based on the observation that overexpression of TFIIA suppresses the synthetic lethality of TBP mutants in *gcn5* deletion strains, we looked for synthetic lethality of *gcn5* and TFIIA. TFIIA has two subunits encoded by the essential *TOA1* and *TOA2* genes. We constructed a *gcn5 toa2* double deletion mutant, kept alive with the YCp-*URA3*-TFIIA (wild type) plasmid. This strain was transformed with YCp-*LEU2* plasmids with various mutant *toa2* genes (51), and we assessed viability of the *gcn5 toa2* strains by plasmid shuffling on 5-FOA medium. (We hereafter refer to these mutant *toa2* genes by the corresponding protein designation, TFIIA.)

We tested seven viable TFIIA mutants with mutations at positions that make important stabilizing contacts with TBP in the TBP-TFIIA-DNA structure (17, 65), and all of the substitutions prevented formation of the TBP-TFIIA-DNA complex in vitro (51). We first determined whether the TFIIA mutants were viable in our strain background by plasmid shuffling in a *GCN5 toa2* strain (Fig. 3A and Table 4). Interestingly, the Y69A mutant and Y69F W76F double mutant that were viable in the BWG1 strain background (51) were lethal in our W303 strain. The substitutions at residues Y69, F71, and F76 were at the interface of TFIIA-TBP interaction. We also examined a Y10G R11Δ mutant (with a glycine substitution at Y10 combined with deletion of R11), as Y10 is predicted to be a protein interaction surface (Paul Lieberman, personal communication).

When tested by plasmid shuffling in the *gcn5 toa2* strain, four of these TFIIA mutants, F71E, W76A, W76F, and Y10G R11Δ, were lethal in the absence of Gcn5 (Fig. 3B). Additionally, the Y69F and F71R mutants were lethal in the absence of

TABLE 4. Synthetic growth defects caused by TFIIA mutants with *gcn5*, *swi2*, or *nhp6ab*

TFIIA mutant	Growth of strain with TFIIA mutant in the following background at the indicated temperature[a]								
	BWG1 (30°C)	W303 *toa2*		W303 *toa2 gcn5*			W303 *toa2 swi2*		W303 *toa2 nhp6ab* (25°C)
		33°C	37°C	25°C	33°C	37°C	25°C	33°C	
Wild type	++++	+++	+++	+++	+++	+++	+++	+++	++
Y69F	+++	+++	+++	++	++	−	+++	+++	++
F71E	++++	+++	+++	+	−	−	+	−	++
F71R	++++	+++	+++	++	++	−	++	++	++
W76A	++	++	−	−	−	−	−	−	−
W76F	++++	+++	+++	+	−	−	−	−	++
Y10G R11Δ	ND	+++	+	−	−	−	−	−	+/−
Y69A	++	Lethal mutation	Lethal mutation						
Y69F W76F	+	Lethal mutation	Lethal mutation						

[a] Growth is rated from ++++, indicating unimpaired growth, to −, indicating no growth, with +/− indicating weaker growth than +. ND, not determined. Data for BWG1 are from Ozer et al. (51). The W303 background strains with the indicated TFIIA mutants were grown on 5-FOA.

Gcn5 at 37°C (Table 4). Thus, the mutations that reduced the ability of TFIIA to form a complex with TBP and DNA were tolerated in a wild-type strain but not in the *gcn5* mutant. This result suggests that histone acetylation contributes to formation of the TBP-TFIIA-DNA complex in vivo.

Because some of the synthetic lethalities of *swi2* and TBP mutants with point mutations could be suppressed by TFIIA overexpression, we next examined whether *swi2* was synthetically lethal with these TFIIA mutants. We constructed an *swi2 toa2* double mutant with the YCp-*URA3*-TFIIA (wild type) plasmid for this plasmid shuffling experiment. The same four TFIIA mutants were unable to support viability at 33°C in the absence of the Swi/Snf chromatin remodeling complex (Fig. 3C and Table 4). We conclude that *swi2* and TFIIA are synthetically lethal, and this result suggests that Swi/Snf facilitates formation of the TBP-TFIIA-DNA complex.

TBP overexpression suppresses synthetic lethality. If our hypothesis that histone acetylation by Gcn5 and chromatin remodeling by Swi/Snf stimulate formation of the TBP-TFIIA-DNA complex is correct, then the *gcn5*-TFIIA and *swi2*-TFIIA synthetic lethalities may be suppressed by overexpression of TBP. To test this idea, the *gcn5 toa2* and *swi2 toa2* strains, with the YCp-*URA3*-TFIIA (wild type) plasmid, were transformed with two plasmids. One was a single-copy plasmid with a mutant TFIIA gene, and the second was a multicopy plasmid, either YEp-TBP or the YEp vector control. As shown in Fig. 4A, overexpression of TBP suppressed the synthetic lethality of *gcn5* and the TFIIA W76F mutant. The YEp-TBP plasmid was not able to suppress the synthetic lethality with *gcn5* for the other three TFIIA mutants. Similarly, a multicopy plasmid with TBP suppressed the *swi2*-TFIIA synthetic lethality for three of the TFIIA mutants (Fig. 4B). We did not observe suppression of the *gcn5*-TFIIA or *swi2*-TFIIA synthetic lethality by multicopy plasmids with either TFIIB or *NHP6A*.

Synthetic lethality of *gcn5* and *swi2*. There are strong synthetic phenotypes when *nhp6ab* mutations are combined with either *swi2* or *gcn5* mutations (Fig. 1A) (72). Additionally, mutants with certain point mutations affecting either TBP or TFIIA that were viable in an otherwise wild-type strain were lethal in either *gcn5* or *swi2* mutants. These results, along with multicopy suppression of these synthetic lethalities by overexpression of either TFIIA or TBP, suggest that the Gcn5 histone acetyltransferase and the Swi/Snf chromatin remodeling factor are involved in a common pathway in transcriptional activation, such as formation of the TBP-TFIIA-DNA complex. Based on these results, we decided to determine whether *gcn5* and *swi2* are synthetically lethal, as *gcn5* is synthetically lethal with an *swi1* mutation affecting a different Swi/Snf component (53).

We constructed a *gcn5*Δ/+ *swi2*Δ/+ doubly heterozygous diploid strain and transformed it with a YCp-*URA3-SWI2* plasmid, and after sporulation we isolated *gcn5 swi2* strains with the YCp-*URA3-SWI2* plasmid. These strains were unable to lose the YCp-*URA3-SWI2* plasmid and grow on 5-FOA (Fig. 4C), and thus *gcn5* and *swi2* were synthetically lethal in the W303 strain background. In contrast, in the S288c strain background, the *swi2 gcn5* double mutant is viable but has a strong synthetic growth defect (57). As a control, we showed that an *swi2 GCN5* strain with the YCp-*URA3-SWI2* plasmid did grow on 5-FOA medium (Fig. 4C), demonstrating that the FOA-sensitive phenotype is dependent upon the *gcn5* mutation. Interestingly, the plating efficiency of the *swi2* strain with the YCp-*URA3-SWI2* plasmid was much lower on FOA than it was on complete medium. The *swi2* strain has a marked growth defect, and apparently this strain infrequently loses the YCp-*URA3-SWI2* plasmid. None of the multicopy plasmids tested were able to suppress the *gcn5-swi2* synthetic lethality (data not shown).

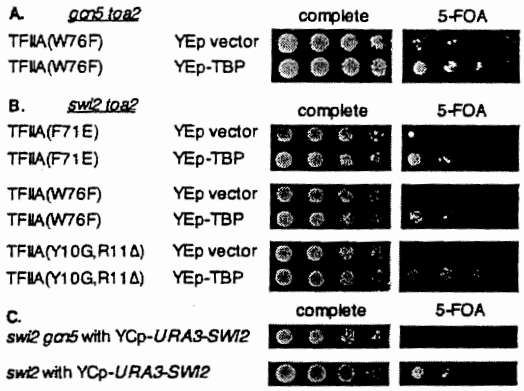

FIG. 4. Overexpression of TBP suppresses *gcn5*-TFIIA and *swi2*-TFIIA lethalities. (A) Strain DY8709 (*gcn5 toa2*) was transformed with two plasmids, a *LEU2* plasmid corresponding to the TFIIA W76F mutant and either pRS327 (YEp-*LYS2* vector) or M4533 (YEp-TBP), dilutions were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for either 2 (complete medium) or 3 (5-FOA) days. (B) Strain DY8811 (*swi2 toa2*) was transformed with two plasmids, a *LEU2* plasmid corresponding to the indicated TFIIA mutant and either YEplac112 (YEp-*TRP1* vector) or M4827 (YEp-TBP), dilutions were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated as follows: plates with TFIIA mutant F71E or W76F, 34°C for 2 (complete medium) or 3 (5-FOA) days, and those with TFIIA mutant Y10G R11Δ, 25°C for 2 (complete medium) or 4 (5-FOA) days. Note that the incubation of the TFIIA Y10G R11Δ mutant on 5-FOA was considerably longer in this experiment than in the one described in the legend to Fig. 3C, and thus tiny colonies are visible when the vector control is grown at 25°C. (C) *gcn5* is synthetically lethal with *swi2*. Dilutions of strains DY8827 (*swi2 gcn5* strain with a YCp-*URA3-SWI2* plasmid) or DY8664 (*swi2* strain with a YCp-*URA3-SWI2* plasmid) were plated at 25°C onto complete medium-containing plates for 3 days or onto FOA-containing plates for 5 days. Note that the TFIIA mutants designated in the figure correspond to substitutions in the Toa2 subunit of TFIIA.

Histone acetylation facilitates TBP binding. While TBP binds readily to a TATA sequence in naked DNA, TBP does not bind to a nucleosomal site. In vitro studies show that TBP, alone or in the presence of TFIIA, is unable to bind to consensus TATA sequences at multiple rotationally phased positions, whether located at the dyad, side, or edge of a mononucleosome particle (19, 24). However, the Swi/Snf remodeling complex stimulates TBP and TFIIA binding to a nucleosomal TATA site (24), consistent with our genetic results showing that mutations that impaired TBP-TFIIA interactions were lethal in an *swi2* mutant. Our genetic studies suggest an in vivo role for histone acetylation by Gcn5 in stimulating DNA binding by TBP and thus forming a TBP-TFIIA-DNA complex. To address whether histone acetylation plays a role in TBP bind-

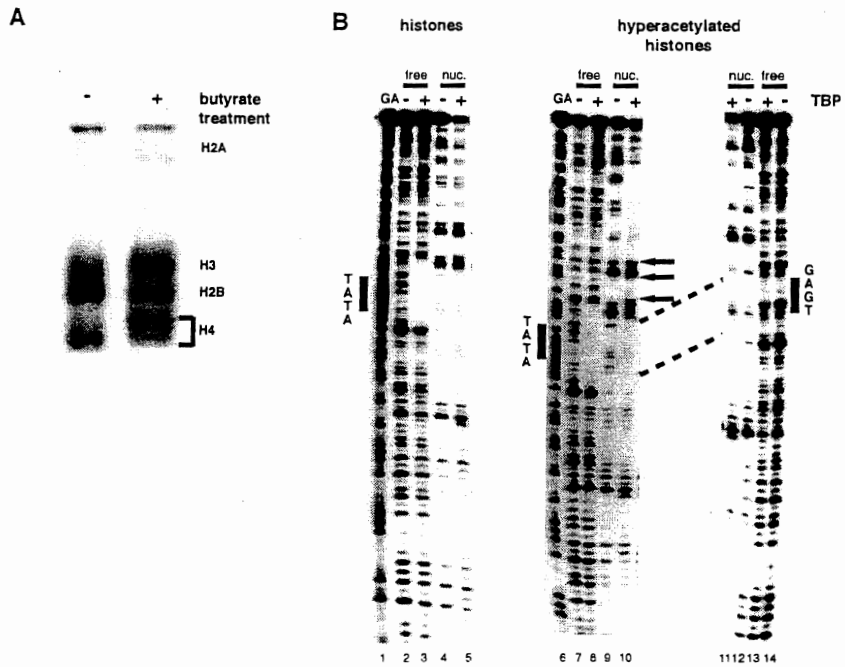

FIG. 5. TBP binds to acetylated nucleosomes. (A) Twenty micrograms of HeLa histones or hyperacetylated HeLa histones were loaded onto a 15% TAU gel and stained with Coomassie brilliant blue following electrophoresis. (B) TBP binding was assessed by DNase I digestions by using free DNA (lanes 2, 3, 7, 8, 13, and 14) or nucleosomes (nuc.) assembled with regular histones (lanes 4 and 5) or hyperacetylated histones (lanes 9 to 12). The DNA template for lanes 11 to 14 has mutations at the TATA sequence. Lanes 1 and 6 contain G+A sequencing ladders. Addition of TBP to the binding reaction mixtures is indicated by +. The data in lanes 1 to 5 are reprinted from *Nature* (24) with permission of the publisher. The arrows indicate hypersensitive DNase I cleavages 5′ to the TATA sequence.

ing in vitro, mononucleosome particles were assembled with a template containing a rotationally phased TATA sequence positioned at the dyad by using either normal histones or hyperacetylated histones. The hyperacetylated histones were prepared from HeLa cells treated with sodium butyrate, a deacetylase inhibitor. TAU gel electrophoresis, which can resolve histones based on their acetylation state, showed that most of the H4 histone purified from butyrate-treated HeLa cells was tri- or tetra-acetylated and that this histone preparation differed significantly from the preparation isolated from the untreated cells (Fig. 5A). Mononucleosome particles assembled from hyperacetylated histones showed no significant changes in DNase I or micrococcal nuclease sensitivity relative to nucleosomes assembled with histones that were not hyperacetylated (data not shown). TBP was unable to bind to the template assembled with normal HeLa histones (Fig. 5B, lane 5) (24) but showed clear protection of the TATA sequence when the template contained hyperacetylated histones (Fig. 5B, lane 10). Hypersensitive cleavages immediately upstream of the TATA sequences were also observed. In contrast, a template containing a mutated TATA box in the same rotational position did not bind to TBP (Fig. 5B, lane 11). Thus, hyperacetylation of histones sufficiently alters nucleosome structure such that the TATA sequence, at least in some locations, can become accessible to TBP binding.

Interactions between Nhp6 and TFIIA. We next tested whether the TFIIA mutants were lethal in the absence of Nhp6. We constructed an *nhp6ab toa2* strain with the YCp-*URA3*-TFIIA (wild type) plasmid and transformed this strain with plasmids carrying the various TFIIA mutations. Several TFIIA mutants were viable in the absence of Nhp6 (Table 4). However, the Y10G R11Δ TFIIA mutant showed a marked growth defect in the absence of Nhp6, and the *nhp6ab* strain with TFIIA mutant W76A was unable to grow on plates with 5-FOA (Fig. 6A). We note that the W76A mutant resulted in a growth defect in an otherwise wild-type strain when the strain was grown at either 33 or 37°C (Fig. 3A and Table 4). However, the W76A mutant did not show this growth defect at 25°C, the incubation temperature used in this experiment (Fig. 6B). These results suggest that *nhp6ab* was synthetically lethal with the TFIIA mutant W76A. We note that the *nhp6ab* and TFIIA W67A mutants each had a growth defect, and thus the observed synthetic lethality may simply be an additive effect.

This genetic interaction of Nhp6 with both TFIIA and TBP (13) suggests that Nhp6 may function to promote interaction between TBP and TFIIA. To test this idea, we performed in vitro binding experiments with purified, bacterially expressed TBP, TFIIA, and Nhp6 (Fig. 6C). We used a small amount of TBP in the gel shift assay so that only a small amount of TBP-DNA complex was formed (lanes 3 and 10). TFIIA did

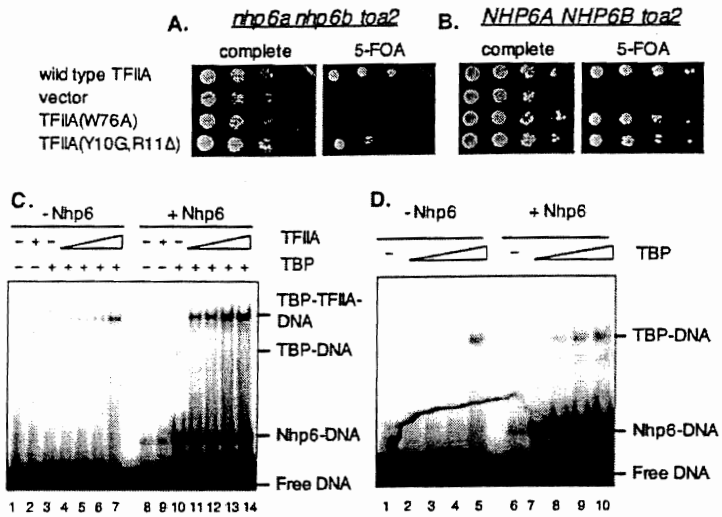

FIG. 6. Nhp6 interacts with TBP and TFIIA. (A) *nhp6ab* is synthetically lethal with TFIIA mutants. Dilutions of strain DY8510 (*nhp6ab toa2*) carrying the YCp-*URA3*-TFIIA (wild type) plasmid and transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 25°C for 4 days. (B) Dilutions of strain DY 8541 (*NHP6A NHP6B toa2*) transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 25°C for 3 days. Note that the TFIIA mutants designated in the figure correspond to substitutions in the Toa2 subunit of TFIIA. (C) Nhp6 stimulates formation of the TBP-TFIIA-DNA complex. TBP (144 nM) was added to lanes 3 to 7 and 10 to 14, and Nhp6 (70 nM) was added to lanes 8 to 14. TFIIA was added to reaction mixtures in the following amounts: 0.15 nM, lanes 4 and 11; 0.3 nM, lanes 5 and 12; 0.6 nM, lanes 6 and 13; and 1.2 nM, lanes 2, 7, 9, and 14. +, present; −, absent. (D) Nhp6 stimulates formation of the TBP-DNA complex. Nhp6 (70 nM) was added to lanes 6 to 10, and TBP was added to lanes 2 to 4 and 7 to 10 in the following amounts: 96 nM, lanes 2 and 7; 192 nM, lanes 3 and 8; 288 nM, lanes 4 and 9; and 384 nM, lanes 5 and 10.

not bind DNA on its own (lane 2), but in the presence of TBP it formed the TBP-TFIIA-DNA complex in a highly cooperative fashion (lanes 4 to 7). However, addition of Nhp6 to the binding reaction mixture affected the amount of TBP-TFIIA-DNA complex formed (lanes 11 to 14). Quantitation shows that Nhp6 caused a three- to fivefold increase in formation of the TBP-TFIIA-DNA complex. Nhp6 had no effect on recruitment of TFIIB to the TBP-TFIIA-DNA complex in our assays (data not shown). This experiment shows that Nhp6 stimulates formation of the TBP-TFIIA-DNA complex.

The results shown in Fig. 6C suggest that Nhp6 modestly stimulates the binding of TBP to DNA, in the absence of TFIIA (compare lanes 3 to 10). To test this idea, we performed a gel shift experiment by varying the amount of TBP, without TFIIA, in the presence or absence of Nhp6 (Fig. 6D). Nhp6 moderately stimulated the formation of the TBP-DNA complex (compare lanes 2 to 5 with lanes 7 to 10). Quantitation shows that Nhp6 could stimulate formation of the TBP-DNA complex by twofold. This result is consistent with the synthetic lethality of TBP mutants in strains lacking Nhp6 (13). In summary, these in vitro binding experiments show that Nhp6 can facilitate the in vitro interaction of TBP with DNA, especially in the presence of TFIIA.

DISCUSSION

Transcriptional activation by RNA polymerase II requires promoter binding by TBP and general transcription factors TFIIA and TFIIB, even for promoters lacking a TATA element (55). Formation of the TBP-TFIIA-TFIIB-DNA complex is the limiting event in transcriptional activation, and much of the transcriptional regulatory machinery is devoted to regulating promoter binding by these factors (36, 37). Activation-defective TBP mutants can be suppressed by overexpression of TFIIA or by point mutations in TFIIA (40), emphasizing the importance of TBP-TFIIA interactions in transcriptional activation. The work described in this paper supports the idea that transcriptional coactivators, such as the Swi/Snf chromatin remodeling complex, the Gcn5 histone acetyltransferase, and the Nhp6 architectural transcription factor, promote transcription by facilitating the interaction of TBP and TFIIA on promoter DNA. A previous study identified viable substitution mutations in TBP that are lethal in an *nhp6ab* strain (13), and many of these TBP mutations are lethal in strains with disruptions of *SWI2* or *GCN5*. Overexpression of TFIIA can suppress some of these lethal genetic interactions, suggesting that these coactivators promote formation of the TBP-TFIIA complex on DNA. Mutations in the Toa2 subunit of TFIIA that eliminate interaction with TBP are lethal in *swi2 gcn5* and *nhp6ab* strains. These TFIIA mutants are viable in an otherwise wild-type strain, suggesting that decreased affinity between TFIIA and TBP is tolerated as long as Swi/Snf, Gcn5, and Nhp6 are present. The fact that TBP overexpression can suppress the lethality of the TFIIA mutants in these strains suggests that these coactivators function to promote formation of a TBP-TFIIA complex on DNA.

The Swi/Snf chromatin remodeling complex, the Gcn5 histone acetyltransferase, and the Nhp6 architectural transcription factor all contribute to transcriptional activation. Microar-

ray experiments show that mutations in the genes encoding these factors reduce expression of many genes (35, 47, 64), but increased expression of some genes suggests that the mutations can also repress transcription (16, 44). Inactivating any two of these pathways in the *swi2 gcn5*, *swi2 nhp6ab*, or *gcn5 nhp6ab* mutant causes either lethality or a severe growth defect (Fig. 1A and 4C) (72). This type of synthetic lethality from combining null mutations (gene deletions) can be interpreted as the result of two genes' having overlapping functions (54). While gene deletions eliminating *SWI2*, *GCN5*, or *NHP6AB* are tolerated, we suggest that combining these mutations results in sufficiently reduced expression of some critical genes to affect viability. Similarly, mutants with point mutations in TBP or TFIIA are viable, but reduced expression of critical target genes may cause the TBP or TFIIA mutants to be lethal in the *swi2*, *gcn5*, or *nhp6ab* strain.

What is the overlapping function of Swi/Snf, Gcn5, and Nhp6? One possibility is promoting DNA binding by transcription factors. Swi/Snf uses the energy of ATP to alter nucleosome structure, exposing binding sites for factors and thus facilitating factor binding (8, 31). Acetylation of histones also facilitates access of transcription factors to their binding sites (33, 68). Nhp6 is a member of the HMGB family of architectural transcription factors, and mammalian HMGB proteins have been shown to enhance DNA binding by various transcription factors (26, 49, 73, 76). Our genetic data suggest that Swi/Snf, Gcn5, and Nhp6 may all be acting to promote formation of the TBP-TFIIA complex on DNA. TBP bends DNA upon binding, and this may explain the difficulty TBP has in binding to a nucleosomal site (24). Alteration of nucleosome structure by the Swi/Snf complex has been shown to allow binding of TBP and TFIIA (24), and we show that histone acetylation promotes TBP binding (Fig. 5B). We also show that Nhp6 stimulates formation of the TBP-TFIIA-DNA complex (Fig. 6C) and modestly stimulates formation of the TBP-DNA complex (Fig. 6D).

Paull et al. (52) previously examined in vitro interactions of Nhp6 with TBP, TFIIA, and TFIIB, but they obtained different results. They did not find Nhp6 stimulating formation of the TBP-TFIIA-DNA complex, but instead they observed that Nhp6 promoted inclusion of TFIIB into the complex. However, there are two important methodological differences between their studies and ours. First, they used human basal factors and we used yeast TBP and TFIIA. More importantly, they used "core" TBP and we used full-length TBP. Full-length TBP binds DNA slowly, and kinetic analysis suggests a two-step model of binding (23). In contrast, core TBP, lacking the unconserved N-terminal region, binds DNA with higher affinity than full-length TBP (29, 39). Recent work suggests that TBP rapidly forms an unstable complex with unbent DNA and then slowly forms a stable complex containing bent DNA (74). We suggest that DNA bending by Nhp6 may facilitate DNA association with TBP and TFIIA. Nhp6 may act as a shape chaperone by bending DNA briefly, facilitating the adoption of shapes that are energetically allowed but kinetically unlikely (58). There is no evidence either in our experiments or that of Paull et al. (52) that Nhp6 remains associated with any type of TBP-DNA complex. In contrast to the situation with yeast Nhp6, mammalian HMGB proteins stimulate TBP binding to DNA and remain associated in an HMGB-TBP-DNA complex (9).

We believe that Swi/Snf, Gcn5, and Nhp6 act in similar fashions to promote transcription in the same way, via TBP-TFIIA interactions on DNA. In vivo, Swi/Snf facilitates TBP binding to the beta interferon promoter (1, 41), and histone acetylation stimulates TBP binding to the estrogen-responsive pS2 promoter (59). We find that the synthetic lethality of either coactivator mutation, *swi2* or *gcn5*, and a mutant basal factor, either TBP or TFIIA, can be suppressed by overexpression of the other basal factor. This suggests that Swi/Snf activity is absolutely required when there are mutations that affect TBP-TFIIA interaction. Similarly, these TBP or TFIIA mutants may have difficulty in binding DNA at certain promoters when the template is underacetylated in a *gcn5* mutant.

ACKNOWLEDGMENTS

We thank Karen Arndt, Steve Buratowski, Steve Hahn, Mike Hampsey, Tetsuro Kokubo, Paul Lieberman, Laurie Stargell, and Fred Winston, who provided plasmids, and Tim Formosa, who provided Nhp6 protein. We also thank Tim Formosa, Paul Lieberman, and Warren Voth for helpful discussions and Bob Kingston for valuable input and support for the TBP-nucleosome binding experiments.

This work was supported by a grant from the National Institutes of Health awarded to Bob Kingston, A.N.I., and D.J.S.

REFERENCES

1. **Agalioti, T., S. Lomvardas, B. Parekh, J. Yie, T. Maniatis, and D. Thanos.** 2000. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. Cell **103**:667–678.
2. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription in vivo. EMBO J. **14**:1490–1497.
3. **Arndt, K. M., C. R. Wobbe, S. Ricupero-Hovasse, K. Struhl, and F. Winston.** 1994. Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. Mol. Cell. Biol. **14**:3719–3728.
4. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
5. **Brewster, N. K., G. C. Johnston, and R. A. Singer.** 2001. A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol. Cell. Biol. **21**:3491–3502.
6. **Buratowski, S., and H. Zhou.** 1992. Transcription factor IID mutants defective for interaction with transcription factor IIA. Science **255**:1130–1132.
7. **Costigan, C., D. Kolodrubetz, and M. Snyder.** 1994. *NHP6A* and *NHP6B*, which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. Mol. Cell. Biol. **14**:2391–2403.
8. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265**:53–60.
9. **Das, D., and W. M. Scovell.** 2001. The binding interaction of HMG-1 with the TATA-binding protein/TATA complex. J. Biol. Chem. **276**:32597–32605.
10. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. **6**:1319–1331.
11. **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.
12. **Emerson, B. M.** 2002. Specificity of gene regulation. Cell **109**:267–270.
13. **Eriksson, P., D. Biswas, Y. Yu, J. M. Stewart, and D. J. Stillman.** 2004. TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol. Cell. Biol. **24**:6419–6429.
14. **Eriksson, P., L. R. Thomas, A. Thorburn, and D. J. Stillman.** 2004. pRS yeast vectors with a *LYS2* marker. BioTechniques **36**:212–213.
15. **Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman.** 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. **20**:3506–3517.
16. **Fragiadakis, G. S., D. Tzamarias, and D. Alexandraki.** 2004. Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for *FRE2* transcriptional activation. EMBO J. **23**:333–342.
17. **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.
18. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74**:527–534.

19. **Godde, J. S., Y. Nakatani, and A. P. Wolffe.** 1995. The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. Nucleic Acids Res. **23**:4557–4564.
20. **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.
21. **Hampsey, M.** 1998. Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. **62**:465–503.
22. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2**:163–167.
23. **Hoopes, B. C., J. F. LeBlanc, and D. K. Hawley.** 1992. Kinetic analysis of yeast TFIID-TATA box complex formation suggests a multi-step pathway. J. Biol. Chem. **267**:11539–11547.
24. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature **370**: 481–485.
25. **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.
26. **Jayaraman, L., N. C. Moorthy, K. G. Murthy, J. L. Manley, M. Bustin, and C. Prives.** 1998. High mobility group protein-1 (HMG-1) is a unique activator of p53. Genes Dev. **12**:462–472.
27. **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.
28. **Kruppa, M., R. D. Moir, D. Kolodrubetz, and I. M. Willis.** 2001. Nhp6, an HMG1 protein, functions in *SNR6* transcription by RNA polymerase III in *S. cerevisiae*. Mol. Cell **7**:309–318.
29. **Kuddus, R., and M. C. Schmidt.** 1993. Effect of the non-conserved N-terminus on the DNA binding activity of the yeast TATA binding protein. Nucleic Acids Res. **21**:1789–1796.
30. **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.
31. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature **370**:477–481.
32. **Larschan, E., and F. Winston.** 2001. The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. **15**:1946–1956.
33. **Lee, D. Y., J. L. Hayes, D. Pruss, and A. P. Wolffe.** 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell **72**:73–84.
34. **Lee, M., and K. Struhl.** 1997. A severely defective TATA-binding protein–TFIIB interaction does not preclude transcriptional activation in vivo. Mol. Cell. Biol. **17**:1336–1345.
35. **Lee, T. I., H. C. Causton, F. C. Holstege, W. C. Shen, N. Hannett, E. G. Jennings, F. Winston, M. R. Green, and R. A. Young.** 2000. Redundant roles for the TFIID and SAGA complexes in global transcription. Nature **405**: 701–704.
36. **Lee, T. I., and R. A. Young.** 2000. Transcription of eukaryotic protein-coding genes. Annu. Rev. Genet. **34**:77–137.
37. **Lemon, B., and R. Tjian.** 2000. Orchestrated response: a symphony of transcription factors for gene control. Genes Dev. **14**:2551–2569.
38. **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.
39. **Lieberman, P. M., M. C. Schmidt, C. C. Kao, and A. J. Berk.** 1991. Two distinct domains in the yeast transcription factor IID and evidence for a TATA box-induced conformational change. Mol. Cell. Biol. **11**:63–74.
40. **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.
41. **Lomvardas, S., and D. Thanos.** 2001. Nucleosome sliding via TBP DNA binding in vivo. Cell **106**:685–696.
42. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14**:953–961.
43. **Lopez, S., M. Livingstone-Zatchej, S. Jourdain, F. Thoma, A. Sentenac, and M. C. Marsolier.** 2001. High-mobility-group proteins NHP6A and NHP6B participate in activation of the RNA polymerase III SNR6 gene. Mol. Cell. Biol. **21**:3096–3104.
44. **Martens, J. A., and F. Winston.** 2002. Evidence that Swi/Snf directly represses transcription in S. cerevisiae. Genes Dev. **16**:2231–2236.
45. **Martens, J. A., and F. Winston.** 2003. Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr. Opin. Genet. Dev. **13**: 136–142.
46. **Martin, M. P., V. L. Gerlach, and D. A. Brow.** 2001. A novel upstream RNA polymerase III promoter element becomes essential when the chromatin structure of the yeast U6 RNA gene is altered. Mol. Cell. Biol. **21**:6429–6439.
47. **Moreira, J. M., and S. Holmberg.** 2000. Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6A and NHP6B. EMBO J. **19**:6804–6813.
48. **Narlikar, G. J., H. Y. Fan, and R. E. Kingston.** 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell **108**:475–487.
49. **Onate, S. A., P. Prendergast, J. P. Wagner, M. Nissen, R. Reeves, D. E. Pettijohn, and D. P. Edwards.** 1994. The DNA-bending protein HMG-1 enhances progesterone receptor binding to its target DNA sequences. Mol. Cell. Biol. **14**:3376–3391.
50. **Orphanides, G., T. Lagrange, and D. Reinberg.** 1996. The general transcription factors of RNA polymerase II. Genes Dev. **10**:2657–2683.
51. **Ozer, J., L. E. Lezina, J. Ewing, S. Audi, and P. M. Lieberman.** 1998. Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18**:2559–2570.
52. **Paull, T. T., M. Carey, and R. C. Johnson.** 1996. Yeast HMG proteins *NHP6A/B* potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev. **10**:2769–2781.
53. **Pollard, K. J., and C. L. Peterson.** 1997. Role for *ADA/GCN5* products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell. Biol. **17**:6212–6222.
54. **Prelich, G.** 1999. Suppression mechanisms: themes from variations. Trends Genet. **15**:261–266.
55. **Pugh, B. F., and R. Tjian.** 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. Genes Dev. **5**:1935–1945.
56. **Ranish, J. A., W. S. Lane, and S. Hahn.** 1992. Isolation of two genes that encode subunits of the yeast transcription factor IIA. Science **255**:1127–1129.
57. **Roberts, S. M., and F. Winston.** 1997. Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. Genetics **147**:451–465.
58. **Ross, E. D., P. R. Hardwidge, and L. J. Maher III.** 2001. HMG proteins and DNA flexibility in transcription activation. Mol. Cell. Biol. **21**:6598–6605.
59. **Sewack, G. F., T. W. Ellis, and U. Hansen.** 2001. Binding of TATA binding protein to a naturally positioned nucleosome is facilitated by histone acetylation. Mol. Cell. Biol. **21**:1404–1415.
60. **Sherman, F.** 1991. Getting started with yeast. Methods Enzymol. **194**:1–21.
61. **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.
62. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators in vivo. Science **269**:75–78.
63. **Sterner, D. E., and S. L. Berger.** 2000. Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64**:435–459.
64. **Sudarsanam, P., V. R. Iyer, P. O. Brown, and F. Winston.** 2000. Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **97**:3364–3369.
65. **Tan, S., Y. Hunziker, D. F. Sargent, and T. J. Richmond.** 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature **381**:127–151.
66. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56**:619–630.
67. **Travers, A. A.** 2003. Priming the nucleosome: a role for HMGB proteins? EMBO Rep. **4**:131–136.
68. **Vettese-Dadey, M., P. A. Grant, T. R. Hebbes, C. Crane-Robinson, C. D. Allis, and J. L. Workman.** 1996. Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J. **15**:2508–2518.
69. **von Schwedler, U. K., M. Stuchell, B. Muller, D. M. Ward, H. Y. Chung, E. Morita, H. E. Wang, T. Davis, G. P. He, D. M. Cimbora, A. Scott, H. G. Krausslich, J. Kaplan, S. G. Morham, and W. I. Sundquist.** 2003. The protein network of HIV budding. Cell **114**:701–713.
70. **Workman, J. L., I. C. Taylor, R. E. Kingston, and R. G. Roeder.** 1991. Control of class II gene transcription during in vitro nucleosome assembly. Methods Cell Biol. **35**:419–447.
71. **Yu, Y., P. Eriksson, L. T. Bhoite, and D. J. Stillman.** 2003. Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. Mol. Cell. Biol. **23**:1910–1921.
72. **Yu, Y., P. Eriksson, and D. J. Stillman.** 2000. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol. Cell. Biol. **20**:2350–2357.
73. **Zappavigna, V., L. Falciola, M. Helmer-Citterich, F. Mavilio, and M. E. Bianchi.** 1996. HMG1 interacts with HOX proteins and enhances their DNA binding and transcriptional activation. EMBO J. **15**:4981–4991.
74. **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.
75. **Zweidler, A.** 1978. Resolution of histones by polyacrylamide gel electrophoresis in presence of nonionic detergents. Methods Cell Biol. **17**:223–233.
76. **Zwilling, S., H. Konig, and T. Wirth.** 1995. High mobility group protein 2 functionally interacts with the POU domains of octamer transcription factors. EMBO J. **14**:1198–1208.

CHAPTER 4

GENETIC INTERACTIONS BETWEEN Nhp6 AND Gcn5 WITH Mot1 AND THE Ccr4/Not COMPLEX THAT REGULATE BINDING TATA-BINDING PROTEIN IN SACCHAROMYCES CEREVISIAE

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DOI: 10.1534/genetics.105.050245

Genetic Interactions Between Nhp6 and Gcn5 With Mot1 and the Ccr4–Not Complex That Regulate Binding of TATA-Binding Protein in *Saccharomyces cerevisiae*

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Manuscript received August 29, 2005
Accepted for publication October 20, 2005

ABSTRACT

Our previous work suggests that the Nhp6 HMGB protein stimulates RNA polymerase II transcription via the TATA-binding protein TBP and that Nhp6 functions in the same functional pathway as the Gcn5 histone acetyltransferase. In this report we examine the genetic relationship between Nhp6 and Gcn5 with the Mot1 and Ccr4–Not complexes, both of which have been implicated in regulating DNA binding by TBP. We find that combining either a *nhp6ab* or a *gcn5* mutation with *mot1*, *ccr4*, *not4*, or *not5* mutations results in lethality. Combining *spt15* point mutations (in TBP) with either *mot1* or *ccr4* also results in either a growth defect or lethality. Several of these synthetic lethalities can be suppressed by overexpression of TFIIA, TBP, or Nhp6, suggesting that these genes facilitate formation of the TBP–TFIIA–DNA complex. The growth defect of a *not5* mutant can be suppressed by a *mot1* mutant. *HO* gene expression is reduced by *nhp6ab*, *gcn5*, or *mot1* mutations, and the additive decreases in *HO* mRNA levels in *nhp6ab mot1* and *gcn5 mot1* strains suggest different modes of action. Chromatin immunoprecipitation experiments show decreased binding of TBP to promoters in *mot1* mutants and a further decrease when combined with either *nhp6ab* or *gcn5* mutations.

TRANSCRIPTIONAL activation by RNA polymerase II (pol II) requires the assembly of a complex of general transcription factors at a promoter (Hampsey 1998; Dvir *et al.* 2001). It is believed that transcriptional coactivators function by stimulating DNA binding by the general transcription factors TBP (TATA-binding protein), TFIIA, and TFIIB. Additionally, there are transcription factors that have been shown to negatively regulate binding of TBP to promoter DNA (reviewed by Lee and Young 1998; reviewed by Pugh 2000). Factors such as TAF1 and NC2 interact with TBP and inhibit its activity (Goppelt *et al.* 1996; Mermelstein *et al.* 1996; Bai *et al.* 1997; Kokubo *et al.* 1998). In contrast, Mot1 can disassociate TBP from DNA (Auble *et al.* 1994), and the Ccr4–Not complex may inhibit the recruitment of other general factors by TBP (Collart 1996; Badarinarayana *et al.* 2000). Additionally, two TBP molecules can dimerize to create a form that does not bind DNA (Coleman and Pugh 1997).

Mot1 is thought to inhibit transcription of certain genes by inhibiting TBP binding (for review see Pereira *et al.* 2003). *In vitro*, the Mot1 protein binds to TBP–DNA complexes and uses the energy of ATP to dissociate TBP from the DNA (Auble *et al.* 1994; Darst *et al.* 2003). Mot1 is an essential gene, and the fact that *mot1* mutations cause derepression of specific genes is consistent with a proposed role as a negative regulator (Auble *et al.* 1994). However, Mot1 also functions as a positive regulator of transcription, as *mot1* mutations reduce expression of certain genes (Andrau *et al.* 2002; Dasgupta *et al.* 2002). There are strong genetic interactions between *MOT1* and *SPT15* (encoding TBP) and with other basal factors, including *TOA1* and *TOA2* (encoding TFIIA), *SPT3*, and the Ccr4–Not complex (Collart 1996; Madison and Winston 1997). It has been suggested that Mot1 can stimulate transcription by inhibiting the association of NC2, a TBP inhibitor, with promoters (Geisberg *et al.* 2002) and that the Mot1–TBP complex delivers TBP to TAF-independent genes (Gumbs *et al.* 2003). Additionally, Mot1 is required for nucleosome remodeling at the *GAL1* promoter (Topalidou *et al.* 2004). In normally growing cells, Mot1 co-occupies promoters with TBP, but not with TFIIB, TFIIA, or TAFs (Geisberg and Struhl 2004).

The Ccr4–Not complexes have multiple roles in gene regulation, including regulation of transcriptional initiation, elongation, and mRNA degradation (for reviews see Collart 2003; for reviews see Denis and Chen 2003). Ccr4–Not has been implicated as both a positive and a negative regulator of transcription (Liu *et al.* 1998), and the Gcn4 DNA-binding activator can recruit Ccr4–Not to promoters (Swanson *et al.* 2003). Some of the genes encoding subunits of this protein complex have been found to interact both physically and genetically with TBP, TAFs, and regulators of TBP binding, and it has been suggested that Ccr4–Not represses transcription

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TABLE 1

Strain list

DY 150	*MAT*a	*ade2 can1 his3 leu2 trp1 ura3*
DY5265	*MAT*a	*gcn5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7139	*MAT*a	*nhp6a::KanMX nhp6b::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7176	*MAT*a	*ccr4::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7441	*MAT*a	*ccr4::LEU2 nhp6a::KanMX nhp6b::ADE2 NHP6B(YCp-URA3) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7462	*MAT*a	*mot1(R1243I) ade2 can1 his3 leu2 trp1 ura3*
DY7463	*MAT*α	*mot1(R1243I) ade2 can1 his3 leu2 trp1 ura3*
DY7841	*MAT*a	*gcn5::TRP1 mot1(R1243I) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7847	*MAT*a	*mot1(R1243I) nhp6a::KanMX nhp6b::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8237	*MAT*a	*mot1(R1243I) spt15::LEU2 SPT15(YCp-URA3) ade2 can1 his3 leu2 trp1 ura3*
DY8563	*MAT*a	*ccr4::LEU2 gcn5::TRP1 GCN5(YCp-URA3) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8617	*MAT*α	*not4::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY8618	*MAT*a	*gcn5::HIS3 not4::LEU2 GCN5(YCp-URA3) ade2 can1 his3 leu2 trp1 ura3*
DY8625	*MAT*a	*nhp6a::KanMX nhp6b::ADE2 not5::LEU2 NHP6A(YCp-URA3) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8626	*MAT*a	*not5::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8627	*MAT*α	*not5::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8628	*MAT*a	*gcn5::HIS3 not5::LEU2 GCN5(YCp-URA3) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9348	*MAT*a	*ccr4::LEU2 spt15::ADE2 SPT15(YCp-URA3) ade2 can1 his3 leu2 trp1 ura3*
DY9383	*MAT*a	*mot1(R1243I) spt15::LEU2 SPT15(YCp-URA3) ade2 can1 leu2 lys2 trp1 ura3*
DY9384	*MAT*a	*ccr4::LEU2 spt15::ADE2 SPT15(YCp-URA3) ade2 can1 leu2 lys2 trp1 ura3*
DY9470	*MAT*a	*ccr4::LEU2 mot1(R1243I) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9545	*MAT*a	*mot1(R1243I) not4::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9582	*MAT*a	*mot1(R1243I) not5::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*

by inhibiting DNA binding by TBP (COLLART 1996; BADARINARAYANA *et al.* 2000; LEMAIRE and COLLART 2000; DELUEN *et al.* 2002).

We have studied the regulation of the yeast *HO* gene, and our studies suggest that Gcn5 and Nhp6 function in parallel to activate expression of this gene (YU *et al.* 2000, 2003). Gcn5 is the histone acetyltransferase present in the yeast SAGA complex, and histone acetylation by Gcn5 is required for expression of many yeast genes (STERNER and BERGER 2000). Nhp6 is related to the HMGB family of small, abundant chromatin proteins that bend DNA sharply and modulate gene expression (TRAVERS 2003). Nhp6 is encoded by two genes, *NHP6A* and *NHP6B*. No phenotype is seen in *nhp6a* and *nhp6b* single mutants, while the *nhp6a nhp6b* double mutant (which we will describe as *nhp6ab*) is temperature sensitive for growth (COSTIGAN *et al.* 1994) and shows transcriptional defects (PAULL *et al.* 1996; YU *et al.* 2000; FRAGIADAKIS *et al.* 2004). Nhp6 also functions with Spt16 and Pob3, as part of the yeast FACT complex, to promote transcriptional elongation (FORMOSA *et al.* 2001), and Nhp6 is important for expression of the *SNR6* gene, transcribed by RNA polymerase III (KRUPPA *et al.* 2001; LOPEZ *et al.* 2001; MARTIN *et al.* 2001).

Our data suggest that Gcn5 and Nhp6 function to promote assembly of the TBP–TFIIA–DNA complex (YU *et al.* 2003; BISWAS *et al.* 2004; ERIKSSON *et al.* 2004a). Viable mutations affecting TBP or TFIIA (*spt15* or *toa2*, respectively) are lethal in *gcn5* or *nhp6ab* mutant strains. TBP overexpression suppresses the temperature-sensitive growth defect of *nhp6ab* strains and certain transcriptional defects of either *nhp6ab* or *gcn5* mutants. Additionally, the *gcn5 nhp6ab* triple mutant displays a strong synthetic growth defect, but this phenotype can be suppressed by mutations in the *SPT3* gene. Spt3, which is part of the SAGA complex with Gcn5 (STERNER *et al.* 1999), interacts with TBP both physically and genetically (EISENMANN *et al.* 1992). We find that an *spt3* mutation can suppress a number of *gcn5* and *nhp6ab* defects, including reduced *HO* expression, temperature-sensitive growth, and synthetic lethality with TBP mutants. Chromatin immunoprecipitation experiments show that Spt3 regulates TBP binding *in vivo*, inhibiting TBP binding to the *HO* promoter while stimulating TBP binding to *GAL1* (DUDLEY *et al.* 1999; YU *et al.* 2003).

In this study we use genetic tools to examine the relationship of Mot1 and Ccr4–Not to Gcn5 and Nhp6. Spt3, Mot1, and Ccr4–Not all regulate binding of TBP to DNA, and *spt3* mutations suppress many *gcn5* and *nhp6ab* defects. However, instead of suppression, we find synthetic lethal interactions between Mot1 and Ccr4–Not with Gcn5 and Nhp6. Multicopy suppression experiments support a critical role of these factors in facilitating formation of the TBP–TFIIA complex on DNA. Additive effects on *HO* gene transcription suggest that Mot1 functions differently from either Nhp6 or Gcn5. Chromatin immunoprecipitation (ChIP) experiments show that TBP binding to promoters is reduced in *mot1* mutants, with an additive decrease when combined with *nhp6ab* or *gcn5*.

MATERIALS AND METHODS

Strains and media: All yeast strains used are listed in Table 1 and are isogenic in the W303 background (THOMAS and

TABLE 2

Plasmids

Plasmid	Description	Source
pRS425	YEp–*LEU2* vector	CHRISTIANSON *et al.* (1992)
pRS327	YEp–*LYS2* vector	ERIKSSON *et al.* (2004b)
YEplac195	YEp–*URA3* vector	GIETZ and SUGINO (1988)
pRS314	YCp–*TRP1* vector	SIKORSKI and HIETER (1989)
M2661	26-kb genomic fragment with *MOT1* in YCp50	JIANG and STILLMAN (1996)
M2719	M2661 with 3.5-kb *Bam*HI fragment deleted	This work
M5099	*mot1(R1243I)* in YCp50	This work
M4252	*GCN5* in YEp–*LEU2* plasmid	This work
M3000	*MOT1* in YCp–*URA3* plasmid	JIANG and STILLMAN (1996)
M4462	*NHP6A* in YEp–*LEU2* plasmid	This work
M4797	*NHP6A* in YEp–*LYS2* plasmid	BISWAS *et al.* (2004)
M4221	*NHP6A* in YEp–*URA3* plasmid	BISWAS *et al.* (2004)
pRS426–*SNR6*	*SNR6* in YEp–*URA3* plasmid	ERIKSSON *et al.* (2004a)
pSH346	TFIIA (*TOA1* and *TOA2*) in YEp–*LEU2* plasmid	ERIKSSON *et al.* (2004a)
M4793	TFIIA (*TOA1* and *TOA2*) in YEp–*LYS2* plasmid	BISWAS *et al.* (2004)
M3415	TFIIB (*SUA7*) in YEp–*URA3* plasmid	Mike Hampsey
M4480	TBP wild-type (*SPT15*) in YEp–*LEU2* plasmid	This work
M4533	TBP wild-type (*SPT15*) in YEp–*LYS2* plasmid	BISWAS *et al.* (2004)
M4403	TBP wild-type (*SPT15*) in YEp–*URA3* plasmid	This work
pTM8	TBP(wild-type) in YCp–*TRP1* plasmid	KOBAYASHI *et al.* (2001)
M4471	TBP(E93G) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4325	TBP(L114F) in YCp–*TRP1* plasmid	ARNDT *et al.* (1994)
M4642	TBP(K133R) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4475	TBP(G147W) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4470	TBP(C164W) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4474	TBP(L172P) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4482	TBP(G174E) in YCp–*TRP1* plasmid	EISENMANN *et al.* (1992)
M4472	TBP(F227L) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4473	TBP(F237L) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4653	TBP(K239T) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4468	TBP(K97R, L193S) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4655	TBP(I103T, K239Stop) in YCp–*TRP1* plasmid	ERIKSSON *et al.* (2004a)
M4550	TBP(K133L, K145L) in YCp–*TRP1* plasmid	BURATOWSKI and ZHOU (1992)
M4404	TBP(K138T, Y139A) in YCp–*TRP1* plasmid	STARGELL and STRUHL (1995)

ROTHSTEIN 1989). Standard genetic methods were used for strain construction (ROTHSTEIN 1991; SHERMAN 1991). W303 strains with disruptions in *gcn5*, *nhp6a*, and *nhp6b* have been described (YU *et al.* 2000, 2003) and the *mot1(R1243I)* allele was identified in a screen for Spt– mutations (JIANG and STILLMAN 1996). The *ccr4* disrupted strain was provided by Clyde Denis, and the *not4* and *not5* disrupted strains by Martine Collart. These strains were then crossed to generate the strains used here. Strain DY9384 was constructed by disrupting the *LYS2* gene in strain DY9348 with the D588 *lys2::HIS3* marker swap plasmid (VOTH *et al.* 2003). Cells were grown at the indicated temperature in YEPD medium (SHERMAN 1991), except where synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids, as appropriate, but lacking essential components, was used to select for plasmids. 5-FOA medium was prepared as described (BOEKE *et al.* 1984).

Plasmids: The multicopy plasmids used are listed in Table 2. Plasmid M4252 was constructed by moving a 1.8-kb *Sac*I–*Xho*I fragment with *GCN5* from plasmid pRS315–*GCN5* (MARCUS *et al.* 1994) into pRS425 (CHRISTIANSON *et al.* 1992). Plasmid M4462 was constructed by moving a 0.95-kb *Hin*dIII–*Sac*I fragment with *NHP6A* from plasmid M4221 (BISWAS *et al.* 2004) into pRS325 (SIKORSKI and HIETER 1989). A 2.25-kb *Bam*HI–*Pst*I fragment with *SPT15* from pSH2223 (YU *et al.* 2003) was cloned into YEplac195 (GIETZ and SUGINO 1988) and pRS425 (CHRISTIANSON *et al.* 1992), constructing M4403 and M4480, respectively. Plasmid M2661 was isolated from a YCp50 genomic library as complementing the temperature-sensitive phenotype of the *mot1* mutant (JIANG and STILLMAN 1996), and M2719 was constructed from M2661 by deleting a 3.5-kb *Bam*HI fragment within the *MOT1* gene. The *mot1(R1243I)* allele was cloned by transforming DY7463 [*mot1(R1243I)*] with plasmid M2719, which had been cleaved with *Sac*I, yielding plasmid M5099, which was then sequenced.

RNA analysis: RNA levels were determined with S1 nuclease protection assays as described (BHOITE and STILLMAN 1998). The sequences of the S1 primers are as follows: *CLN2*, TAC AACCGCCCCAAGTTTTAGCAGCCAACCAGAGACAAGTAG CGACAACCAATTTGGCTTGGTCCCGTAACACGATTCTCG GTTCC; TBP (*SPT15*), ACGCATGATGACAGCAGCAAAACG CTTGGGGTTATATTCTGCATTACGGGCATGTAGCGCTTGA CA; TFIIB (*SUA7*), TCCTTGCCACTTCAGCACGTCTGCAAC CAATCAGTATGGATGCAGCCATTTATGAG; TFIIA (*TOA1*), ATCTGCTCCTTTTCCTTGCGGGTTTTTTCCACGTCCTCC TCCTTTTCCTCGTCGTCTTTCAAGAGT; and TFIIA (*TOA2*), GGAGGCGTCGCGGTGGCTGTCCTCAACAGTAACCTGACA ATTTTTTACGAATTTTC. The *HO*, *CMD1*, and tRNA-Trp primers have been described (BHOITE and STILLMAN 1998; OZER *et al.* 1998).

ChIP analysis: Chromatin immunoprecipitation was performed as described (Biswas *et al.* 2005), with the PCR amplifications performed in triplicate. The sequences of the PCR primers are as follows: *ELP3*, TGCCGCTTTCATTGTTTAATC ATTTCACCTT and TCCATGACGAGCCATCTTTGTCAGGG; *HXT4*, TTAGTGGTGAAAAGCTTCAACACTGG and TTCAA AACCAAACCTTGATAAGAGGC; *RPS5*, AGGCTTAGTGGA GGTCTCACTGAA and GACTGGGGTGAATTCTTCAACAA; *URA1*, CCGAAGGTTATTTCACGA and CTGGCTGTCATGTT TGGT. The PCR primers for *SER3* and intragenic V (used as internal control) have been described (Biswas *et al.* 2005).

RESULTS

Genetic interactions of Nhp6 and Gcn5 with Mot1: Deletion of the *SPT3* gene suppresses both temperature-sensitive growth and transcriptional defects caused by the absence of Nhp6 (Yu *et al.* 2003). As Spt3 interacts with TBP (Eisenmann *et al.* 1992), we wanted to ask whether mutations in other factors that interact with TBP would suppress the *nhp6ab* mutation. Mot1 has also been shown to interact with TBP, and Mot1 inhibits TBP binding *in vitro* (Auble *et al.* 1994). Additionally, strong genetic links have been established for Mot1 with both Spt3 and TBP (Collart 1996; Madison and Winston 1997). *MOT1* is an essential gene, but viable alleles have been identified (Abate *et al.* 1990; Prelich and Winston 1993; Madison and Winston 1997; Darst *et al.* 2003). We isolated a viable *mot1* allele in a genetic screen (Jiang and Stillman 1996). We cloned this *mot1* allele from the genome by allele rescue with a gapped *MOT1* plasmid (Rothstein 1991) and sequenced the gene. The mutation has an arginine-to-isoleucine substitution at residue 1243. R1243 is highly conserved among Mot1 proteins, and when it is not arginine this position is usually lysine, also a basic amino acid. We crossed the *mot1(R1243I)* allele to a *nhp6ab* strain and isolated a *nhp6a nhp6b mot1(R1243I)* triple mutant. Instead of finding genetic suppression, we were surprised to find strong synthetic phenotypes. The *nhp6ab mot1(R1243I)* triple-mutant strain showed a strong growth defect at 25° and was lethal at 30° on YEPD medium (Figure 1A).

We next asked whether multicopy plasmids could suppress the growth defect (Figure 1B). As expected, the *MOT1* and *NHP6A* plasmids complemented, but the YEp–TFIIA and YEp–*GCN5* plasmids exacerbated the growth defect at 25°. However, at 30°, YEp–*SNR6* or YEp–TBP strongly suppressed the growth defect, and YEp–TFIIB showed moderate suppression. *nhp6ab* mutants are defective in expressing *SNR6*, a pol III transcribed gene encoding the U6 splicing RNA (Lopez *et al.* 2001; Martin *et al.* 2001). YEp–*SNR6* suppresses the temperature-sensitive growth defect seen in *nhp6ab* mutants, and it is suggested that decreased *SNR6* RNA contributes to the poor growth at elevated temperatures (Kruppa *et al.* 2001). The suppression of the *nhp6ab mot1(R1243I)* synthetic lethality by YEp–TBP and YEp–TFIIB suggests that this mutant strain is defective in building the TBP–TFIIB complex at promoters of pol II transcribed genes. It is less clear why overexpression of TFIIA or Gcn5 exacerbates the growth defect in the *nhp6ab mot1(R1243I)* strain.

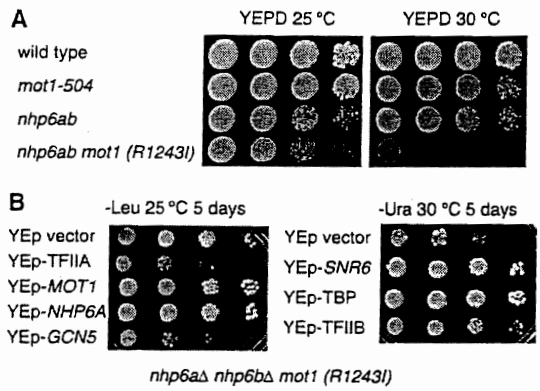

FIGURE 1.—Genetic interactions of *MOT1* with *NHP6*. (A) *nhp6ab mot1(R1243I)* is lethal at 30°. Strains DY150 (wild type), DY7139 (*nhp6ab*), DY7463 [*mot1(R1243I)*], and DY7847 [*nhp6ab mot1(R1243I)*] were plated on YEPD medium for 4 days at 25° or for 2 days at 30°. (B) Strain DY7847 [*nhp6ab mot1 (R1243I)*] was transformed with the indicated multicopy plasmids at 25°, and dilutions were plated on the indicated selective medium for 5 days at the indicated temperature.

We next looked for genetic interactions between *GCN5* and *MOT1*, since Nhp6 and Gcn5 function in the same pathway for transcriptional activation of *HO* (Yu *et al.* 2000). We constructed the *gcn5 mot1(R1243I)* double mutant and found that it too has a strong growth defect at 25° and is nearly inviable at 30° on YEPD medium (Figure 2A). Interestingly, the growth of the *nhp6a nhp6b mot1(R1243I)* triple mutant at 25° is much worse than that for the *gcn5 mot1(R1243I)* double mutant. Figure 2B shows the effects of multicopy plasmids on growth of the *gcn5 mot1(R1243I)* strain. Note that while the *gcn5 mot1(R1243I)* strain is lethal on complete YEPD medium at 30°, it is able to grow, although poorly, on selective plates at 30°. Plasmids with *MOT1* or *GCN5* complemented, as expected, while multicopy plasmids with TFIIA, TFIIB, or *SNR6* did not affect growth of the *gcn5 mot1(R1243I)* strain (data not shown). Interestingly, overexpression of TBP or Nhp6 significantly exacerbated the growth defect of the *gcn5 mot1(R1243I)* mutant at 30°, supporting the idea that Gcn5 and Mot1 play an active role in regulating TBP binding.

We note that the multicopy suppression results are quite different, with YEp–TBP suppressing the *nhp6ab mot1(R1243I)* mutant but exacerbating the growth defect in the *gcn5 mot1(R1243I)* mutant. This suggests that the defects caused by the *nhp6ab* deletion and the *mot1(R1243I)* mutation are quite different.

Genetic interactions of Nhp6 and Gcn5 with the Ccr4–Not complex: The Ccr4–Not complex has roles in

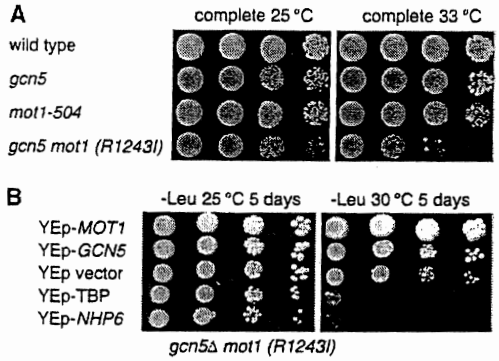

FIGURE 2.—Genetic interactions of *MOT1* with *GCN5*. (A) *gcn5 mot1(R1243I)* is lethal at 33°. Strains DY150 (wild type), DY5265 (*gcn5*), DY7463 [*mot1(R1243I)*], and DY7841 [*gcn5 mot1(R1243I)*] were plated on complete medium for 3 days at either 25° or 33°. (B) Strain DY7841 [*gcn5 mot1(R1243I)*] was transformed with the indicated multicopy plasmids at 25°, and dilutions were plated on selective medium for 5 days at the indicated temperature.

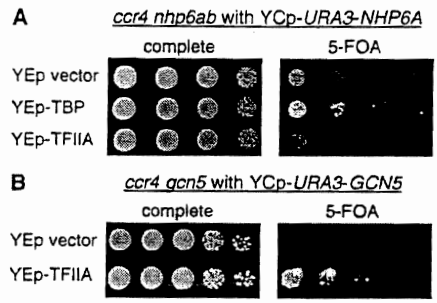

FIGURE 3.—Genetic interactions of *CCR4* with *GCN5* and *NHP6*. (A) The *nhp6ab ccr4* synthetic lethality is suppressed by TBP overexpression. Strain DY7441 (*nhp6ab ccr4* with a YCp–*URA3–NHP6B* plasmid) was transformed with the indicated *LYS2* multicopy plasmids, and dilutions were plated at 30° for 2 days (complete) or for 6 days (5-FOA). (B) The *gcn5 ccr4* synthetic lethality is suppressed by TFIIA overexpression. Strain DY8563 [(*gcn5 ccr4*) with a YCp–*URA3–GCN5* plasmid] was transformed with either YEp–TFIIA or the YEp–*LYS2* vector, and dilutions were plated at 33° for 2 days (complete) or for 5 days (5-FOA).

regulating transcriptional initiation, elongation, and mRNA degradation (DENIS and CHEN 2003). A number of experiments have shown that Ccr4–Not represses transcription through direct contacts with TBP, inhibiting TBP binding to DNA (COLLART 1996; BADARINARAYANA *et al.* 2000; LEMAIRE and COLLART 2000; DELUEN *et al.* 2002). We therefore performed genetic crosses to determine whether a *ccr4* mutation might suppress *nhp6ab* or *gcn5* defects. In the first cross we were unable to recover a viable *nhp6ab ccr4* spore. To verify this apparent synthetic lethality, we constructed a *nhp6aΔ/+ nhp6bΔ/+ ccr4Δ/+* triply heterozygous diploid strain and transformed it with a YCp–*URA3–NHP6A* plasmid. The diploids were induced to undergo meiosis, tetrads were dissected, and we isolated haploid strains with the *nhp6a nhp6b ccr4* genotype containing the YCp–*URA3–NHP6A* plasmid. These strains were unable to grow on media containing 5-FOA (Figure 3A), indicating that the YCp–*URA3–NHP6A* plasmid cannot be lost. We next asked whether multicopy plasmids could suppress this synthetic lethality. For *nhp6ab ccr4*, the YEp–TBP plasmid partially suppressed the synthetic lethality, but YEp–TFIIA did not (Figure 3A). This is an important result, as both Ccr4 and Nhp6 have roles in transcriptional initiation and elongation, but the suppression by TBP overexpression suggests that a defect in initiation contributes to the *nhp6ab ccr4* synthetic lethality. We also determined that *ccr4* is synthetic lethal with a *gcn5* mutation. We constructed a *gcn5 ccr4* strain, containing a YCp–*URA3–GCN5* plasmid, which is unable to grow on 5-FOA (Figure 3B). This synthetic lethality is partially suppressed by YEp–TFIIA, but not by YEp–TBP, YEp–TFIIB, or YEp–*NHP6A* (Figure 3B; data not shown).

In addition to regulating TBP binding, Ccr4 is the catalytic subunit of a cytoplasmic mRNA deadenylase (CHEN *et al.* 2002; TUCKER *et al.* 2002). Although the Not proteins are associated with the cytoplasmic form of the Ccr4–Not complex, mutations in the *NOT* genes have only modest effects on the rate of deadenylation (TUCKER *et al.* 2002), suggesting that the Not proteins and Ccr4 may have important functional differences. We therefore asked whether there are genetic interactions between *nhp6ab* or *gcn5* and *not4* and *not5*. For example, a haploid *nhp6a nhp6b* strain was crossed to a *not5* mutant, and the resulting diploid was transformed with a YCp–*URA3–NHP6A* plasmid. After sporulation and tetrad dissection, a *nhp6ab not5* triple mutant with the YCp–*URA3–NHP6A* plasmid was isolated. This strain was unable to grow on 5-FOA, demonstrating the synthetic lethality of *nhp6ab* with *not5*. In this way we were able to show that the *nhp6ab not4*, *nhp6ab not5*, *gcn5 not4*, and *gcn5 not5* mutant combinations were all synthetic lethal (Figure 4A; data not shown). Multicopy suppression experiments showed that YEp–TFIIA could suppress the *gcn5 not5* synthetic lethality (Figure 4B), but multicopy suppression was not seen with YEp–TFIIB, YEp–TBP, or YEp–*NHP6A*.

We observed synthetic lethality of *gcn5* with all three members of the Ccr4–Not complex that we tested: *ccr4*, *not4*, and *not5*. In contrast, MAILLET *et al.* (2000) did not observe synthetic lethality in *gcn5 ccr4* or *gcn5 not5* mutants and saw only a synthetic slow-growth defect in the *gcn5 not4* double mutant. We used W303 strains, while their studies utilized a different strain background, and strain differences could be responsible for the different results.

The *not5* single mutant shows a growth defect at 30° and is unable to grow at the higher temperature of 33°. Thus, we asked whether overexpression of other factors

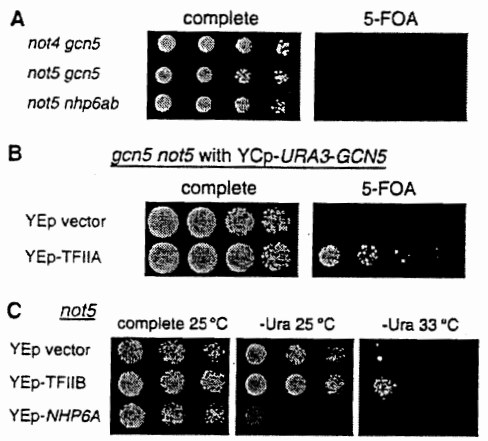

FIGURE 4.—Genetic interactions of *NOT* genes with *GCN5* and *NHP6*. (A) A *not5* mutation is synthetic lethal with *gcn5* and with *nhp6*, and *not4* is synthetic lethal with *gcn5*. Dilutions of strains DY8618 (*not4 gcn5*), DY8628 (*not5 gcn5*), and DY8625 (*not5 nhp6ab*), each carrying a YCp–URA3 plasmid with either *GCN5* or *NHP6A*, were plated on the indicated medium at 25° for 3 days. (B) The *gcn5 not5* synthetic lethality is suppressed by TFIIA overexpression. Strain DY8628 [(*gcn5 not5*) with a YCp–*URA3–GCN5* plasmid] was transformed with either YEp–TFIIA or the YEp–*LYS2* vector and plated at 25° for 2 days on complete medium or for 5 days on 5-FOA plates. (C) Growth of the *not5* mutant is affected by TFIIB or Nhp6 overexpression. Strain DY8626 (*not5*) was transformed with the indicated *URA3* multicopy plasmids, and dilutions were plated for 4 days (complete at 25°), 2 days (−Ura at 25°), or 6 days (−Ura at 33°).

affected growth of the *not5* mutant. The *not5* strain was transformed with multicopy plasmids and then growth at various temperatures was assessed. While multicopy plasmids with TBP or TFIIA did not affect growth of the *not5* mutant, YEp–TFIIB improved growth at 25° and partially suppressed the temperature-sensitive growth defect (Figure 4). In contrast, overexpression of Nhp6 exacerbated the *not5* growth defect, even at 25° (Figure 4; data not shown). This exacerbation of the *not5* growth defect by the multicopy plasmid with *NHP6A* reinforces the role of Nhp6 in RNA pol II transcription.

***spt3* is synthetic lethal with *mot1* or *ccr4*:** Spt3 physically interacts with TBP, and Spt3 acts to either promote or inhibit TBP binding, depending on the promoter (EISENMANN *et al.* 1992; DUDLEY *et al.* 1999; BELOTSERKOVSKAYA *et al.* 2000; BHAUMIK and GREEN 2002; BARBARIC *et al.* 2003; YU *et al.* 2003). Additionally, we have observed that an *spt3* mutation can suppress growth defects in both *nhp6a nhp6b* and *gcn5 nhp6a nhp6b* strains (YU *et al.* 2003) and the synthetic lethality of TBP mutants in *gcn5* or *nhp6ab* strains (BISWAS *et al.* 2004; ERIKSSON *et al.* 2004a). On the basis of these results, we tested whether an *spt3* gene disruption can suppress the synthetic lethality of a *mot1* mutation with *gcn5* or *nhp6ab*. A *mot1(R1243I)* mutant was crossed to a *gcn5 spt3* strain, and we found that *spt3 mot1(R1243I)* double mutants are synthetic lethal, consistent with an earlier report using a different *mot1* allele (MADISON and WINSTON 1997). We also crossed the *mot1(R1243I)* mutant to a *nhp6ab spt3* strain but we were unable to isolate a *mot1(R1243I) nhp6ab spt3* strain. Thus *spt3* cannot suppress these synthetic lethalities with *mot1*.

We next asked whether *spt3* could suppress the synthetic lethality of a *ccr4* mutation with either *gcn5* or *nhp6ab*. In these crosses we did not recover any viable *ccr4 spt3* strains, irrespective of the *GCN5* or *NHP6* genotype, suggesting that *ccr4* and *spt3* are synthetically lethal. To test this idea, we transformed a +/*ccr4* +/*spt3* heterozygous diploid strain with a YCp–*URA3* plasmid with either *CCR4* or *SPT3*, and haploid *ccr4 spt3* segregants with either YCp–*URA3–CCR4* or YCp–*URA3–SPT3* were isolated. These haploid strains were unable to grow on 5-FOA, demonstrating the *ccr4 spt3* synthetic lethality. This result disagrees with that of BADARINARAYANA *et al.* (2000), who found the *ccr4 spt3* double mutant viable in their strain background.

Synthetic lethality of TBP mutants with *mot1* and *ccr4*: We recently conducted a screen to identify TBP mutants that are viable, but lethal in the absence of Nhp6 (ERIKSSON *et al.* 2004a). Many of these TBP mutants are also lethal in a *gcn5* mutant (BISWAS *et al.* 2004), and we decided to test whether *mot1* or *ccr4* mutations affected viability of these TBP mutants. We constructed a *mot1(R1243I) spt15Δ* double mutant, kept alive by the wild-type *SPT15* (TBP) gene on a YCp–*URA3* plasmid. This strain was transformed with 14 TBP mutants on YCp–*TRP1* plasmids, and we used plasmid shuffling to assess the viability of the *mot1(R1243I) spt15* strains on 5-FOA media at 25°, 30°, and 35°, where the YCp–*URA3*—TBP (wild-type) plasmid must be lost for cells to grow (Table 3). Three TBP mutants were synthetic lethal with *mot1(R1243I)* at all temperatures tested, and 9 others either were synthetic lethal or showed very poor growth at 35°. All of these TBP mutants grew well at 35° in a *MOT1* strain (data not shown). None of these *mot1(R1243I)* TBP synthetic interactions could be suppressed by a multicopy plasmid with *NHP6A* (Table 3). However, the synthetic lethality at 35° between *mot1(R1243I)* and the G174E substitution in TBP [*spt15*(G174E)] could be suppressed by overexpression of TFIIA (Figure 5A). Two conclusions result from these genetic experiments. First, most of these TBP mutants show a major growth defect when combined with *mot1(R1243I)*. Second, overexpression of TFIIA can suppress the *mot1(R1243I) spt15*(G174E) lethality, suggesting that Mot1 may contribute to formation of the TBP–TFIIA–DNA complex.

We next constructed a *ccr4 spt15* double-deletion mutant with the wild-type *SPT15* (TBP) gene on a YCp–*URA3* plasmid. This strain was transformed with the same 14 TBP mutants and the ability of these transformants to grow at various temperatures on 5-FOA

TABLE 3

Synthetic lethality of TBP mutants with *mot1* and *ccr4*

spt15 (TBP) mutant	Phenotype in *mot1(R1243I)*	Suppression of *mot1(R1243I) spt15* by:		Phenotype in *ccr4*	Suppression of *ccr4 spt15* by:	
		YEp–TFIIA	YEp–*NHPA*		YEp–TFIIA	YEp–*NHPA*
E93G	S.L. 35°	No effect	No effect	S.L.	Supp.	No effect
L114F	S.L.	No effect	No effect	S.L.	No effect	No effect
K133R	S.L.	No effect	No effect	Viable		
G147W	Viable			S.L.	Supp.	No effect
C164W	Poor growth 35°	No effect	No effect	Viable		
L172P	S.L. 35°	No effect	No effect	Poor growth	Supp.	Supp.
G174E	S.L. 35°	Supp.	No effect	Poor growth	ND	ND
F227L	S.L. 35°	No effect	No effect	Viable		
F237L	S.L. 35°	No effect	No effect	Viable		
K239T	S.L. 35°	No effect	No effect	Viable		
K97R, L193S	S.L. 35°	No effect	No effect	Poor growth	ND	ND
I103T, K239Stop	S.L. 35°	No effect	No effect	Poor growth	ND	ND
K133L,K145L	Viable			Viable		
K138T,Y139A	S.L.	No effect	No effect	S.L.	No effect	No effect

S.L., synthetic lethal at all temperatures; S.L. 35°, viable at 25° and 30°, but lethal at 35°; Supp., suppression; ND, not determined.

without the wild-type TBP gene was assessed (Table 3). Four TBP mutants were synthetic lethal at all temperatures in the *ccr4* mutant, and four other TBP mutants showed poor growth at all temperatures in the *ccr4* mutant. To assess multicopy suppression, the *ccr4 spt15* YCp–*URA3* TBP (wild-type) strain was transformed with the TBP mutants and YEp–TFIIA, YEp–*NHP6A*, or the YEp vector control. In several instances, overexpression of TFIIA or *NHP6A* suppressed the synthetic growth defects (Table 3; Figure 5B). For example, the *ccr4 spt15*(G147W) synthetic lethality is suppressed by YEp–TFIIA, and the *ccr4 spt15*(L172P) growth defect is suppressed by overexpression of either TFIIA or *NHP6A*. The synthetic lethality between *ccr4* and TBP mutants, along with suppression by overexpression of TFIIA, strongly supports a role for Ccr4 either in facilitating the interaction between TBP and TFIIA or in TBP binding at promoters.

Interestingly, the pattern of synthetic lethality is different for *mot1(R1243I)* and *ccr4*. For example, the K133R substitution in TBP [*spt15*(K133R)] is lethal in *mot1(R1243I)* but viable in *ccr4*, while *spt15* (G147W) shows an opposite pattern. This result suggests that Mot1 and Ccr4/Not have nonidentical roles in

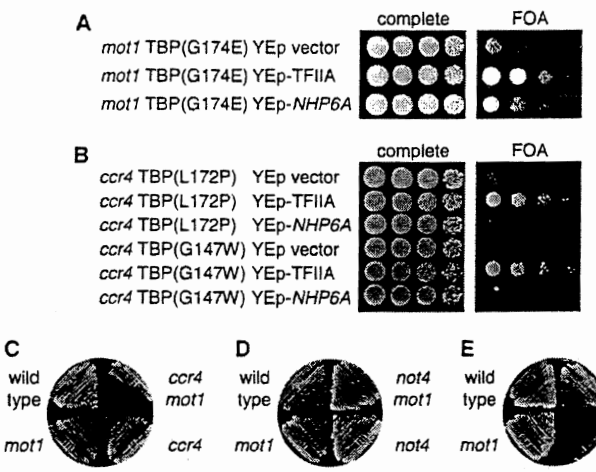

FIGURE 5.—Suppression of *mot1 spt15* and *ccr4 spt15* synthetic lethality. (A) The *mot1(R1243I) spt15*(G174E) [TBP(G174E)] synthetic lethality is suppressed by TFIIA or Nhp6 overexpression. Strain DY9383 [*mot1(R1243I) spt15Δ* with a YCp–*URA3–SPT15*(wild-type) plasmid] was transformed with the YCp–*TRP1*–TBP(G174E) plasmid and the indicated *LYS2* multicopy plasmids and grown for 3 days on complete medium at 25° or on 5-FOA medium at 34°. (B) The *ccr4 spt15* synthetic lethalities are suppressed by TFIIA overexpression. Strain DY9384 [*ccr4 spt15Δ* with a YCp–*URA3–SPT15*(wild-type) plasmid] was transformed with either the YCp–*TRP1*–TBP(G147W) or the YCp–*TRP1*–TBP(L172P) plasmid and the indicated *LYS2* multicopy plasmids and grown at 35° on complete medium for 2 days or on 5-FOA medium for 3 days. (C) Synthetic growth defect in the *ccr4 mot1* double mutant. Strains DY150 (wild type), DY7462 [*mot1(R1243I)*], DY7176 (*ccr4*), and DY9470 [*ccr4 mot1(R1243I)*] were grown on complete medium at 30° for 2 days. (D) No additive effect in the *not4 mot1* double mutant. Strains DY150 (wild type), DY7462 [*mot1(R1243I)*], DY8617 (*not4*), and DY9545 [*not4 mot1(R1243I)*] were grown on complete medium at 30° for 4 days. (E) *mot1* suppresses the *not5* growth defect. Strains DY150 (wild type), DY7462 [*mot1(R1243I)*], DY8627 (*not5*), and DY9582 [*not5 mot1(R1243I)*] were grown on complete medium at 30° for 3 days.

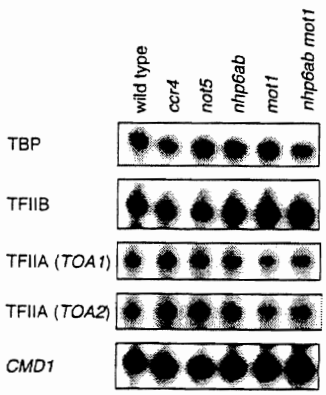

FIGURE 6.—Basal factor expression is not affected by mutants. RNA was prepared from strains DY150 (wild type), DY7176 (*ccr4*), DY8626 (*not5*), DY7139 (*nhp6a*), DY7463 [*mot1 (R1243I)*], and DY7847 [*nhp6ab mot1(R1243I)*] grown at 25° and used for S1 nuclease protection assays to measure TBP (*SPT15*), TFIIB (*SUA7*), and TFIIA subunits one (*TOA1*) and two (*TOA2*) and *CMD1* (internal control) RNA levels.

regulating TBP. To test this idea, we crossed a *mot1 (R1243I)* mutant to three strains with mutations in *CCR4*, *NOT4*, or *NOT5* and examined the growth of double-mutant strains. The *ccr4 mot1(R1243I)* double mutant shows a growth defect, compared to either single mutant (Figure 5C), and the *not4 mot1(R1243I)* double mutant shows no additive effect (Figure 5D). The results with the *not5 mot1(R1243I)* double mutant (Figure 5E) are quite striking. The *not5* mutant is essentially unable to grow at 30°, but this growth defect is completely suppressed in the *not5 mot1(R1243I)* double mutant. This suppression strongly argues that Mot1 and Ccr4/Not have quite different roles in transcriptional regulation.

Overexpression of basal transcription factors suppresses some genetic defects involving *nhp6*, *gcn5*, *mot1(R1243I)*, *ccr4*, and *not5* (Figures 1–4). One explanation for these results is that expression of basal factors is reduced in these mutants, and thus overexpression suppresses growth defects. To address this question, we determined mRNA levels for TBP (*SPT15* mRNA), TFIIB (*SUA7* mRNA), and TFIIA (two subunits, *TOA1* and *TOA2* mRNA). The results in Figure 6 show that these mutations in *nhp6*, *gcn5*, *mot1(R1243I)*, *ccr4*, and *not5* do not significantly affect mRNA levels for basal transcription factors.

Additive effects on *HO* expression in double mutants: As both Gcn5 and Nhp6 are required for full activation of the *HO* gene (Yu *et al.* 2003), we determined whether a *mot1(R1243I)* mutation affected *HO* expression. HO mRNA levels are reduced to ~40% of wild type in the *mot1(R1243I)* strain grown at 25° and reduced to 9% when grown at 30° (Figure 7A). *HO* is cell cycle regulated, and thus a defect in cell cycle

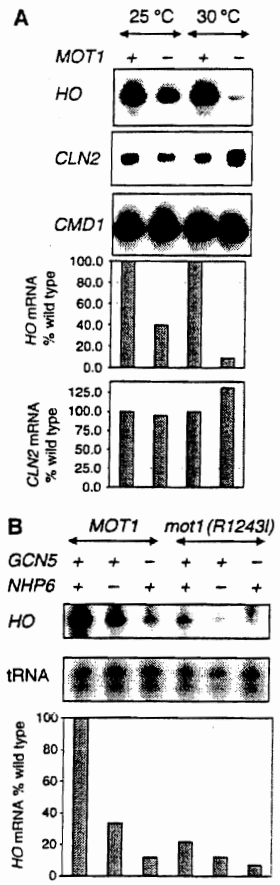

FIGURE 7.—*HO* expression is reduced in mutant strains. (A) RNA was prepared from strains DY150 (wild type) and DY7462 [*mot1(R1243I)*] grown at either 25° or 30° and used for S1 nuclease protection assays to measure *HO*, *CLN2*, and *CMD1* (internal control) RNA levels. (B) RNA was prepared from strains DY150 (wild type), DY7463 [*mot1(R1243I)*], DY5265 (*gcn5*), DY7841 [*gcn5 mot1(R1243I)*], DY7139 (*nhp6ab*), and DY7847 [*nhp6ab mot1(R1243I)*] grown at 25° and used for S1 nuclease protection assays to measure *HO* and tRNA-Trp (internal control) RNA levels.

progression could reduce the fraction of cells in late G_1, when HO is expressed. To address this question, we also measured *CLN2* mRNA levels; *CLN2* is expressed in late G_1, coincident with *HO*. The *mot1(R1243I)* mutation does not affect *CLN2* levels, and thus an alteration in the cell cycle does not cause the decreased *HO* expression. In contrast to *mot1(R1243I)*, a *ccr4* mutation does not affect *HO* expression (data not shown).

On the basis of the additive growth defect in *gcn5 mot1(R1243I)* and *nhp6a nhp6b mot1(R1243I)* mutants, we looked for additive effects in transcriptional activation at *HO*. Cells were grown at 25°, as some of the strains have severe growth defects at higher temperatures, and

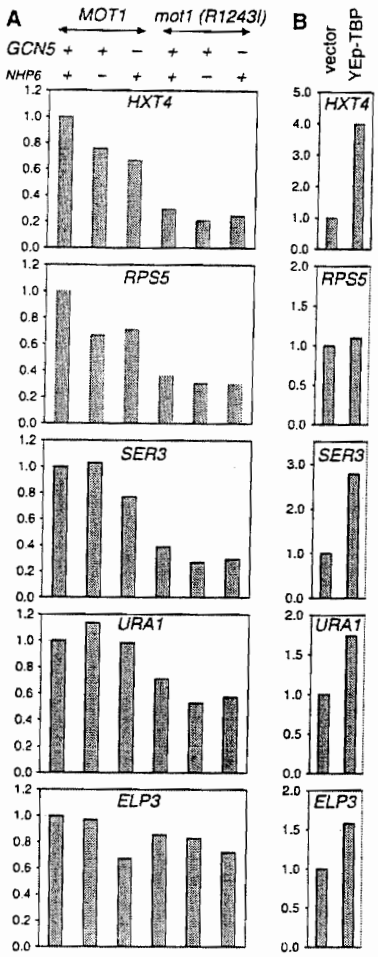

FIGURE 8.—Mutations affect TBP binding to promoters. TBP occupancy at the indicated promoters was determined by chromatin immunoprecipitation with polyclonal anti-TBP antisera and quantitative PCR, using cells that had been grown at 25° and then shifted to 37° for 3 hr. Relative binding is shown, after normalization to an intergenic V internal control. The average of replicate PCR amplifications is shown. (A) TBP binding is reduced in mutants. Strains DY150 (wild type), DY7463 [*mot1(R1243I)*], DY5265 (*gcn5*), DY7841 [*gcn5 mot1(R1243I)*], DY7139 (*nhp6ab*), and DY7847 [*nhp6ab mot1(R1243I)*] were grown on YEPD media. (B) Multicopy TBP plasmid restores TBP binding in the *nhp6ab mot1(R1243I)* strain. DY7847 [*nhp6ab mot1(R1243I)*] with either the YEp vector control or a YEp plasmid with the gene encoding TBP were grown on selective medium.

RNA was isolated for S1 nuclease protection assays. As shown previously, *HO* expression is reduced in the *nhp6ab* and *gcn5* strains (Figure 7B). Interestingly, there is an additive decrease in *HO* mRNA levels in the *nhp6ab mot1(R1243I)* triple mutant, compared to the *nhp6ab* and *mot1(R1243I)* strains. There is a similar additive effect in the *gcn5 mot1(R1243I)* double mutant compared to the corresponding single mutants. These results are consistent with the idea that Nhp6, Gcn5, and Mot1 function through distinct mechanisms, although the effects may be on a common target. We have previously shown that the defect in *HO* expression in *nhp6ab* and *gcn5* mutants can be suppressed by overexpression of TBP (YU *et al.* 2003).

Effects of mutations on TBP binding at promoters: We used ChIP assays to measure TBP binding to promoters in mutants. Cells were grown at 25°, shifted to 37° for 3 hr, and then treated with formaldehyde for crosslinking. After immunoprecipitation with anti-TBP antibody and reversal of crosslinks, TBP binding to various promoters was measured by real time PCR. As shown in Figure 8A, there is decreased TBP binding to the *RPS5*, *HXT4*, *SER3*, and *URA1* promoters in the *nhp6*, *gcn5*, and *mot1(R1243I)* mutants. Other *mot1* mutations have previously been shown to affect TBP binding to *HXT4* and *URA1* (DASGUPTA *et al.* 2005; VAN OEVELEN *et al.* 2005). Importantly, not all promoters are affected so strongly, for example, *ELP3*. When we look at the multiply mutant strains, such as *nhp6ab mot1 (R1243I)* and *gcn5 mot1(R1243I)*, there are additive defects in TBP binding, although the additivity is modest. Overexpression of TBP suppresses the growth defect of *nhp6ab mot1(R1243I)* cells. We therefore examined TBP binding in *nhp6ab mot1(R1243I)* cells with the YEp–TBP plasmid (Figure 8B); the control for this experiment is the same strain with the YEp vector without an insert. TBP overexpression results in a significant increase in TBP binding at several promoters in these cells. These results support the idea that a defect in TBP binding to promoters contributes to the growth defect seen in these multiply mutant strains.

DISCUSSION

We have previously shown that the Nhp6 architectural transcription factor and the Gcn5 histone acetyltransferase function in parallel pathways in activation of the yeast *HO* gene (YU *et al.* 2000), and our data suggest that both Nhp6 and Gcn5 could affect DNA binding by TBP (BISWAS *et al.* 2004; ERIKSSON *et al.* 2004a). To further explore the roles of these factors, in this report we have examined the effect of combining *nhp6ab* or *gcn5* gene disruptions with mutations affecting known regulators of DNA binding by TBP. Both biochemical and genetic experiments show Mot1 regulates TBP binding to DNA, and the *mot1(R1243I)* allele is lethal when combined with either *nhp6ab* or *gcn5*. The Ccr4–Not complex has multiple roles in gene regulation, and genetic experiments suggest one role in regulating TBP binding. We tested gene disruptions affecting three members of the Ccr4–Not complex, *ccr4*, *not4*, and *not5*, and all three were synthetically lethal when combined with either

nhp6ab or *gcn5*. We have recently isolated point mutations in TBP that are viable in wild-type strains but lethal in *nhp6ab* or *gcn5* mutants (BISWAS *et al.* 2004; ERIKSSON *et al.* 2004a). We have tested 14 of these TBP mutants in *mot1* or *ccr4* mutants, and most of them show synthetic growth defects or lethality when combined with *mot1* or *ccr4*. A *not5* mutation has a severe growth defect at 30°, but this is suppressed by a *mot1* mutation. Interestingly, many of the synthetic lethal phenotypes described in this report can be suppressed by overexpression of TFIIA, suggesting that these various regulators all work to stimulate either TBP binding or the interaction of TBP and TFIIA with DNA.

In vitro studies show that the Mot1 protein is able to remove TBP from binding sites, in an ATP-dependent fashion (AUBLE *et al.* 1994; DARST *et al.* 2003), and that *in vivo* Mot1 protein is present in a complex with TBP (POON *et al.* 1994). Chromatin immunoprecipitation experiments show that Mot1 associates with promoters (ANDRAU *et al.* 2002; DASGUPTA *et al.* 2002) and that a *mot1* mutation affects TBP binding to promoters *in vivo* (LI *et al.* 1999; GEISBERG *et al.* 2002). Mot1 co-occupies promoters with TBP, but not with TFIIB, TFIIA, or pol II under normal conditions, suggesting that Mot1 functions as a repressor (GEISBERG and STRUHL 2004). Expression profiling studies show that *mot1* mutations reduce expression of some genes and derepress others (ANDRAU *et al.* 2002; DASGUPTA *et al.* 2002; GEISBERG *et al.* 2002), arguing that Mot1 functions as either an activator or a repressor at different promoters. However, GEISBERG and STRUHL (2004) show that when cells are heat-shocked or stressed Mot1 does co-occupy promoters with TFIIB and RNA pol II, suggesting that these preinitiation complexes contain Mot1. They suggest the stress response resulting from thermal inactivation of mutant Mot1 indirectly causes decreased expression of some genes in the microarray studies. It is intriguing that under stress conditions Mot1 and TFIIA do not co-occupy promoters, suggesting that these preinitiation complexes contain Mot1 instead of TFIIA (GEISBERG and STRUHL 2004). Interestingly, there are data suggesting that Mot1 and TFIIA have opposing effects both *in vivo* and *in vitro* (AUBLE and HAHN 1993; MADISON and WINSTON 1997; CHICCA *et al.* 1998). Finally, DASGUPTA *et al.* (2005) recently showed that TBP is bound to Mot1-activated genes following Mot1 inactivation, but other basal factors are not bound. This results suggests Mot1 mediates repression by displacing TBP from chromatin.

There are several ways to explain the observed *mot1 nhp6ab* and *mot1 gcn5* synthetic lethalities. One explanation is that full Mot1 activity is required for efficient expression of specific genes during stress response, and either the *nhp6ab* or *gcn5* mutations reduce expression of these genes. However, expression profiles of *nhp6ab* and *gcn5* mutants do not show decreased expression of stress response genes (LEE *et al.* 2000; MOREIRA and HOLMBERG 2000; our unpublished observations). We favor another explanation where Mot1, Nhp6, and Gcn5 all function in the same pathway, that of affecting TBP binding to DNA at some genes. In support of this hypothesis, we note that the *mot1 nhp6ab* synthetic lethality is suppressed by TBP overexpression (Figure 1) and that the *mot1 gcn5* defect is much worse when either TBP or Nhp6 is overexpressed (Figure 2). Additionally, the lethality resulting from combining TBP point mutations with either *mot1* or *gcn5* can be suppressed by overexpression of TFIIA (Figure 5) (BISWAS *et al.* 2004). ChIP experiments show that *nhp6ab*, *gcn5*, and *mot1* mutations all lead to reduced TBP binding to promoters (Figure 8).

Genetic and biochemical studies suggest that the Ccr4–Not complex is a regulator of TBP binding, along with roles in transcriptional elongation and mRNA degradation. Mutations in different genes encoding subunits of Ccr4–Not have different phenotypes, suggesting that different subunits make contributions toward different functions (COLLART 2003). For example, Ccr4 is part of the cytoplasmic mRNA deadenylase (TUCKER *et al.* 2001), and while *ccr4* mutations have a major impact on deadenylation activity, *not* mutations have small effects on deadenylation (TUCKER *et al.* 2002). Additionally, the Ccr4 protein, but not other members of the Ccr4–Not complex, is associated with the Paf1 complex that travels with elongating RNA polymerase (CHANG *et al.* 1999). A *ccr4 paf1* double mutant is lethal, but combining any of the *not* mutations with *paf1* is viable (CHANG *et al.* 1999; MAILLET *et al.* 2000). Additionally, Not4 has been recently shown to be a ubiquitin ligase (ALBERT *et al.* 2002), although further work is needed to identify the targets of ubiquitylation and to determine how ubiquitylation affects transcriptional regulation.

The *not* mutations were isolated as global repressors that affected TBP binding at TATA-less promoters (COLLART and STRUHL 1994). The Not1, Not2, and Not5 proteins physically interact with TBP or TAFs, the TBP-associated factors present in TFIID (BADARINARAYANA *et al.* 2000; LEMAIRE and COLLART 2000; SANDERS *et al.* 2002), and *not4* and *not5* mutations show synthetic lethality in combination with *taf* mutations (LEMAIRE and COLLART 2000). Additionally, *not4* mutations suppress the transcriptional defect caused by Ty insertions into the *HIS4* promoter (BADARINARAYANA *et al.* 2000), a phenotype also seen in *spt15* (TBP), *spt3*, and *mot1* mutants (JIANG and STILLMAN 1996; MADISON and WINSTON 1997; WINSTON and SUDARSANAM 1998). Mutations in genes encoding the Ccr4–Not complex affect binding of TBP and TAF1 to promoters (LENSSEN *et al.* 2005).

Thus the evidence linking the *NOT* genes to regulation of TBP is quite strong. Our genetic data bring Nhp6 and Gcn5 into the same pathway as the Ccr4–Not complex in regulating TBP binding. We believe that

the synthetic lethality caused by combining a *ccr4, not4,* or a *not5* mutation with either *gcn5* or *nhp6ab* results from a dysregulation of TBP binding. The fact that overexpression of TBP or TFIIA can suppress some of these synthetic lethalities supports this idea.

Both the Motl and the Ccr4/Not complex regulate TBP binding, but it is not clear whether they do so in the same or different pathways. We find that the *ccr4 motl(R1243I)* double mutant shows a growth defect, and more significantly, *motl(R1243I)* suppresses *not5* growth defects. The *not5* mutant is unable to grow at 30°, but the *not5 motl(R1243I)* double mutant does grow (Figure 5E). This suggests that the *not5* mutant is defective in some aspect of transcriptional activation and that the *motl(R1243I)* allele has properties that overcome this defect. We also note that the *not5* growth defect can be partially suppressed by overexpression of TFIIB (Figure 4C). We suggest that the Motl and Ccr4/Not complexes function in distinct pathways in regulating TBP.

The Spt3 component of the SAGA complex interacts both physically and genetically with TBP (EISENMANN *et al.* 1992). Spt3 is required for TBP recruitment to the *GAL1* and *PHO5* promoters *in vivo* (DUDLEY *et al.* 1999; BARBARIC *et al.* 2003), but Spt3 inhibits TBP binding to the *HO* promoter (YU *et al.* 2003). *spt3* and *motl* are synthetically lethal, and this synthetic lethality can be suppressed by overexpression of TFIIA (MADISON and WINSTON 1997). Interestingly, both *spt3* and *motl* are synthetic lethal with substitutions in the Toa1 subunit of TFIIA (MADISON and WINSTON 1997). Both Spt3 and Motl are required for nucleosome remodeling at Gal4-dependent promoters (TOPALIDOU *et al.* 2004). Moreover, Spt3 is required for Motl to bind to the *GAL1* promoter under inducing conditions, and Motl is similarly required for Spt3 binding (TOPALIDOU *et al.* 2004). We note a number of synthetic lethalities or growth defects among these genes: *motl spt3* (MADISON and WINSTON 1997), *ccr4 spt3,* and *ccr4 motl.* We attribute these additive genetic defects to a common target, TBP.

motl mutations reduce TBP binding to certain promoters (ANDRAU *et al.* 2002), while TBP binding to the *INO1* promoter was unaffected by a *motl* mutation (DASGUPTA *et al.* 2005). We chose to study TBP binding in strains with a *motl* mutation alone or in combination with *gcn5* and *nhp6ab* mutations. Our results show that TBP binding at selected promoters is significantly reduced in a *motl* mutant (Figure 8). TBP binding is further reduced, although modestly, when *motl* is combined with either *gcn5* or *nhp6ab.* The *motl nhp6ab* strain shows reduced binding of basal transcription factors, and suppression of this defect by overexpression of TBP further supports our hypothesis that the *motl* and *nhp6ab* mutations cause defects in TBP binding. RNA analysis shows that *HO* expression is reduced in a *motl* strain and is further reduced when combined with other mutations such as *gcn5* or *nhp6ab* (Figure 7).

An *spt3* gene deletion suppresses several *nhp6ab* defects, including reduced *HO* expression, temperature-sensitive growth, and synthetic lethality with TBP mutants (YU *et al.* 2003; ERIKSSON *et al.* 2004a). *spt3* also suppresses the synthetic lethality resulting from combining *gcn5* with *nhp6ab* and the reduced *HO* expression in a *gcn5* mutant. Additionally, either a *spt3* mutation or a TBP mutation that disrupts the TBP–Spt3 interaction can suppress the temperature sensitivity of *not1-2* (COLLART 1996).

The genetic analyses involving TBP, TFIIA, Nhp6, Gcn5, Motl, Ccr4–Not, and Spt3 show both synthetic lethality and genetic suppression. Taken together, these genetic interactions strongly support a role for these factors in regulating DNA binding of TBP and TFIIA. Further work, particularly at the biochemical level, will be needed to understand exactly how these factors regulate TBP–TFIIA binding to promoters.

We thank David Auble, Martine Collart, Clyde Denis, Steve Hahn, Mike Hampsey, and Tony Weil, who provided plasmids, strains, or antibodies. We also acknowledge Yiwei Jiang who constructed plasmid M2719. We thank David Auble and Clyde Denis for comments on the manuscript. This work was supported by a grant from the National Institutes of Health awarded to D.J.S.

LITERATURE CITED

ABATE, C., D. LUK, R. GENTZ, F. J. RAUSCHER, III and T. CURRAN, 1990 Expression and purification of the leucine zipper and DNA-binding domains of Fos and Jun: both Fos and Jun contact DNA directly. Proc. Natl. Acad. Sci. USA **87:** 1032–1036.

ALBERT, T. K., H. HANZAWA, Y. I. LEGTENBERG, M. J. DE RUWE, F. A. VAN DEN HEUVEL *et al.*, 2002 Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. EMBO J. **21:** 355–364.

ANDRAU, J. C., C. J. VAN OEVELEN, H. A. VAN TEEFFELEN, P. A. WEIL, F. C. HOLSTEGE *et al.*, 2002 Motlp is essential for TBP recruitment to selected promoters during in vivo gene activation. EMBO J. **21:** 5173–5183.

ARNDT, K. M., C. R. WOBBE, S. RICUPERO-HOVASSE, K. STRUHL and F. WINSTON, 1994 Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. Mol. Cell. Biol. **14:** 3719–3728.

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

AUBLE, D. T., K. E. HANSEN, C. G. MUELLER, W. S. LANE, J. THORNER *et al.*, 1994 Motl, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev. **8:** 1920–1934.

BADARINARAYANA, V., Y. C. CHIANG and C. L. DENIS, 2000 Functional interaction of CCR4-NOT proteins with TATAA-binding protein (TBP) and its associated factors in yeast. Genetics **155:** 1045–1054.

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.

BARBARIC, S., H. REINKE and W. HORZ, 2003 Multiple mechanistically distinct functions of SAGA at the PHO5 promoter. Mol. Cell. Biol. **23:** 3468–3476.

BELOTSERKOVSKAYA, R., D. E. STERNER, M. DENG, M. H. SAYRE, P. M. LIEBERMAN *et al.*, 2000 Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol. Cell. Biol. **20:** 634–647.

Bhaumik, S. R., and M. R. Green, 2002 Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol. Cell. Biol. **22:** 7365–7371.

Bhoite, L. T., and D. J. Stillman, 1998 Residues in the Swi5 zinc finger protein that mediate cooperative DNA-binding with the Pho2 homeodomain protein. Mol. Cell. Biol. **18:** 6436–6446.

Biswas, D., A. N. Imbalzano, P. Eriksson, Y. Yu and D. J. Stillman, 2004 Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TATA-binding protein-TFIIA-DNA complex. Mol. Cell. Biol. **24:** 8312–8321.

Biswas, D., Y. Yu, M. Prall, T. Formosa and D. J. Stillman, 2005 The yeast FACT complex has a role in transcriptional initiation. Mol. Cell. Biol. **25:** 5812–5822.

Boeke, J. D., F. LaCroute and G. R. Fink, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–346.

Buratowski, S., and H. Zhou, 1992 Transcription factor IID mutants defective for interaction with transcription factor IIA. Science **255:** 1130–1132.

Chang, M., D. French-Cornay, H. Y. Fan, H. Klein, C. L. Denis *et al.*, 1999 A complex containing RNA polymerase II, Paf1p, Cdc73p, Hpr1p, and Ccr4p plays a role in protein kinase C signaling. Mol. Cell. Biol. **19:** 1056–1067.

Chen, J., Y. C. Chiang and C. L. Denis, 2002 CCR4, a 3′-5′ poly(A) RNA and ssDNA exonuclease, is the catalytic component of the cytoplasmic deadenylase. EMBO J. **21:** 1414–1426.

Chicca, J. J., III, D. T. Auble and B. F. Pugh, 1998 Cloning and biochemical characterization of TAF-172, a human homolog of yeast Mot1. Mol. Cell. Biol. **18:** 1701–1710.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.

Coleman, R. A., and B. F. Pugh, 1997 Slow dimer dissociation of the TATA binding protein dictates the kinetics of DNA binding. Proc. Natl. Acad. Sci. USA **94:** 7221–7226.

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

Collart, M. A., 2003 Global control of gene expression in yeast by the Ccr4-Not complex. Gene **313:** 1–16.

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.

Costigan, C., D. Kolodrubetz and M. Snyder, 1994 *NHP6A* and *NHP6B,* which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. Mol. Cell. Biol. **14:** 2391–2403.

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

Dasgupta, A., 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. USA **99:** 2666–2671.

Dasgupta, A., S. A. Juedes, R. O. Sprouse and D. T. Auble, 2005 Mot1-mediated control of transcription complex assembly and activity. EMBO J. **24:** 1717–1729.

Deluen, C., N. James, L. Maillet, M. Molinete, G. Theiler *et al.*, 2002 The Ccr4-Not complex and yTAF1 (yTaf(II)130p/yTaf(II)145p) show physical and functional interactions. Mol. Cell. Biol. **22:** 6735–6749.

Denis, C. L., and J. Chen, 2003 The CCR4-NOT complex plays diverse roles in mRNA metabolism. Prog. Nucleic Acid Res. Mol. Biol. **73:** 221–250.

Dudley, A. M., C. Rougeulle and F. Winston, 1999 The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. **13:** 2940–2945.

Dvir, A., J. W. Conaway and R. C. Conaway, 2001 Mechanism of transcription initiation and promoter escape by RNA polymerase II. Curr. Opin. Genet. Dev. **11:** 209–214.

Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney and F. Winston, 1992 SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae.* Genes Dev. **6:** 1319–1331.

Eriksson, P., D. Biswas, Y. Yu, J. M. Stewart and D. J. Stillman, 2004a TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol. Cell. Biol. **24:** 6419–6429.

Eriksson, P., L. R. Thomas, A. Thorburn and D. J. Stillman, 2004b pRS yeast vectors with a *LYS2* marker. BioTechniques **36:** 212–213.

Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu *et al.*, 2001 Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome- binding factor SPN. EMBO J. **20:** 3506–3517.

Fragiadakis, G. S., D. Tzamarias and D. Alexandraki, 2004 Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for *FRE2* transcriptional activation. EMBO J. **23:** 333–342.

Geisberg, J. V., and K. Struhl, 2004 Cellular stress alters the transcriptional properties of promoter-bound Mot1-TBP complexes. Mol. Cell **14:** 479–489.

Geisberg, J. V., Z. Moqtaderi, 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.

Gietz, R. D., and A. Sugino, 1988 New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:** 527–534.

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

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.

Hampsey, M., 1998 Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. **62:** 465–503.

Jiang, Y. W., and D. J. Stillman, 1996 Epigenetic effects on yeast transcription caused by mutations in an actin-related protein present in the nucleus. Genes Dev. **10:** 604–619.

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.

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.

Kruppa, M., R. D. Moir, D. Kolodrubetz and I. M. Willis, 2001 Nhp6, an HMG1 protein, functions in *SNR6* transcription by RNA polymerase III in *S. cerevisiae.* Mol. Cell **7:** 309–318.

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

Lee, T. I., H. C. Causton, F. C. Holstege, W. C. Shen, N. Hannett *et al.*, 2000 Redundant roles for the TFIID and SAGA complexes in global transcription. Nature **405:** 701–704.

Lemaire, M., and M. A. Collart, 2000 The TATA-binding protein-associated factor yTafII19p functionally interacts with components of the global transcriptional regulator Ccr4-Not complex and physically interacts with the Not5 subunit. J. Biol. Chem. **275:** 26925–26934.

Lenssen, E., N. James, I. Pedruzzi, F. Dubouloz, E. Cameroni *et al.*, 2005 The Ccr4-Not complex independently controls both Msn2-dependent transcriptional activation—via a newly identified Glc7/Bud14 type I protein phosphatase module—and TFIID promoter distribution. Mol. Cell. Biol. **25:** 488–498.

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.

Liu, H. Y., V. Badarinarayana, D. C. Audino, J. Rappsilber, M. Mann *et al.*, 1998 The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. EMBO J. **17:** 1096–1106.

Lopez, S., M. Livingstone-Zatchej, S. Jourdain, F. Thoma, A. Sentenac *et al.*, 2001 High-mobility-group proteins NHP6A and NHP6B participate in activation of the RNA polymerase III SNR6 gene. Mol. Cell. Biol. **21:** 3096–3104.

MADISON, J. M., and F. WINSTON, 1997 Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TATA-binding protein to confer promoter-specific transcriptional control in Saccharomyces cerevisiae. Mol. Cell. Biol. **17:** 287–295.

MAILLET, L., C. TU, Y. K. HONG, E. O. SHUSTER and M. A. COLLART, 2000 The essential function of Not1 lies within the Ccr4-Not complex. J. Mol. Biol. **303:** 131–143.

MARCUS, G. A., N. SILVERMAN, S. L. BERGER, J. HORIUCHI and L. GUARENTE, 1994 Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. EMBO J. **13:** 4807–4815.

MARTIN, M. P., V. L. GERLACH and D. A. BROW, 2001 A novel upstream RNA polymerase III promoter element becomes essential when the chromatin structure of the yeast U6 RNA gene is altered. Mol. Cell. Biol. **21:** 6429–6439.

MERMELSTEIN, F., K. YEUNG, J. CAO, J. A. INOSTROZA, H. ERDJUMENT-BROMAGE *et al.*, 1996 Requirement of a corepressor for Dr1-mediated repression of transcription. Genes Dev. **10:** 1033–1048.

MOREIRA, J. M., and S. HOLMBERG, 2000 Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6A and NHP6B. EMBO J. **19:** 6804–6813.

OZER, J., L. E. LEZINA, J. EWING, S. AUDI and P. M. LIEBERMAN, 1998 Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:** 2559–2570.

PAULL, T. T., M. CAREY and R. C. JOHNSON, 1996 Yeast HMG proteins *NHP6A/B* potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev. **10:** 2769–2781.

PEREIRA, L. A., M. P. KLEJMAN and H. T. TIMMERS, 2003 Roles for BTAF1 and Mot1p in dynamics of TATA-binding protein and regulation of RNA polymerase II transcription. Gene **315:** 1–13.

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:** 23135–23140.

PRELICH, G., and F. WINSTON, 1993 Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription *in vivo*. Genetics **135:** 665–676.

PUGH, B. F., 2000 Control of gene expression through regulation of the TATA-binding protein. Gene **255:** 1–14.

ROTHSTEIN, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:** 281–302.

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.

SHERMAN, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 1–21.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

STARGELL, L. A., and K. STRUHL, 1995 The TBP-TFIIA interaction in the response to acidic activators in vivo. Science **269:** 75–78.

STERNER, D. E., and S. L. BERGER, 2000 Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:** 435–459.

STERNER, D. E., P. A. GRANT, S. M. ROBERTS, L. J. DUGGAN, R. BELOTSERKOVSKAYA *et al.*, 1999 Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA- binding protein interaction. Mol. Cell. Biol. **19:** 86–98.

SWANSON, M. J., H. QIU, L. SUMIBCAY, A. KRUEGER, S. J. KIM *et al.*, 2003 A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. Mol. Cell. Biol. **23:** 2800–2820.

THOMAS, B. J., and R. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

TOPALIDOU, I., M. PAPAMICHOS-CHRONAKIS, G. THIREOS and D. TZAMARIAS, 2004 Spt3 and Mot1 cooperate in nucleosome remodeling independently of TBP recruitment. EMBO J. **23:** 1943–1948.

TRAVERS, A. A., 2003 Priming the nucleosome: A role for HMGB proteins? EMBO Rep. **4:** 131–136.

TUCKER, M., M. A. VALENCIA-SANCHEZ, R. R. STAPLES, J. CHEN, C. L. DENIS *et al.*, 2001 The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in Saccharomyces cerevisiae. Cell **104:** 377–386.

TUCKER, M., R. R. STAPLES, M. A. VALENCIA-SANCHEZ, D. MUHLRAD and R. PARKER, 2002 Ccr4p is the catalytic subunit of a Ccr4p/Pop2p/Notp mRNA deadenylase complex in Saccharomyces cerevisiae. EMBO J. **21:** 1427–1436.

VAN OEVELEN, C. J., H. A. VAN TEEFFELEN and H. T. TIMMERS, 2005 Differential requirement of SAGA subunits for Mot1p and Taf1p recruitment in gene activation. Mol. Cell. Biol. **25:** 4863–4872.

VOTH, W. P., Y. W. JIANG and D. J. STILLMAN, 2003 New 'marker swap' plasmids for converting selectable markers on budding yeast gene disruptions and plasmids. Yeast **20:** 985–993.

WINSTON, F., and P. SUDARSANAM, 1998 The SAGA of Spt proteins and transcriptional analysis in yeast: past, present, and future. Cold Spring Harbor Symp. Quant. Biol. **63:** 553–561.

YU, Y., P. ERIKSSON and D. J. STILLMAN, 2000 Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol. Cell. Biol. **20:** 2350–2357.

YU, Y., P. ERIKSSON, L. T. BHOITE and D. J. STILLMAN, 2003 Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. Mol. Cell. Biol. **23:** 1910–1921.

Communicating editor: F. WINSTON

CHAPTER 5

THE yFACT COMPLEX HAS A ROLE IN TRANSCRIPTIONAL INITIATION

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Molecular and Cellular Biology 2004 Jul;24(14):6419-29.

MOLECULAR AND CELLULAR BIOLOGY, July 2005, p. 5812–5822 Vol. 25, No. 14
0270-7306/05/$08.00+0 doi:10.1128/MCB.25.14.5812–5822.2005
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The Yeast FACT Complex Has a Role in Transcriptional Initiation†

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Received 9 March 2005/Returned for modification 29 March 2005/Accepted 19 April 2005

A crucial step in eukaryotic transcriptional initiation is recognition of the promoter TATA by the TATA-binding protein (TBP), which then allows TFIIA and TFIIB to be recruited. However, nucleosomes block the interaction between TBP and DNA. We show that the yeast FACT complex (yFACT) promotes TBP binding to a TATA box in chromatin both in vivo and in vitro. The *SPT16* gene encodes a subunit of yFACT, and we show that certain *spt16* mutations are synthetically lethal with TBP mutants. Some of these genetic defects can be suppressed by TFIIA overexpression, strongly suggesting a role for yFACT in TBP-TFIIA complex formation in vivo. Mutations in the *TOA2* subunit of TFIIA that disrupt TBP-TFIIA complex formation in vitro are also synthetically lethal with *spt16*. In some cases this *spt16 toa2* lethality is suppressed by overexpression of TBP or the Nhp6 architectural transcription factor that is also a component of yFACT. The Spt3 protein in the SAGA complex has been shown to regulate TBP binding at certain promoters, and we show that some *spt16* phenotypes can be suppressed by *spt3* mutations. Chromatin immunoprecipitations show TBP binding to promoters is reduced in single *spt16* and *spt3* mutants but increases in the *spt16 spt3* double mutant, reflecting the mutual suppression seen in the genetic assays. Finally, in vitro studies show that yFACT promotes TBP binding to a TATA sequence within a reconstituted nucleosome in a TFIIA-dependent manner. Thus, yFACT functions in establishing transcription initiation complexes in addition to the previously described role in elongation.

One of the critical steps in the formation of a preinitiation complex is the assembly of the TATA-binding protein (TBP)-TFIIA-TFIIB complex at the TATA site at promoters. In vitro studies suggest sequential recruitment of TBP followed by TFIIA and TFIIB, with transcriptional coactivators simulating TBP-TFIIA-TFIIB complex formation in vivo (33). For example, some DNA-binding factors, including TBP, are unable to access a binding site within a nucleosome, but Swi/Snf, one of a number of chromatin remodeling complexes that use ATP to change chromatin structure (5), enhances factor binding in chromatin (15, 24, 31, 61).

The yeast FACT complex (yFACT; facilitator of chromatin transactions) also changes chromatin structure, but its activity does not require ATP (20) and does not result in DNA movement relative to the nucleosomal histone core (43). Human FACT was first identified as a factor that stimulates RNA polymerase II elongation through chromatin templates (38). Human FACT promotes the displacement of one H2A-H2B dimer from a nucleosome, and the resulting partial nucleosome is less inhibitory to the elongating RNA polymerase (6). Genetic studies in *Saccharomyces cerevisiae* suggest that yFACT may be able to partially disrupt nucleosomes as well as restore the nucleosome to its normal state (21).

The human FACT complex is composed of two proteins, p140 and SSRP1 (39), whose yeast homologs are Spt16 (or Cdc68) and Pob3, respectively. SSRP1 contains a DNA-binding motif of the HMGB family, but this motif is absent in Pob3 and is instead provided by a separate small protein, Nhp6. Spt16 and Pob3 form a stable heterodimeric SP complex that weakly associates with Nhp6 (11, 20). A role for yFACT in transcriptional elongation is supported by biochemical studies showing that yFACT associates with known elongation factors (30, 50, 51), by chromatin immunoprecipitation (ChIP) and immunolocalization studies showing association of FACT with elongating RNA polymerase II (36, 45), as well as genetic interactions with mutations affecting elongation factors (21, 51). However, *Drosophila* FACT associates with the GAGA factor, stimulating chromatin remodeling at the promoter (49), and Spt16 inactivation in yeast results in reduced binding of TBP and TFIIB at promoters (36). These results suggest a role for FACT in transcription initiation in addition to the known role in elongation.

The Nhp6 subunit of yFACT has roles in both transcriptional initiation and elongation, and we recently identified TBP point mutants that are viable in a *NHP6* strain but lethal in the *nhp6a/b* mutant (19). Many of these TBP mutants are also lethal when combined with mutations affecting the Swi/Snf ATP-dependent chromatin remodeling factor or the Gcn5 histone acetyltransferase (9). Additionally, mutations in TFIIA that affect its interaction with TBP are also lethal with *nhp6a/b*, *swi2*, and *gcn5* (9). These results suggest that Nhp6, Swi/Snf, and Gcn5 enhance assembly of the TBP-TFIIA-DNA complex. In this report we present evidence suggesting that yFACT also facilitates interaction of TBP with TFIIA on nucleosomal DNA and therefore has a direct role in initiation of transcription.

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† Supplemental material for this article may be found at http://mcb.asm.org/.

MATERIALS AND METHODS

Strains and media. All yeast strains used are listed in Table S1 in the supplemental material and are isogenic with W303 (59). Standard genetic methods were used for strain construction (48). Cells were grown in yeast extract-peptone-

dextrose (YEPD) medium (48) at 30°C, except where other temperatures are noted, or in synthetic complete medium (48) with 2% glucose and supplemented with adenine, uracil, and amino acids, as appropriate, to select for plasmids. 5-Fluoroorotic acid (5-FOA) medium was prepared as described previously (10).

Plasmids. The plasmids used are listed in Table S2 in the supplemental material. Plasmid M4806 was constructed by moving a 4.2-kb SalI fragment with *TOA1* and *TOA2* from pSH346, provided by Steve Hahn, into YEplac195 (23). Plasmid M4761 was constructed by moving a 937-bp BamHI-SacI fragment with *NHP6A* from plasmid M4221 (9) into YEplac195 (23).

In vitro binding experiments. Spt16-Pob3 was overexpressed in yeast and purified to apparent homogeneity as described previously using standard chromatographic methods (63) or using a cleavable histidine tag/Ni affinity procedure that produces a similarly pure preparation. Both preparations produce identical results. No ATP is added to the reactions, and yFACT activity is unaffected by the addition of apyrase, ruling out a contribution to the activity from potential contamination with ATP-dependent factors such as remodelers. Recombinant Nhp6 with the native protein sequence was purified from bacteria as described previously (44). Plasmids PH-MLT(+3), PH-MLT(+3)-Mu, PH-MLT(0), and PH-MLT(+6) (24) were PCR amplified using oligonucleotides CCCGGATCC CCCGGGGTTACAAG and GGGCCCGGGTTCGTGATACGAGC, and the resulting PCR products were radiolabeled with polynucleotide kinase and [γ-^{32}P]ATP. Following restriction digestion with BamHI to remove the label at one end, the 157-nucleotide templates were assembled into mononucleosomes by slow dialysis from a high-salt solution in the presence of histone octamers (20). Partial DNase I digestion was performed as described previously (43). The purified Spt16-Pob3, Nhp6, TBP, and TFIIA proteins were used in binding reactions at the following concentrations: 58 nM Spt16-Pob3, 5 uM Nhp6, 3.1 uM TBP, and 0.85 uM TFIIA.

Chromatin immunoprecipitations. Chromatin immunoprecipitations were performed as described previously (3) using a polyclonal anti-TBP sera generously provided by Tony Weil, except that the wash buffers included 1% Sarkosyl and 0.1% sodium dodecyl sulfate (46). Real-time PCR and calculations were performed as described previously (19), using the Intergenic V primer set (29) as the internal control. The sequences of the PCR primers are as follows: *ELP3*, TGCCGCTTTCATTGTTTA and TGTTGTTCCCGAGGTTAAAG; *SER3*, GAGTAATTACTTTGTTGGAAGG and AGTAAAATCTTCATATCACCCG; and Intergenic V, GGCTGTCAGAATATGGGGCCGTAGTA and CACCCC GAAGCTGCTTTCACAATAC.

RESULTS

Genetic interaction between Spt16 and TBP. We have identified TBP mutants that support viability in an otherwise wild-type strain but are lethal in the absence of Nhp6 (19). As Nhp6 is part of the yFACT complex, along with Spt16 and Pob3 (11, 20), we wanted to determine whether the viability of these TBP mutants is reduced when *SPT16* is also mutated. We constructed a strain in which both the *SPT15* gene (encoding TBP) and the *SPT16* gene are disrupted. (To avoid confusion, we will refer to the *SPT15* gene by the protein name, TBP.) The genes encoding TBP and Spt16 are both essential for viability, so the strain is kept alive by a YCp-*URA3* plasmid containing the wild-type genes for both TBP and Spt16. This strain was transformed with two plasmids, YCp-*TRP1* plasmids with various TBP mutants and YCp-*LEU2* plasmids with various Spt16 mutants. Cells that lose the YCp-*URA3*-TBP-Spt16 plasmid can grow on 5-FOA. Thus, after transformation with the mutant TBP and Spt16 plasmids, the ability to grow on 5-FOA reflects the ability of these TBP and Spt16 mutants to sustain growth in the absence of the wild-type genes.

We tested 16 TBP mutants for synthetic lethality in combination with seven *spt16* mutations. The various *spt16* alleles showed subtle differences in the pattern of synthetic lethalities with TBP mutants (Table 1). The *spt16-11* (T828I, P859S) allele shows the strongest effects, with eight TBP mutants showing synthetic lethality or a significant growth defect (Fig. 1A and Table 1). Four of the TBP mutants, E93G, K138T/Y139A, G147W, and G174E, showed very strong synthetic defects in most of the *spt16* mutants. Our previous genetic analysis of these mutants showed some interesting common features among these same four mutants (9). These TBP mutants are lethal in a *gcn5* strain, and the TBP *gcn5* synthetic lethality for all four can be suppressed by overexpression of TFIIA. Similarly, these four TBP mutants are all lethal in a *swi2* mutant, and TFIIA overexpression suppresses the TBP *swi2* synthetic lethality for three of these TBP mutants. All four TBP mutants are lethal in a *nhp6a/b* strain, and for three of the TBP mutants, this synthetic lethality can be suppressed by a *spt3* mutation. These TBP mutants that cannot be tolerated along with mutation of *SPT16* are therefore generally sensitive to changes that lead to decreased probability of transcription initiation.

These four TBP mutations also have interesting effects on the binding of TBP to other factors. TBP interacts with Spt3, and the TBP(G174E) substitution causes reduced coimmunoprecipitation of TBP with Spt3 (18). (We show below that *spt3* mutations also affect a number of *spt16* phenotypes.) The TBP(K138T/Y139A) double substitution mutant was identified as an activation-defective TBP mutant that lost the ability to interact with TFIIA in vitro (53). Despite this defect, the TBP(K138T/Y139A) mutant is viable; we suggest that other factors present in vivo, but not in vitro, facilitate formation of the TBP-TFIIA-DNA complex and consequently overcome this defect. Thus, the observed lethality of TBP(K138T/Y139A) with a Spt16 mutant could indicate that yFACT facilitates the TBP-TFIIA interaction. The G147W substitution is on the upper surface of TBP, near K138/Y139, and E93G is on the lower surface of TBP, in close contact with TFIIA in the cocrystal structure (57). These three mutants are in positions that likely affect TBP-TFIIA interaction.

These TBP mutants could be defective in interacting with TFIIA, and the synthetic defects could reflect a role for yFACT in promoting assembly of the TBP-TFIIA-DNA complex. Overexpression of TFIIA might then allow these TBP mutants to interact with TFIIA despite the *spt16* mutation. In fact, TFIIA overexpression did suppress the *spt16* TBP lethality for 9 of 12 mutant combinations tested, and overexpressing TFIIB suppressed it in one instance (Fig. 1B and Table 2), strongly suggesting that the *spt16* mutation exacerbates the defective TBP-TFIIA interaction. Nhp6 overexpression did not suppress any synthetic lethality, but it strongly inhibited growth of several mutant combinations (Fig. 1C and Table 2). Nhp6 may enhance TBP binding more globally (9), and thus excess Nhp6 could be inhibitory because it promotes formation of TBP-containing complexes at nonproductive sites. These results support a role for Spt16 in facilitating formation of the TBP-TFIIA complex in vivo.

Genetic interaction between Spt16 and TFIIA. To determine directly whether Spt16 and TFIIA contribute to the same pathway, we next determined whether a *spt16-11* mutation is synthetic lethal with TFIIA mutants. TFIIA is composed of two subunits, Toa1 and Toa2, and we tested Toa2 mutants which disrupt formation of the TBP-TFIIA complex in vitro (40). We constructed two strains with a *toa2* gene disruption, with either wild-type *SPT16* or *spt16-11*. *TOA2* is an essential gene, so these strains contained YCp-*URA3-TOA2*(wild type) for viability. These strains were transformed with YCp-*LEU2* plas-

TABLE 1. Genetic interactions between TBP and Spt16 mutants[a]

TBP mutant	Effect with *spt16* allele and substitution(s)[b]							Note(s)[c]
	spt16-11 (T828I, P859S)	*spt16-2* (G132D)	*spt16-9A* (G836S, P838S)	*spt16-12* (A417T, G568S, R569K, P599L)	*spt16-22* (A417V)	*spt16-24* (T434I)	*spt16-16A* (R204W, A273V, C290V, D318V)	
E93G	Synthetic lethal	Growth defect 33°	Growth defect	Growth defect	Growth defect 33°	Growth defect 33°	Growth defect 30° 33°	a, b, c
K97R, L193S	Growth defect 33°	No effect	No effect	No effect	ND	No effect	No effect	b, d, e
I103T, K239Stop	No effect	Growth defect 33°	Growth defect	No effect	No effect	No effect	No effect	b, d, e
L114F	No effect	No effect	No effect	No effect	No effect	No effect	No effect	b, c, f, g
E129G	No effect	No effect	No effect	No effect	No effect	No effect	No effect	b, d, e
K133R	No effect	Growth defect 33°	No effect	No effect	No effect	No effect	No effect	b
K133L, K145L	Growth defect 33°	No effect	No effect	No effect	No effect	No effect	Synthetic lethal 33°	b, e, h, i
K138T, Y139A	Synthetic lethal	Synthetic lethal	Synthetic lethal	Synthetic lethal	Synthetic lethal	Synthetic lethal	Growth defect	a, b, c, g, h, i
G147W	Synthetic lethal 33°	Growth defect 33°	Growth defect 33°	Growth defect 33°	Growth defect 33°	Growth defect 33°	Growth defect 33°	a, b, c
C164W	Growth defect 33°	No effect	No effect	No effect	Growth defect	No effect	No effect	b
L172P	Synthetic lethal 33°	No effect	Growth defect	No effect	No effect	No effect	No effect	b, c, d, g
G174E	Synthetic lethal	Synthetic lethal	Synthetic lethal	Synthetic lethal	Synthetic lethal	ND	No effect	b, d, e, j
E186M	No effect	No effect	No effect	No effect	No effect	No effect	No effect	b, d, k
F227L	No effect	No effect	No effect	No effect	No effect	No effect	No effect	b
F237L	No effect	No effect	No effect	No effect	No effect	No effect	Growth defect 33°	b, g, h, k
K239T	No effect	No effect	No effect	No effect	No effect	No effect	No effect	b, l

[a] Strain DY8552, with the genes for both TBP and Spt16 deleted but with the YCp-*URA3*-TBP-Spt16 plasmid, was transformed with the appropriate YCp-*TRP1* plasmids with TBP mutants and YCp-*LEU2* plasmids with Spt16 mutants. The transformants with the various TBP and Spt16 mutants were then grown on 5-FOA at 25°C, 30°C, or 33°C, to assess the ability to grow in the absence of the YCp-*URA3*-TBP-Spt16 plasmid with the wild-type genes. A synthetic genetic defect is indicated by either no growth or poor growth of 5-FOA. As a control, each of the TBP mutants grows on 5-FOA in a strain with YCp-*LEU2*-Spt16(wild type) *SPT16*, and the *spt16* mutants are viable in the presence of wild-type TBP.

[b] Synthetic lethal, no growth at 25°C, 30°C, or 33°C; synthetic lethal 33°, no growth at 33°C; growth defect, poor growth at 25°C, 30°C, or 33°C; growth defect 33°, poor growth at 33°C; growth defect 30° 33°, poor growth at 30°C or 33°C; ND, not determined.

[c] Notes: a, synthetic lethal with *gcn5* (9); b, synthetic lethal with *nhp6ab* (19); c, synthetic lethal with *swi2* (9); d, growth defect with *gcn5* (9); e, growth defect with *swi2* (9); f, Spt$^-$ phenotype (2); g, mutations at this residue are activation defective (27, 37, 53, 64); h, mutation reduces interaction with TFIIA (13, 28, 52, 53); i, mutation reduces interaction with Mot1 (1); j, mutation reduces interaction with Spt3 (18); k, mutation reduces interaction with TFIIB (52, 58); l, mutations at this residue suppress *sua7* (TFIIB) mutations (12).

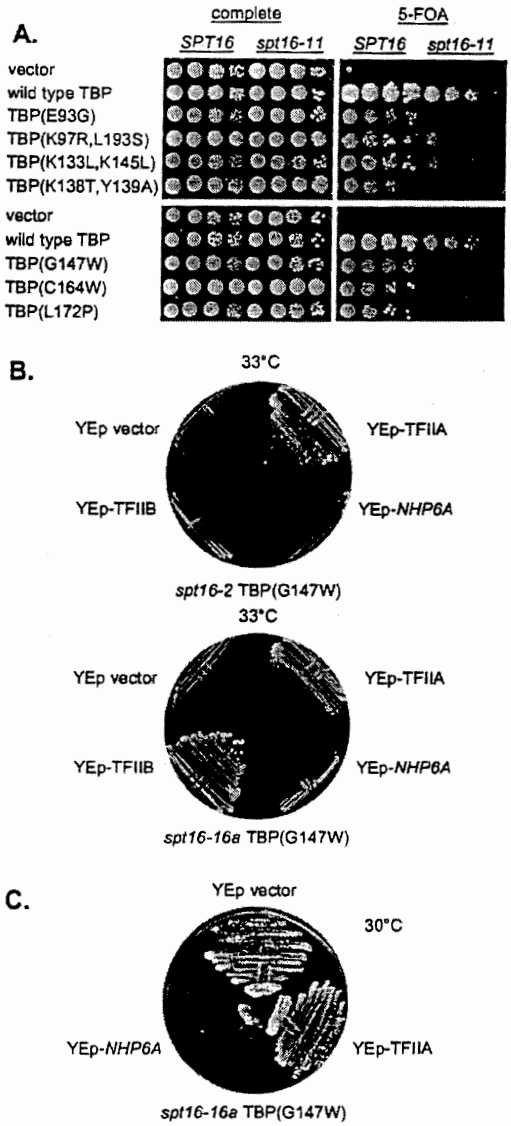


FIG. 1. *spt16* synthetic lethality with TBP mutants. A. Strain DY8552 (*spt15*Δ *spt16*Δ + YCp-*URA3*-TBP-Spt16) was transformed with two plasmids, a YCp-*TRP1* plasmid with a TBP mutant and a YCp-*LEU2* plasmid with either wild-type SPT16 or *spt16-11*, and dilutions were plated on complete or 5-FOA medium at 33°C for 3 days. B. Strains DY8969 and DY9452 were transformed with either YEp-TFIIA, YEp-TFIIB, YEp-*NHP6A*, or the vector and were plated on selective medium at 33°C for 3 days. C. Strain DY8969 was transformed with either YEp-TFIIB. YEp-*NHP6A*, or the vector, and dilutions were plated on selective medium at 30°C for 2 days.

mids with the wild-type *TOA2* gene or a mutant *toa2* gene, or the plasmid vector. Growth on 5-FOA medium was assessed to determine the ability of these transformants to grow without wild-type *TOA2*. The *toa2* mutants shown in Fig. 2A are lethal in the *spt16-11* strain but viable in the otherwise wild-type strain. Interestingly, the same *toa2* mutants that are lethal with *spt16-11* also showed synthetic lethality with *swi2* and *gcn5* (9). The TFIIA residues affected in these *toa2* mutants make important contacts with TBP, and the lethality with *spt16-11*, *swi2*, or *gcn5* suggests that yFACT, Swi/Snf, and the Gcn5 histone acetyltransferase may all be involved in the same pathway stimulating formation of the TBP-TFIIA-DNA complex.

Some of these *spt16-11 toa2* synthetic lethalities can be suppressed by overexpression of TBP or Nhp6. The *spt16-11 toa2*(Y10G,R11Δ) synthetic lethality is suppressed by YEp-*NHP6A*, while weaker suppression by YEp-TBP is seen. In contrast, the *spt16-11 toa2*(W76A) synthetic lethality is only suppressed by YEp-TBP (Fig. 2B). These results support the idea of a role for the yFACT complex in TBP-TFIIA-DNA complex formation.

Deletion of SPT3 suppresses *spt16* phenotypes. Spt3 and Spt8 are components of the SAGA transcriptional coactivator complex (54). Spt3 physically interacts with TBP, and Spt8 makes direct contact with TFIIA (18, 62). We have observed that disruption of either *SPT3* or *SPT8* suppresses synthetic lethalities along with transcriptional and growth defects associated with mutations affecting Nhp6, Gcn5, and TBP (9, 65). This suggests that Spt3 and Spt8 act in opposition to these transcription initiation factors at some genes important for growth. In support of this, *SPT3* and *SPT8* have been shown to negatively regulate expression of specific promoters (7, 65). Based on these observations, we constructed *spt16-11 spt3*Δ and *spt16-11 spt8*Δ double mutant strains and found that *spt3*Δ or *spt8*Δ suppress the temperature sensitivity of an *spt16* mutant (Fig. 3A). This mutual suppression suggests that Spt16 and Spt3/8 oppose each other while regulating TBP binding to promoters in vivo.

Deletion of SPT3 suppresses the synthetic lethality between *spt16-11* and other transcription factors. We have shown that a *spt16-11* mutation shows a strong synthetic growth defect when combined with *gcn5* or *rpd3* mutations (21). Since deletion of the *SPT3* gene suppresses other *spt16* defects, we asked whether a *spt3*Δ mutation would also suppress these synthetic growth defects. The *spt16-11 gcn5* double mutant grows poorly at 25°C and is lethal at 30°C, but both of these defects are suppressed by deletion of the *SPT3* gene (Fig. 3B). The Gcn5 histone acetyltransferase and the Rpd3 histone deacetylase act in opposition, and thus it is surprising that *spt16-11* shows synthetic growth defects with both *gcn5* and *rpd3*. The *spt16-11 rpd3* strain is lethal at 34°C, but a *spt3*Δ mutation suppresses this synthetic lethality (Fig. 3C). In interpreting these results, we note that the Gcn5 and Rpd3 factors have been implicated primarily as regulators acting at promoter regions. Moreover, histone acetylation promotes in vivo binding by TBP (47) and in vitro binding by TBP and TFIIA to nucleosomal templates (9). We propose that at some promoters both the yFACT complex and histone acetylation influence DNA binding by TBP and TFIIA and that either the proper level of acetylation or the ability to remove this modification at the appropriate time is an important component of the effect of acetylation.

The Nhp6 architectural transcription factor is required for the Spt16-Pob3 complex to bind to nucleosomes in vitro (20). An *nhp6ab* strain, with deletions of both of the genes that encode Nhp6, *NHP6A* and *NHP6B*, is viable, as is the *spt16-11*

TABLE 2. Multicopy suppression of TBP *spt16* synthetic lethality[a]

Strain	TBP mutant	Spt16 mutant	Effect with plasmid		
			YEp-TFIIA	YEp-TFIIB	YEp-*NHP6A*
DY9446	TBP(E93G)	*spt16-2*	Suppression	No effect	No effect
DY9447	TBP(E93G)	*spt16-11*	Suppression	No effect	Inhibition
DY9450	TBP(E93G)	*spt16-16a*	Suppression	No effect	No effect
DY9448	TBP(E93G)	*spt16-22*	Suppression	No effect	Inhibition
DY9449	TBP(E93G)	*spt16-24*	Suppression	No effect	No effect
DY9451	TBP(K133L,K145L)	*spt16-16a*	Inhibition	No effect	No effect
DY9452	TBP(G147W)	*spt16-2*	Suppression	No effect	No effect
DY9453	TBP(G147W)	*spt16-9a*	Suppression	No effect	No effect
DY8970	TBP(G147W)	*spt16-12*	Suppression	ND	No effect
DY8969	TBP(G147W)	*spt16-16a*	Suppression	Suppression	No effect
DY8971	TBP(G147W)	*spt16-22*	Suppression	No effect	Inhibition
DY8972	TBP(G147W)	*spt16-24*	Suppression	ND	No effect
DY9456	TBP(L172P)	*spt16-11*	No effect	No effect	No effect
DY9454	TBP(F237L)	*spt16-16a*	No effect	No effect	No effect

[a] Strain DY8552, with the genes for both TBP and Spt16 deleted but with the YCp-*URA3*-TBP-Spt16 plasmid, was transformed with the appropriate YCp-*TRP1* plasmids with TBP mutants and YCp-*LEU2* plasmids with Spt16 mutants and then passed over 5-FOA at 25°C to isolate the indicated strain without the wild-type TBP and Spt16 genes. These strains were then transformed with the YEp-*URA3* multicopy plasmids with TFIIA, TFIIB, or *NHP6A*, or the vector control, and growth was assessed after 3 days at 33°C on selective medium. ND, not done.

mutant. However, combining these viable mutations in the *spt16-11 nhp6ab* strain results in synthetic lethality at 33°C (20). However, a *spt3*Δ mutation also suppresses the synthetic lethality of the *spt16-11 nhp6ab* double mutant (Fig. 3D). Nhp6 has been shown to stimulate formation of the TBP-TFIIA-DNA complex in vitro (9), and the suppression of *spt16-11 nhp6ab* synthetic lethality by *spt3*Δ supports the idea that yFACT is involved in regulating TBP-TFIIA-DNA complex formation.

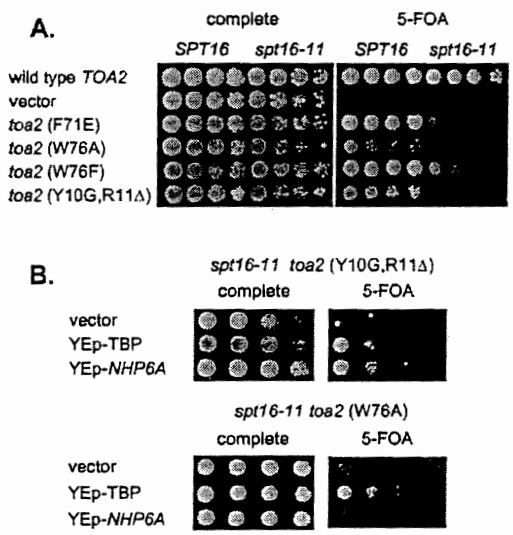


FIG. 2. *spt16* synthetic lethality with TFIIA mutants. A. Strains DY8541 (*toa2*Δ) and DY8699 (*spt16-11 toa2*Δ) transformed with the indicated TFIIA mutant plasmids were plated on complete or 5-FOA plates and incubated at 33°C for 3 days. Two other *toa2* mutants (Y69F and F71R) were viable in the *spt16-11* mutant (data not shown). B. Strain DY8700 (*spt16-11 toa2*Δ) was transformed with a YCp-*LEU2* plasmid with the indicated *toa2* mutant and a multicopy *URA3* plasmid with either TFIIA, *NHP6A*, or the vector, and dilutions were plated on complete or 5-FOA medium at 33°C [*toa2*(Y10G,R11Δ)] or 30°C [*toa2*(W76A)].

The *spt16* phenotype depends on a functional interaction between TBP and Spt3. The Spt3 component of the SAGA complex has been shown to interact with TBP, and the TBP(G174E) mutant shares phenotypes with *spt3* mutations (18). Additionally, allele-specific suppression between TBP and Spt3 mutations suggests a direct interaction between TBP and Spt3 (18). TBP binding to the *GAL1* promoter is lost in either a TBP(G174E) or a Spt3(E240K) mutant (32). However, TBP binding to *GAL1* is restored in the TBP(G174E) Spt3(E240K) double mutant, demonstrating a functional interaction between TBP and Spt3. We have shown that Spt3 inhibits TBP binding to the *HO* promoter (66). Additionally, the reduced *HO* expression caused by a *gcn5* mutation can be suppressed by either a TBP(G174E) or a Spt3(E240K) mutant. The presence of both TBP(G174E) and Spt3(E240K) reduces *HO* expression in the *gcn5* mutant to the same level as observed with wild-type TBP and Spt3, again demonstrating the functional interaction between TBP(G174E) and Spt3(E240K), in this case to maintain repression of a hypoacetylated promoter.

Based on these results, we wanted to test whether the phenotypes of the *spt16-11* strain are dependent on the functional interaction between Spt3 and TBP, using the TBP(G174E) and Spt3(E240K) mutants. Isogenic strains were constructed that differ at *SPT16*, TBP, and *SPT3*. However, we were unable to isolate a *spt16-11* TBP(G174E) *SPT3* strain in our crosses, suggesting that this combination was lethal. A plasmid shuffle experiment shows that *spt16-11* and TBP(G174E) are synthetically lethal, showing no growth on 5-FOA (Fig. 3E), while the TBP(G174E) mutant grows well in an *SPT16* strain (65). However, introduction of either a *spt3*Δ gene disruption or the *spt3*(E240K) mutation allows the *spt16-11* TBP(G174E) strain to grow (Fig. 3F, lines 6 and 7). The *spt16-11* strain grows poorly at a semipermissive temperature of 34°C. This defect is suppressed by a *spt3*Δ gene disruption, and the suppression is even stronger with the *spt3*(E240K) mutation (Fig. 3F, com-

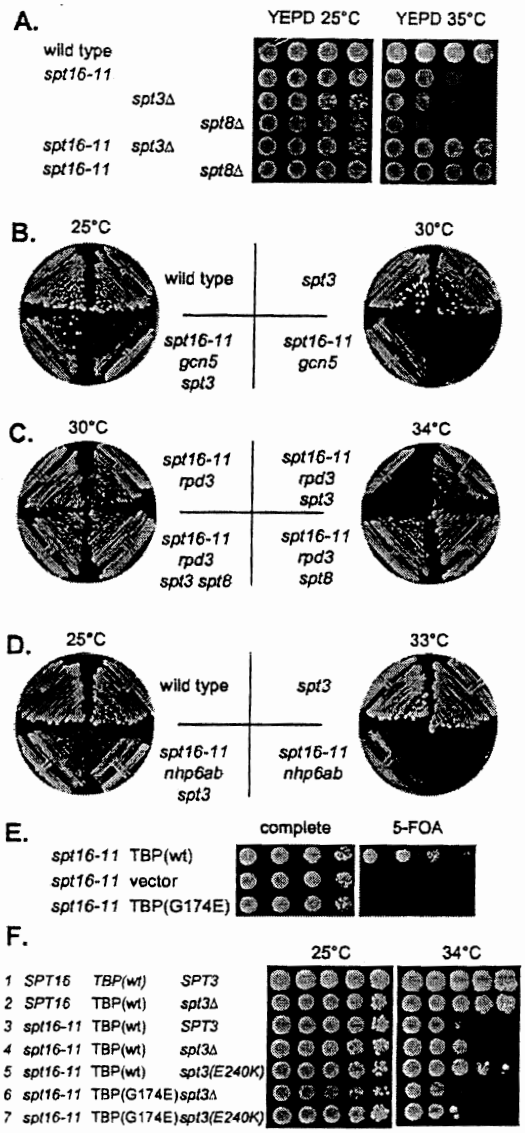


FIG. 3. Suppression of *spt16-11* by *spt3*. A. Dilutions of strains DY3398 (wild type), DY8788 (*spt16-11*), DY8980 (*spt3*), DY8981 (*spt8*). DY8977 (*spt16-11 spt3*), and DY8978 (*spt16-11 spt8*) were incubated on YEPD medium for 2 days at either 25°C or 35°C. B. Disruption of *SPT3* suppresses the *spt16-11 gcn5* synthetic lethality. Strains DY150 (wild type), DY6220 (*spt3*), DY8154 (*spt16-11 gcn5*), and DY9071 (*spt16-11 gcn5 spt3*) were grown on YEPD plates at 25°C for 4 days or at 30°C for 2 days. C. Disruption of *SPT3* or *SPT8* suppresses the *spt16-11 rpd3* synthetic lethality. Strains DY8941 (*spt16-11 rpd3*), DY8946 (*spt16-11 rpd3 spt3*), DY8948 (*spt16-11 rpd3 spt8*), and DY8950 (*spt16-11 rpd3 spt3 spt8*) were grown on YEPD plates at 30°C for 3 days or at 34°C for 3 days. D. Disruption of *SPT3* suppresses the *spt16-11 nhp6ab* synthetic lethality. Strains DY150 (wild type), DY8808 (*spt16-11 nhp6ab*), DY6220 (*spt3*), and DY8985 (*spt16-11 nhp6ab spt3*) were grown on YEPD plates at 25°C for 4 days or at 33°C for 2 days. E. *spt16-11* is synthetic lethal with TBP(G174E). pare lines 3, 4, and 5). Importantly, when the allelic interaction is restored with Spt3(E240K) in the presence of TBP(G174E), the strain once again becomes sensitive to growth at a higher temperature (Fig. 3F, compare lines 5 and 7). We conclude that the ability of Spt3 to bind to TBP is required for Spt3 to perform the activity that opposes yFACT action with TBP.

***spt16* and *spt3* mutations affect TBP binding in vivo.** ChIP experiments have shown that mutations in Spt16 and Spt3 both affect TBP binding to promoters in vivo. Inactivation of Spt16 results in reduced binding of TBP and TFIIB to promoters (36). Spt3 is required for TBP binding to some promoters (8, 17). In light of these results, and our results showing mutual suppression between *spt16-11* and *spt3* (Fig. 3A), we decided to examine whether this suppression is also seen in terms of TBP binding.

Four isogenic strains differing at the *SPT16* and *spt3* loci were grown at 25°C to early log phase and then shifted to 37°C for 3 h, and then TBP binding was analyzed by ChIP. Following cross-linking, immunoprecipitation with anti-TBP antibody, and reversal of cross-links, TBP binding to various promoters was measured by real-time PCR. After the shift to 37°C, TBP binding to the *ELP3* and *SER3* promoters was markedly reduced in both the *spt16-11* and *spt3* mutant strains (Fig. 4). Moreover, TBP binding in the *spt16-11 spt3* double mutant is higher than in either single mutant, approaching that seen in the wild type. These results show that the improved growth in the *spt16-11 spt3* double mutant compared to the single mutants (Fig. 3A) is reflected by changes in TBP binding to multiple promoters.

yFACT stimulates TBP-TFIIA binding to a nucleosome in vitro. We next used purified Spt16-Pob3 and Nhp6 in an in vitro assay to determine whether yFACT could promote formation of TBP-TFIIA-DNA. TBP can bind to DNA containing a TATA element but cannot bind when the TATA is within a nucleosome (24). Importantly, the Swi/Snf chromatin remodeler facilitates TBP binding to a nucleosomal TATA in the presence of TFIIA (24). We used the PH-MLT(+3) template (24), containing a nucleosome positioning sequence that places the TATA element at the center of the positioned histone octamer, to test whether yFACT could promote TBP binding to TATA. The PH-MLT(+3) template was radiolabeled, assembled into a nucleosome, and used for DNase I digestion experiments (Fig. 5). The nucleosome shows the expected periodicity in DNase I protection (lanes 1 to 3), while yFACT enhances sensitivity at specific sites (lanes 4 to 6), particularly in the dyad region, consistent with previous observations (20, 43). DNase I is able to access the region containing the TATA sequence in free nucleosomes (see the TATA region in lanes 1 to 3 in Fig. 5) or in nucleosomes bound by yFACT (lanes 4 to

Strain DY8552 (*spt15Δ spt16Δ* + YCp-*URA3*-TBP-Spt16) was transformed with a YCp-*LEU2-spt16-11* plasmid and a YCp-*TRP1* plasmid with either TBP(wild type [wt]). TBP(G174E), or the empty YCp-*TRP1* vector, and dilutions were plated on complete or 5-FOA medium at 33°C for 2 days. F. Spt3(E240K) suppresses *spt16-11* TBP(G174E) synthetic lethality. Strains DY150 (wild type), DY6220 (*spt3*), DY8107 (*spt16-11*), DY8903 (*spt16-11 spt3Δ*), DY9036 [*spt16-11* Spt3(E240K)], DY9038 [*spt16-11* TBP(G174E)], and DY9040 [*spt16-11* TBP(G174E) Spt3(E240K)] were plated on complete or 5-FOA medium at 25°C for 3 days or at 34°C for 4 days.

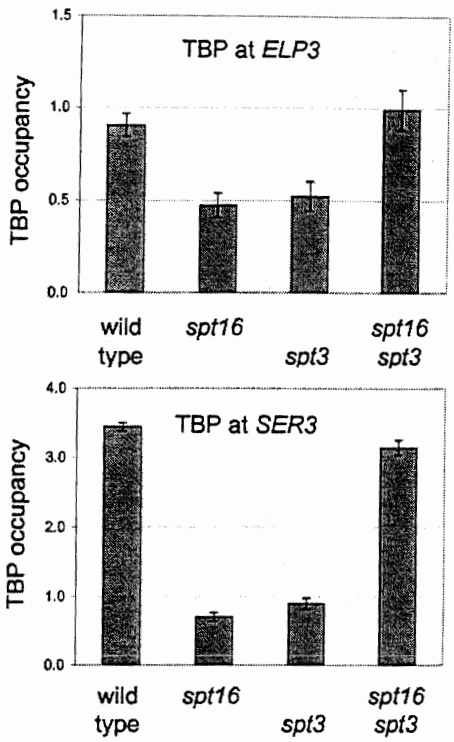


FIG. 4. *spt16* and *spt3* mutations affect TBP binding. TBP occupancy at the *ELP3* and *SER3* promoters was determined by chromatin immunoprecipitation with polyclonal anti-TBP antisera and quantitative PCR, using cells that had been grown at 25°C and then shifted to 37°C for 3 h. Relative binding is shown, after normalization to an Intergenic V internal control. Error bars reflect variance among replicate PCRs. Strains DY3398 (wild type), DY8788 (*spt16-11*), DY8980 (*spt3*), and DY8977 (*spt16-11 spt3*) were used.

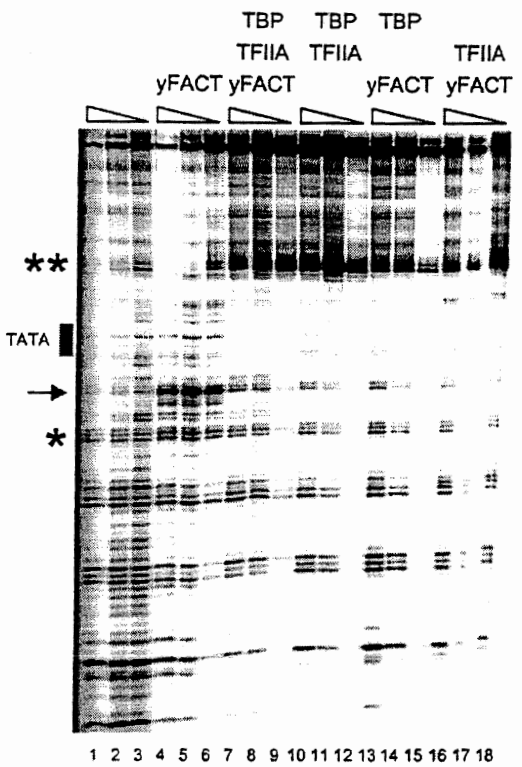


FIG. 5. yFACT stimulates TBP and TFIIA binding to a nucleosomal TATA site. The PH-MLT(+3) template with a nucleosome positioning sequence and a TATA element near the dyad (24) was radiolabeled and assembled into nucleosomes, and the structure of these nucleosomes was assessed by partial DNase I digestion followed by electrophoresis and phosphorimager analysis. Each set of three lanes has twofold decreases in the amount of DNase I. Lanes 1 to 3 (nucleosome only), the DNase I digestion pattern shows the 10-bp periodicity of a rotationally phased nucleosome. Lanes 4 to 6, addition of yFACT to the binding reaction results in changes in the pattern of DNase I protection, particularly near the dyad (marked with an arrow), demonstrating that yFACT reorganizes the structure of the nucleosome. The changes in the DNase I digestion pattern of the PH-MLT(+3) sequence due to yFACT are different from those seen with either the 5S or 601 nucleosome positioning sequences previously examined with yFACT (20, 43), but as in those cases increased access to DNase I is observed near the dyad axis. This is consistent with the previous conclusion that the effects of nucleosome reorganization induced by yFACT are focused in specific regions of the nucleosome structure but the specific sites digested are strongly influenced by the DNA sequence. Lanes 7 to 18, with added TBP and/or TFIIA, as indicated. The position of the TATA element is indicated. The regions marked with single or double asterisks are discussed in the text. The single asterisk indicates the sequences that display constant accessibility to DNase I (showing that protection is specific to the TATA region). The double asterisks indicate a site within the nucleosomes in which the combination of TBP and TFIIA strongly enhances DNase I sensitivity.

6). However, the region is protected from DNase I digestion when TBP is present, but only when yFACT and TFIIA are both also added (compare the TATA region in lanes 7 to 9 to the same region in adjacent lanes 3 to 6 and 10 to 11 in Fig. 5). This protection is specific to the TATA region, as other nearby sequences display constant accessibility to DNase I (Fig. 5). Further, the presence of TBP or TFIIA reverses the enhanced DNase I sensitivity induced by yFACT near the dyad. These changes indicate that TBP binds to its cognate site in the nucleosome only if yFACT and TFIIA are present, demonstrating that the reorganization of the nucleosome by yFACT promotes accessibility of this site for assembling a TBP-TFIIA complex.

The combination of TBP and TFIIA also strongly enhances DNase I sensitivity at a site within these nucleosomes (Fig. 5). This effect occurs away from the TATA site and is independent of yFACT. yFACT therefore only enables TBP-TFIIA effects on the accessibility of the nucleosomal DNA to DNase I at the appropriate TATA site. We used a nucleosome with a mutant TATA to further demonstrate binding specificity (Fig. 6A). Protection is seen when yFACT, TBP, and TFIIA are incubated with the nucleosome with the wild-type TATA, while no binding is seen with the mutant TATA (compare lanes 5 to 6 to lanes 7 to 8), showing that TBP-TFIIA binding to the nu-

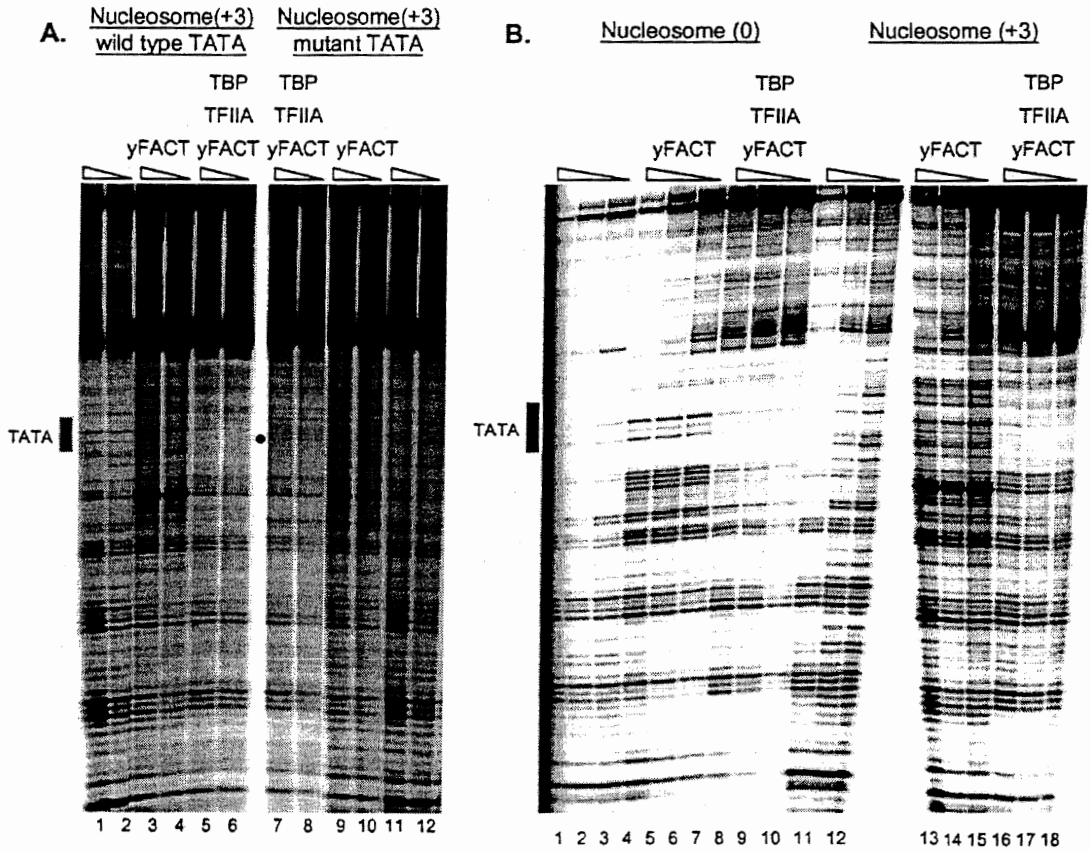


FIG. 6. TBP and TFIIA binding to a nucleosome is affected by the integrity and rotational phase of the TATA site. A. An intact TATA box is required for TBP-TFIIA binding. Nucleosomes were assembled onto either the PH-MLT(+3) template with a wild-type TATA or the PH-MLT(+3)-Mu template with a mutated TATA (24), and TBP-TFIIA binding was examined as described for Fig. 5. B. The position of the TATA sequence within the nucleosome affects binding. Nucleosomes were assembled onto either the PH-MLT(+3) template or the PH-MLT(0) template which has a 3-nucleotide change in the rotational position of the TATA sequence relative to the histone core (24), and TBP-TFIIA binding was examined as described for Fig. 5.

cleosome is TATA sequence specific. More significantly, TBP binding to a nucleosomal TATA requires both yFACT and TFIIA. Imbalzano et al. (24) showed that the orientation of the TATA element relative to the histone core was critical for TBP-TFIIA binding to a nucleosome in the presence of Swi/Snf. Using two additional templates, PH-MLT(0) and PH-MLT(+6), which differ in the rotational position of the TATA sequence relative to the positioning sequence, they showed that only one orientation of the TATA relative to the histone core could be made accessible to TBP binding by Swi/Snf. We found that yFACT stimulates TBP-TFIIA binding to the PH-MLT(0) nucleosome, although not as well as to PH-MLT(+3) (Fig. 6B), but was unable to stimulate TBP-TFIIA binding to PH-MLT(+6) (data not shown). We conclude that both Swi/Snf and yFACT can stimulate TBP-TFIIA binding to PH-MLT(+3), but only yFACT stimulates binding to the PH-MLT(0) template, suggesting that yFACT may provide accessibility to a broader range of sites within nucleosomes than Swi/Snf.

DISCUSSION

Although there is substantial evidence that yFACT participates in transcriptional elongation (6, 26, 30, 36, 38, 51), *SPT16* was first characterized because its overexpression or mutation cause the Spt$^-$ phenotype, which results from aberrant TATA site utilization (35). This suggests that Spt16 functions in transcriptional initiation, but a recent report provided an alternative explanation: the Spt$^-$ phenotype can be caused by a defect in reestablishing chromatin structure after passage of the elongating polymerase (25). While replacing nucleosomal components may be an important component of the function of Spt16, here we have presented the results of in vitro and in vivo experiments indicating that yFACT directly promotes forma-

tion of TBP-TFIIA-DNA complexes on promoters. We find strong genetic interactions between yFACT and basal transcription factors, and biochemical studies show that purified yFACT stimulates TBP-TFIIA binding to a nucleosomal template.

Support for a direct role of yFACT in initiation also derives from our experiments showing genetic interactions between *spt16* and *spt3* mutations. Unlike yFACT, which also has a role in transcriptional elongation, the available evidence indicates that Spt3 functions solely at promoters (4, 7, 8, 14, 32, 34, 60, 65), and Spt3 interacts with TBP physically and genetically (18). The suppression of *spt16* defects by a *spt3* gene deletion is consistent with the idea that yFACT stimulates TBP binding to certain promoters while Spt3 usually functions as a TBP inhibitor. In contrast, our ChIP experiments suggest Spt3 promotes TBP binding, as TBP occupancy at selected promoters is reduced in the *spt3* mutant. These results are not inconsistent, as Spt3 can either stimulate or inhibit TBP binding, depending on the promoter (7, 8, 17, 65). In any case, we see mutual suppression in both experiments, with reduced growth at 35°C and reduced TBP binding in the *spt16* and *spt3* single mutants and suppression of both effects in the *spt16 spt3* double mutant. Finally, ChIP experiments show that yFACT binds both to promoters and to coding regions (36), and *Drosophila* FACT associates with the GAGA factor, stimulating chromatin remodeling at promoters (49). This large set of interactions, many of which depend on allele-specific interactions among proteins known to interact directly, strongly implicates yFACT directly in promoting transcription initiation. The ability of yFACT to promote TBP binding in vitro using purified components demonstrates that this activity is direct.

Genetic analyses show many similarities in the relationships between yFACT and Swi/Snf with TBP-TFIIA, suggesting that each facilitates formation of TBP-TFIIA-TATA complexes. For example, we have shown that mutating yFACT causes synthetic lethality with both TBP and TFIIA mutants, and we previously showed that *swi2* is also synthetically lethal with the same TBP and TFIIA mutants (9). Moreover, TFIIA overexpression suppresses the *spt16* TBP and *swi2* TBP synthetic lethalities, and TBP overexpression suppresses the *spt16* TFIIA and *swi2* TFIIA synthetic lethalities. Additionally, histone acetylation by Gcn5 is also important for TBP binding. Much like the results with Swi/Snf and yFACT, a *gcn5* mutation is synthetically lethal with TBP and TFIIA mutants, and these lethal interactions were suppressed by overexpression of the other partner in the TBP-TFIIA complex (9). Further, acetylation of histones also promotes TBP-TFIIA binding to TATA sites within nucleosomes (9). Thus, chromatin remodeling by Swi/Snf, histone acetylation by Gcn5, and now nucleosome reorganization by yFACT have all been shown to contribute independently to formation of the TBP-TFIIA-DNA complex in a native chromatin context.

The mechanisms used by each of these factors to promote formation of the preinitiation complex are likely to be distinct. Swi/Snf is the archetypical ATP-dependent chromatin remodeling factor and appears to use ATP hydrolysis to translocate DNA relative to the histone octamer (5). This could either place the TATA site in an accessible linker location or on the surface of a repositioned nucleosome such that it is more available for binding. The strong dependence of TBP binding on rotational phasing in the presence of Swi/Snf is most consistent with the latter interpretation.

In contrast, reorganization of nucleosomes by yFACT alters chromatin structure without hydrolyzing ATP and does not involve movement of the histone octamer core relative to the DNA sequence (20, 43). Specific sites become more accessible to DNase I and to some restriction endonucleases in the reorganized nucleosomes, even though they can be recovered subsequently in a largely intact form (43). We have proposed that yFACT promotes an internal rearrangement of the nucleosomal components and that while this may lead to displacement of some components, it is instead normally a rapidly reversible process that leaves the nucleosome unaffected afterwards (21). We propose that this ability to reversibly reorganize nucleosome structure to an alternate form is important during both initiation and elongation by RNA polymerase II. One possibility is that reorganization assists the formation of TBP-TFIIA-TATA complexes required for initiation by making the binding site at least temporarily more accessible, and it also promotes elongation by making nucleosomes less inhibitory to polymerase passage.

yFACT contains Nhp6, Spt16, and Pob3. Mutations in *NHP6* are also synthetically lethal with either TBP or TFIIA mutants (19), and thus one might think that *spt16* mutants should show similar genetic interactions. However, there are important functional differences between Nhp6 and Spt16-Pob3. Strains with *nhp6ab* gene disruptions are viable, while *SPT16* and *POB3* are essential genes. Nhp6 does not bind tightly to Spt16-Pob3 (11, 20), and multiple Nhp6 molecules are needed to allow Spt16-Pob3 binding to a nucleosome (44). Nhp6 bends DNA sharply (42), and we believe that multiple Nhp6 molecules are needed to destabilize the nucleosome to promote binding by other factors such as Spt16-Pob3 (44). Nhp6 is a very abundant protein and has been shown to interact with a number of important chromatin proteins, including Spt16-Pob3, Swi/Snf, RSC, and Ssn6/Tup1 (9, 20, 22, 56). Nhp6 and other HMG proteins have been previously shown to interact with basal transcription factors like TBP (9, 16, 41, 55) but Spt16-Pob3 has not. Thus, while Nhp6 supports the function of Spt16-Pob3 in the context of yFACT, it is not simply a subunit of yFACT but instead has roles in other contexts. In this view, it is not surprising that phenotypes are not always shared among mutants in Nhp6, Spt16, and Pob3. However, Nhp6 and Spt16-Pob3 each function directly during initiation of transcription. Further work is needed to understand the mechanisms by which yFACT facilitates binding of factors to chromatin.

ACKNOWLEDGMENTS

We thank Mike Hampsey, Tony Imbalzano, and Paul Lieberman for providing plasmids, Tony Weil for antibody, and Susan Ruone for technical assistance.

This work was supported by grants awarded to T.F. and D.J.S. from the National Institutes of Health.

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**:11883–11894.
2. **Arndt, K. M., S. L. Ricupero, D. M. Eisenmann, and F. Winston.** 1992. Biochemical and genetic characterization of a yeast TFIID mutant that alters

transcription in vivo and DNA binding in vitro. Mol. Cell. Biol. 12:2372–2382.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. E. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology. Wiley and Sons, New York, N.Y.
4. **Barbaric, S., H. Reinke, and W. Horz.** 2003. Multiple mechanistically distinct functions of SAGA at the PHO5 promoter. Mol. Cell. Biol. 23:3468–3476.
5. **Becker, P. B., and W. Horz.** 2002. ATP-dependent nucleosome remodeling. Annu. Rev. Biochem. 71:247–273.
6. **Belotserkovskaya, R., S. Oh, V. A. Bondarenko, G. Orphanides, V. M. Studitsky, and D. Reinberg.** 2003. FACT facilitates transcription-dependent nucleosome alteration. Science 301:1090–1093.
7. **Belotserkovskaya, R., D. E. Sterner, M. Deng, M. H. Sayre, P. M. Lieberman, and S. L. Berger.** 2000. Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol. Cell. Biol. 20:634–647.
8. **Bhaumik, S. R., and M. R. Green.** 2002. Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol. Cell. Biol. 22:7365–7371.
9. **Biswas, D., A. N. Imbalzano, P. Eriksson, Y. Yu, and D. J. Stillman.** 2004. Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TATA-binding protein-TFIIA-DNA complex. Mol. Cell. Biol. 24:8312–8321.
10. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. 197:345–346.
11. **Brewster, N. K., G. C. Johnston, and R. A. Singer.** 2001. A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol. Cell. Biol. 21:3491–3502.
12. **Buratowski, R. M., J. Downs, and S. Buratowski.** 2002. Interdependent interactions between TFIIB, TATA binding protein, and DNA. Mol. Cell. Biol. 22:8735–8743.
13. **Buratowski, S., and H. Zhou.** 1992. Transcription factor IID mutants defective for interaction with transcription factor IIA. Science 255:1130–1132.
14. **Collart, M. A.** 1996. The *NOT*, *SPT3*, and *MOT1* genes functionally interact to regulate transcription at core promoters. Mol. Cell. Biol. 16:6668–6676.
15. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–60.
16. **Das, D., and W. M. Scovell.** 2001. The binding interaction of HMG-1 with the TATA-binding protein/TATA complex. J. Biol. Chem. 276:32597–32605.
17. **Dudley, A. M., C. Rougeulle, and F. Winston.** 1999. The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. 13:2940–2945.
18. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. 6:1319–1331.
19. **Eriksson, P., D. Biswas, Y. Yu, J. M. Stewart, and D. J. Stillman.** 2004. TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol. Cell. Biol. 24:6419–6429.
20. **Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman.** 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. 20:3506–3517.
21. **Formosa, T., S. Ruone, M. D. Adams, A. E. Olsen, P. Eriksson, Y. Yu, A. R. Rhoades, P. D. Kaufman, and D. J. Stillman.** 2002. Defects in SPT16 or POB3 (yFACT) in Saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway. Polymerase passage may degrade chromatin structure. Genetics 162:1557–1571.
22. **Fragiadakis, G. S., D. Tzamarias, and D. Alexandraki.** 2004. Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for *FRE2* transcriptional activation. EMBO J. 23:333–342.
23. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene 74:527–534.
24. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature 370:481–485.
25. **Kaplan, C. D., L. Laprade, and F. Winston.** 2003. Transcription elongation factors repress transcription initiation from cryptic sites. Science 301:1096–1099.
26. **Kim, M., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski.** 2004. Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. EMBO J. 23:354–364.
27. **Kim, T. K., S. Hashimoto, R. J. Kelleher III, P. M. Flanagan, R. D. Kornberg, M. Horikoshi, and R. G. Roeder.** 1994. Effects of activation-defective TBP mutations on transcription initiation in yeast. Nature 369:252–255.
28. **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.
29. **Komarnitsky, P., E. J. Cho, and S. Buratowski.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. 14:2452–2460.
30. **Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt.** 2002. RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol. Cell. Biol. 22:6979–6992.
31. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature 370:477–481.
32. **Larschan, E., and F. Winston.** 2001. The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. 15:1946–1956.
33. **Lee, T. I., and R. A. Young.** 2000. Transcription of eukaryotic protein-coding genes. Annu. Rev. Genet. 34:77–137.
34. **Madison, J. M., and F. Winston.** 1997. Evidence that Spt3 functionally interacts with Mot1, TFIIA, and TATA-binding protein to confer promoter-specific transcriptional control in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 17:287–295.
35. **Malone, E. A., C. D. Clark, A. Chiang, and F. Winston.** 1991. Mutations in *SPT68/CDC68* suppress *cis*- and *trans*-acting mutations that affect promoter function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 11:5710–5717.
36. **Mason, P. B., and K. Struhl.** 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol. Cell. Biol. 23:8323–8333.
37. **Moreira, J. M., and S. Holmberg.** 1998. Nucleosome structure of the yeast CHA1 promoter: analysis of activation-dependent chromatin remodeling of an RNA-polymerase-II-transcribed gene in TBP and RNA pol II mutants defective in vivo in response to acidic activators. EMBO J. 17:6028–6038.
38. **Orphanides, G., G. LeRoy, C. H. Chang, D. S. Luse, and D. Reinberg.** 1998. FACT, a factor that facilitates transcript elongation through nucleosomes. Cell 92:105–116.
39. **Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey, and D. Reinberg.** 1999. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature 400:284–288.
40. **Ozer, J., L. E. Lezina, J. Ewing, S. Audi, and P. M. Lieberman.** 1998. Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18:2559–2570.
41. **Paull, T. T., M. Carey, and R. C. Johnson.** 1996. Yeast HMG proteins *NHP6A/B* potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev. 10:2769–2781.
42. **Paull, T. T., and R. C. Johnson.** 1995. DNA looping by *Saccharomyces cerevisiae* high mobility group proteins *NHP6A/B*. Consequences for nucleoprotein complex assembly and chromatin condensation. J. Biol. Chem. 270:8744–8754.
43. **Rhoades, A. R., S. Ruone, and T. Formosa.** 2004. Structural features of nucleosomes reorganized by yeast FACT and its HMG box component, Nhp6. Mol. Cell. Biol. 24:3907–3917.
44. **Ruone, S., A. R. Rhoades, and T. Formosa.** 2003. Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. J. Biol. Chem. 278:45288–45295.
45. **Saunders, A., J. Werner, E. D. Andrulis, T. Nakayama, S. Hirose, D. Reinberg, and J. T. Lis.** 2003. Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. Science 301:1094–1096.
46. **Schroeder, S. C., B. Schwer, S. Shuman, and D. Bentley.** 2000. Dynamic association of capping enzymes with transcribing RNA polymerase II. Genes Dev. 14:2435–2440.
47. **Sewack, G. F., T. W. Ellis, and U. Hansen.** 2001. Binding of TATA binding protein to a naturally positioned nucleosome is facilitated by histone acetylation. Mol. Cell. Biol. 21:1404–1415.
48. **Sherman, F.** 1991. Getting started with yeast. Methods Enzymol. 194:1–21.
49. **Shimojima, T., M. Okada, T. Nakayama, H. Ueda, K. Okawa, A. Iwamatsu, H. Handa, and S. Hirose.** 2003. Drosophila FACT contributes to Hox gene expression through physical and functional interactions with GAGA factor. Genes Dev. 17:1605–1616.
50. **Simic, R., D. L. Lindstrom, H. G. Tran, K. L. Roinick, P. J. Costa, A. D. Johnson, G. A. Hartzog, and K. M. Arndt.** 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. 22:1846–1856.
51. **Squazzo, S. L., P. J. Costa, D. L. Lindstrom, K. E. Kumer, R. Simic, J. L. Jennings, A. J. Link, K. M. Arndt, and G. A. Hartzog.** 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J. 21:1764–1774.
52. **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.
53. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators in vivo. Science 269:75–78.
54. **Sterner, D. E., P. A. Grant, S. M. Roberts, L. J. Duggan, R. Belotserkovskaya, L. A. Pacella, F. Winston, J. L. Workman, and S. L. Berger.** 1999. Functional organization of the yeast SAGA complex: distinct components

involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell. Biol. 19:86–98.
55. **Sutrias-Grau, M., M. E. Bianchi, and J. Bernues.** 1999. High mobility group protein 1 interacts specifically with the core domain of human TATA box-binding protein and interferes with transcription factor IIB within the pre-initiation complex. J. Biol. Chem. 274:1628–1634.
56. **Szerlong, H., A. Saha, and B. R. Cairns.** 2003. The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. EMBO J. 22:3175–3187.
57. **Tan, S., Y. Hunziker, D. F. Sargent, and T. J. Richmond.** 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature 381:127–151.
58. **Tansey, W. P., and W. Herr.** 1997. Selective use of TBP and TFIIB revealed by a TATA-TBP-TFIIB array with altered specificity. Science 275:829–831.
59. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell 56:619–630.
60. **Topalidou, I., M. Papamichos-Chronakis, G. Thireos, and D. Tzamarias.** 2004. Spt3 and Mot1 cooperate in nucleosome remodeling independently of TBP recruitment. EMBO J. 23:1943–1948.
61. **Utley, R. T., J. Cote, T. Owen-Hughes, and J. L. Workman.** 1997. SWI/SNF stimulates the formation of disparate activator-nucleosome complexes but is partially redundant with cooperative binding. J. Biol. Chem. 272:12642–12649.
62. **Warfield, L., J. A. Ranish, and S. Hahn.** 2004. Positive and negative functions of the SAGA complex mediated through interaction of Spt8 with TBP and the N-terminal domain of TFIIA. Genes Dev. 18:1022–1034.
63. **Wittmeyer, J., L. Joss, and T. Formosa.** 1999. Spt16 and Pob3 of Saccharomyces cerevisiae form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. Biochemistry 38:8961–8971.
64. **Yamamoto, T., M. Horikoshi, J. Wang, S. Hasegawa, P. A. Weil, and R. G. Roeder.** 1992. A bipartite DNA binding domain composed of direct repeats in the TATA box binding factor TFIID. Proc. Natl. Acad. Sci. USA 89:2844–2848.
65. **Yu, Y., P. Eriksson, L. T. Bhoite, and D. J. Stillman.** 2003. Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. Mol. Cell. Biol. 23:1910–1921.
66. **Yu, Y., P. Eriksson, and D. J. Stillman.** 2000. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol. Cell. Biol. 20:2350–2357.

Table 5.3. Strain list for yFACT and TBP study

DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY3398	*MAT***a** *ade2 can1 his3 leu2 trp1*
DY6220	*MAT*α *spt3::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8107	*MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8154	*MAT***a** *spt16-11 gcn5::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY8541	*MAT***a** *toa2::His3MX + TOA2(YCp-URA3) ade2 can1 his3 leu2 trp1 ura3*
DY8552	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP,*SPT16(YCp-URA3) ade2 can1 his3 leu2 trp1 ura3*
DY8699	*MAT***a** *spt16-11 toa2::His3MX + TOA2(YCp-URA3) ade2 can1 his3 leu2 ura3 met15*
DY8700	*MAT***a** *spt16-11 toa2::His3MX + TOA2(YCp-URA3) ade2 can1 his3 leu2 met15 trp1 ura3*
DY8788	*MAT*α *spt16-11 ade2 can1 his3 leu2 met15*
DY8808	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 spt16-11 ade2 can1 his3 leu2 trp1 ura3*
DY8903	*MAT***a** *spt16-11 spt3::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY8941	*MAT***a** *rpd3::LEU2 spt16-11 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8946	*MAT***a** *rpd3::LEU2 spt3::TRP1 spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8948	*MAT***a** *rpd3::LEU2 spt8::KanMX spt16-11 ade2 can1 his3 leu2 trp1 ura3*
DY8950	*MAT*α *rpd3::LEU2 spt3::TRP1 spt8::KanMX spt16-11 ade2 can1 his3 leu2 lys2 trp1 ura3*

Table 5.3. Cont.

DY8969	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp-*TRP1*) + *spt16-16a* (YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY8970	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-12*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY8971	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-22*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY8972	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-24*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY8977	*MAT***a** *spt16-11 spt3::TRP1 ade2 can1 his3 leu2 trp1*
DY8978	*MAT*α *spt16-11 spt8::KanMX ade2 can1 his3 leu2 lys2 trp1*
DY8980	*MAT***a** *spt3::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY8981	*MAT*α *spt8::KanMX ade2 can1 his3 leu2 lys2 met15 trp1*
DY8985	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 spt16-11 spt3::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY9036	*MAT***a** *spt16-11 spt3::ADE2 URA3::spt3-401 ade2 can1 his3 leu2 lys2 trp1*
DY9038	*MAT***a** *spt16-11 spt3::ADE2 spt15::LEU2* + TBP(G174E)(YCp- *TRP1*) *ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9040	*MAT*α *spt16-11 spt3::ADE2 URA3::spt3-401 spt15::LEU2* + TBP(G174E)(YCp- *TRP1*) *ade2 can1 his3 leu2 lys2 met15 trp1*
DY9071	*MAT***a** *spt16-11 gcn5::HIS3 spt3::TRP1 ade2 can1 his3 leu2 trp1 ura3*

Table 5.3. Cont.

DY9398	***MATa*** *spt16-11 spt3::ADE2 spt15::LEU2* + TBP(G174E)(YCp- *TRP1*) *ade2 can1 his3 leu2 lys2 trp1*
DY9446	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(E93G)(YCp- *TRP1*) + *spt16-2*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9447	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(E93G)(YCp- *TRP1*) + *spt16-11*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9448	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(E93G)(YCp- *TRP1*) + *spt16-22*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9449	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(E93G)(YCp- *TRP1*) + *spt16-24*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9450	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(E93G)(YCp- *TRP1*) + *spt16-16a*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9451	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(K133L,K145L)(YCp- *TRP1*) + *spt16-16a*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9452	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-2*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9453	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-9a*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9454	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(F237L)(YCp- *TRP1*) + *spt16-16a*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*
DY9455	***MATa*** *spt15::ADE2 spt16::HIS3* + TBP(G147W)(YCp- *TRP1*) + *spt16-9a*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*

Table 5.3. Cont.

DY9456	*MAT***a** *spt15::ADE2 spt16::HIS3* + TBP(L172P)(YCp- *TRP1*) + *spt16-11*(YCp-*LEU2*) *ade2 can1 his3 leu2 trp1 ura3*

Table 5.4. Plasmid list for yFACT and TBP study

Plasmid	Description	Source
pRS314	YCp-*TRP1* Vector	(Sikorski and Hieter, 1989b)
pRS315	YCp-*LEU2* Vector	(Sikorski and Hieter, 1989b)
YCplac111	YCp-*LEU2* Vector	(Gietz and Sugino, 1988)
YEplac112	YEp-*TRP1* Vector	(Gietz and Sugino, 1988)
YEplac195	YEp-*URA3* Vector	(Gietz and Sugino, 1988)
M3415	TFIIB (*SUA7*) in YEp-*URA3* plasmid	Mike Hampsey
M4806	TFIIA (*TOA1* and *TOA2*) in YEp-*URA3* plasmid	this work
M4221	*NHP6A* in YEp-*URA3* plasmid	(Biswas et al., 2004)
M4761	*NHP6A* in YEp-*TRP1* plasmid	this work
M4827	TBP (wild type) in YEp-*TRP1* plasmid	(Biswas et al., 2004)
pTM8	TBP(wild type) in YCp-*TRP1* plasmid	(Kobayashi et al., 2001)
M4471	TBP(E93G) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4468	TBP(K97R, L193S) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4655	TBP(I103T, K239Stop) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4325	TBP(L114F) in YCp-*TRP1* plasmid	(Arndt et al., 1994)
M4640	TBP(E129G) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4642	TBP(K133R) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4550	TBP(K133L,K145L) in YCp-*TRP1* plasmid	(Buratowski and Zhou, 1992)
M4404	TBP(K138T,Y139A) in YCp-*TRP1* plasmid	(Stargell and Struhl, 1995)

Table 5.4. Cont.

Plasmid	Description	Source
M4475	TBP(G147W) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4470	TBP(C164W) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4474	TBP(L172P) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4482	TBP(G174E) in YCp-*TRP1* plasmid	(Eisenmann et al., 1992)
M4511	TBP(E186M) in YCp-*TRP1* plasmid	(Biswas et al., 2004)
M4472	TBP(F227L) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4473	TBP(F237L) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4653	TBP(K239T) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
pTF128	*SPT16*(wild type) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-02	*spt16-2*(G132D) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-09a	*spt16-9a*(G836S, P838S) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-11	*spt16-11*(T828I, P859S) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-12	*spt16-12*(A417T, G568S, R569K, P599L) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-16a	*spt16-16a*(R204W, A273V, C290V, D318V) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-24	*spt16-24*(T434I) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
M4614	*toa2*(wild type) in YCp -*LEU2* plasmid	(Ozer et al., 1998)
M4606	*toa2*(Y10G,R11Δ)) in YCp -*LEU2* plasmid	(Biswas et al., 2004)

Table 5.4. Cont.

Plasmid	Description	Source
M4599	*toa2*(F71E) in YCp-*LEU2* plasmid	(Ozer et al., 1998)
M4601	*toa2*(W76A) in YCp -*LEU2* plasmid	(Ozer et al., 1998)
M4603	*toa2*(W76F) in YCp -*LEU2* plasmid	(Ozer et al., 1998)

References

Arndt, K.M., Wobbe, C.R., Ricupero-Hovasse, S., Struhl, K. and Winston, F. (1994) Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. *Mol Cell Biol*, **14**, 3719-3728.

Biswas, D., Imbalzano, A.N., Eriksson, P., Yu, Y. and Stillman, D.J. (2004) Role for Nhp6, Gcn5, and the Swi/Snf Complex in Stimulating Formation of the TATA-Binding Protein-TFIIA-DNA Complex. *Mol Cell Biol*, **24**, 8312-8321.

Buratowski, S. and Zhou, H. (1992) Transcription factor IID mutants defective for interaction with transcription factor IIA. *Science*, **255**, 1130-1132.

Eisenmann, D.M., Arndt, K.M., Ricupero, S.L., Rooney, J.W. and Winston, F. (1992) SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. *Genes Dev*, **6**, 1319-1331.

Eriksson, P., Biswas, D., Yu, Y., Stewart, J.M. and Stillman, D.J. (2004) TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. *Mol Cell Biol*, **24**, 6419-6429.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y. and Stillman, D.J. (2001) Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome- binding factor SPN. *Embo Journal*, **20**, 3506-3517.

Gietz, R.D. and Sugino, A. (1988) New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene*, **74**, 527-534.

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

Ozer, J., Lezina, L.E., Ewing, J., Audi, S. and Lieberman, P.M. (1998) Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **18**, 2559-2570.

Sikorski, R.S. and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 19-27.

Stargell, L.A. and Struhl, K. (1995) The TBP-TFIIA interaction in the response to acidic activators in vivo. *Science*, **269**, 75-78.

CHAPTER 6

OPPOSING ROLES FOR Set2 AND yFACT IN REGULATING TBP BINDING AT PROMOTERS

Abstract

Previous work suggested that histone methylation by Set2 regulates transcriptional elongation. yFACT (Spt16-Pob3 and Nhp6), reorganizes nucleosomes and functions in both transcriptional initiation and elongation. We show that growth defects caused by *spt16* or *pob3* mutations can be suppressed by deleting *SET2*, suggesting that Set2 and yFACT have opposing roles. Set2 methylates K36 of histone H3, and K36 substitutions also suppress yFACT mutations. In contrast, *set1* enhances yFACT mutations, and methylation at H3 K4 by Set1 is required for *set2* to suppress yFACT defects. RNA and ChIP assays fail to detect an elongation defect in yFACT mutants. Instead, *pob3* mutants display reduced binding of both pol II and TBP to the *GAL1* promoter. Importantly, both *GAL1* transcription and promoter binding of pol II and TBP are significantly restored in the *pob3 set2* double mutant. Defects caused by an *spt16* mutation are enhanced by either TBP or TFIIA mutants. These synthetic defects are suppressed by *set2*, demonstrating that yFACT and Set2 oppose one another during transcriptional initiation at a step involving DNA-binding by TBP and TFIIA.

Introduction

Eukaryotic DNA is packaged into a highly compacted structure called chromatin, that limits the accessibility of transcription factors to DNA. There are several ways that the DNA sequences within chromatin can be made accessible to transcription factors. First, ATP-dependent chromatin remodeling factors can use the energy of ATP to move nucleosomes and expose the DNA sequences for transcription factor binding. Second, nucleosomes can be altered by posttranslational modification of histone proteins, including acetylation, methylation and ubiquitylation of lysine residues and

phosphorylation of serine residues. These modifications may directly change the properties of chromatin, thereby aiding factor binding, and they may create recognition sites for other factors such as bromodomain and chromodomain containing proteins that recognize acetylated and methylated lysines, respectively.

We have been studying a third mechanism that enhances accessibility of binding sites, the ATP-independent reorganization of nucleosomes by yFACT. Mammalian FACT contains two subunits, hSpt16 and SSRP1, which are similar throughout their lengths with the yeast orthologs, Spt16 and Pob3, except that SSRP1 has a single HMGB DNA binding motif at its C-terminus that is missing from Pob3. The yeast Nhp6 protein, essentially a single HMGB domain, supports the ability of Spt16-Pob3 to function as yFACT both *in vitro* and *in vivo*. There is substantial evidence linking yFACT to transcriptional elongation. Some yFACT mutants are sensitive to the elongation inhibitor 6-azauracil and show genetic interactions with known elongation factors. yFACT associates with known elongation factors, human FACT facilitates pol II elongation through a chromatin template *in vitro*, and ChIP and immunolocalization studies show association of FACT with elongating RNA pol II. However, experiments also suggest that yFACT has a role in regulating transcriptional initiation. An *spt16* mutation can change the site of transcriptional initiation, and *Drosophila* FACT associates with the GAGA factor and stimulates chromatin changes at promoters. Spt16 inactivation in yeast results in reduced binding of TBP and TFIIB at promoters, *spt16* mutants show strong genetic interactions with mutations affecting TBP and TFIIA, and yFACT facilitates TBP and TFIIA binding to nucleosomal binding sites *in vitro*. yFACT can therefore enhance the accessibility of DNA sequences in chromatin, and this is an important component of

transcriptional regulation both during initiation and elongation. yFACT also interacts with DNA polymerase α, MCM proteins, and plays and important role in DNA replication. Histone proteins are methylated by SET domain containing proteins, and histone methylation can regulate transcription. In yeast, histone H3 is methylated at K4, K36 and K79 by the Set1, Set2, and Dot1 histone methyl transferases, respectively. It has been suggested that K4 methylation by Set1 facilitates transcriptional elongation, as K4 methylation is enriched in the transcribed regions of actively transcribed genes, and Set1 is recruited to elongating RNA polymerase complexes. These observations suggest that Set1 is a positive elongation factor.

Di- and tri-methylation at K36 by Set2 is also found at transcribed open reading frames. Set2 also associates with the elongating form of RNA polymerase. Moreover, *set2* mutants show synthetic growth defects with genes implicated in elongation, consistent with Set2 also being a positive elongation factor. However, several observations are more consistent with a negative role for Set2 in regulating transcription initiation. K36 methylation by Set2 is required to recruit the Rpd3S histone deacetylase complex through its Eaf3 chromodomain subunit, and deacetylation by Rpd3S may be required to restore chromatin to the repressed pretranscribed state. Set2 represses transcription when tethered to a heterologous promoter, indicating a direct negative effect on initiation. Additionally, expression of a mutant *GAL4* promoter lacking its UAS element is very low, but can be increased either by a *set2* mutation or a histone H3 K36R substitution, suggesting that modification of H3 by Set2 inhibits initiation. Importantly, although several studies have shown greater K36 methylation at open reading frames, it is

clear that K36 methylation also occurs in promoter regions. Set2 therefore has complex and perhaps opposite effects on different stages of transcriptional regulation.

In this report we show that yFACT and K36 methylation by Set2 have opposing roles in regulating transcription. To our surprise, we find no evidence that mutations affecting yFACT and Set2 influence transcriptional elongation. Instead, our results show that yFACT and histone methylation by Set2 regulate, in opposite ways, binding of both RNA pol II and TBP to promoters.

Results

Set1 methylation at histone H3 K4 supports the function of yFACT

SPT16 and *POB3* are essential genes, and mutant alleles with distinct phenotypes have been isolated. We chose the *spt16-11* and *pob3(L78R)* alleles for these studies because they display the Spt- phenotype from inappropriate TATA element usage, and they are sensitive to elevated temperatures, to the dNTP synthesis inhibitor hydroxyurea (HU), and to the transcription elongation inhibitor 6-azauracil (6-AU). Thus the phenotypes of the *spt16-11* and *pob3(L78R)* alleles suggest that they have defects in transcriptional initiation, transcriptional elongation, as well as in replication of DNA.

We previously showed that some yFACT mutations are synthetically lethal with some mutations in histone H3 and H4, including deletions of the N-terminal tails and mutations of certain acetylatable lysine residues. Here, we look for genetic interactions between yFACT mutations and H3 mutations in methylated lysines K4 and K79, and acetylated site K23. Strains with deletions of both sets of chromosomal genes encoding histone H3 and H4, and carrying a YCp-*URA3* plasmid with the wild type *HHT2-HHF2* genes (encoding histone H3 and H4 respectively) were constructed. Plasmids with either wild

type or mutant *HHT2-HHF2* alleles were introduced into these strains by transformation, and the ability of transformants to grow on media with 5-FOA was assessed. *URA3+* strains cannot grow on 5-FOA, and thus growth demonstrates that the wild type histone genes on the YCp-*URA3* plasmid can be lost with the introduced plasmid supporting viability. As shown in Fig 6.1A, introducing plasmids with wild type histones, H3(K4R), H3(K23R), or H3(K79R) into a wild type strain results in healthy growth, while the empty vector does not. We conclude that these H3 mutations support viability in a wild type strain. In contrast, the H3(K23R) mutation shows a modest growth defect in combination with either a *spt16-11* (Fig 6.1B) or a *pob3(L78R)* (Fig 6.1C) mutation. The H3(K4R) mutation has a more striking effect, showing a strong synthetic defect when combined with either *spt16* or *pob3*. Lysine 4 of histone H3 is methylated by the Set1 enzyme, and thus we predict a similar effect from a *set1* mutation. We constructed a *spt16 set1* double mutant and found it to be viable at 25°C, but lethal at 33°C (Fig 6.1D). We were unable to construct a *pob3 set1* double mutant, as it was lethal at all temperatures tested. We conclude that the function of yFACT is strongly dependent on methylation of histone H3 at K4 by Set1.

Absence of Set2 methylation at histone H3 K36 suppresses temperature sensitivity caused by yFACT mutations

In contrast with our results with the K4R mutation, we found that mutations at histone H3 K36 suppress growth defects associated with yFACT mutations. The *spt16-11* mutant does not grow at 35°C, as evidenced by its failure to grow on 5-FOA when containing a plasmid with wild type histone genes (Fig 6.2A). However the *spt16* mutant grows on 5-FOA if the plasmid contains either a K36R or a K36A mutation in histone H3. Similarly,

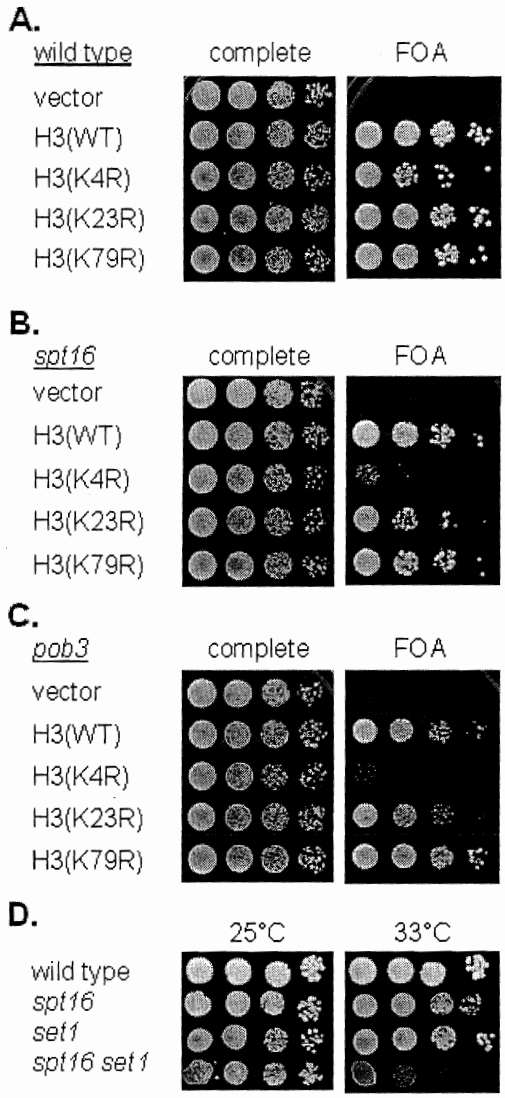


Fig 6.1. Histone H3(K4R) substitutions enhance the defects caused by *spt16* and *pob3* mutations.

A. Strain DY7803 was transformed with a YCp-TRP1 plasmid with wild type histone H4 gene and the indicated histone H3 mutation, and dilutions were plated on the indicated medium for 2 days at 33°C.

B. As in panel A, except the strain is DY7809.

C. As in A except the strain is DY7818 and dilutions were incubated for 3 days at 25°C.

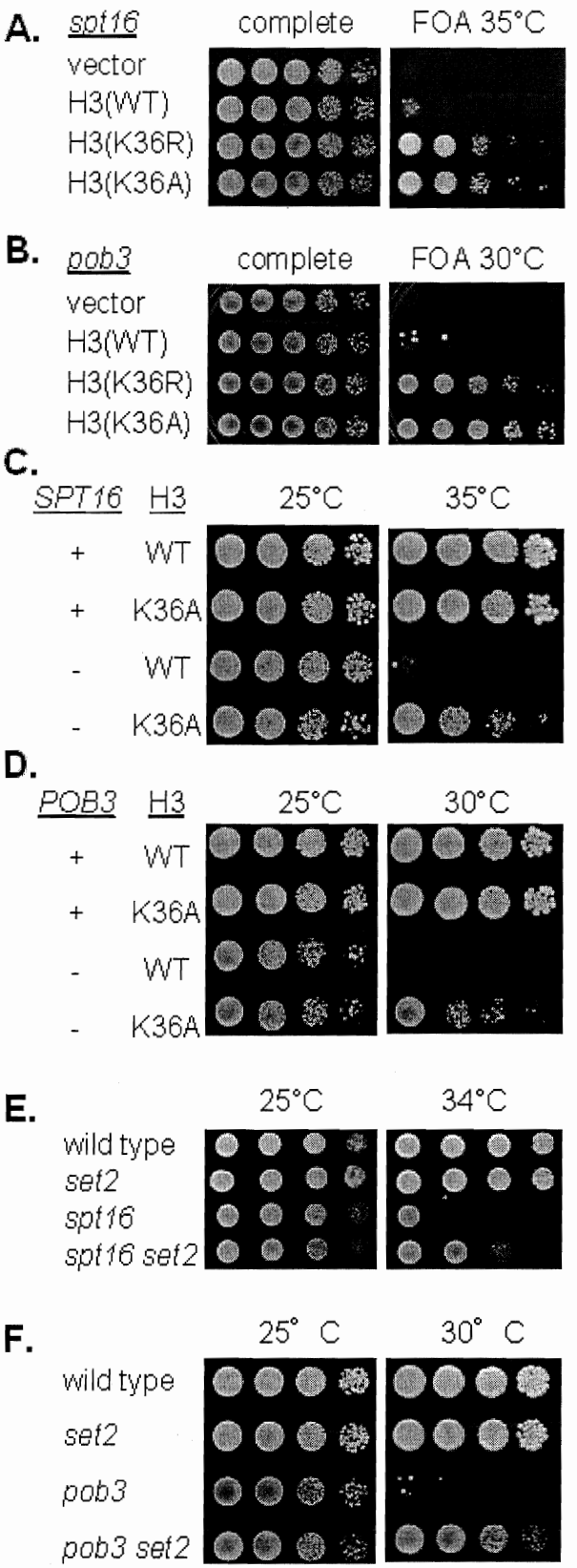
D. Dilutions of strains DY150, DY8788, DY8875, and DY9206 were plated on complete medium at 25°C for 3 days or at 33°C for 2 days.

a strain with the *pob3(L78R)* allele is unable to grow at 30°C, but the H3 K36R or K36A mutations suppress this growth defect (Fig 6.2B). To verify that this apparent growth suppression was not an artifact of growth on 5-FOA-containing medium, we used a plasmid shuffle at 25°C to evict the YCp-*URA3* plasmid, obtaining strains with a YCp-*TRP1* plasmid with either wild type histone genes, or a derivative with the H3(K36A) substitution. The *spt16* mutant with wild type histone H3 is unable to grow at 35°C, but the K36A substitution suppresses (Fig 6.2C). Similarly, histone H3(K36A) suppresses the *pob3* mutant (Fig 6.2D). The Set2 enzyme methylates K36 of histone H3, and thus a *set2* mutation should have a similar phenotype to an H3 K36 mutation if methylation at this site by Set2 is the cause of the suppression. As shown in Fig 6.2E and 6.2F, a *set2* gene deletion also suppress the temperature sensitivity of the *spt16* and *pob3* strains. These observations clearly indicate that Set2 methylation of histone H3 at K36 has an opposing role to that of yFACT in supporting viability.

Opposing roles of Set1 and Set2 methyl transferases

The temperature sensitive growth defect in an *spt16* mutant is affected by *set1* and *set2* mutations, but in opposite directions. To examine the epistasis relationships, we constructed a *spt16 set1 set2* triple mutant strain. The results in Fig 6.3A show that the triple mutant has a marked growth defect, although not quite as severe as the *spt16* mutant or the *spt16 set1* double mutant. We next constructed a plasmid with both histone H3 K4R and K36R mutations and tested it in the *spt16* and *pob3* mutants. The results in Fig 6.3B show that the H3(K4R,K36R) double mutant is synthetically lethal with both *spt16* and *pob3*, the same phenotype seen with K4R. Thus, the K4R mutation is epistatic

Fig 6.2. Histone H3(K36) substitutions and *set2* mutations suppress *spt16* and *pob3* mutations.
A. Strain DY7809 was transformed with a YCp-TRP1 plasmid with wild type histone H4 gene and the indicated histone H3 mutant, and dilutions were plated on complete medium (2 days) or FOA medium (3 days) at 35°C.
B. As in A, except the strain is DY7818 and dilutions were plated on complete medium (3 days) or FOA medium (5 days) at 30°C.
C. Dilutions of Strains DY8862, DY8864, DY8865, and DY8867 were plated on complete medium at the indicated temperature for 3 days.
D. Dilutions of strains DY8862, DY8864, DY10468, and DY10469 were plated on complete medium at the indicated temperature for 3 days.
E. Dilutions of strains DY150, DY8690, DY8787, and DY8790 were plated on complete medium at the indicated temperature for 2 days.
F. Dilutions of strains DY150, DY8690, DY8881, and DY8878 were plated on complete medium at 25°C for 2 days or at 33°C for 3 days.


A.
spt16
complete
FOA 35°C
vector
H3(WT)
H3(K36R)
H3(K36A)
B.
pob3
complete
FOA 30°C
vector
H3(WT)
H3(K36R)
H3(K36A)
C.
SPT16
H3
25°C
35°C
+ WT
+ K36A
- WT
- K36A
D.
POB3
H3
25°C
30°C
+ WT
+ K36A
- WT
- K36A
E.
25°C
34°C
wild type
set2
spt16
spt16 set2
F.
25° C
30° C
wild type
set2
pob3
pob3 set2

to K36R. We conclude that the absence of Set2 methylation at H3 K36 can only suppress the yFACT defects when methylation by Set1 occurs at H3 K4.

Histone mutations affect growth in *nhp6ab* mutant strains

In addition to Spt16 and Pob3, yFACT contains Nhp6, a small HMG protein required for nucleosomal binding by Spt16-Pob3. Nhp6 is encoded by two redundant genes, *NHP6A* and *NHP6B*, and the *nhp6ab* double mutant shows growth defects. Using an *nhp6ab* strain suitable for shuffling in plasmids with wild type or mutant histone H3 genes, we examined the effect of histone mutations (Fig 6.3C). Similar to the results with *spt16* and *pob3* mutants, the H3(K4R) mutation caused a severe synthetic defect with *nhp6ab*. However, the H3(K36) substitutions had a markedly different effect in the *nhp6ab* mutant; instead of the suppression seen with *spt16* and *pob3*, the K36 mutationsinhibited growth of the *nhp6ab* strain. Interestingly, the H3(K36R) substitution, arginine residue maintaining the basic charge of the unmodified lysine, has a more severe effect than mutation to the neutral alanine residue. A synthetic growth defect is also seen when a *set2* mutation is introduced into a *nhp6ab* strain (data not shown). Mutations at K79 did not inhibit growth in the *nhp6ab* strain (data not shown). In addition to its role in yFACT, Nhp6 has been shown to interact with other chromatin proteins, including Swi/Snf, RSC, and Ssn6/Tup1 and to play a role in transcription by RNA polymerase III. These additional roles for Nhp6 could explain why the H3(K36) mutations have such markedly different effects in the *nhp6ab* strain compared to the *spt16* and *pob3* mutants.

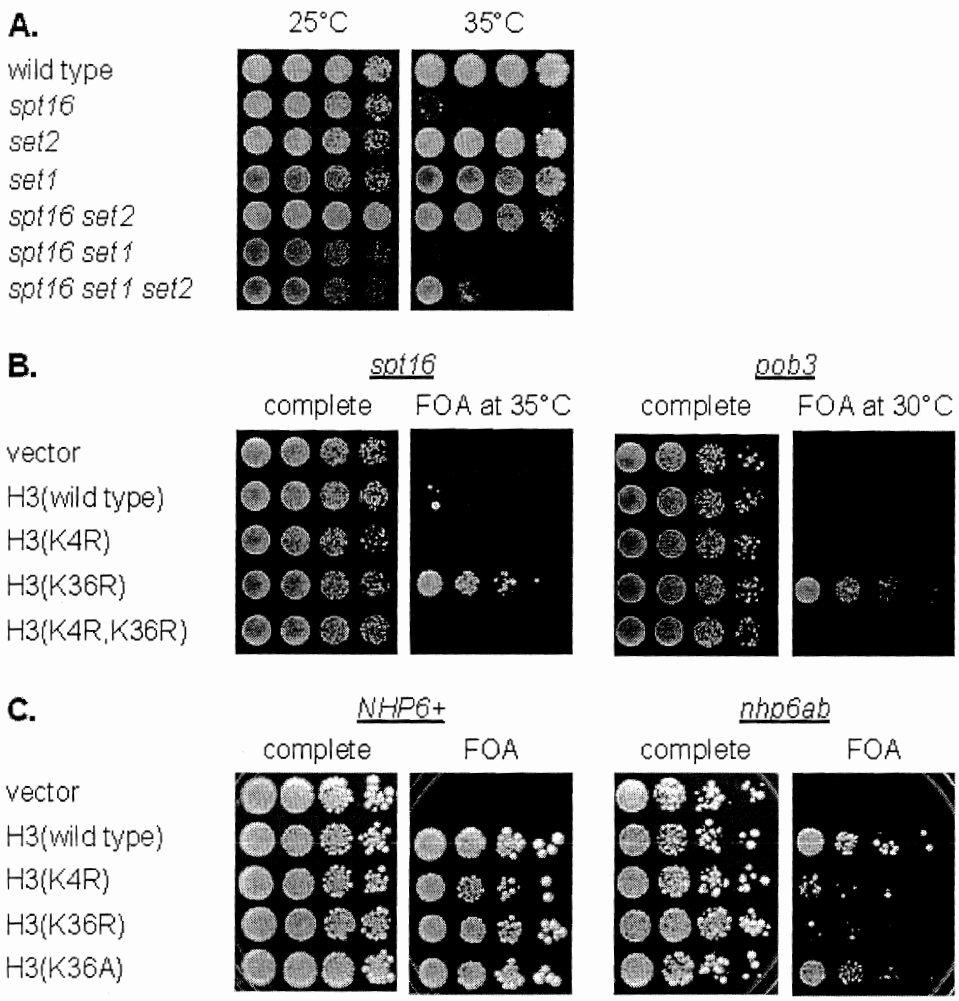


Fig 6.3. *set1* is epistatic to *set2* in genetic interactions with *spt16*.
A. Dilutions of strains DY150, DY8787, DY8690, DY8875, DY8777, DY9178, and DY9180 were plated on complete medium at 25°C for 2 days or at 35°C for 3 days.
B. Strains DY7809 and DY7818 were transformed with a YCp-TRP1 plasmid with wild type histone H4 gene and the indicated histone H3 mutant, and dilutions were plated and incubated as follows: *spt16* on complete, 2 days at 25°C, *spt16* on FOA, 4 days at 35°C, *pob3* on complete, 3 days at 25°C, and *pob3* on FOA, 5 days at 30°C.
C. As in B, except the strains are DY7803 and DY7142, and dilutions were plated on the indicated medium at 33°C for 4 days.

A variety of yFACT mutant defects are suppressed by *set2*

spt16-11 mutants are defective for growth on media containing 6-azauracil (6-AU). 6-AU is a uracil analog that causes imbalances in the pools of rNTPs, and many strains with defects in transcriptional elongation are sensitive to 6-AU. We determined whether the 6- AU sensitivity caused by *spt16* could be suppressed either by a histone mutation or by a *set2* mutation. As shown in Fig 6.4A, the 6-AU sensitivity of the *spt16* strain (line 4) is suppressed by a K36A substitution in histone H3 (line 6). A *set2* mutation similarly suppresses the 6-AU sensitivity caused by *spt16* (Fig 6.4B). We note that *set2* mutants display slightly higher 6-AU resistance than wild type strains, consistent with previous reports. Thus, *spt16* and *set2* mutants have opposite responses to 6-AU, suggesting that yFACT and Set2 have opposing roles in transcriptional elongation. However, sensitivity to 6-AU does not necessarily demonstrate a role in transcriptional elongation; a mutation in the *SNR6* promoter that reduces expression of the U6 small nuclear RNA causes 6-AU sensitivity.

Based on the observation that a *set2* mutation suppresses the temperature- and 6-AU-sensitive phenotypes associated with yFACT mutations, we tested whether *set2* can also suppress other synthetic defects observed with yFACT mutants. An *spt16* mutation displays synthetic defects with mutations in either the *GCN5* or the *ELP3* histone acetyltransferase genes. Strains with either a *spt16* or a *gcn5* mutation grow well at 30°C (Fig 6.5A). However, the *spt16 gcn5* double mutant strain grows poorly at 25°C and is lethal at 30°C. Importantly, this *spt16 gcn5* lethality can be suppressed by deletion of *SET2* in this strain. Similarly, the *spt16 elp3* double mutant cannot grow at 34°C, but *set2* suppresses this defect (Fig 6.5B). An *spt16 nhp6a nhp6b* triple mutant also shows

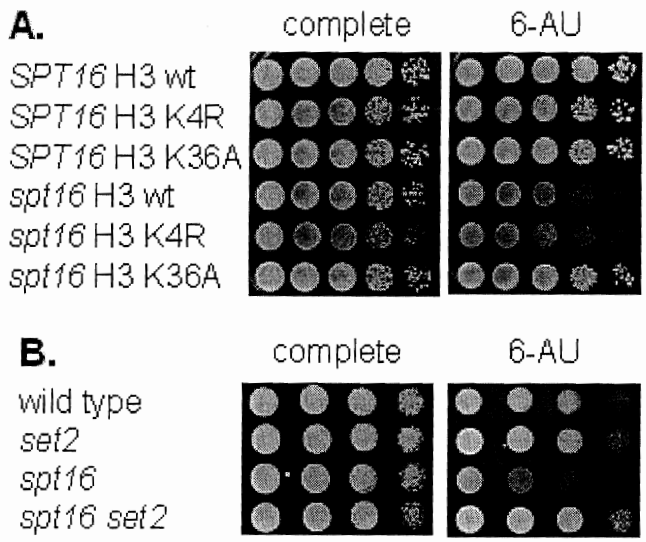


Fig 6.4. H3(K36A) and *set2* suppress the 6-AU sensitivity caused by a *spt16* mutation.
A. Dilutions of strains DY8883, DY8884, DY8885, DY8886, DY8887, and DY8888 were plated at 25°C on complete medium for 2 days or on medium lacking uracil containing 50 μg/ml 6-azauracil for 4 days.
B. As in A, except the strains are DY3398, DY8789, DY8788, and DY8790.

synthetic growth defects, and this can also be suppressed by *set2* (Fig 6.5C). The H2A.Z histone variant of H2A in yeast, encoded by *HTZ1* gene, has diverse functions. We constructed a *spt16 htz1* double mutant strain, and observed synthetic lethality at 33°C (Fig 6.5D). This growth defect is also suppressed by a *set2* mutation. Importantly, genome wide studies show that the Htz1 protein localizes preferentially at the promoter regions of genes. This promoter localization of Htz1, and the genetic interactions seen here, suggest that Set2 and yFACT might have opposing roles at promoter regions, in addition to their proposed elongation functions.

Specificity of *set2* suppression

We also tested whether *set1* or *set2* mutations also affect other factors thought to be involved in transcriptional elongation. We constructed double mutant strains, combining either a *set1* or a *set2* mutation with disruptions in *PAF1*, *CDC73*, *DST1*, *SPT4* or *ELP3* (Fig. 6.6A-G). The double mutants with *set1* or *set2* were examined for growth phenotypes, including sensitivity to temperature and 6-AU. There are some instances of suppression and some of synthetic defects (Fig 6.6A-G). However, these elongation mutants do not all show suppression with *set2* and synthetic defects with *set1*, and thus the effect appears to be specific to *spt16* and *pob3*. *spt16* mutants also cause an Spt- phenotype, altering transcription start sites from the *his4-912δ* and *lys2-128δ* alleles and conferring a His+ Lys+ phenotype. Interestingly, *spt16 set2* strains are still Spt-, and thus *set2* does not suppress this phenotype (data not shown). Similarly, the synthetic growth defect seen in a *spt16 rpd3* double mutant is not suppressed by *set2* (data not shown). However, we do see suppression of the Spt- phenotype seen in a *gcn5* mutant with the *lys2-173R2* allele strain is Lys+, showing suppression of the Spt- phenotype (Fig 6.7A).

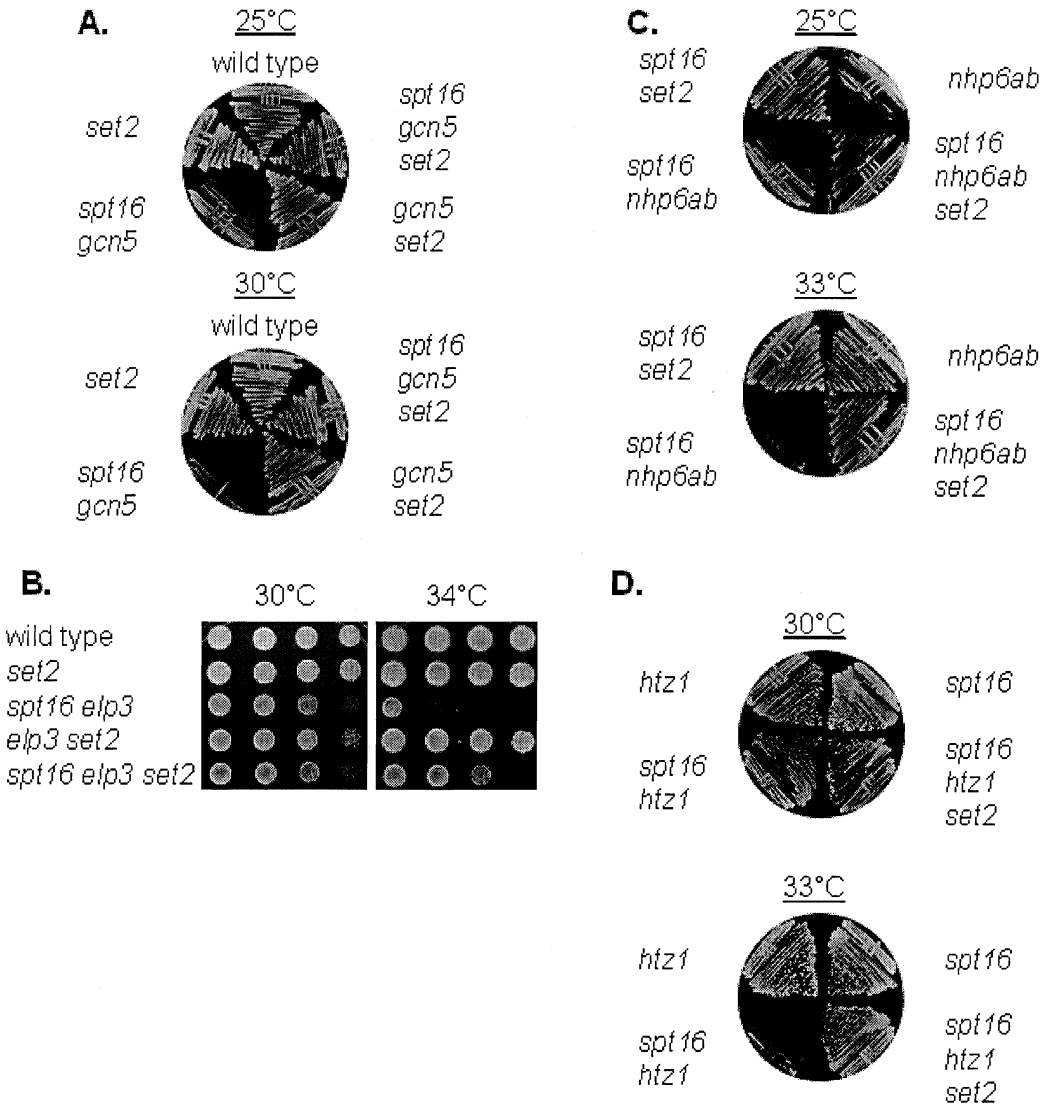
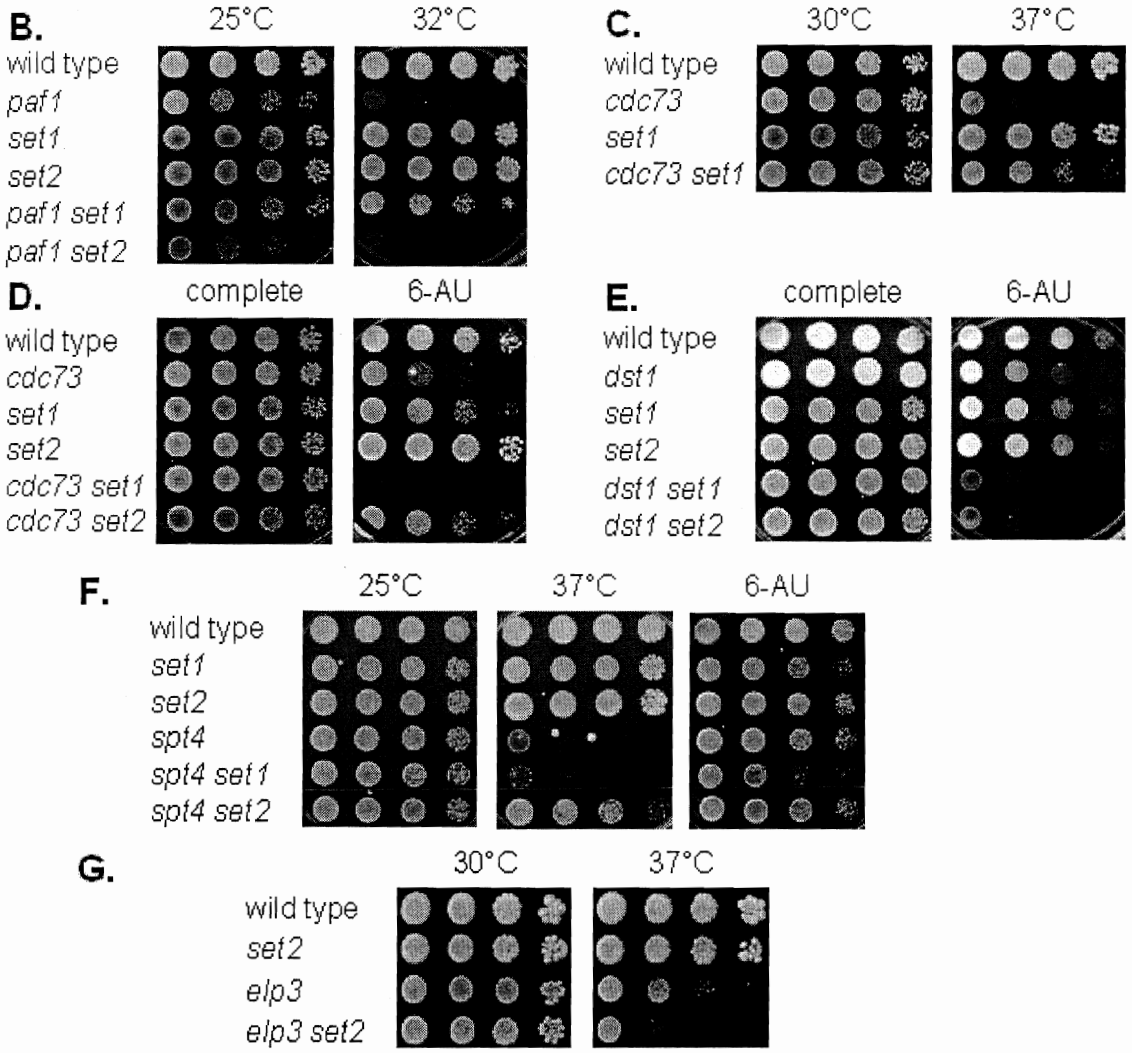


Fig 6.5. A *set2* mutation suppresses the synthetic defects between *spt16* and other transcription factor mutations.
A. Strains DY150, DY8780, DY8821, DY8155, and DY8820 were plated on complete medium at 25°C for 3 days or at 30°C for 2 days.
B. Dilutions of strains DY150, DY8780, DY8153, DY8837, and DY8833 were plated on complete medium at 30°C for 2 days or at 34°C for 4 days.
C. Strains DY8779, DY8808, DY8810, and DY7588 were plated on completed medium at the indicated temperature for 2 days.
D. Strains DY7836, DY9808, DY9805, and DY8107 were plated on complete medium at the indicated temperature for 2 days.

Figure 6.6. Genetic interactions of *set1* and *set2* with various mutations.
A. Table summarizing phenotypes of mutants.
B. A *paf1* mutant is suppressed by *set1*. Dilutions of strains DY3398 (wild type), DY7014 (*paf1*), DY8917 (*set1*), DY8919 (*set2*), DY8911 (*paf1 set1*), andDY8913 (*paf1 set2*), were plated on complete medium at 25°C for 3 days or at 32°C for 2 days.
C. A *cdc73* mutant is suppressed by *set1*. Dilutions of strains DY3398 (wild type), DY8870 (*cdc73*), DY8917 (*set1*), and DY8923 (*cdc73 set1*), were plated on complete medium at 25°C for 3 days or at 32°C for 2 days.
D. The 6-AU sensitivity of a *cdc73* mutant is oppositely affected by *set1* and *set2* mutations. Dilutions of strainsDY3398 (wild type), DY8870 (*cdc73*), DY8917 (*set1*), DY8919 (*set2*), DY8923 (*cdc73 set1*), and DY8925 (*cdc73 set2*), were plated at 30°C on complete medium for 2 days or on medium lacking uracil containing 100 ug/ml 6-azauracil for 6 days.
E. The 6-AU sensitivity of a *dst1*mutant is exacerbated by a *set1* or a *set2* mutation. Dilutions of strains DY3398 (wild type), DY8872 (*dst1*), DY8917 (*set1*), DY8919 (*set2*), DY8930 (*dst1 set1*), and DY8933 (*dst1 set2*), were plated at 25°C on complete medium for 2 days or on medium lacking uracil containing 25 ug/ml 6-azauracil for 4 days.
F. Phenotypes of a spt4 mutant are affected by a *set1* or a *set2* mutation. Dilutions of strains DY3398 (wild type), DY8917 (*set1*), DY8919 (*set2*), DY9050 (*spt4*), DY9051 (*spt4 set1*), and DY9052 (*spt4 set2*), were plated on complete medium for 2 days, at 37°C on complete medium for 3 days, or on medium lacking uracil containing 50 ug/ml 6-azauracil for 4 days.
G. An *elp3* mutation synthetic lethal with *set2*. Dilutions of strains DY150 (wild type), DY8690 (*set2*), DY8156 (*elp3*), and DY8837 (*elp3 set2*), were plated on complete medium at the indicted temperature for 4 days.

A.

	Temperature sensitivity			6-AU sensitivity		
Mutant	*SET+*	*set1*	*set2*	*SET+*	*set1*	*set2*
WT	Ts+	no change	no change	resistant	sensitive	increased resistance
paf1	Ts-	Ts+ (suppression)	no change	sensitive	no change	no change
cdc73	Ts-	Ts+ (suppression)	no change	sensitive	increased sensitivity	suppression
dst1	Ts+	no change	no change	resistant	increased sensitivity	additive
spt4	Ts-	more severe Ts-	Ts+ (suppression)	resistant	sensitive	no change
elp3	weak Ts-	no change	increased Ts-	sensitive	not determined	not determined
spt16	Ts-	more severe Ts-	Ts+ (suppression)	sensitive	no change	suppression


B.
25°C
32°C
wild type
paf1
set1
set2
paf1 set1
paf1 set2
C.
30°C
37°C
wild type
cdc73
set1
cdc73 set1
D.
complete
6-AU
wild type
cdc73
set1
set2
cdc73 set1
cdc73 set2
E.
complete
6-AU
wild type
dst1
set1
set2
dst1 set1
dst1 set2
F.
25°C
37°C
6-AU
wild type
set1
set2
spt4
spt4 set1
spt4 set2
G.
30°C
37°C
wild type
set2
elp3
elp3 set2

It has been proposed that the Isw1 chromatin remodeling has a role in transcriptional elongation, and we find a synthetic growth defect in *spt16 isw1* double mutants at 33°C (Fig 6.7B). Interestingly, a *set2* mutation suppresses this synthetic growth defect (Fig 6.7B). Thus suppression supports the idea that yFACT and Set2 have opposing roles. *spt16* mutants also cause an Spt- phenotype, altering transcription start sites from the *his4-912δ* and *lys2-128δ* alleles and conferring a His+ Lys+ phenotype. Interestingly, *spt16 set2* strains are still Spt-, and thus *set2* does not suppress this phenotype (data not shown). Similarly, the synthetic growth defect seen in a *spt16 rpd3* double mutant is not suppressed by *set2* (data not shown). However, we do see suppression of the Spt- phenotype seen in a *gcn5* mutant with the *lys2-173R2* allele (Fig 6.7A). A wild type strain with the *lys2-173R2* allele is Lys+, but the *gcn5* mutant is Lys-, an Spt- phenotype. The *gcn5 set2 lys2-173R2* strain is Lys+, showing suppression of the Spt- phenotype (Fig 6.7A). It has been proposed that the Isw1 chromatin remodeling has a role in transcriptional elongation, and we find a synthetic growth defect in *spt16 isw1* double mutants at 33°C (Fig 6.7B). Interestingly, a *set2* mutation suppresses this synthetic growth defect (Fig 6.7B). Thus suppression supports the idea that yFACT and Set2 have opposing roles.

Pob3 and Set2 regulate *GAL1* induction in opposing ways

Mason and Struhl used a *GAL1-YLR454w* reporter, with the *GAL1* promoter inserted upstream of the nonessential 8 kb *YLR454w* gene, to show that yFACT associates with open reading frames during transcription. We constructed *pob3* and *set2* strains with this *GAL1-YLR454w* allele and measured *YLR454w* mRNA levels by S1 nuclease protection assays following induction of the *GAL1* promoter. The results in Fig 6.8A show a rapid

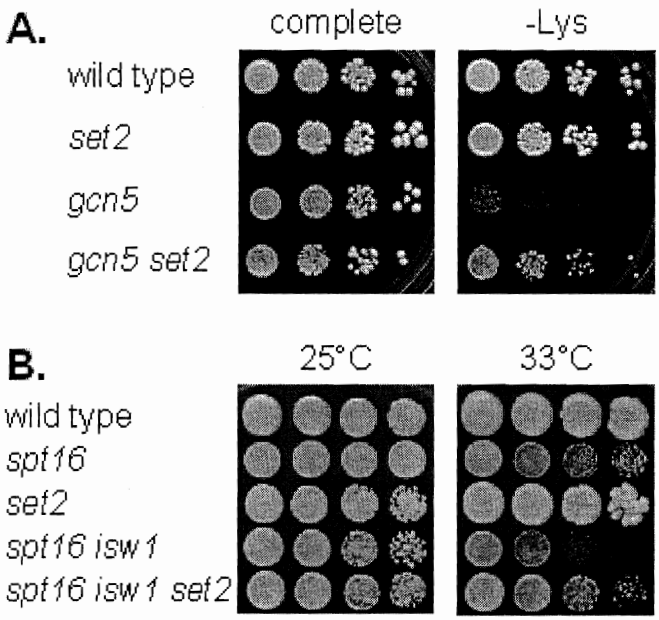


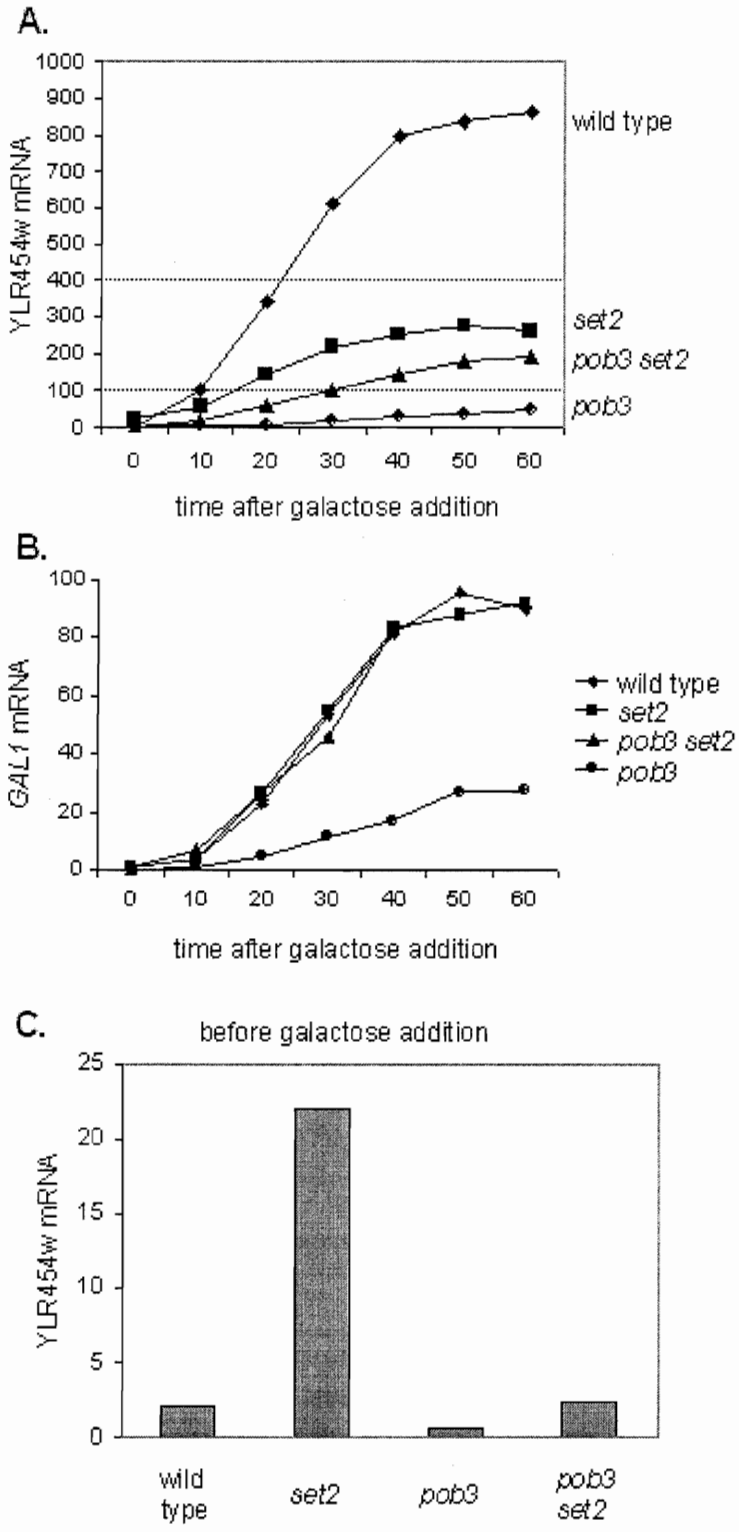
Figure 6.7. A *set2* mutation affects the Spt- phenotype of *gcn5* and suppresses the *spt16 isw1* synthetic lethality.
A. A *set2* mutation reverses the Spt- phenotype of a *gcn5* mutant. Dilutions of strains DY7467 (*his4-917∂ lys2-173R2),* DY9413 (*set2 his4-917∂ lys2-173R2*), DY6077 (*gcn5 lys2-173R2*), and DY9412 (*gcn5 set2 lys2-173R2*), were plated at 30°C on complete medium for 4 days or on medium lacking lysine for 3 days.
B. A *set2* mutation suppresses the synthetic lethality of the *spt16 isw1* double mutant. Dilutions of strains DY150 (wild type), DY8107 (*spt16*), DY8690 (*set2*), DY9022 (*spt16 isw1*), andDY9029 (*spt16 isw1 set2*), were plated on complete medium at the indicted temperature for 3 days.

increase in *YLR454w* mRNA in the wild type strain, as expected. The *pob3* mutant strain is markedly defective in inducing *YLR454w* mRNA from the *GAL1* promoter (Fig 6.8A), and a similar defect is seen in induction of the native *GAL1* gene in the *pob3* mutant (Fig 6.8B). Importantly, deletion of *SET2* significantly ameliorates the transcriptional defect at *GAL1-YLR454w* (Fig 6.8A), and completely at *GAL1* (Fig 6.8B) (compare *pob3* and *pob3 set2*). Additionally, we examined other genes and found either a *spt16* or *pob3* mutation could reduce expression, and a *set2* mutation partially suppresses these defects (as discussed later). These observations imply that Set2 and yFACT have opposing roles in regulating transcription. While a *set2* mutant caused decreased induction of *GAL1-YLR454w*, it displayed increased expression before induction (Fig 6.8C). Set2 is therefore needed both for repression of the *GAL1* promoter in this context and for normal induction of expression, underscoring the complexity of the role of Set2 in transcription. Notably, Set2 is not needed for full induction of the native *GAL1* message (Fig 6.8B). It is possible that the large size of the 8 kb *YLR454w* transcription unit places a larger demand on Set2 function than the 1.6 kb *GAL1* gene.

Defect in pol II binding to the *GAL1* promoter in *pob3* mutants

We used the *GAL1-YLR454w* reporter to assess the rate of pol II elongation in wild type and mutant strains. We took RNA samples every 10 min. following galactose induction, and used probes for S1 nuclease protection assays specific to the 5' and 3' ends of the gene. The time between appearance of mRNA sequences corresponding to the 5' and 3' ends gives an indication of how long it takes for pol II to traverse the 8 kb gene. We find that in a wild type strain RNA pol II takes about 4 min. to traverse the 8 kb long gene, consistent with previous work. Importantly, this rate is not altered by the *set2*

Fig 6.8. A *set2* mutation reverses the poor induction of *GAL1* caused by a *pob3* mutation. Strains DY9591, DY9976, DY9972, and DY9974 were grown on YP medium with 2% raffinose. Galactose was added to 2% concentration, and samples were taken at 10 min. intervals and mRNA measured by S1 nuclease protection.
A. *YLR454w* mRNA levels from the *GAL1-YLR454w* allele.
B. *GAL1* mRNA levels.
C. *YLR454w* mRNA levels before galactose induction.


A.
YLR454w mRNA
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
time after galactose addition
wild type
set2
pob3 set2
pob3
B.
GAL1 mRNA
100
80
60
40
20
0
0
10
20
30
40
50
60
time after galactose addition
wild type
set2
pob3 set2
pob3
C.
before galactose addition
YLR454w mRNA
25
20
15
10
5
0
wild type
set2
pob3
pob3 set2

mutation, the *pob3* mutation, or the *pob3 set2* double mutation (data not shown). This is a surprising result since earlier reports have suggested that yFACT has a positive role in transcriptional elongation, but the marked differences we observe in *GAL1-YLR454w* expression in *pob3* and *pob3 set2* strains do not appear to be explained by altered rates of Pol II elongation.

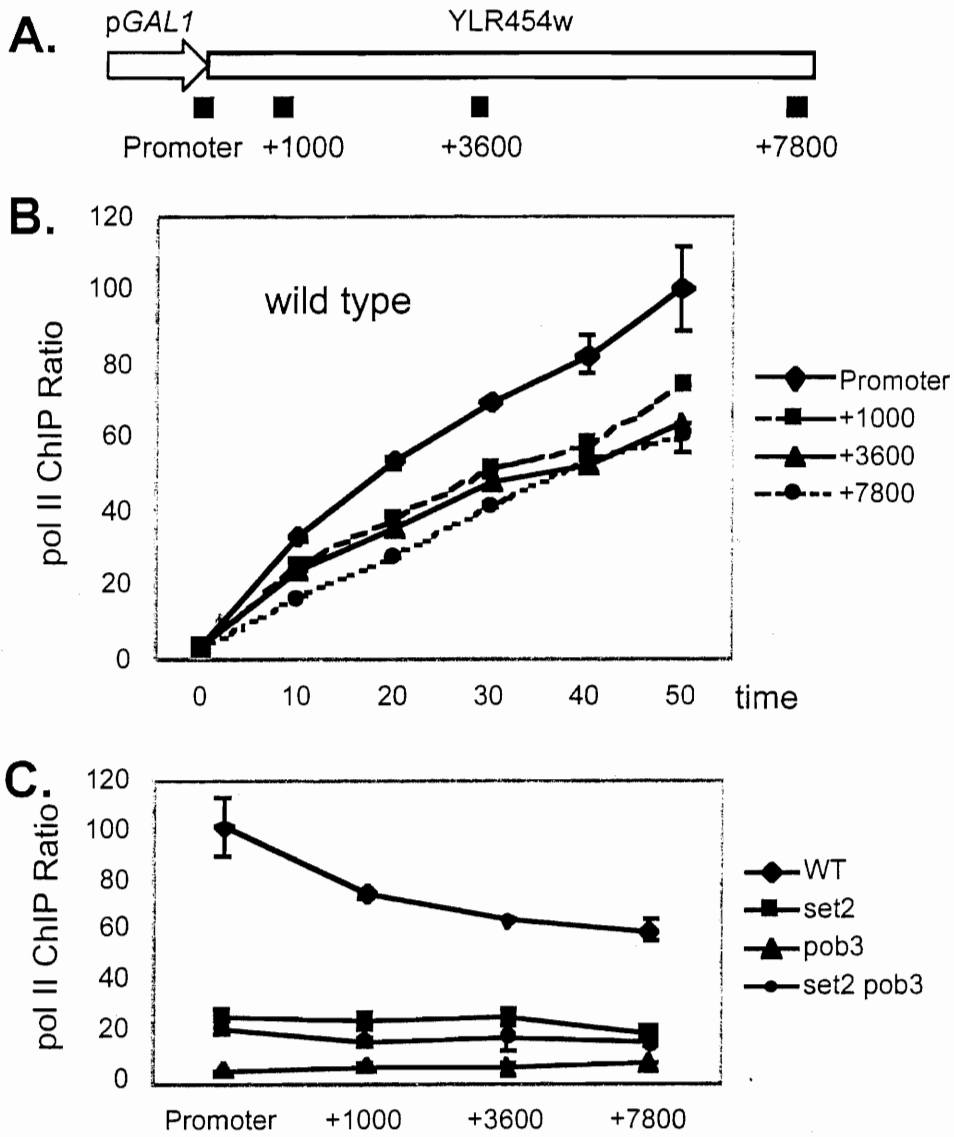
As an alternative method to measure RNA pol II elongation, we used chromatin immunoprecipitation (ChIP) assays to measure pol II levels along the *YLR454w* gene following galactose induction. Fig 6.9A shows a map of the *GAL1-YLR454w* reporter, and four regions amplified with specific primers corresponding to the *GAL1-YLR454w* promoter, 1 kb downstream of start codon, the middle of the *YLR454w* ORF (+3600), and the 3' end of the gene (+7800). At time intervals following galactose induction, samples were harvested, treated with formaldehyde to crosslink, and processed for ChIP. The results of the pol II ChIP from the wild type strain are shown in Fig 6.9B. Pol II occupancy increases throughout the gene with time of induction, as expected. The increase in Pol II displays the expected delay between upstream and downstream sites, reflecting the amount of time required to progress through the ORF. For example, occupancy reaches a 30-fold ratio after about 14 minutes at +1000 and after about 22 minutes at +7800. This is also true for a *pob3* mutant, although the overall occupancy is much lower (Fig 6.9C). This suggests that the rates of elongation are similar in these strains, as well as for the other strains (data not shown). Examination of the wild type strain shows that occupancy at the promoter is much higher than it is at any point within different strains. ChIP values were normalized to binding at t = 0. Error bars show the ORF. Plotting the Pol II occupancy at this time point alone for all four strains reveals that

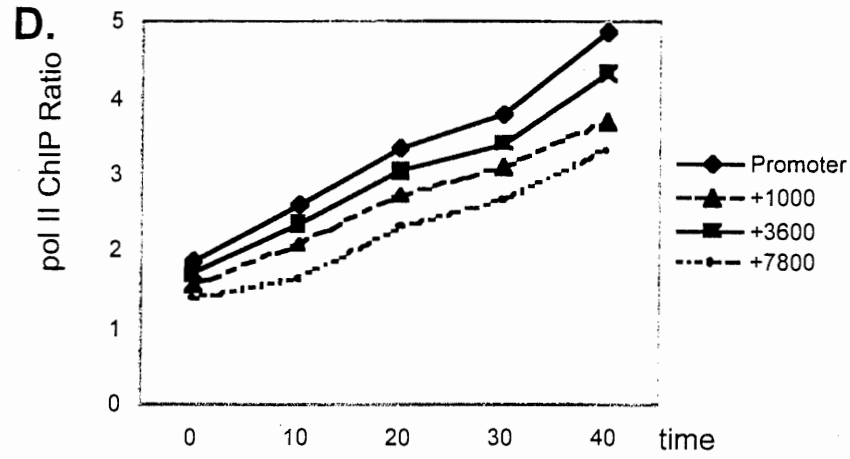
this difference is only observed with the wild type (Fig 6.9D). The greater occupancy at the promoter suggests that the transition from initiation to elongation is a slow step, even under strongly inducing conditions. However this kinetic barrier is not observed in *set2*, *pob3*, or *set2 pob3* mutants. It is possible that the barrier represents a bottleneck through which only a limited number of Pol II molecules can pass per unit of time; such a barrier would be lacking in the mutants simply because overall attempts to pass this site are lower. Alternatively, Set2 and yFACT could be involved in creating this barrier. Importantly, while a *pob3* strain has a severe defect in Pol II occupancy at the promoter, the *set2 pob3* double mutant has a less severe defect. similar to the *set2* single mutant. The same effect is seen at the native *GAL1* promoter, where the *set2* mutation does not by itself cause diminished Pol II occupancy (Fig 6.9E). Deletion of *SET2* therefore at least partially restores the ability of a *pob3* mutant to recruit Pol II to a promoter.

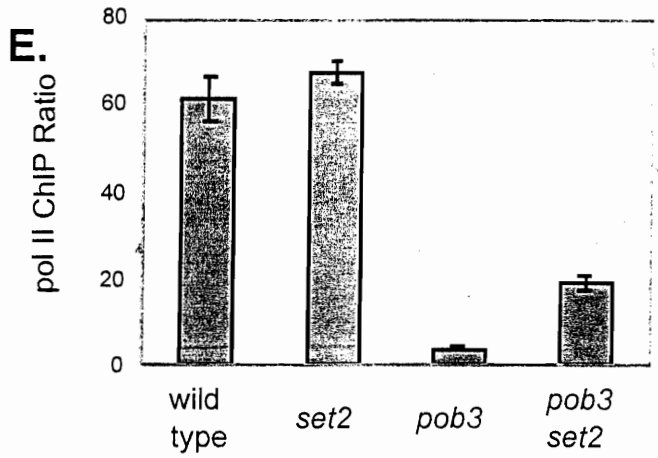
Defective TBP binding in *pob3* mutants is suppressed by *set2*

RNA polymerase II is typically recruited to promoters by the TATA-binding protein TBP. We have previously shown that yFACT can play a role in transcriptional initiation through regulation of TBP binding, and we therefore examined TBP binding to the *GAL1-YLR454w* promoter in these mutants. As shown in Fig 6.9F, TBP binding to *GAL1-YLR454w* is essentially eliminated in the *pob3* mutant but is largely restored in the *pob3 set2* double mutant strain. We also found reduced RNA expression and TBP binding at other promoters in a *pob3* mutant (Fig 6.10 A-B). An increased RNA expression and TBP binding is seen in the *pob3 set2* strain compared to the pob3 single mutant (Fig 6.10 A-B). Thus, TBP binding to the *GAL1* promoter is stimulated by yFACT, and the *pob3* defect can be suppressed by a *set2* mutation.

Fig 6.9. A *pob3* mutation reduces pol II and TBP binding, and binding is restored in a *pob3 set2* strain.
Strains DY9591, DY9976, DY9972, and DY9974 were grown on YP medium with 2% raffinose. Galactose was added to 2%, and samples were taken at 10 min. intervals and processed for ChIP analysis to measure pol II and TBP binding.
A. Map of the *GAL1-YLR454w* allele showing the positions of the PCR primers at the promoter and within the gene.
B. Kinetics of pol II binding following galactose induction at different *GAL1-YLR454w* regions in a wild type strain. Error bars show variance among replicate PCRs.
C. Distribution of pol II at 50 min. following galactose induction at different *GAL1-YLR454w* regions in four different strains. Error bars show variance among replicate PCRs.
D. Kinetics of pol II binding following galactose induction at different *GAL1-YLR454w* regions in a *pob3* strain.
E. Pol II binding to the native *GAL1* promoter at 30 min. following galactose induction in four different strains. Error bars show variance among replicate PCRs.
F. TBP binding to the *GAL1-YLR454w* promoter following galactose induction in four variance among replicate PCRs.


A.
pGAL1
YLR454w
Promoter
+1000
+3600
+7800
B.
wild type
pol II ChIP Ratio
120
100
80
60
40
20
0
0
10
20
30
40
50
time
Promoter
+1000
+3600
+7800
C.
pol II ChIP Ratio
120
100
80
60
40
20
0
Promoter
+1000
+3600
+7800
WT
set2
pob3
set2 pob3


D.
pol II ChIP Ratio
5
4
3
2
1
0
0
10
20
30
40
time
Promoter
+1000
+3600
+7800


E.
pol II ChIP Ratio
80
60
40
20
0
wild type
set2
pob3
pob3 set2

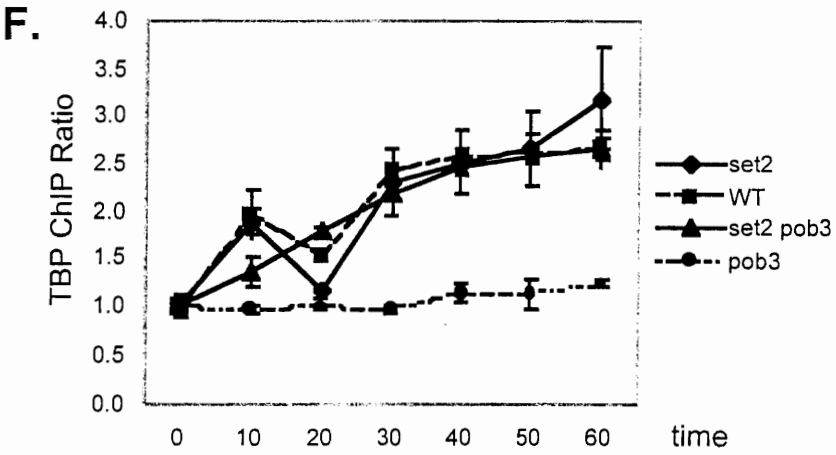
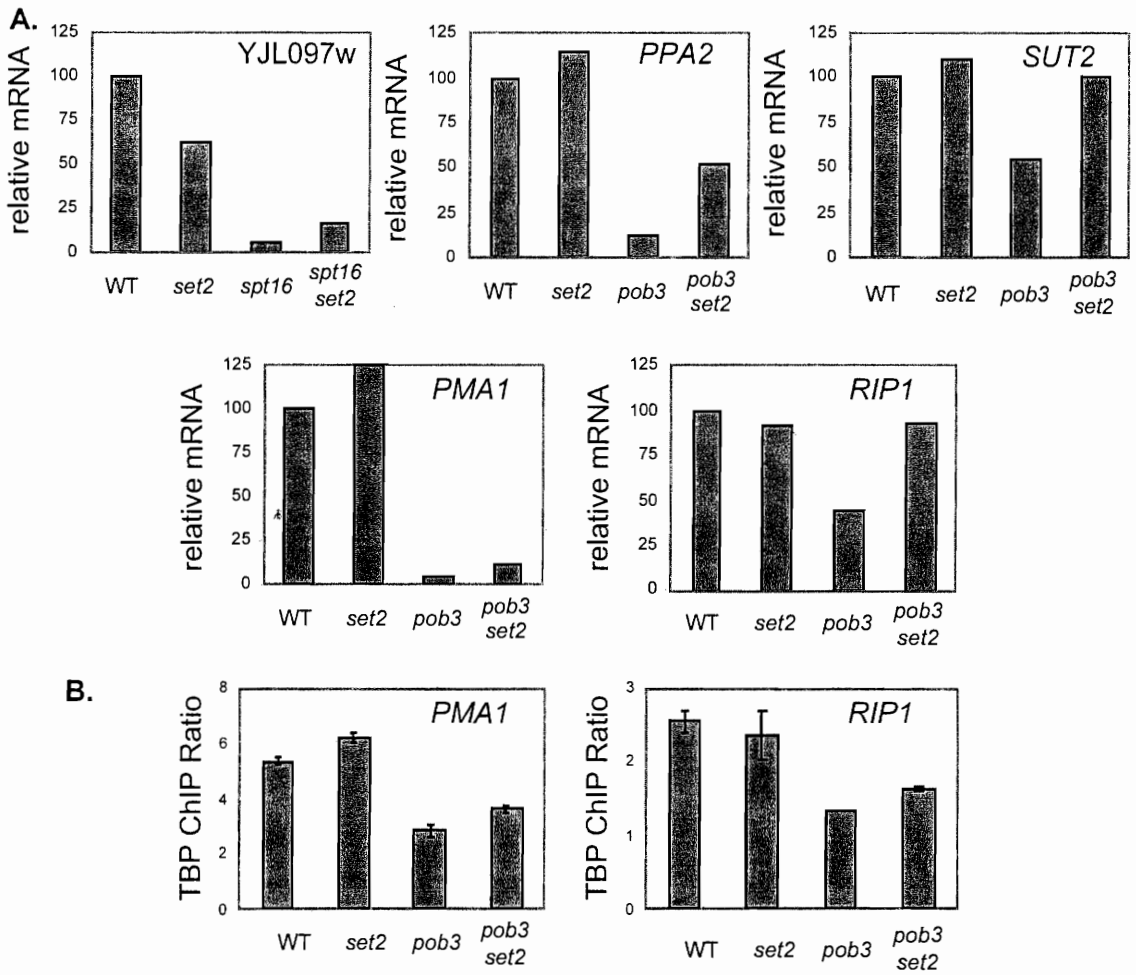

F.
TBP ChIP Ratio
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
time
set2
WT
set2 pob3
pob3

Fig 6.10. Reduced expression and TBP binding for some genes in a *pob3* mutant and suppression by *set2*.
A. RNA was isolated from strains DY150 (wild type), DY8690 (*set2*), DY8107 (*spt16*), DY8777 (*spt16 set2*), DY8881 (*pob3*), and DY8877 (*pob3 set2*), and mRNA levels for specific genes measured by S1 nuclease protection.
B. TBP binding to promoters from strains DY150 (wild type), DY8690 (*set2*), DY8881 (*pob3*), and DY8877 (*pob3 set2*), were measured by ChIP. Error bars show variance among replicate PCRs.


A.
relative mRNA
YJL097w
PPA2
SUT2
PMA1
RIP1
125
100
75
50
25
0
WT
set2
spt16
spt16 set2
pob3
pob3 set2
B.
TBP ChIP Ratio
8
6
4
2
0
3
2
1

The synthetic lethality between *spt16* and either TBP or TFIIA mutations is suppressed by *set2*

We previously demonstrated genetic interactions between *spt16* and TBP mutations. We showed that combining mutant Spt16 and mutant TBP proteins in the same cell results in lethality, using a plasmid shuffle assay to introduce mutant alleles. We used a strain with disruptions in both the *SPT15* gene (encoding TBP) and the *SPT16* gene. (To avoid confusion, we will refer to the *SPT15* gene by the protein name, TBP.) As TBP and Spt16 are both essential for viability, the strain is kept alive by a YCp-*URA3* plasmid containing wild type TBP and wild type *SPT16* genes. After transforming this strain with a YCp-*TRP1* plasmid with a TBP mutant and a YCp-*LEU2* plasmid with a Spt16 mutant, cells were plated on 5-FOA media to assess the ability of these TBP and Spt16 mutants to sustain growth in the absence of the wild type genes. We have repeated this experiment, now including a *spt15Δ spt16Δ set2* YCp-*URA3*-TBP-Spt16 strain. As shown in Fig 6.11A, certain combinations of Spt16 and TBP result in lethality in *SET2* strains, but a *set2* mutation allows these combinations of Spt16 and TBP to be viable. For example, cells with *spt16-11* and TBP(E93G) cannot lose the YCp-*URA3*-TBP-Spt16 plasmid with the wild type genes, as evidenced by the failure to grow on FOA, demonstrating the synthetic lethality. In contrast, the *set2* mutant with *spt16-11* and TBP(E93G) can grow on FOA. Thus, *set2* suppresses the synthetic lethality between *spt16* and TBP.

We also showed synthetic lethality between *spt16* and TFIIA mutations. Yeast TFIIA protein is composed of two subunits, Toa1 and Toa2. Based on the TBP-TFIIA crystal structure, Ozer et al. generated *toa2* mutants at the TBP-TFIIA interface. Although these mutations in *TOA2* eliminate TBP-TFIIA interactions with *in vitro* binding assays, the

toa2 mutants are viable, presumably because other factors present in cells facilitate TBP-TFIIA interaction and DNA-binding. For example, Toa2(W76) is required for cooperative DNA-binding with TBP *in vitro*, but a *toa2*(W76A) mutant is viable in an otherwise wild type strain. The *toa2*(W76A) allele is lethal in a *spt16* mutant (Fig 6.11B). However, *toa2*(W76A) is viable in the *spt16 set2* strain, and thus *set2* suppresses the synthetic lethality between *spt16* and TFIIA mutations. Suppression of the synthetic lethality between *spt16* and TBP or TFIIA mutants by deletion of *SET2* strongly supports our hypothesis that yFACT and Set2 have opposing roles in binding of TBP and TFIIA to promoters.

Discussion

Spt16 and Pob3, along with Nhp6, comprise the yFACT complex that can reorganize chromatin structure. *SPT16* and *POB3* are essential genes, and *spt16* and *pob3* point mutants have been isolated with phenotypes including temperature sensitive growth, sensitivity to 6-AU, and synthetic lethality with certain transcription factor mutations. We find that a *set2* deletion, eliminating the enzyme that methylates lysine 36 on histone H3, suppresses all of these phenotypes. Additionally, a histone H3 mutation, replacing the lysine at position 36 with either an alanine or an arginine residue, also suppresses *spt16* and *pob3* mutations. We conclude that methylation on histone H3 at K36 by Set2 acts in opposition to the chromatin changes facilitated by yFACT.

Both Set2 and yFACT have been implicated in regulating transcriptional elongation. Both Set2 and yFACT localize preferentially to transcribed regions compared to promoters, Set2 associates with hyperphosphorylated pol II, and several studies have shown greater K36 methylation at open reading frames compared to promoters.

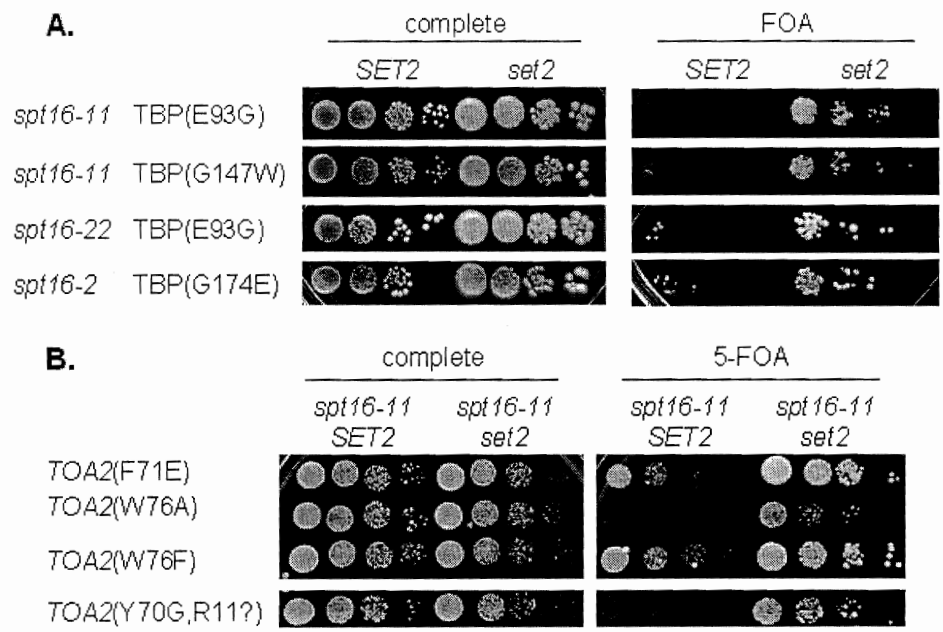


Fig 6.11. A *set2* mutation suppresses the synthetic lethality of an *spt16* mutation with either TBP or TFIIA mutations.
A. Strains DY8552 (indicated as "*SET2*") and DY10065 (indicated as "*set2*") were transformed with two plasmids, a YCp-*TRP1* plasmid encoding a TBP mutant and a YCp-*LEU2* plasmid with either wild type *SPT16* or *spt16* mutations, and dilutions were plated on complete or FOA medium at 33°C for three days.
B. Strains DY8700 (indicated as "*SET2*") and DY10212 (indicated as "*set2*") were transformed with a YCp-*LEU2* plasmid with the indicated *toa2* mutant, and dilutions were plated on complete medium at 25°C for 2 days and on FOA medium at 30°C for 4 days, except the *TOA2*(Y10G,R11Δ) strains were incubated on FOA medium at 33°C.

Additionally, both *spt16* and *set2* mutations show genetic interactions with known elongation factors. We looked for defects in transcriptional elongation in a *pob3* mutant, using a strain with the *GAL1* promoter inserted upstream of the nonessential *YLR454w* gene. We measured polymerase progression down the 8 kb *YLR454w* gene following galactose induction, but we found no evidence for a transcriptional elongation defect in *pob3* mutants *in vivo*. We did find reduced expression of both *GAL1* and *GAL1-YLR454w* in the *pob3* mutant. Importantly, expression was restored in the *pob3 set2* double mutant.

ChIP experiments indicate that yFACT and Set2 act at the *GAL1* promoter at the level of transcriptional initiation. Following galactose induction, there is reduced pol II binding at the *GAL1* promoter in the *pob3* mutant, and pol II binding is increased in the *pob3 set2* strain. We also examined TBP binding at the *GAL1* promoter, and again in a *pob3* mutant TBP binding is markedly reduced, and increased in the *pob3 set2* strain. These experiments suggest that yFACT facilitates TBP binding, at least at some promoters, and Set2 opposes this effect. How does Set2 inhibit TBP binding at *GAL1*? It is possible that histone methylation by Set2 inhibits binding of a transcriptional co-activator complex that stimulates TBP binding. The SAGA complex is recruited to *GAL1* and promotes TBP binding. We have analyzed SAGA binding at the *GAL1 UAS* by ChIP, but we did not observe any significant difference in SAGA binding in strains differing at the *POB3* and *SET2* loci (data not shown). Chromodomains bind to methylated histone residue, and it is possible that methylated K36 residues at the *GAL1* promoter encourage binding of a chromodomain containing transcription factor that regulates TBP binding.

Our results strongly support a role for K36 methylation by Set2 in decreasing TBP binding to promoters. There are two earlier studies that support a repressive role for Set2

at promoters. A Set2-LexA fusion protein strongly represses transcription when tethered to a promoter with a lexA binding site. This repression by the Set2-LexA fusion protein was reduced by point mutations in the Set2 catalytic domain. In a separate study, the weak expression from a *GAL4* promoter lacking its UAS element could be suppressed by either a *set2* mutation or a K36R substitution in histone H3. This suggests that Set2 can repress transcription from promoters.

Set2 and yFACT are believed to function in transcriptional elongation, but our results strongly suggest that both Set2 and yFACT function at initiation of transcription by regulating DNA-binding by TBP. We previously demonstrated that *spt16* mutants are synthetically lethal with point mutations in either TBP or TFIIA. Importantly, a *set2* mutation can suppress this synthetic lethal interaction. Additionally, *in vitro* studies show that yFACT can facilitate binding of TBP and TFIIA to a nucleosomal TATA site that is normally refractory to binding. Biochemical studies have shown that the TFIIS factor (encoded by *DST1* in yeast) facilitates elongation by pol II. However, it was recently shown that TFIIS binds to the *GAL1* promoter. Additionally, a *dst1* mutation affected the kinetics of *GAL1* induction, and reduced the association of both TBP and pol II to the *GAL1* promoter, and a further decrease in promoter occupancy by basal factors is seen when a *dst1* mutant is treated with 6-AU. It has been suggested that a decrease in elongation slows promoter clearance, leading to destabilization of the preinitiation complex. It is intriguing that TFIIS, Set2 and yFACT, all proposed as elongation factors, regulate binding of TBP at *GAL1*.

Although a *set2* mutation suppresses defects caused by yFACT mutations, we find that combining a *set1* mutation with either *spt16* or *pob3* results in synthetic defects. Set1

and Set2 methylate histone H3 at different residues, K4 and K36, respectively. A histone H3 K4 substitution enhances yFACT defects, while a substitution at K36 suppresses these defects. We examined the epistasis relationships by constructing a *spt16 set1 set2* triple mutant, and by testing a histone H3(K4R, K36R) double mutant in *spt16* and *pob3* strains. The results show that the absence of K36 methylation is not sufficient to suppress the yFACT mutants. It is the combination of methylated K4 and unmethylated K36 at histone H3 that suppresses the yFACT defects.

How do the presence or absence of methylated lysine residues on histone H3 have such marked effects on growth of cells with a partially defective yFACT complex? Chromodomain containing proteins can bind to methylated lysine residues. One group has reported that yeast Chd1 binds to methylated K4, while another group finds that human, but not yeast Chd1, is capable of binding to methylated K4. It is possible that in the *spt16* H3(K4)R mutant, the lack of binding of a chromodomain protein, possibly Chd1, is toxic in the presence of the defective yFACT complex. The Eaf3 chromodomain protein has been shown to bind methylated H3 K36. Eaf3 is present in two complexes, NuA4 and Rpd3S, and thus it is possible that the absence of one of these complexes and their associated enzymatic activities suppresses the growth defects of the *spt16* and *pob3* mutant strains. It has also been shown that K36 methylation by Set2 recruits the Rpd3S histone deacetylase complex to the 3' portions of coding regions. It is possible that in *set2* or H3(K36R) mutants there is a redistribution of Rpd3S from coding regions to promoters, and thus the effect of *set2* on TBP binding could be indirect. Further work will be needed to decipher the molecular mechanisms of how the loss of H3 K36 methylation suppresses yFACT mutants.

Materials and methods

Yeast strains are listed in Table 6.1 Cells were grown in YPD medium at 30°C, except where other temperatures are noted, or in synthetic complete medium with 2% glucose and supplemented with adenine, uracil and amino acids, as appropriate, to select for plasmids. For the galactose induction experiments cells were grown at 25˚C in YP medium supplemented with 2% raffinose to mid log, shifted to 30˚C for growth for 2 hours, and then galactose was added to a final concentration of 2%. Plasmids are listed in Table 6.2.

RNA levels were determined with S1 nuclease protection assays as described using probes listed in Table 6.3. Chromatin immunoprecipitations were performed as described using the 8WG16 monoclonal antibody against the pol II C-terminal repeat, and a polyclonal anti-TBP sera generously provided by Tony Weil. Real Time PCR and calculations were performed as described, using of the ORF free chromosome I region as the internal control.

Table 6.1. Strain list for yFACT and Set2 study

DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY3398	*MAT***a** *ade2 can1 his3 leu2 trp1*
DY7014	*MAT***a** *paf1::URA3 ade2 can1 his3 leu2 trp1 ura3*
DY7142	*MAT*α *nhp6a::KanMX nhp6b::ADE2 hht1-hhf1::LEU2 hht2-hhf2::HIS3 + YCp-URA3(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7588	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7803	*MAT***a** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-URA3(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7809	*MAT***a** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-URA3(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7818	*MAT***a** *pob3(L78R) hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-URA3(HHT2-HHF2) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY7836	*MAT*α *htz1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8107	*MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8153	*MAT*α *spt16-11 elp3::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8155	*MAT*α *spt16-11 gcn5::HIS3 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8156	*MAT*α *elp3::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8552	*MAT***a** *spt15::ADE2 spt16::HIS3 + YCp-URA3(SPT15, SPT16) ade2 can1 his3 leu2 trp1 ura3*
DY8690	*MAT***a** *set2::KanMX ade2 can1 his3 leu2 trp1 ura3*
DY8700	*MAT***a** *spt16-11 toa2::His3MX + YCp-URA3(TOA2) ade2 can1 his3 leu2 met15 trp1 ura3*

Table 6.1. Cont.

DY8777	*MAT***a** *spt16-11 set2::KanMX ade2 can1 his3 leu2 ura3*
DY8779	*MAT*a *spt16-11 set2::KanMX ade2 can1 his3 leu2 trp1 ura3*
DY8780	*MAT***a** *set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY8787	*MAT*α *spt16-11 ade2 can1 his3 leu2 met15 ura3*
DY8788	*MAT*α *spt16-11 ade2 can1 his3 leu2 met15*
DY8789	*MAT***a** *set2::KanMX ade2 can1 his3 leu2 trp1*
DY8790	*MAT***a** *spt16-11 set2::KanMX ade2 can1 his3 leu2*
DY8808	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 spt16-11 ade2 can1 his3 leu2 trp1 ura3*
DY8810	*MAT*α *nhp6a::URA3 nhp6b::ADE2 spt16-11 set2::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8820	*MAT*α *spt16-11 gcn5::HIS3 set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY8821	*MAT***a** *gcn5::HIS3 set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY8833	*MAT*α *spt16-11 elp3::LEU2 set2::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8837	*MAT*α *elp3::LEU2 set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY8862	*MAT***a** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3* + *YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8864	*MAT***a** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3* + YCp-TRP(HHT2[K*36A*]-*HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*

Table 6.1. Cont.

DY8865	***MATa*** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8867	***MATa*** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K36A]-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8870	***MATa*** *cdc73::HIS3 ade2 can1 his3 leu2 trp1*
DY8872	***MATa*** *dst1::HIS3 ade2 can1 his3 leu2 trp1*
DY8875	***MATa*** *set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8877	***MATa*** *pob3(L78R) set2::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8878	***MATa*** *pob3(L78R) set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY8881	***MATa*** *pob3(L78R) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8883	***MATa*** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1*
DY8884	***MATa*** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K4R]-HHF2) ade2 can1 his3 leu2 lys2 trp1*
DY8885	***MATa*** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K36A]-HHF2) ade2 can1 his3 leu2 lys2 trp1*
DY8886	***MATa*** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1*
DY8887	***MATa*** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K4R]-HHF2) ade2 can1 his3 leu2 lys2 trp1*

Table 6.1. Cont.

DY8888	*MAT***a** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2 [K36A]-HHF2) ade2 can1 his3 leu2 lys2 trp1*
DY8911	*MAT*α *paf1::URA3 set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8913	*MAT***a** *paf1::URA3 set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8917	*MAT***a** *set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY8919	*MAT*α *set2::ADE2 ade2 can1 his3 leu2 lys2 trp1*
DY8923	*MAT***a** *cdc73::HIS3 set1::TRP1 ade2 can1 his3 leu2 met15 trp1*
DY8925	*MAT*α *cdc73::HIS3 set2::ADE2 ade2 can1 his3 leu2 lys2 trp1*
DY8930	*MAT***a** *dst1::HIS3 set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY8933	*MAT***a** *dst1::HIS3 set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9022	*MAT***a** *spt16-11 isw1::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9029	*MAT*α *spt16-11 isw1::ADE2 set2::KanMX ade2 can1 his3 leu2 trp1 ura3*
DY9050	*MAT*α *spt4::HIS3 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9051	*MAT***a** *spt4::HIS3 set1::TRP1 ade2 can1 his3 leu2 lys2 trp1*
DY9052	*MAT*α *spt4::HIS3 set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9178	*MAT***a** *spt16-11 set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9180	*MAT***a** *spt16-11 set1::TRP1 set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9206	*MAT***a** *spt16-11 set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9591	*MAT***a** *HIS3::GAL1::YLR454w ade2 can1 his3 leu2 met15 trp1 ura3*
DY9805	*MAT*α *spt16-11 htz1::KanMX set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

Table 6.1. Cont.

DY9808	*MATα spt16-11 htz1::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9972	*MAT***a** *pob3(L78R) HIS3::GAL1::YLR454w ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9974	*MAT***a** *pob3(L78R) set2::KanMX HIS3::GAL1::YLR454w ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9976	*MAT***a** *set2::KanMX HIS3::GAL1::YLR454w ade2 can1 his3 leu2 met15 trp1 ura3*
DY10065	*MAT***a** *spt15::ADE2 spt16::HIS3 set2::KanMX + YCp-URA3(SPT15, SPT16) ade2 can1 his3 leu2 met15 trp1 ura3*
DY10212	*MAT***a** *spt16-11 toa2::His3MX + YCp-URA3(TOA2) set2::KanMX ade2 can1 his3 leu2 met15 trp1 ura3*
DY10468	*MAT***a** *pob3(L78R) hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY10469	*MAT***a** *pob3(L78R) hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K36A]-HHF2) ade2 can1 his3 leu2 lys2 met15 trp1*
FY167	*MATα his4-917∂ lys2-173R2 leu2 trp1 ura3*
FY1292	*MATα gcn5::HIS3 lys2-173R2 arg4 his3 leu2 trp1 ura3*
DY9412	*MATα gcn5::HIS3 lys2-173R2 set2::TRP1 arg4 his3 leu2 trp1 ura3*
DY9413	*MATα his4-917∂ lys2-173R2 set2::TRP1 leu2 trp1 ura3*

Table 6.2. Plasmid list for yFACT and Set2 study

Plasmid	Description	Source
pRS314	YCp-*TRP1* Vector	(Sikorski and Heiter 1989)
M4471	TBP(E93G) in YCp-*TRP1* plasmid	(Eriksson et.al. 2004)
M4475	TBP(G147W) in YCp-*TRP1* plasmid	(Eriksson et.al. 2004)
pDE58-1	TBP(G174E) in YCp-*TRP1* plasmid	(Eriksson et.al. 2004)
M4599	*toa2*(F71E) in YCp-*LEU2* plasmid	(Ozer et.al. 1998)
M4601	*toa2*(W76A) in YCp-*LEU2* plasmid	(Ozer et.al. 1998)
M4603	*toa2*(W76F) in YCp-*LEU2* plasmid	(Ozer et.al. 1998)
M4606	*toa2*(Y10G,R11Δ) in YCp -*LEU2* plasmid	(Biswas et.al. 2004)
pTF128-02	*spt16-2*(G132D) in YCp-*LEU2* plasmid	(Formosa et.al. 2001)
pTF128-11	*spt16-11*(T828I, P859S) in YCp-*LEU2* plasmid	(Formosa et.al. 2001)
pTF128-22	*spt16-11*(A417V) in YCp-*LEU2* plasmid	(Formosa et.al. 2001)
M4817	Histone H3 (wild type) and histone H4 (wild type in YCp-*TRP1*	this work
M4818	Histone H3 (K4R) and histone H4 (wild type in YCp-*TRP1*)	this work
M4819	Histone H3 (K23R) and histone H4 (wild type in YCp-*TRP1*)	this work
M4821	Histone H3 (K36R) and histone H4 (wild type in YCp-*TRP1*)	this work

Table 6.2. Cont.

Plasmid	Description	Source
M4822	Histone H3 (K36A) and histone H4 (wild type in YCp-*TRP1*)	this work
M4823	Histone H3 (K79R) and histone H4 (wild type in YCp-*TRP1*)	this work
M4821	Histone H3 (K4R,K36R) and histone H4 (wild type in YCp-*TRP1*)	this work

Table 6.3. Oligonucelotide list for yFACT and Set2 study

F764	*GAL1*	CCTTTGCGCTAGAATTGAACTCAGGTACAATCACTTCT TCTGAATGAGATTTAGTCATGCGCGCGC
F878	tRNA control	GGAATTTCCAAGATTTAATTGGAGTCGAAAGCTCGCCT TA
F1310	*PMA1*	CAAACCACAGATAACACCGAAATCGACCCAATCGGACA AACCGGCAGCCAGCGCGA
F1324	*PPA2*	CCCATGTTTGGGGAATAGCACCATAGTTGTGTATATAG CCGTGATAGGGCCCGGC
F1325	*SUT2*	CGGTTGTTTCGCCGGAAGAAACAGTTTTATAGTATTCAG TGTACTCTGTTGGGCAACCCCACTT
F1431	*YLR454w*	GGATTCATAATTTCACCAATTAGTAAGTTTGTATTCACT ATAAAAATGGCATCAGCAATGATGGTCTGGCCCTGTTG
F1463	*RIP1*	GAACACAACCTAAGTGAGTACAAATACCCAGCATAATT AACCATTGAGGGAGAGGA
F1464	*YJL097W*	GTATCTGACGATCTCAGTTATAGACCATGCCAGTAATAA TGATATGTAAACAACTGATTGGGAGGT
F1363	*GAL1*	GGTAATTAATCAGCGAAGCGATGATTT
F1364	*GAL1*	TGCGCTAGAATTGAACTCAGGTAC
F1539	*PMA1*	CTTTCTTTCCTATAACACCAATAGTG
F1540	*PMA1*	ATGAAGAGGATGATGTATCAGTCATA
F1457	*RIP1*	ACTCCTGAACGGGTATCG
F1548	*RIP1*	CCTTCAACCATTTGTCGA

Table 6.3. Cont.

F1593	-192 to +168	GGGGTAATTAATCAGCGAAGCGATG
F1594	-192 to +168	CCTGACGGTACCATCTTCTAAGGATAAAC
F1410	+ 945 to +1147	CAATACCAACAGGTTCAGAAATGAGATGC
F1411	+ 945 to +1147	GAGAGAACAAATTGGTTTCGCCAAATATCG
F1232	+ 3540 to +3705	CTCCAACGCAGCCAAACTTT
F1233	+ 3540 to +3705	CTCGAAATGATGCGGTATGG
F1412	+ 7701 to +7850	GAGGGTCACAGATCTATTACTTGCCC
F1413	+ 7701 to +7850	GTTGTGAGTTGCTTCAGTGGTGAAGT

References

Adhvaryu, K.K., Morris, S.A., Strahl, B.D. and Selker, E.U. (2005) Methylation of histone H3 lysine 36 is required for normal development in Neurospora crassa. *Eukaryot Cell*, **4**, 1455-1464.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.E., Seidman, J.G., Smith, J.A. and Struhl, K. (1987) *Current Protocols in Molecular Biology*. Wiley and Sons, New York.

Bhoite, L.T. and Stillman, D.J. (1998) Residues in the Swi5 zinc finger protein that mediate cooperative DNA-binding with the Pho2 homeodomain protein. *Mol Cell Biol*, **18**, 6436-6446.

Biswas, D., Imbalzano, A.N., Eriksson, P., Yu, Y. and Stillman, D.J. (2004) Role for Nhp6, Gcn5, and the Swi/Snf Complex in Stimulating Formation of the TATA-Binding Protein-TFIIA-DNA Complex. *Mol Cell Biol*, **24**, 8312-8321.

Biswas, D., Yu, Y., Prall, M., Formosa, T. and Stillman, D.J. (2005) The Yeast FACT Complex Has a Role in Transcriptional Initiation. *Mol Cell Biol*, **25**, 5812-5822.

Brewster, N.K., Johnston, G.C. and Singer, R.A. (2001) A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. *Mol Cell Biol*, **21**, 3491-3502.

Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y., Winston, F. and Allis, C.D. (2001) Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes Dev*, **15**, 3286-3295.

Cairns, B.R. (2005) Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr Opin Genet Dev*, **15**, 185-190.

Carrozza, M.J., Li, B., Florens, L., Suganuma, T., Swanson, S.K., Lee, K.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P. and Workman, J.L. (2005) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell*, **123**, 581-592.

Dryhurst, D., Thambirajah, A.A. and Ausio, J. (2004) New twists on H2A.Z: a histone variant with a controversial structural and functional past. *Biochem Cell Biol*, **82**, 490-497.

Dudley, A.M., Rougeulle, C. and Winston, F. (1999) The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. *Genes Dev*, **13**, 2940-2945.

Eriksson, P., Biswas, D., Yu, Y., Stewart, J.M. and Stillman, D.J. (2004) TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. *Mol Cell Biol*, **24**, 6419-6429.

Exinger, F. and Lacroute, F. (1992) 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. *Curr Genet*, **22**, 9-11.

Formosa, T. (2003) Changing the DNA landscape: putting a SPN on chromatin. *Curr Top Microbiol Immunol*, **274**, 171-201.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y. and Stillman, D.J. (2001) Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome- binding factor SPN. *Embo Journal*, **20**, 3506-3517.

Formosa, T., Ruone, S., Adams, M.D., Olsen, A.E., Eriksson, P., Yu, Y., Rhoades, A.R., Kaufman, P.D. and Stillman, D.J. (2002) Defects in SPT16 or POB3 (yFACT) in *Saccharomyces cerevisiae* Cause Dependence on the Hir/Hpc Pathway. Polymerase passage may degrade chromatin structure. *Genetics*, **162**, 1557-1571.

Fragiadakis, G.S., Tzamarias, D. and Alexandraki, D. (2004) Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for *FRE2* transcriptional activation. *Embo Journal*, **23**, 333-342.

Gambus, A., Jones, R.C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R.D. and Labib, K. (2006) GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat Cell Biol.*

Joshi, A.A. and Struhl, K. (2005) Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. *Mol Cell*, **20**, 971-978.

Kassavetis, G.A. and Steiner, D.F. (2006) NHP6 is a transcriptional initiation fidelity factor for RNA polymerase III transcription in vitro and in vivo. *J Biol Chem.*

Keogh, M.C., Kurdistani, S.K., Morris, S.A., Ahn, S.H., Podolny, V., Collins, S.R., Schuldiner, M., Chin, K., Punna, T., Thompson, N.J., Boone, C., Emili, A., Weissman, J.S., Hughes, T.R., Strahl, B.D., Grunstein, M., Greenblatt, J.F., Buratowski, S. and Krogan, N.J. (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell*, **123**, 593-605.

Kizer, K.O., Phatnani, H.P., Shibata, Y., Hall, H., Greenleaf, A.L. and Strahl, B.D. (2005) A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K36 methylation with transcript elongation. *Mol Cell Biol*, **25**, 3305-3316.

Krogan, N.J., Kim, M., Ahn, S.H., Zhong, G., Kobor, M.S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S. and Greenblatt, J.F. (2002) RNA polymerase II elongation

factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. *Mol Cell Biol*, **22**, 6979-6992.

Krogan, N.J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D.P., Beattie, B.K., Emili, A., Boone, C., Shilatifard, A., Buratowski, S. and Greenblatt, J. (2003) Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol*, **23**, 4207-4218.

Landry, J., Sutton, A., Hesman, T., Min, J., Xu, R.M., Johnston, M. and Sternglanz, R. (2003) Set2-catalyzed methylation of histone H3 represses basal expression of GAL4 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **23**, 5972-5978.

Li, B., Howe, L., Anderson, S., Yates, J.R., 3rd and Workman, J.L. (2003) The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J Biol Chem*, **278**, 8897-8903.

Li, B., Pattenden, S.G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J. and Workman, J.L. (2005) Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc Natl Acad Sci U S A*, **102**, 18385-18390.

Malone, E.A., Clark, C.D., Chiang, A. and Winston, F. (1991) Mutations in *SPT68/CDC68* suppress *cis*- and *trans*-acting mutations that affect promoter function in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **11**, 5710-5717.

Martin, C. and Zhang, Y. (2005) The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol*, **6**, 838-849.

Mason, P.B. and Struhl, K. (2003) The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol*, **23**, 8323-8333.

Mason, P.B. and Struhl, K. (2005) Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. *Mol Cell*, **17**, 831-840.

Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N. and Mellor, J. (2003) Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell*, **115**, 425-435.

Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2003) Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell*, **11**, 709-719.

Orphanides, G., LeRoy, G., Chang, C.H., Luse, D.S. and Reinberg, D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell*, **92**, 105-116.

Orphanides, G., Wu, W.H., Lane, W.S., Hampsey, M. and Reinberg, D. (1999) The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature*, **400**, 284-288.

Ozer, J., Lezina, L.E., Ewing, J., Audi, S. and Lieberman, P.M. (1998) Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **18**, 2559-2570.

Peterson, C.L. and Laniel, M.A. (2004) Histones and histone modifications. *Curr Biol*, **14**, R546-551.

Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E., Zeitlinger, J., Lewitter, F., Gifford, D.K. and Young, R.A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell*, **122**, 517-527.

Prather, D.M., Larschan, E. and Winston, F. (2005) Evidence that the elongation factor TFIIS plays a role in transcription initiation at GAL1 in *Saccharomyces cerevisiae*. *Mol Cell Biol*, **25**, 2650-2659.

Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, **433**, 434-438.

Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J. and Madhani, H.D. (2005) Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell*, **123**, 233-248.

Rao, B., Shibata, Y., Strahl, B.D. and Lieb, J.D. (2005) Dimethylation of histone H3 at lysine 36 demarcates regulatory and nonregulatory chromatin genome-wide. *Mol Cell Biol*, **25**, 9447-9459.

Saunders, A., Werner, J., Andrulis, E.D., Nakayama, T., Hirose, S., Reinberg, D. and Lis, J.T. (2003) Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. *Science*, **301**, 1094-1096.

Schlesinger, M.B. and Formosa, T. (2000) POB3 is required for both transcription and replication in the yeast *Saccharomyces cerevisiae*. *Genetics*, **155**, 1593-1606.

Schroeder, S.C., Schwer, B., Shuman, S. and Bentley, D. (2000) Dynamic association of capping enzymes with transcribing RNA polymerase II. *Genes Dev*, **14**, 2435-2440.

Shaw, R.J. and Reines, D. (2000) *Saccharomyces cerevisiae* transcription elongation mutants are defective in PUR5 induction in response to nucleotide depletion. *Mol Cell Biol*, **20**, 7427-7437.

Sherman, F. (1991) Getting started with yeast. *Meth. Enzymol.*, **194**, 1-21.

Shimojima, T., Okada, M., Nakayama, T., Ueda, H., Okawa, K., Iwamatsu, A., Handa, H. and Hirose, S. (2003) Drosophila FACT contributes to Hox gene expression through physical and functional interactions with GAGA factor. *Genes Dev*, **17**, 1605-1616.

Simic, R., Lindstrom, D.L., Tran, H.G., Roinick, K.L., Costa, P.J., Johnson, A.D., Hartzog, G.A. and Arndt, K.M. (2003) Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo Journal*, **22**, 1846-1856.

Sims, R.J., 3rd, Chen, C.F., Santos-Rosa, H., Kouzarides, T., Patel, S.S. and Reinberg, D. (2005) Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *J Biol Chem*, **280**, 41789-41792.

Squazzo, S.L., Costa, P.J., Lindstrom, D.L., Kumer, K.E., Simic, R., Jennings, J.L., Link, A.J., Arndt, K.M. and Hartzog, G.A. (2002) The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. *Embo Journal*, **21**, 1764-1774.

Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41-45.

Strahl, B.D., Grant, P.A., Briggs, S.D., Sun, Z.W., Bone, J.R., Caldwell, J.A., Mollah, S., Cook, R.G., Shabanowitz, J., Hunt, D.F. and Allis, C.D. (2002) Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. *Mol Cell Biol*, **22**, 1298-1306.

Szerlong, H., Saha, A. and Cairns, B.R. (2003) The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *Embo Journal*, **22**, 3175-3187.

VanDemark, A.P., Blanksma, M., Ferris, E., Heroux, A., Hill, C.P. and Formosa, T. (2006) The structure of the yFACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. *Mol Cell*, **In Press**.

Wittmeyer, J., Joss, L. and Formosa, T. (1999) Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. *Biochemistry*, **38**, 8961-8971.

Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H. and Strahl, B.D. (2003) Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes Dev*, **17**, 654-663.

Zhang, H., Roberts, D.N. and Cairns, B.R. (2005) Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell*, **123**, 219-231.

CHAPTER 7

A NEGATIVE ROLE FOR Chd1 IN REGULATING yFACT MEDIATED TBP BINDING AT PROMOTER

Abstract

ATP-dependent chromatin remodelers utilize the energy of ATP to either remodel the chromatin structure or assemble chromatin in a regularly spaced structure. The yeast Chd1 is a *SNF2*-like DEAD/H ATPase that shows ATP-dependent chromatin remodeling and chromatin assembly activity *in vitro*. The genetic and biochemical evidence suggests that Chd1 may have a role in regulating the transcriptional elongation step. We present evidence that shows that Chd1 acts as a negative regulator during the initiation step of transcription. Our evidence shows that Chd1 has a negative role in yFACT mediated TBP binding at the promoter region. The *SPT16* and *POB3* genes encode the Spt16 and Pob3 components of the yFACT complex. Deletion of *CHD1* suppresses the temperature sensitive phenotype of *spt16-11* and *pob3*(L78R) mutant strains. Suppression of the 6-AU sensitivity phenotype of *spt16-11* strain by *chd1*Δ suggests that Chd1 has a negative role in transcriptional regulation by yFACT. Deletion of *CHD1* also suppresses synthetic lethalities between *spt16-11* and mutations in other transcription factors that have a role in either the initiation or elongation steps of transcription. A galactose induction experiment shows that a *chd1*Δ restores the defect in transcriptional induction from the *GAL1* promoter in a *pob3*(L78R) strain. ChIP analysis shows that a *pob3*(L78R) strain is defective in TBP binding during galactose induction. Deletion of *CHD1* restores normal TBP binding defect in the *pob3*(L78R) strain. Increased TBP binding results in an increase in RNA polymerase II recruitment at the promoter region and restoration of normal transcriptional in the *pob3*(L78R) strain. Finally, in support of our hypothesis that Chd1 has a negative role in regulating yFACT mediated TBP binding, we show that

deletion of *CHD1* suppresses the synthetic lethality between *spt16* mutations and TBP mutations as well as *spt16-11* mutation and TFIIA mutations.

Introduction

The underlying DNA sequences in eukaryotic chromosomes are not readily available due to the compaction of the chromatin structure. There are three major ways by which these DNA sequences are made available to the DNA binding factors that regulate several processes *in vivo*. First, the post translational histone modifications that either change the properties of the chromatin structure or create a binding site for other transcription factors (Formosa, 2003). These post translational modifications include phosphorylation of serine residues and acetylation, methylation and ubiquitylation of lysine residues (de la Cruz et al., 2005). Several transcription factors have been described that recognize specific histone modifications. For example, the bromodomain containing proteins recognize acetylated lysines (Yang, 2004) of histone proteins whereas chromodomain containing proteins are involved in recognizing the methylation mark on histone proteins (Brehm et al., 2004; de la Cruz et al., 2005). The second way to make the DNA sequence in chromatin structure available is through ATP-dependent chromatin remodeling (Cairns, 2005; Havas et al., 2001; Wang, 2003). These process utilize the energy of ATP to either disrupt the chromatin structure or to assemble a proper chromatin structure (Havas et al., 2001). The third way by which the DNA sequence is made available is by ATP-independent chromatin reorganization that changes the structure of chromatin in a localized manner (Formosa, 2003). For example the yFACT complex changes the structure of chromatin in an ATP-independent manner (Rhoades et al., 2004; Ruone et al., 2003). The reorganization by yFACT is thought to be important for

regulating several processes both *in vivo* and *in vitro* (Biswas et al., 2005; Formosa, 2003; Mason and Struhl, 2003; Shimojima et al., 2003; Simic et al., 2003).

The FACT complex (facilitates chromatin transcription) was first identified as a factor that enhanced RNA polymerase II transcription elongation during an *in vitro* transcription assay using assembled chromatin as a template (Orphanides et al., 1998). The mammalian FACT complex is composed of two subunits, p140 and SSRP1. The homologs of p140 and SSRP1 in yeast are Spt16 and Pob3 respectively (Orphanides et al., 1999). The Spt16 and Pob3 proteins are always present in a heterodimer to form the SP complex in yeast (Wittmeyer et al., 1999). Although the N-terminal DNA binding domain of SSRP1 is absent in Pob3, Nhp6, a high mobility group (HMG) protein is thought to serve as the DNA binding activity of the SP complex to form the yFACT complex (Brewster et al., 2001; Formosa et al., 2001a). Genetic and biochemical evidence suggests that yFACT is involved in regulating both transcription and DNA replication (Belotserkovskaya et al., 2003; Biswas et al., 2005; Formosa et al., 2001a; Formosa et al., 2002; Krogan et al., 2002; Mason and Struhl, 2003; Orphanides et al., 1999; Saunders et al., 2003; Wittmeyer and Formosa, 1997; Wittmeyer et al., 1999). Immunoprecipitation studies have shown physical association between components of the yFACT complex and several elongation factors (Krogan et al., 2002; Simic et al., 2003). An immunolocalization study showed that yFACT associates with an actively transcribed chromatin region (Saunders et al., 2003). In vivo ChIP analysis shows that yFACT travels with elongating RNA polymerase II (Mason and Struhl, 2003). Earlier reports also suggests that the FACT complex has a role in transcription initiation (Biswas et al., 2005; Shimojima et al., 2003). We have shown earlier that yFACT has a role in regulating TBP

binding during the transcriptional initiation step (Biswas et al., 2005). Certain mutations of TBP and TFIIA showed a synthetic lethal interaction with mutations in *SPT16*. TBP binding is also reduced at the promoter region of some genes in a *spt16* mutation strain. An *in vitro* DNase I protection assay showed that yFACT helps TBP binding to a TATA box containing nucleosomal DNA in presence of TFIIA (Biswas et al., 2005).

ATP-dependent chromatin remodeling factors are multisubunit complexes that contain an ATPase subunit belonging to the Snf2-like subfamily of nucleic acid-stimulated DEAD/H ATPases (Havas et al., 2001). Some members of this Snf2-like subfamily are able to space nucleosomes in an ATP-dependent manner during chromatin remodeling. One such example is the chromodomain 1 (Chd1) protein (Lusser et al., 2005; Robinson and Schultz, 2003). Chd1 and other CHD proteins have two chromodomains near the N-terminus, a centrally located Snf2-related helicase/ATPase domain and a Myb-related DNA-binding domain near the C-terminus (Woodage et al., 1997). *In vitro* biochemical evidence suggests that Chd1 has a nucleosome assembly and spacing activity in association with nucleosome assembly protein 1 (Nap1) (Lusser et al., 2005). *In vitro* chromatin assembly reactions using a crude DEAE extract from *chd1*Δ strain produces chromatin that is hyper-sensitive to DNaseI digestion (Robinson and Schultz, 2003). This shows that Chd1 produces an inhibitory chromatin structure that is less sensitive to digestion by DNase I. Genetic interactions have been reported between mutations of *CHD1* and mutations in other transcription elongation factors such as Spt5, Isw1 and Isw2 (Simic et al., 2003; Tsukiyama et al., 1999). Chd1 also physically interacts with several transcription elongation factors such as members of Paf1 complex, Spt4-Spt5 complex and components of yFACT (Kelley et al., 1999; Krogan et al., 2002; Simic et

al., 2003; Tsukiyama et al., 1999). Recently Chd1 has been shown to physically associate with the SAGA/SLIK complex in yeast (Pray-Grant et al., 2005). In this report the authors also show that chromodomain 2 of Chd1 is involved in recognizing histone H3-K4 methylated tail peptides in an *in vitro* reaction. The recognition of a methylated H3-K4 tail by chromodomain 2 is important for SAGA mediated acetylation at the *GAL10* gene. Although yeast Chd1 was first identified as a factor that has a negative role in regulating transcription (Woodage et al., 1997), the mechanism of this negative transcriptional regulation is unknown. Moreover, the functional role of Chd1 *in vivo* in regulating transcription is also unknown. In this report, we show that Chd1 has a negative role in regulating yFACT mediated transcription. We present strong evidence which suggests that Chd1 has a negative role in regulating yFACT mediated TBP binding at the promoter region. An increase in TBP binding in a yFACT mutant strain upon deletion of *CHD1* also results in an increase in RNA polymerase II binding at the promoter region to initiate transcription. Finally, deletion of *CHD1* suppresses synthetic lethalities between *spt16* mutations and TBP mutations as well as between *spt16* and TFIIA mutations. This is the first evidence that shows a functional role of Chd1 in regulating transcription at the promoter region. Our report also provides a mechanistic explanation of earlier observations that implicated a negative role of Chd1 in regulating transcription

Materials and methods

Yeast strains are listed in Table 7.1 Cells were grown in YPD medium at 30°C, except where other temperatures are noted, or in synthetic complete medium with 2% glucose and supplemented with adenine, uracil and amino acids, as appropriate, to select for plasmids. For the galactose induction experiments cells were grown at 25°C in YP

Table 7.1. Strains used for yFACT and Chd1 study

DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY3398	*MAT***a** *ade2 can1 his3 leu2 trp1*
DY5926	*MAT***a** *gcn5::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY6612	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY6957	*MAT***a** *chd1::TRP1 ade2 ade3 can1 his3 leu2 trp1 ura3*
DY7379	*MAT***a** *pob3(L78R) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY7656	*MAT***a** *isw1::ADE2 isw2::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY7836	*MAT*α *htz1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY7836	*MAT*α *htz1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8107	*MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8117	*MAT*α *spt16-11 ade2 can1 his3 leu2 lys2 met15 ura3*
DY8156	*MAT*α *elp3::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8185	*MAT*α *spt16-11 elp3::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1*
DY8552	*MAT***a** *spt15::ADE2 spt16::HIS3 + YCp-URA3(SPT15, SPT16) ade2 can1 his3 leu2 trp1 ura3*
DY8700	*MAT***a** *spt16-11 toa2::His3MX + YCp-URA3(TOA2) ade2 can1 his3 leu2 met15 trp1 ura3*
DY8788	*MAT*α *spt16-11 ade2 can1 his3 leu2 met15*
DY8799	*MAT***a** *spt16-11 set2::ADE2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY8808	*MAT***a** *nhp6a::URA3 nhp6b::ADE2 spt16-11 ade2 can1 his3 leu2 trp1 ura3*

Table 7.1. Cont.

DY8862	*MAT***a** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8863	*MAT***a** *hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K4R]-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8865	*MAT***a** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2-HHF2) ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8866	*MAT***a** *spt16-11 hht1-hhf1::LEU2 hht2-hhf2::kanMX3 + YCp-TRP(HHT2[K4R]-HHF2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8875	*MAT***a** *set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9055	*MAT***a** *spt16-11 isw1::ADE2 ade2 can1 his3 leu2 trp1*
DY9151	*MAT*α *spt16-11 chd1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9152	*MAT***a** *spt16-11 chd1::TRP1 ade2 ade3 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9206	*MAT***a** *spt16-11 set1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9271	*MAT***a** *spt16-11 chd1::LEU2 set1::TRP1 ade2 ade3 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9458	*MAT***a** *pob3(L78R) chd1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9591	*MAT***a** *HIS3::GAL1::YLR454w ade2 can1 his3 leu2 met15 trp1 ura3*
DY9809	*MAT*α *chd1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9811	*MAT*α *spt16-11 chd1::TRP1 htz1::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9816	*MAT*α *isw1::ADE2 ade2 can1 his3 leu2 trp1 ura3*

Table 7.1. Cont.

DY9820	*MATα spt16-11 isw1::ADE2 isw2::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY9827	*MATα chd1::TRP1 isw1::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9831	*MAT***a** *spt16-11 chd1::TRP1 isw1::ADE2 isw2::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY9834	*MATα spt16-11 chd1::TRP1 isw1::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9873	*MATα gcn5::HIS3 chd1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY9959	*MAT***a** *chd1::TRP1 HIS3::GAL1::YLR454w ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9961	*MAT***a** *spt16-11 chd1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9963	*MAT***a** *chd1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1*
DY9965	*MAT***a** *spt16-11 chd1::TRP1 elp3::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9972	*MAT***a** *pob3(L78R) HIS3::GAL1::YLR454w ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY9978	*MAT***a** *spt16-11 nhp6a.URA3 nhp6b.ADE2 chd1.TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY10020	*MAT***a** *pob3(L78R) chd1.TRP1 HIS3.GAL1.YLR454w ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY10141	*MAT***a** *spt15::ADE2 spt16::HIS3 chd1::KanMX + YCp-URA3(SPT15, SPT16) ade2 can1 his3 leu2 met15 trp1 ura3*
DY10214	*MAT***a** *spt16-11 toa2::His3MX + YCp-URA3(TOA2) chd1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

medium supplemented with 2% raffinose to mid log, shifted to 30°C for growth for 2 hours, and then galactose was added to a final concentration of 2%. Plasmids are listed in Table 7.2. RNA levels were determined with S1 nuclease protection assays as described using probes listed in Table 7.3. Chromatin immunoprecipitations were performed as described using the 8WG16 monoclonal antibody against the pol II C-terminal repeat, and a polyclonal anti-TBP sera generously provided by Tony Weil. Real Time PCR and calculations were performed as described, using of the ORF free chromosome I region as the internal control.

Results

Deletion of *CHD1* suppresses phenotypes of yFACT mutant strains

It was previously reported that a *chd1* mutation can suppress the growth defects of a pob3-272 mutation (Costa and Arndt, 2000). We asked whether a *chd1* deletion could suppress the temperature sensitive growth defects of *spt16-11* and *pob3(L78R)* mutations in our strain background. The results in Fig. 7.1A show that *spt16 chd1* and *pob3 chd1* strains grow well under conditions where the *spt16* and *pob3* single mutations are lethal. We also examined growth on media containing 6-azauracil (6-AU), as some *spt16* mutants are sensitive to 6-azauracil (6-AU). 6-AU is a uracil analog that causes imbalance in the pools of rNTPs, and many strains with defects in transcriptional elongation are sensitive to 6-AU. A *chd1* mutation suppresses the 6-AU sensitivity seen in a *spt16* mutant (Fig. 7.1B). Additionally, the *chd1* mutant strain shows slightly higher 6-AU resistance than wild type, as reported previously (Woodage et al., 1997). Thus, *spt16* and *chd1* mutations have opposite effects on sensitivity to 6-AU. However, a *SNR6*

Table 7.2. Plasmids used for yFACT and Chd1 study

Plasmid	Description	Source
pRS316	YCp URA3 vector	(Sikorski and Hieter, 1989a)
M584	YEp24 URA3 vector	(Botstein et al., 1979)
M4294	CHD1 [and ADE3] in YEp24	(Tran et al., 2000)
M4493	TBP(K138T,Y139A) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4471	TBP(E93G) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
M4475	TBP(G147W) in YCp-*TRP1* plasmid	(Eriksson et al., 2004)
pDE58-1	TBP(G174E) in YCp-*TRP1* plasmid	(Eisenmann et al., 1992)
M4599	*toa2*(F71E) in YCp-*LEU2* plasmid	(Ozer et al., 1998)
M4601	*toa2*(W76A) in YCp-*LEU2* plasmid	(Ozer et al., 1998)
M4603	*toa2*(W76F) in YCp-*LEU2* plasmid	(Ozer et al., 1998)
M4606	*toa2*(Y10G,R11Δ) in YCp -*LEU2* plasmid	(Biswas et al., 2004)
pTF128-22	*spt16-11*(A417V) in YCp-*LEU2* plasmid	(Formosa et al., 2001a)
pTF128-24	*spt16-24(T434I) in YCp LEU2* plasmid	(Formosa et al., 2001a)
M4960	pGH269 - HA(3x):CHD1 in pRS316	(Simic et al., 2003)
M4961	pGH271 - HA(3x):CHD1(CD1+2Δ) in pRS316	(Simic et al., 2003)
M4962	HA(3x):CHD1(Y316E CD2) in pRS316	(Pray-Grant et al., 2005)
M4963	HA(3x):CHD1(L314Y CD2) in pRS316	(Pray-Grant et al., 2005)
M4964	HA(3x):CHD1(E220L CD1) in pRS316	(Pray-Grant et al., 2005)
M4986	GHB244 - HA(3x):CHD1(K407R) in pRS316	(Simic et al., 2003)

Table 7.3. Oligonucelotide list for yFACT and Chd1 study

F764	*GAL1*	CCTTTGCGCTAGAATTGAACTCAGGTACAATCACTTC TTCTGAATGAGATTTAGTCATGCGCGCGC
F878	tRNA control	GGAATTTCCAAGATTTAATTGGAGTCGAAAGCTCGCC TTA
F1263	*DSE2*	ATGTACCTGACGATTCGATACTTTGAGATGATTCGATAT CGTATGTAGAGCTAGA GGTACAAC
F1464	*YJL097W*	GTATCTGACGATCTCAGTTATAGACCATGCCAGTAATAA TGATATGTAAACAACTGATTGGGAGGT
F1464	*YRO2*	CTTGACCAGGTTGAATGTAGTTGTCATCAAACTAATAC AAACAACAATGGCAGCACCAATACCAATGGTGTAGTAAC CCCTACGAG
F1593	-192 to +168	GGGGTAATTAATCAGCGAAGCGATG
F1594	-192 to +168	CCTGACGGTACCATCTTCTAAGGATAAAC
F1410	+ 945 to +1147	CAATACCAACAGGTTCAGAAATGAGATGC
F1411	+ 945 to +1147	GAGAGAACAAATTGGTTTCGCCAAATATCG
F1232	+ 3540 to +3705	CTCCAACGCAGCCAAACTTT
F1233	+ 3540 to +3705	CTCGAAATGATGCGGTATGG
F1412	+ 7701 to +7850	GAGGGTCACAGATCTATTACTTGCCC
F1413	+ 7701 to +7850	GTTGTGAGTTGCTTCAGTGGTGAAGTG

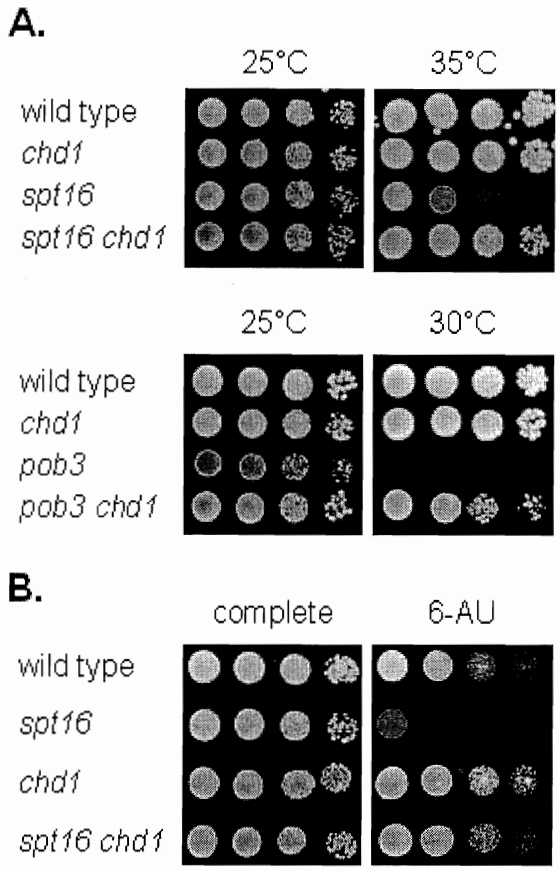


Fig. 7.1. *chd1* suppresses *spt16* and *pob3* phenotypes.
A. Dilutions of strains DY150 (wild type), DY6957 (*chd1*), DY8788 (*spt16*), and DY9151 (*spt16 chd1*) were plated on complete medium at the indicted temperature for 2 days. Dilutions of strains DY150 (wild type), DY6957 (*chd1*), DY7379 (*pob3*), and DY9458 (*pob3 chd1*) were plated on complete medium at the indicted temperature for 3 days.
B. Dilutions of strains DY3398 (wild type), DY8788 (*spt16*), DY9963 (*chd1*), and DY9961 (*spt16 chd1*) were plated for 2 days at 25°C on complete medium or on medium lacking uracil containing 50 ug/ml 6-azauracil.

promoter mutation that reduces expression of the U6 small nuclear RNA causes sensitivity to 6-AU, and thus sensitivity to 6-AU does not necessarily demonstrate a role in transcriptional elongation. In summary, the chd1 suppression of yFACT mutant phenotypes suggest that yFACT and Chd1 have opposing roles in regulating transcription. The yFACT complex, in addition to Spt16 and Pob3, contains the Nhp6 HMGB protein (Formosa et al., 2001a). Nhp6 is encoded by two redundant genes, *NHP6A* and *NHP6B*, and the *nhp6ab* double mutant strain is temperature sensitive for growth. Based on our observation that a *chd1* mutation suppresses the temperature sensitive growth phenotype of the *spt16* and *pob3* strains, we asked whether *chd1* could suppress temperature sensitivity of the *nhp6ab* strain. We constructed a *nhp6ab chd1* triple mutant strain, but this strain failed to display suprpession (data not shown). Nhp6 has a role in transcription by RNA polymerase III (Kassavetis and Steiner, 2006a) and interacts with other chromatin proteins besides yFACT, including Swi/Snf, RSC, and Ssn6/Tup1 (Biswas et al., 2004; Fragiadakis et al., 2004; Szerlong et al., 2003). The ability of a *chd1* mutation to suppress *spt16* and *pob3* but not *nhp6ab* may reflect the additional functions of Nhp6 in the cell.

Deletion of *CHD1* suppresses synthetic lethality between *spt16* and other transcription factors

The ISW1 and ISW2 chromatin complexes have been implicated in both transcriptional elongation and in repressing transcriptional initiation (Kent et al., 2004). Additionally, the *isw1 isw2 chd1* triple mutant shows additive growth defects at elevated temperatures (Tsukiyama et al., 1999). Based on these results, we looked for genetic interactions between *spt16*, *chd1*, *isw1* and *isw2*. The *spt16 isw1* double mutant shows a

significant growth defect at 33°C (Fig. 7.2A), and the *spt16 isw1 isw2* triple is completely dead at 33°C (Fig. 7.2B). These synthetic growth defects suggest that the yFACT chromatin reorganizing complex and the ISW remodeling complexes may perform similar functions in vivo. Importantly, a *chd1* mutation suppresses both the *spt16 isw1* and the *spt16 isw1 isw2* growth defects, supporting the idea that Chd1 acts in opposition to yFACT.

As a *chd1* mutation suppresses a number of *spt16* phenotypes, we asked whether *chd1* can also suppress other synthetic lethal phenotypes seen with *spt16*. *spt16* shows marked growth defects when combined with both *nhp6a* and *nhp6b* mutations (Formosa et al., 2001a), and a *spt16 nhp6ab* strain is lethal at 33°C (Fig. 7.2C). A *chd1* mutation suppresses this synthetic lethality, seen by growth of the *spt16 nhp6ab chd1* strain. *ELP3* encodes a histone acetyltransferase subunit of the elongator complex (Wittschieben et al., 2000) and *elp3* is synthetic lethal with *spt16* (Formosa et al., 2002). The *spt16 elp3* synthetic lethality is suppressed by a *chd1* mutation (Fig. 7.2D). *HTZ1* encodes the yeast H2A.Z histone variant of H2A (Dryhurst et al., 2004), and we recently showed that *htz1* and *spt16* are synthetic lethal (Biswas et al., 2006 submitted). A *chd1* mutation also suppresses the *spt16 htz1* synthetic lethality (Fig. 7.2E). Htz1 is believed to function at promoter regions, as it localizes preferentially at promoter regions of genes, and this suppression suggests that Chd1 might function at promoters. We recently showed that a *set2* mutation can suppress many *spt16* phenotypes (Biswas et al., 2006 submitted). Like *chd1*, *set2* suppresses the *spt16 np6ab*, *spt16 elp3*, and *spt16 htz1* synthetic lethalities. There are differences in suppression, however. While *chd1* suppresses the *spt16 isw1 isw2* lethality, a *set2* mutation does not (data not shown). Conversely, *set2* suppresses

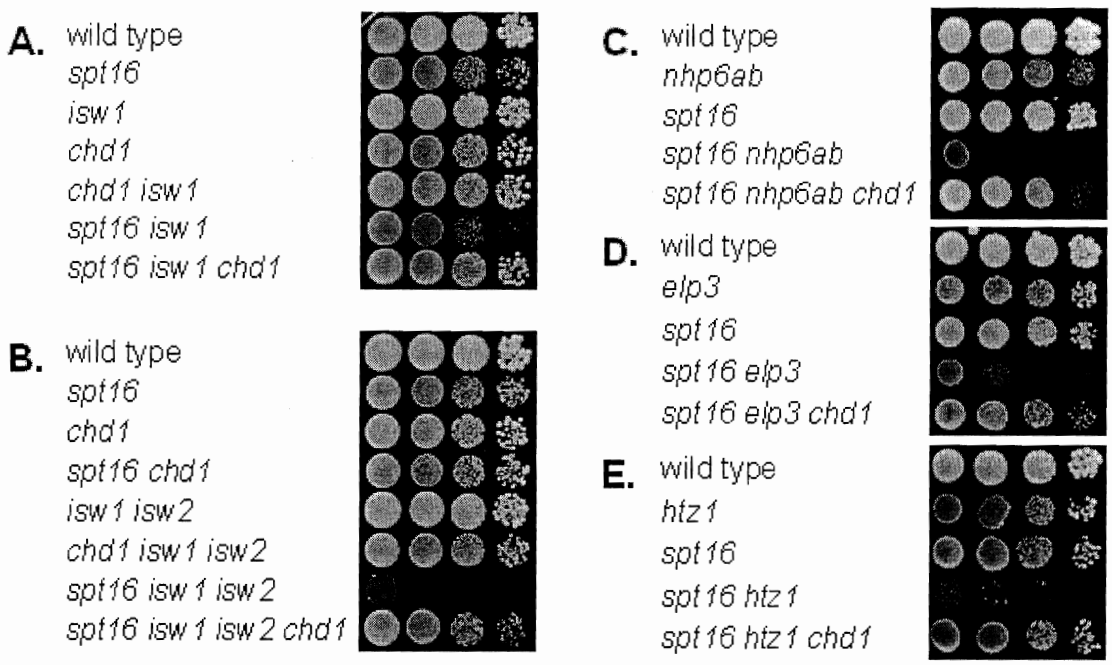


Fig. 7.2. *chd1* suppresses *spt16* synthetic growth defects.
A. Dilutions of strains DY150 (wild type), DY8107 (*spt16*), DY9816 (*isw1*), DY9809 (*chd1*), DY9827 (*chd1 isw1*), DY9055 (*spt16 isw1*), and DY9834 (*spt16 isw1 chd1*) were plated on complete medium at 33°C for 2 days.
B. Dilutions of strains DY150 (wild type), DY8107 (*spt16*), DY9809 (*chd1*), DY9152 (*spt16 chd1*), DY7656 (*isw1 isw2*), DY9823 (*chd1 isw1 isw2*), DY9820 (*spt16 isw1 isw2*), and DY9831 (*spt16 isw1 isw2 chd1*) were plated on complete medium at 33°C for 2 days.
C. Dilutions of strains DY150 (wild type), DY6612 (*nhp6ab*), DY8788 (*spt16*), DY8808 (*spt16 nhp6ab*), and DY9978 (*spt16 nhp6ab chd1*) were plated on complete medium at 33°C for 2 days.
D. Dilutions of strains DY150 (wild type), DY8156 (*elp3*), DY8788 (*spt16*), DY8185 (*spt16 elp3*), and DY9965 (*spt16 elp3 chd1*) were plated on complete medium at 33°C for 2 days.
E. Dilutions of strains DY150 (wild type), DY7836 (*htz1*), DY8788 (*spt16*), DY9808 (*spt16 htz1*), and DY9811 (*spt16 htz1 chd1*) were plated on complete medium at 33°C for 2 days.

synthetic lethality of the *spt16 gcn5* double mutant, but a *chd1* mutation does not (data not shown). In fact, a *gcn5 chd1* double mutant shows a growth defect at 25°C and is synthetic lethal at 35°C (Fig. 7.3). It was recently reported that Chd1 is present in the SAGA/SLIK co-activator complexes (Pray-Grant et al., 2005), and the synthetic effects of combining *gcn5* and *chd1* mutations could reflect distinct functions of these two proteins in the same protein complex.

Mutations in the ATPase domain and in chromodomain 2 of Chd1 suppresses yFACT mutations

The Chd1 protein has ATPase activity (Tran et al., 2000), and it also contains two chromodomains (Woodage et al., 1997). We investigated the roles of the ATPase and chromodomains in the genetic interactions of *chd1* with *spt16*. An *spt16* mutant will not grow at an elevated temperature, but a *spt16 chd1* double mutant does grow (Fig. 7.1A). YCp-*URA3* plasmids with mutations in *CHD1* gene were transformed into *spt16 chd1* and *pob3 chd1* strains, along with the empty vector and the wild type *CHD1* gene as controls, and plated on media lacking uracil (Fig. 7.4A). Strains with the empty vector grow well, while the plasmid with wild type Chd1 inhibits growth. Using this as a complementation assay, we tested the Chd1(K407R) substitution within the consensus ATP binding motif (Simic et al., 2003). The Chd1(K407R) mutant complements poorly (Fig. 7.4A), suggesting that the ATPase activity of Chd1 is required for Chd1 to be toxic in *spt16* mutants. Interestingly, the strain with the Chd1(K407R) plasmid does not grow as well as the empty vector, suggesting that there is residual effect of Chd1(K407R) in this assay.

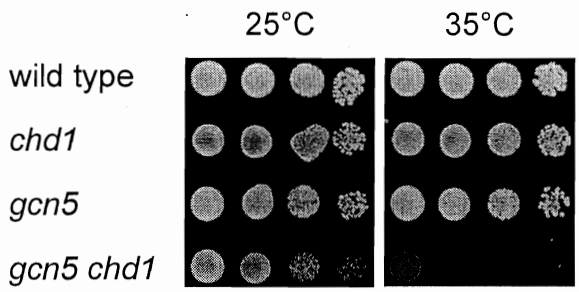


Fig. 7.3. *chd1* and *gcn5* show a synthetic growth defect.
Dilutions of strains DY150 (wild type), DY9809 (*chd1*), DY5926 (*gcn5*), and DY9873 (*gcn5 chd1*) were plated on complete medium at 33°C for 2 days.

Chd1 has two chromodomains, protein domains that bind to methylated lysine residues. Chromodomain 2 of Chd1 was recently shown to recognize methylated K4 in histone H3 *in vitro* (Pray-Grant et al., 2005), although this result has not been confirmed by others (Sims et al., 2005). We transformed plasmids with chromodomain mutations into the *spt16 chd1* and *pob3 chd1* strains and assessed their growth (Fig. 7.4B). The strain with both chromodomains deleted plasmid Chd1(CD1Δ,CD2Δ) fails to complement, and grows nearly as well as the empty vector. We conclude that the chromodomains are required for Chd1 to be toxic in *spt16* mutants. We tested three Chd1 point mutants, E220L in chromodomain 1, and E314Y and Y316E in chromodomain 2. The E220L and E314Y mutations have no effect different from Chd1(wild type), while the Y316E mutation restores growth similar to the plasmid with both chromodomains deleted. Importantly, the Y316E substitution eliminated *in vitro* binding to a peptide containing methylated K4, while the E220L and E314Y mutations had no effect on the *in vitro* assay (Pray-Grant et al., 2005). This suggests that it is the binding of Chd1 to histone H3 via methylated K4 contributes to the toxicity of Chd1 in yFACT mutants; eliminating Chd1 binding to K4-Me by either a null mutation, deletion of the chromodomain, or by the Y316E substitution suppresses the *spt16* and *pob3* mutations. We note that in our assays the Chd1(K407R) ATPase mutant and the two chromodomain mutants, Chd1(CD1Δ,CD2Δ) and Chd1(Y316E), growth is not as robust as seen with the empty vector control. This suggests that the ATPase and the chromodomains each contribute to activity, and that these mutants have some residual activity.

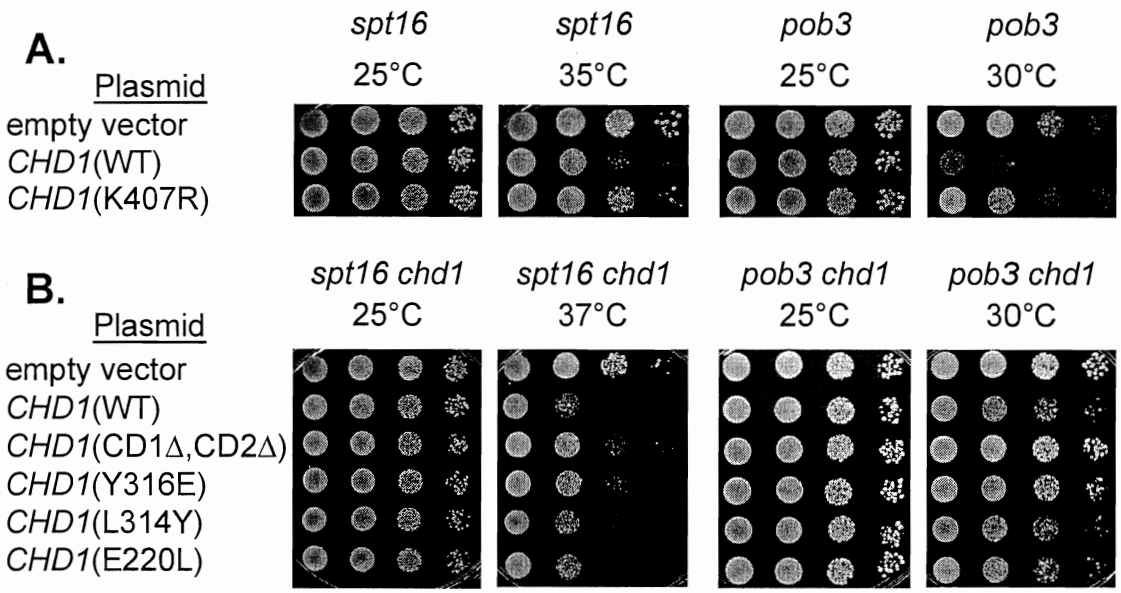


Fig. 7.4. Mutations in either the ATPase or chromodomain of Chd1 suppress the *spt16* growth defect.
A. A mutation in the ATPase domain prevents *CHD1* complementation.
B. Deletion of both chromodomains or the Y316E mutation in chromodomain 2 prevents *CHD1* complementation. Strains DY9152 (*spt16 chd1*) and DY9458 (*pob3 chd1*) were transformed with the indicated YCp-*URA3* plasmid and dilutions were plated on medium lacking uracil at the indicated temperature for 3 days.

A *chd1* mutation suppresses the synthetic lethality of *spt16* with *set1* or histone H3(K4R) mutations

The Chd1(Y316E) mutant has been reported to be unable to bind to H3 K4-Me (Pray-Grant et al., 2005), and that Chd1(Y316E) does not suppress *spt16* suggests that other mutations that prevent this binding by Chd1 should similarly suppress. Such mutations include disruption of the *SET1* gene, eliminating the Set1 methyltransferase that modifies K4 of histone H3 (Briggs et al., 2001), or a substitution at K4 of histone H3 that prevents methylation. However this prediction does not hold true, as we recently demonstrated that a *spt16 set1* double mutant has a synthetic phenotype, lethality at 33°C (Biswas et al., 2006 submitted). Thus, the simple idea that the absence of Chd1 binding via methylated K4 of histone H3 is not sufficient to explain the suppression (see Discussion).

As a *chd1* mutation suppresses many *spt16* phenotypes, including some synthetic lethal interactions, we constructed a *spt16 set1 chd1* triple mutant strain. As shown in Fig. 7.5A, *chd1* suppresses the *spt16 set1* synthetic lethality, similar to the suppression of *spt16 set1* by *set2* (Biswas et al., 2006 submitted). Similar to *set1*, a K4R substitution in histone H3 is synthetic lethal with *spt16* at 33°C, and this is suppressed by a *chd1* mutation (Fig. 7.5B). Similar effects can be seen with *pob3* mutants, where *pob3 set1* and *pob3* H3(K4R) strains are lethal, but can be suppressed by *chd1* (data not shown). The fact that similar genetic effects are seen with either a *set1* or a histone H3(K4R) mutation are consistent with lysine 4 of H3 being the critical target for the Set1 enzyme. This data is also consistent with a recent report showing suppression of the growth defect of *set1*Δ with *chd1*Δ (Zhang et al., 2005b).

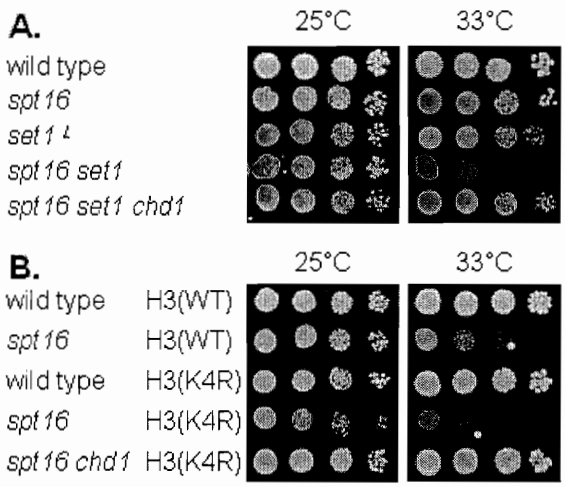


Fig. 7.5. A *chd1* mutation suppresses the synthetic growth defect of *spt16* with *set1* or histone H3(K4R) mutations.

A. *chd1* mutation suppresses the *spt16 set1* synthetic growth defect. Dilutions of strains DY150 (wild type), DY8788 (*spt16*), DY8875 (*set1*), DY9206 (*spt16 set1*), and DY9271 (*spt16 set1 chd1*) were plated on complete medium at 25°C for 3 days or at 33°C for 2 days.

B. *chd1* mutation suppresses the *spt16* histone H3(K4R) synthetic growth defect. Dilutions of strains DY8862 (*hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(wild type)-H4(wild type)), DY8865 (*spt16 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(wild type)-H4(wild type)), DY8863 (*hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)), DY8866 (*spt16 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)), and DY10472 (*spt16 chd1 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)) were plated on complete medium at the indicated temperature for 2 days.

CHD1 overexpression is toxic in yFACT mutant strains

Our experiments suggest that yFACT and Chd1 act oppositely in regulating transcription. Thus, the activity of Chd1 is toxic in cells that have a partially defective yFACT chromatin reorganizing factor, and a *chd1* mutation relieves this toxicity. This model predicts that Chd1 overexpression could be toxic in strains with yFACT mutations. We transformed wild type and *spt16* mutant strains with a multicopy plasmid containing *CHD1*, and assessed growth on selective medium to maintain the plasmid (Fig. 7.6). *CHD1* overexpression has no effect in the wild type stain, but is very toxic in the *spt16* strain. Interestingly, a *set2* mutation partially reverses the toxicity of *CHD1* overexpression in the *spt16* mutant. There is no phenotypic consequence of *CHD1* overexpression in *set1* or *set2* single mutant strains, and thus the effect appears to be specific to yFACT mutant strains. We conclude that the amount of Chd1 is of critical importance in strains with a defect in the yFACT complex.

A *chd1* mutation suppresses a galactose induction defect in a *pob3* strain

Although genetic and biochemical experiments suggests a role for *Chd1* in regulating transcription in eukaryotes, the exact mechanism of Chd1 function is unclear. Chromatin immunoprecipitation (ChIP) experiments showed that Chd1 was bound to the coding region of the *TEF2* and *GAL10* genes, suggesting an elongation function (Simic et al., 2003). However, the ChIP studies also showed Chd1 was recruited to the *GAL10* promoter, consistent with a role in initiation of transcription. We recently showed that a *pob3* mutation reduces expression of a *GAL1-YLR454w* gene fusion, and that the *pob3* mutation reduces binding of both pol II and TBP to the *GAL1* promoter (Biswas et.al

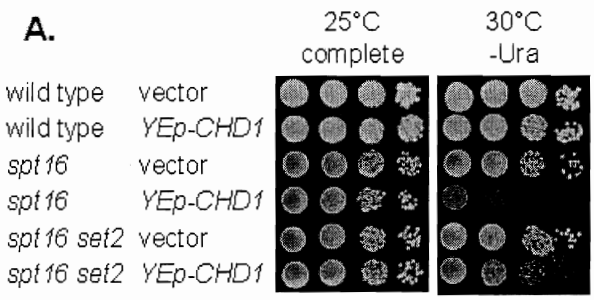


Fig. 7.6. *CHD1* overexpression is toxic an *spt16* mutant.
Strains DY150 (wild type), DY8117 (*spt16*), and DY8799 (*spt16 set2*) were transformed with either YEp-CHD1 or the empty YEp-URA3 vector and plated on complete medium at 25°C for 3days or on medium lacking uracil at 30°C for days.

2006 submitted). We performed similar experiments examining the effect of *pob3* and *chd1* mutations on expression and factor binding at *GAL1-YLR454w* (Fig. 7.7A). Four isogenic strains were first grown in raffinose medium at 25°C, shifted for 2 hours to 30°C, galactose was added to the medium to induce *GAL1-YLR454w* expression, and samples were taken at timed intervals for mRNA and ChIP analyses. There is a rapid rise in *GAL1-YLR454w* mRNA levels following galactose induction in wild type and *chd1* cells (Fig. 7.7B). There is a marked defect in *GAL1-YLR454w* induction in the *pob3* mutant, but this defect is completely suppressed in the *pob3 chd1* double mutant. We conclude that Chd1 has a negative role at the *GAL1* promoter, opposing the yFACT dependent transcriptional activation at this promoter.

To examine the molecular mechanism of suppression of the defect in transcriptional induction in the *pob3* mutant by *chd1*, we used ChIP experiments to measure RNA polymerase II occupancy following galactose induction. Samples were harvested at different time intervals and treated with formaldehyde to crosslink, and processed for ChIP. We used PCR probes specific for four different regions of the 8 kb long *YLR454w* gene, the *GAL1-YLR454w* promoter, 1 kb downstream of start codon, the middle of the *YLR454w* ORF (+3600), and the 3' end of the gene (+7800) (see map in Fig. 7.7A). The ChIP results shown in Fig. 7.7C show pol II occupancy 60 min. after galactose induction at different regions of the *GAL1-YLR454w* gene. A mutation with an elongation defect should cause decreased pol II binding along the gene, but pol II binding at the promoter should not be affected. In contrast, the *pob3* mutation sharply reduces pol II binding at all regions of the gene, including the promoter, suggesting that the *pob3* mutation affects

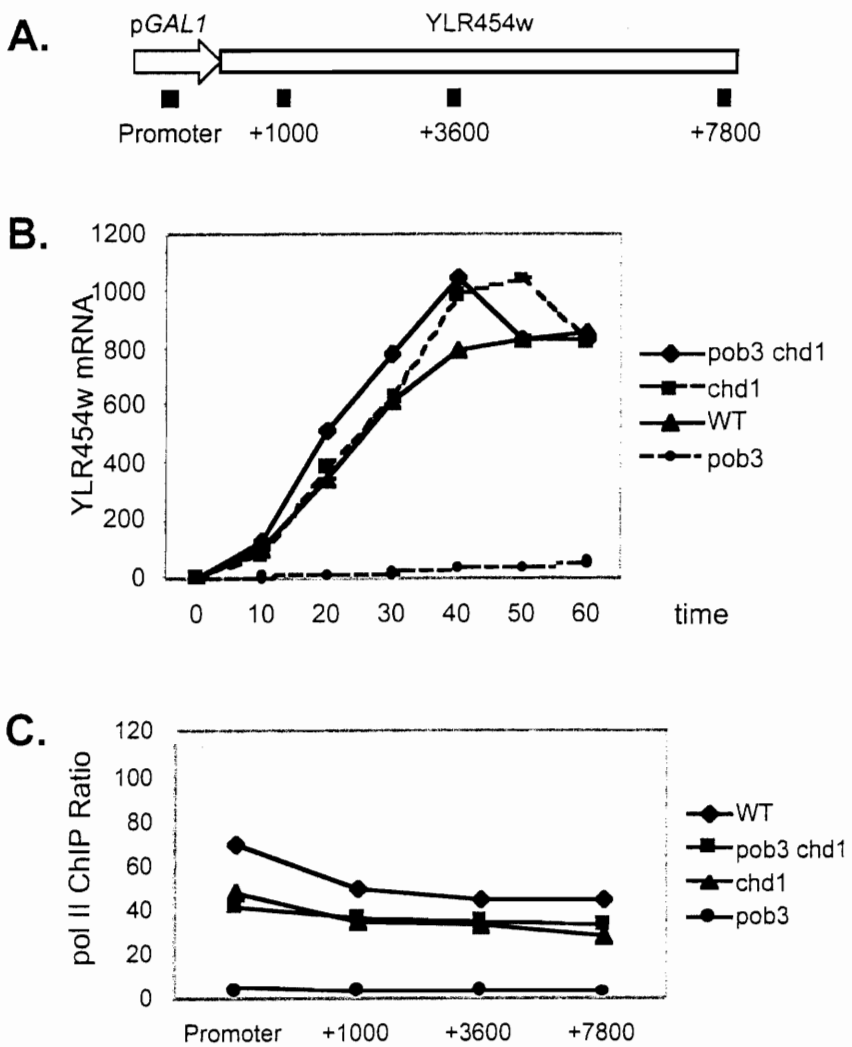
recruitment of pol II to the promoter. Importantly, pol II binding is effectively restored in the *pob3 chd1* double mutant.

Next, we used ChIP assays to measure binding of TBP to the *GAL1-YLR454w* promoter following galactose induction. TBP binding was severely reduced in the *pob3* mutant (Fig. 7.7E) and TBP binding approached wild type levels in the *pob3 chd1* double mutant strain. These results are consistent with our earlier data suggesting that yFACT has a role in facilitating formation of the TBP-TFIIA complex on DNA. The observation that deletion of *CHD1* overcomes the defect in TBP binding in the *pob3* strain suggests that Chd1 has a negative role regulating TBP binding at the *GAL1* promoter region.

Deletion of *CHD1* increases expression of some genes *in vivo*

Our data presented here show that deletion of *CHD1* restores the defective galactose induction in a *pob3*(L78R) mutation strain. We have further analyzed the effect of *chd1*Δ on expression of other genes in yFACT mutant strains during logarithmic growth conditions. Based on our micro-array analysis we have tested expression of several genes in *WT*, *chd1*Δ, *spt16-11* and *spt16-11 chd1*Δ strains by S1 analysis. As shown in Fig 7.8 expression of some of the genes is significantly reduced in a *spt16-11* strain as compared to the wild type strain. Deletion of *CHD1* has no significant effect on expression of these genes. However deletion of *CHD1* in the *spt16-11* strain significantly restores the defect in expression of these genes. This shows that Chd1 has a negative role in expression of some genes *in vivo* during logarithmic growth. The fact that deletion of *CHD1* restores this transcriptional defect suggests once again that perhaps Chd1 negatively regulates expression of some genes mediated by yFACT.

Fig. 7.7. A *chd1* mutation suppresses defects in *GAL1* induction and pol II and TBP binding caused by a *pob3* mutation.
Strains DY9591 (*GAL1-YLR454w*), DY9959 (*chd1 GAL1-YLR454w*), DY9972 (*pob3 GAL1-YLR454w*), and DY10020 (*chd1 set2 GAL1-YLR454w*) were grown on YP medium with 2% raffinose. Galactose was added to 2%, and samples were taken at 10 min. intervals and processed for ChIP analysis to measure pol II and TBP binding.
A. Map of the *GAL1-YLR454w* allele showing the positions of the PCR primers at the promoter and within the gene.
B. *YLR454w* mRNA levels from the *GAL1-YLR454w* allele.
C. Distribution of pol II at 60 min. following galactose induction at different *GAL1-YLR454w* regions in four different strains. Error bars show variance among replicate PCRs.
D. RNA pol II binding to the native *GAL1* promoter at 30 min. following galactose induction in four different strains. Error bars show variance among replicate PCRs.
E. TBP binding to the *GAL1-YLR454w* promoter following galactose induction in four different strains. ChIP values were normalized to binding at t = 0. Error bars show variance among replicate PCRs.
F. SAGA binding to the native *GAL1* promoter at 40 min. following galactose induction in four different strains. Error bars show variance among replicate PCRs.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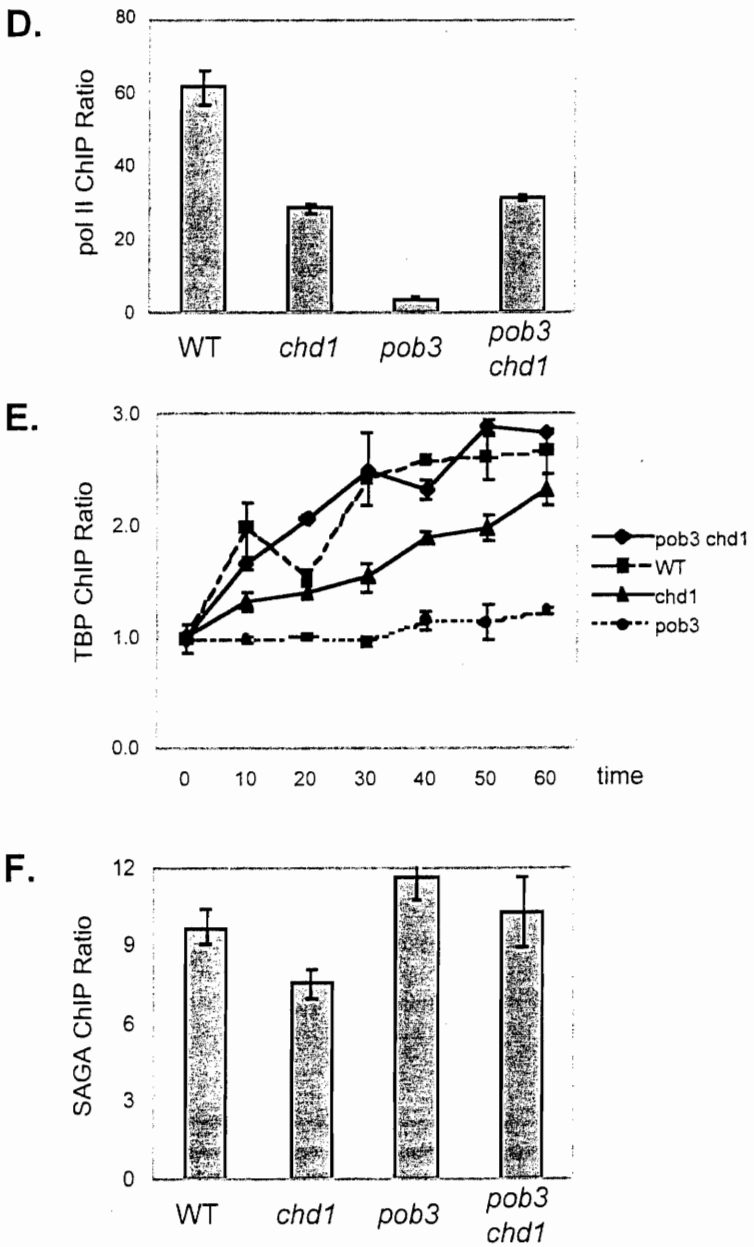
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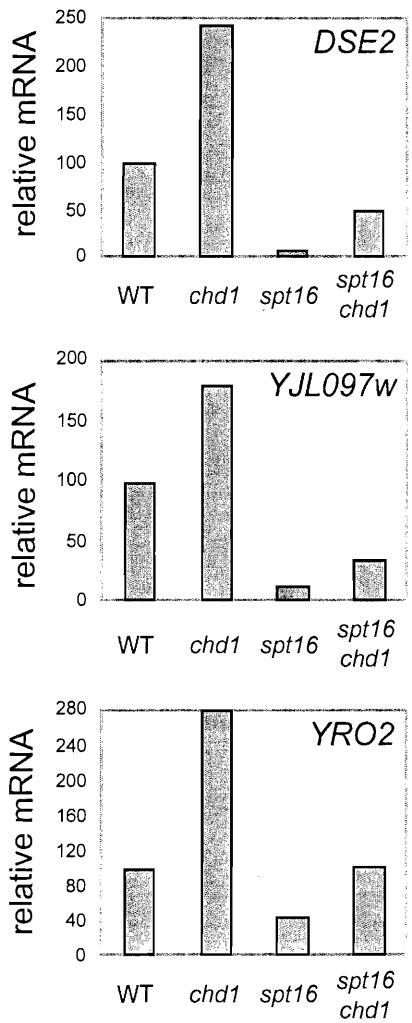

Fig 7.8. Reduced expression for some genes in a *spt16* mutant and suppression by *chd1*. RNA was isolated from strains DY150 (wild type), DY8107 (*spt16*), DY6957 (*chd1*), and DY9152 (*spt16 chd1*), and mRNA levels for specific genes measured by S1 nuclease protection

Deletion of *CHD1* suppresses the synthetic lethality between *spt16-11* and TBP as well as TFIIA mutations

It has been shown that several transcriptional co-activators regulate transcription initiation by regulating TBP-TFIIA complex formation. The Swi/Snf chromatin remodeling complex uses the energy of ATP hydrolysis to regulate TBP binding both *in vivo* and *in vitro* (Biswas et al., 2004; Imbalzano et al., 1994). Our genetic and biochemical data also showed that yFACT has a role in regulating TBP-TFIIA complex biochemical data also showed that yFACT has a role in regulating TBP-TFIIA complex formation. Since we observe restoration of the defect of TBP binding in a yFACT mutant strain by deletion of *CHD1*, we asked whether deletion of *CHD1* would also suppress the synthetic lethalities between TBP mutations and *spt16* mutations that we have described earlier (Biswas et al., 2005). We used a plasmid shuffle assay to address this question. We constructed two isogenic strains containing the wild type *CHD1* gene and deletion of the *CHD1* gene. In both of these strains the TBP gene and *SPT16* genes were disrupted. Since these genes are essential for cell viability, the strains were kept alive by providing these genes on a YCp-*URA3* plasmid. We transformed these strains with the TBP plasmid and *spt16* plasmid combination that showed synthetic lethality in our genetic assay earlier (Biswas et al., 2005). The transformants were grown on media containing 5-FOA so that the strains are required to loose the parental YCp-*URA3* plasmid containing both the wild type TBP gene and *SPT16* for their growth. As shown in Fig 7.9A, the strain transformed with empty vector could not grow on a 5-FOA plate. However these strains transformed with wild type copies of TBP and *SPT16* plasmids could grow on media containing 5-FOA. Introduction of some combinations of TBP mutations and *spt16* mutations resulted

either in synthetic lethality or a synthetic growth defect in a *CHD1* strain background (Fig 7.9A). Deletion of *CHD1* rescued some of these synthetic lethalities or synthetic growth defects (compare *CHD1* with chd1Δ on 5-FOA plate). This in vivo evidence strongly suggests that Chd1 has a negative role in yFACT mediated TBP binding. A deletion of this negative factor rescues the synthetic lethal or synthetic growth defect phenotypes associated with *spt16* mutations and TBP mutations.

During the initiation transcription, TBP binding is followed by TFIIA binding to form a stable TBP-TFIIA complex on DNA. Yeast TFIIA is a heterodimer composed of a larger subunit Toa1 and a smaller subunit Toa2. Some *toa2* mutations were described that abolished the TFIIA interaction with TBP in *in vitro* binding reactions (Ozer et al., 1998). We have earlier shown that some of these *toa2* mutations are synthetically lethal with the *spt16-11* mutation (Biswas et al., 2005). Since our data presented here strongly suggest that Chd1 has a negative role in regulating TBP binding *in vivo*, we asked whether a *chd1Δ* would also suppress the synthetic lethal interactions between *spt16-11* and *toa2* mutations. Two isogenic strains, *spt16-11 toa2* and *spt16-11 toa2 chd1Δ* were constructed. Since *TOA2* is an essential gene for the cell viability, the strains were kept alive by providing the *TOA2* gene on a YCp-*URA3* plasmid. Both these strains are transformed with plasmids containing *toa2* mutations that showed a synthetic growth defect or synthetic lethal phenotype with *spt16-11*. The transformants were grown on a 5-FOA plate so that the strains are required to lose the parental YCp-*URA3-TOA2* plasmid and depend on the mutant *toa2* plasmid for their growth. As shown in Fig 7.9B, the transformant containing empty vector could not grow on the 5-FOA plate. However transformants containing wild type *TOA2* could loose the YCp-*URA3-TOA2* plasmid on

5-FOA in the *spt16-11* strain. Some *toa2* mutations showed synthetic lethal phenotype with *spt16-11* mutation in the presence of wild type *CHD1*. Importantly, deletion of *CHD1* rescued these synthetic lethalities between *spt16-11* and *toa2* mutations (Fig 7.9B). We also have observed a synthetic growth defect with some *toa2* mutations in combination with *spt16-11* mutation. Deletion of *CHD1* also restored this synthetic growth defect between *spt16-11* and *toa2* mutations (Fig 7.9B). Collectively, these data once again strongly suggest a negative role played by Chd1 in yFACT mediated TBP binding during the transcriptional initiation step.

Discussion

The chromatin remodeling complexes that have a Snf2-related helicase/ATPase have roles in either chromatin reassembly or chromatin remodeling activities in an ATP-dependent manner (Havas et al., 2001). These complexes are broadly classified into three major classes. Members of the Swi2 group contain a bromodomain, those in the IswI group contain a SANT domain, and chromodomain (CHD)-type enzymes are characterized by chromodomains. The Swi/Snf complex is the widely characterized bromodomain containing chromatin remodeling complex. Our report and reports from other labs have shown that one of the functions of the Swi/Snf chromatin remodeling complex is to regulate TBP binding (Biswas et al., 2004; Imbalzano et al., 1994). The Chd1 protein in yeast is a chromodomain containing ATPase. Previous studies have suggested that Chd1 is a transcription elongation factor. Several genetic interactions are reported between *chd1* mutations and mutations in several transcriptional elongation factors. Biochemical evidence showed that Chd1 has chromatin remodeling activity in an ATP-dependent manner (Tran et al., 2000). Studies on *Drosophila* Chd1 have shown that

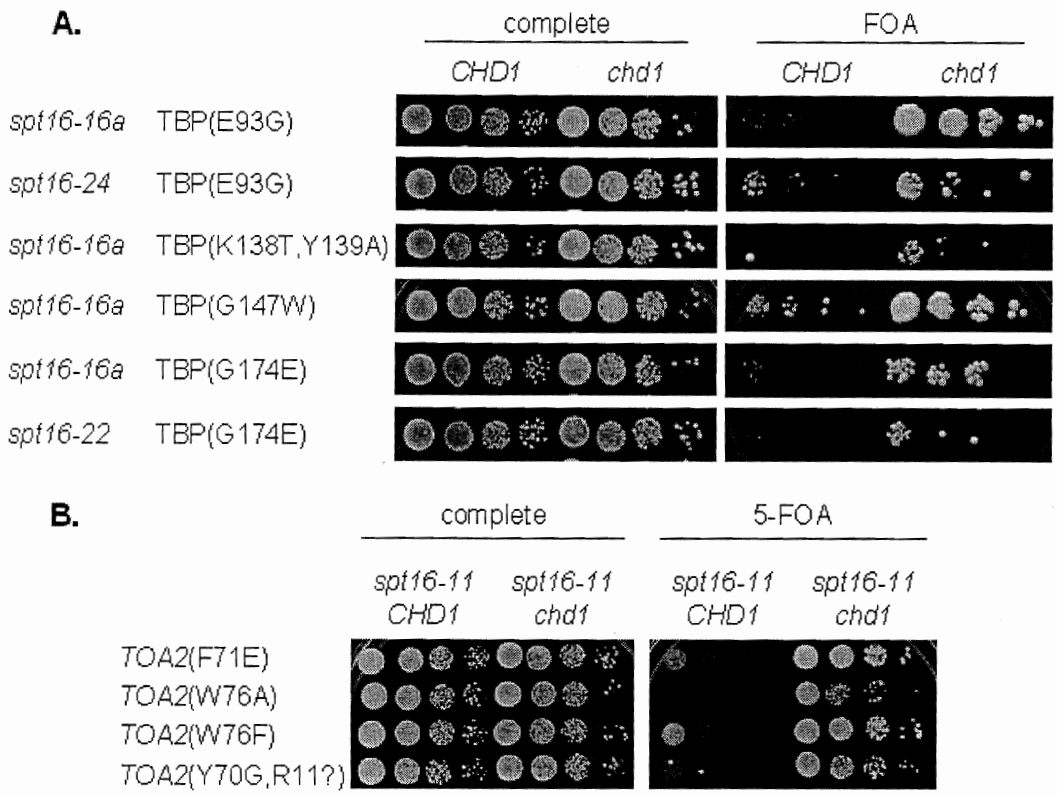

Fig. 7.9. A *chd1* mutation suppresses the synthetic lethality of an *spt16* mutation with either TBP or TFIIA mutations.
A. Strains DY8552 (*spt15Δ spt16Δ* + YCp-*URA3*-TBP-Spt16) (indicated as "*CHD1*") and DY10141 (*spt15Δ spt16Δ chd1* + YCp-*URA3*-TBP-Spt16) (indicated as "*chd1*")were transformed with two plasmids, a YCp-*TRP1* plasmid encoding a TBP mutant and a YCp-*LEU2* plasmid with either wild type *SPT16* or *spt16* mutations, and dilutions were plated at 33°C on either complete for 2 days or on FOA medium for 3 days.
B. Strains DY8700 (*spt16-11 toa2Δ* + YCp-*URA3-TOA2*) (indicated as "*CHD1*") and DY10214 (*spt16-11 chd1toa2Δ* + YCp-*URA3-TOA2*) (indicated as "*chd1*") were transformed with a YCp-*LEU2* plasmid with the indicated *toa2* mutant, and dilutions were plated for 2 days on complete medium at 25°C and on FOA medium at 30°C.

Chd1 acts as an ATP-dependent histone assembly factor that requires Nap1. ChIP analysis showed that Chd1 is recruited to the actively transcribed region in a transcription dependent manner. The yeast Chd1 protein was first reported as a negative regulator of transcription. A *chd1*Δ strain showed a hyper-resistant phenotype to growth on 6-AU containing media (Woodage et al., 1997). A *chd1*Δ suppresses the cold-sensitivity phenotype of *spt5* and temperature sensitivity phenotype of an allele of *POB3* (Simic et al., 2003). All of these reports have suggested that Chd1 might have a negative role in regulating transcription in yeast. Physical interactions have been reported between the components of yFACT and Chd1 (Krogan et al., 2002; Simic et al., 2003). However the functional consequence of this interaction and the mechanism of the negative role of Chd1 are unclear. Here we present evidence that indicates a negative role of Chd1 in regulating yFACT mediated TBP binding at the promoter region. Deletion of *CHD1* suppresses lethal phenotypes associated with yFACT mutations. A *chd1*Δ also suppresses various synthetic lethalities between *spt16-11* and mutations in components of other transcription factors that have a role in either transcription initiation or elongation. In vivo ChIP analysis showed that deletion of *CHD1* restores the defect of TBP binding at the promoter region in a *pob3*(L78R) mutant strain at the *GAL1* promoter. This enhanced TBP binding results in enhanced RNA polymerase II binding at the promoter region to restore the transcriptional defect in the *pob3*(L78R) mutation strain. Finally deletion of *CHD1* suppresses the synthetic lethalities between *spt16* mutations and TBP mutations as well as *spt16-11* and *toa2* mutations.

yFACT is involved in regulating both the transcriptional elongation and the initiation stage of transcription. During the initiation step, yFACT regulates TBP-TFIIA complex

formation at the promoter region (Biswas et al., 2005). The current model suggests that the FACT complex partially or fully dissociates the histone H2A and H2B dimer to form a nucleosome structure that is less inhibitory to RNA polymerase II progression during transcription (Belotserkovskaya et al., 2003). Since Chd1 has a role in proper chromatin assembly (Lusser et al., 2005; Robinson and Schultz, 2003), it is quite possible that Chd1 and yFACT have opposing roles in regulating essential functions in vivo in context of chromatin. We show here that Chd1 and yFACT have an opposing role in regulating TBP binding at the promoter region. Deletion of *CHD1* suppresses synthetic lethalities between *spt16-11* and other transcription factors that have roles either in initiation or in elongation step. In support of a negative role of Chd1 on yFACT, we have shown that over-expression of Chd1 is toxic in a yFACT mutant strain. This toxic effect is yFACT mutant specific since we have not observed the same on other mutation strains that we have studied. In support of this specific genetic interaction, a functional interaction has also been reported between yFACT and Chd1.

Recently, it has been reported that Chd1 is present in the SAGA/SLIK transcriptional coactivator complex in yeast (Pray-Grant et al., 2005). The chromodomain 2 of Chd1 has been shown to recognize histone H3-K4 methylated tail peptides *in vitro*. This recognition of the methylated histone H3-K4 residue is important for SAGA complex mediated acetylation by Gcn5. By complementation assay, we have shown that a chromodomain 2 mutation that impairs the histone H3-K4 methylated tail peptide recognition does not complement like wild type in our assay. However complementation by mutation in chromodomain 1 is close to wild type. Therefore the negative role of Chd1 in yFACT mediated transcription is at least partially dependent on recognition of the

histone H3-K4 methylated residue. Indeed in support of this hypothesis, we have found that a *chd1*Δ also suppresses the synthetic growth defect between *spt16-11* and *set1*Δ. It possible that the chromodomain 1 of Chd1 may recognize unknown histone lysine methylation modification which also has a role in negative regulation of yFACT mediated transcription. Although a *chd1*Δ suppressed several synthetic lethalities between *spt16-11* and other transcription factors, it could not suppress the synthetic lethality between *spt16-11* and *gcn5*Δ. In fact the data presented here shows that *chd1*Δ and *gcn5*Δ are synthetically lethal. This shows that perhaps Chd1 may have a positive role in histone acetylation by Gcn5 in the SAGA complex to regulate transcription of some genes.

Chd1 has been shown to be recruited at the promoter region as well as coding region in a transcription dependent manner (Simic et al., 2003). The association between Chd1 and SAGA/SLIK complex indicates that Chd1 may have a role at the promoter region. We show here that Chd1 has a negative role in yFACT mediated TBP binding. The *pob3*(L78R) strain is defective in TBP binding during galactose induction. Deletion of *CHD1* restores TBP binding in this *pob3*(L78R) strain. This also results in an increase in RNA polymerase II binding and thereby an increase in transcriptional induction in the *pob3*(L78R) strain. This data first demonstrates a functional role of Chd1 in regulating eukaryotic transcription. Other strong evidence for the role of Chd1 in TBP binding came from the observation that deletion of *CHD1* suppresses synthetic lethalities between TBP mutations and *spt16* mutations as well as *spt16-11* and *toa2* mutations.

How does Chd1 negatively regulate TBP binding at the promoter region? The current evidence shows that Chd1 has a chromatin remodeling and chromatin assembly property

in vitro. The *Drosophila* Chd1 produces a regularly spaced nucleosome that is less inhibitory to digestion by micrococcal nuclease (MNase) assay (Lusser et al., 2005). The crude DEAE extract from a *chd1*Δ strain showed hypersensitivity to digestion by MNase (Robinson and Schultz, 2003). All this evidence shows that the *in vivo* activity of Chd1 produces a repressive chromatin structure that may be inhibitory to binding for other transcription factors such as TBP. It is also possible that Chd1 has a direct role in regulating recruitment of a transcription factor that has a negative role in TBP binding. It has been reported that Chd1 is coimmunoprecipitated with the NCoR transcriptional corepressor (Kelley et al., 1999). NCoR is associated with a histone deacetylase which has a repressive activity during transcription (Tai et al., 2003). A similar mechanism may also exist in yeast whereby Chd1 has a role in recruiting an unknown histone deacetylase at the promoter region. We have shown that histone acetylation has a positive role in TBP binding. Thereby, recruitment of a histone deacetylase complex may explain the negative regulation of TBP binding by Chd1. Further biochemical and genetic experiments are needed to decipher the exact mechanism of the role of Chd1 in TBP binding in association with yFACT.

References

Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M., and Reinberg, D. (2003). FACT facilitates transcription-dependent nucleosome alteration. *Science 301*, 1090-1093.

Biswas, D., Imbalzano, A. N., Eriksson, P., Yu, Y., and Stillman, D. J. (2004). Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TATA-binding protein-TFIIA-DNA complex. *Mol Cell Biol 24*, 8312-8321.

Biswas, D., Yu, Y., Prall, M., Formosa, T., and Stillman, D. J. (2005). The yeast FACT complex has a role in transcriptional initiation. *Mol Cell Biol 25*, 5812-5822.

Biswas, D., Dutta-Biswas, R., Mitra, D., Shibata, Y ., Strahl, B.D., Formosa, T., and Stillman, D. J. (2006). Opposing roles for Set2 and yFACT in regulating TBP binding at promoters. *Submitted*

Botstein, D., Falco, S. C., Stewart, S. E., Brennan, M., Scherer, S., Stinchcomb, D. T., Struhl, K., and Davis, R. W. (1979). Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. *Gene 8*, 17-24.

Brehm, A., Tufteland, K. R., Aasland, R., and Becker, P. B. (2004). The many colours of chromodomains. *Bioessays 26*, 133-140.

Brewster, N. K., Johnston, G. C., and Singer, R. A. (2001). A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. *Mol Cell Biol 21*, 3491-3502.

Briggs, S. D., Bryk, M., Strahl, B. D., Cheung, W. L., Davie, J. K., Dent, S. Y., Winston, F., and Allis, C. D. (2001). Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes Dev 15*, 3286-3295.

Cairns, B. R. (2005). Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr Opin Genet Dev 15*, 185-190.

Costa, P. J., and Arndt, K. M. (2000). Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. *Genetics 156*, 535-547.

de la Cruz, X., Lois, S., Sanchez-Molina, S., and Martinez-Balbas, M. A. (2005). Do protein motifs read the histone code? *Bioessays 27*, 164-175.

Dryhurst, D., Thambirajah, A. A., and Ausio, J. (2004). New twists on H2A.Z: a histone variant with a controversial structural and functional past. *Biochem Cell Biol 82*, 490-497.

Eisenmann, D. M., Arndt, K. M., Ricupero, S. L., Rooney, J. W., and Winston, F. (1992). SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. *Genes* Dev *6*, 1319-1331.

Eriksson, P., Biswas, D., Yu, Y., Stewart, J. M., and Stillman, D. J. (2004). TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. *Mol Cell Biol 24*, 6419-6429.

Formosa, T. (2003). Changing the DNA landscape: putting a SPN on chromatin. *Curr Top Microbiol Immunol 274*, 171-201.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y., and Stillman, D. J. (2001). Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *Embo Journal 20*, 3506-3517.

Formosa, T., Ruone, S., Adams, M. D., Olsen, A. E., Eriksson, P., Yu, Y., Rhoades, A. R., Kaufman, P. D., and Stillman, D. J. (2002). Defects in SPT16 or POB3 (yFACT) in *Saccharomyces cerevisiae* cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. *Genetics 162*, 1557-1571.

Fragiadakis, G. S., Tzamarias, D., and Alexandraki, D. (2004). Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for FRE2 transcriptional activation. *Embo Journal 23*, 333-342.

Havas, K., Whitehouse, I., and Owen-Hughes, T. (2001). ATP-dependent chromatin remodeling activities. *Cell Mol Life Sci 58*, 673-682.

Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481-485.

Kassavetis, G. A., and Steiner, D. F. (2006). NHP6 is a transcriptional initiation fidelity factor for RNA polymerase III transcription in vitro and in vivo. *J Biol Chem.*

Kelley, D. E., Stokes, D. G., and Perry, R. P. (1999). CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. *Chromosoma 108*, 10-25.

Kent, N. A., Eibert, S. M., and Mellor, J. (2004). Cbf1p is required for chromatin remodeling at promoter-proximal CACGTG motifs in yeast. *J Biol Chem 279*, 27116-27123.

Krogan, N. J., Kim, M., Ahn, S. H., Zhong, G., Kobor, M. S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., and Greenblatt, J. F. (2002). RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. *Mol Cell Biol 22*, 6979-6992.

Lusser, A., Urwin, D. L., and Kadonaga, J. T. (2005). Distinct activities of CHD1 and ACF in ATP-dependent chromatin assembly. *Nat Struct Mol Biol 12*, 160-166.

Mason, P. B., and Struhl, K. (2003). The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol 23*, 8323-8333.

Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S., and Reinberg, D. (1998). FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell 92*, 105-116.

Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature 400*, 284-288.

Ozer, J., Lezina, L. E., Ewing, J., Audi, S., and Lieberman, P. M. (1998). Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. *Mol Cell Biol 18*, 2559-2570.

Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates, J. R., 3rd, and Grant, P. A. (2005). Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature 433*, 434-438.

Rhoades, A. R., Ruone, S., and Formosa, T. (2004). Structural features of nucleosomes reorganized by yeast FACT and its HMG box component, Nhp6. *Mol Cell Biol 24*, 3907-3917.

Robinson, K. M., and Schultz, M. C. (2003). Replication-independent assembly of nucleosome arrays in a novel yeast chromatin reconstitution system involves antisilencing factor Asf1p and chromodomain protein Chd1p. *Mol Cell Biol 23*, 7937-7946.

Ruone, S., Rhoades, A. R., and Formosa, T. (2003). Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. *J Biol Chem 278*, 45288-45295.

Saunders, A., Werner, J., Andrulis, E. D., Nakayama, T., Hirose, S., Reinberg, D., and Lis, J. T. (2003). Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. *Science 301*, 1094-1096.

Shimojima, T., Okada, M., Nakayama, T., Ueda, H., Okawa, K., Iwamatsu, A., Handa, H., and Hirose, S. (2003). Drosophila FACT contributes to Hox gene expression through physical and functional interactions with GAGA factor. *Genes Dev 17*, 1605-1616.

Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19-27.

Simic, R., Lindstrom, D. L., Tran, H. G., Roinick, K. L., Costa, P. J., Johnson, A. D., Hartzog, G. A., and Arndt, K. M. (2003). Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo Journal 22*, 1846-1856.

Sims, R. J., 3rd, Chen, C. F., Santos-Rosa, H., Kouzarides, T., Patel, S. S., and Reinberg, D. (2005). Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *J Biol Chem 280*, 41789-41792.

Szerlong, H., Saha, A., and Cairns, B. R. (2003). The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *Embo Journal 22*, 3175-3187.

Tai, H. H., Geisterfer, M., Bell, J. C., Moniwa, M., Davie, J. R., Boucher, L., and McBurney, M. W. (2003). CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins. *Biochem Biophys Res Commun 308*, 170-176.

Tran, H. G., Steger, D. J., Iyer, V. R., and Johnson, A. D. (2000). The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *Embo Journal 19*, 2323-2331.

Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999). Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev 13*, 686-697.

Wang, W. (2003). The SWI/SNF family of ATP-dependent chromatin remodelers: similar mechanisms for diverse functions. *Curr Top Microbiol Immunol 274*, 143-169.

Wittmeyer, J., and Formosa, T. (1997). The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol Cell Biol 17*, 4178-4190.

Wittmeyer, J., Joss, L., and Formosa, T. (1999). Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. *Biochemistry 38*, 8961-8971.

Wittschieben, B. O., Fellows, J., Du, W., Stillman, D. J., and Svejstrup, J. Q. (2000). Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo. *Embo Journal 19*, 3060-3068.

Woodage, T., Basrai, M. A., Baxevanis, A. D., Hieter, P., and Collins, F. S. (1997). Characterization of the CHD family of proteins. *Proc Natl Acad Sci U S A 94*, 11472-11477.

Yang, X. J. (2004). Lysine acetylation and the bromodomain: a new partnership for signaling. *Bioessays 26*, 1076-1087.

Zhang, L., Schroeder, S., Fong, N., and Bentley, D. L. (2005). Altered nucleosome occupancy and histone H3K4 methylation in response to 'transcriptional stress'. *Embo Journal 24*, 2379-2390.

CHAPTER 8

CONCLUSIONS

Summary

The Nhp6 architectural transcription factor may have a role in promoting formation of multiprotein complexes that activate transcription. The initial observation that over-expression of TBP suppresses temperature sensitivity and defective *HO* expression in an *nhp6ab* strain suggested that Nhp6 may have a role in regulating DNA binding by TBP. TBP binding to TATA-box containing DNA is kinetically a two step process and upon binding TBP bends DNA (Zhao and Herr, 2002). The first step of the TBP binding is slow and the second step of bending DNA and forming a stable complex with DNA follows rapid kinetics. TBP binds more rapidly to bent DNA than to linear DNA. The Nhp6 protein also bends DNA upon binding, and thus Nhp6 may stimulate TBP binding by handing off bent DNA to TBP, thus promoting rapid binding. Nhp6 may have a role in regulating some essential functions *in vivo* in collaboration with other accessory transcription factors. It was unknown about the nature of these accessory factors that are required for Nhp6 functions *in vivo*.

The histone acetyl transferase Gcn5 in the SAGA complex may function as an accessory factor that regulates some essential functions *in vivo* in collaboration with Nhp6 (Yu et al., 2000). Our experiments showed that both the *nhp6ab* and *gcn5* strains are defective in *HO* expression *in vivo* (Yu et al., 2000) and a triple mutant *nhp6ab gcn5* strain is very sick and shows a temperature sensitive lethal phenotype at 30°C (Yu et al., 2000). Overexpression of TBP from a multicopy plasmid partially rescues the defect of *HO* expression in both the *nhp6ab* and *gcn5* strains (Yu et al., 2003). This indicated that perhaps one of the functions of both Gcn5 and Nhp6 is to regulate TBP binding *in vivo*.

To decipher the mechanisms by which Nhp6 regulates TBP binding we performed a genetic analysis to identify the TBP mutants that are synthetically lethal in the absence of Nhp6. We hoped these TBP mutants would prove valuable in studying other transcriptional regulators that also affect TBP binding. We isolated several TBP mutations that are scattered over the surface of TBP and form five different clusters on the TBP surface. Some of these regions of TBP have been previously shown to interact with other transcription factors. For example, some point mutations of TBP were isolated in the region where the Spt3 transcription factor interacts with TBP. Spt3 is a part of the SAGA complex (Grant et al., 1997). We showed that deletion of *SPT3* suppresses some phenotypes associated with the *nhp6ab* mutation. Deletion of *SPT3* also suppressed some of the synthetic lethalities between *nhp6ab* and TBP mutations. This result shows a functional interaction between Nhp6, Spt3, and TBP.

Nhp6 may also work with other transcription factors that remodel the structure of chromatin. Our genetic experiment showing that a *swi2 nhp6ab* triple mutant is lethal demonstrates the Swi/Snf factor that has a role in regulating some essential functions along with Nhp6 *in vivo*. *In vitro* studies show that chromatin remodeling by the Swi/Snf chromatin remodeling complex helps in TBP binding to a nucleosomal TATA sequence (Imbalzano et al., 1994). Strong genetic and biochemical studies described in Chapter 3 show that Nhp6 in association with histone acetylation by Gcn5 and chromatin remodeling by the Swi/Snf chromatin remodeling complex plays a role in regulating formation of the TBP-TFIIA-DNA complex both *in vivo* and *in vitro*.

We have also studied the functional relationship between Nhp6 and Gcn5 with the negative regulators of TBP binding such as Mot1 and the Ccr4/Not complex.

Unexpectedly, as described in Chapter 4, we observe that mutations in either *MOT1* or the components of the Ccr4/Not complex show synthetic lethal interactions with both *nhp6ab* and *gcn5*. Synthetic lethal interactions are also observed between TBP mutants and mutations in either *mot1* or *ccr4*. Suppression of some of these synthetic lethalities by overexpression of TFIIA indicates that these TBP regulators also have a positive role in regulating TBP binding *in vivo*. In fact, Mot1 has been implicated in both positive and negative regulation of transcription. This positive regulation of TBP binding by Mot1 may explain the mechanism of positive regulation of Mot1 in transcription. With this study, we have found two other positive regulators of formation of the TBP-TFIIA complex *in vivo,* along with Nhp6, Gcn5 and the Swi/Snf chromatin remodeling complex.

Our *in vitro* binding experiments described in Chapter 3 show that Nhp6 stimulates formation of the TBP-TFIIA complex *in vitro* when a TATA box within naked DNA is used in the reaction. Since TBP does not bind to a TATA box within nucleosomal DNA, we were interested in testing whether Nhp6 could help in TBP binding on the nucleosomal TATA. *In vitro* DNaseI protection assays showed that Nhp6 does not promote TBP binding on the nucleosomal TATA (Biswas and Stillman; unpublished data). Since loading of Spt16-Pob3 proteins onto a nucleosome by Nhp6 reorganizes the chromatin structure, we were interested in testing whether this nucleosome reorganization by yFACT promotes DNA-binding by TBP and TFIIA. As discussed in Chapter 5 of this thesis, the yFACT complex has a role in the formation of the TBP-TFIIA complex both *in vivo* and *in vitro*. Strong synthetic lethal or synthetic growth defect interactions are observed between *spt16* mutations and mutations in either TBP or TFIIA. Suppression experiments strongly suggest that these synthetic lethalities are a result of defects in

formation of the TBP-TFIIA complex. ChIP experiments examining TBP binding show reduced TBP binding at promoters of some genes in a *spt16* strain. *In vitro* DNaseI protection assays showed that yFACT promotes TBP binding to a nucleosomal TATA in the presence of TFIIA. These results suggest the role of Nhp6 in promoting formation of the TBP-TFIIA complex *in vivo* is mediated through yFACT. This is the first experimental observation suggesting that the yFACT complex also has a role in promoting TBP binding at promoters, along with its previously described function in transcriptional elongation. In fact in consistent with this idea, the *Drosophila* FACT complex has been shown to regulate chromatin remodeling by the GAGA factor at Hox gene promoters (Shimojima et al., 2003).

Post translational modification of histones plays an important role in regulating eukaryotic transcription (Strahl and Allis, 2000). Our earlier genetic experiments indicated that post translational histone acetylation has a role in regulating transcription by yFACT (Formosa et al., 2002). We were also interested in testing the role of histone methylation in transcriptional regulation by yFACT. Results discussed in Chapter 6 show that histone methylation at H3-K4 and H3-K36 have opposing roles in regulating yFACT mediated transcription. Combining mutations affecting yFACT with a mutation in histone H3 that prevents modification at K4 results in severe growth defects. This result shows that the histone methylation at H3-K4 and yFACT may work together to regulate some essential functions *in vivo*. In contrast, mutations preventing histone methylation at H3-K36 suppress yFACT mutant defects, suggesting opposing roles. Results in Chapter six show that yFACT stimulates TBP and RNA pol II binding at the GAL1 promoter, and histone methylation at H3-K36 has a negative or opposing role. This negative effect of

methylated H3-K36 on yFACT mediated functions at the promoters could be an indirect effect, caused by changes in activity or association of the Rpd3(S) complex at the 3' end of the genes. Although the experimental evidence described in Chapter six does not rule out this possibility, it provides a mechanistic explanation of earlier observations suggesting a negative regulatory role for histone methylation by Set2 at the promoters.

The final experimental chapter of this thesis, Chapter 7, describes the functional relationship between yFACT and the chromatin remodeling factor, Chd1. Earlier reports have shown physical and genetic interactions between Chd1 and components of the yFACT complex. However, the functional consequence of these interactions was not known. Our results strongly indicate that chromatin remodeling by Chd1 also acts in opposition to that of yFACT in promoting TBP and RNA pol II binding at promoters *in vivo*. Combining a yFACT mutation with either a *set2* or a *chd1* mutation results in strikingly similar effects, either suppression of growth defects or effects on TBP and pol II binding at promoters. This indicates that perhaps both Set2 and Chd1 may regulate the functions of yFACT at the promoter region in the same pathway. It is possible that Chd1 recognizes the histone H3-K36 methylation through one of its chromodomain motifs. In support of this idea, ChIP analysis shows a reduced Chd1 binding in a set2Δ strain as compared to a wild type strain. However, a triple mutant *spt16 chd1 set2* strain grows more slowly than either a *spt16 set2* or a *spt16 chd1* strain. This observation contradicts the proposed hypothesis. Collectively, the results presented in this thesis show a diverse and complicated functional relationship between yFACT other transcriptional regulators in regulating TBP and RNA pol II binding at promoter regions.

yFACT as a global transcription regulator at promoter region

The mammalian FACT complex was first identified as an elongation factor helping RNA polymerase II elongate through a nucleosome *in vitro* (Orphanides et al., 1998). Several subsequent reports have shown a role of the FACT complex in transcriptional elongation. One model for the mechanism of action of the FACT complex suggests that the FACT complex dissociates histones H2A and H2B from a nucleosome to facilitate passage for RNA polymerase II during transcriptional elongation (Belotserkovskaya et al., 2003). The *SPT16* gene was first identified as a transcription factor that causes the Spt$^-$ phenotype upon mutation or overexpression (Malone et al., 1991). The Spt$^-$ phenotype results from aberrant TATA site utilization at the promoter of a specific gene (Simchen et al., 1984; Winston et al., 1984). This was the first evidence suggesting a role for yFACT at promoter regions. However a defect in reestablishing the proper chromatin structure after transcription by the RNA polymerase II may also cause the Spt$^-$ phenotype (Kaplan et al., 2003). Our experimental results clearly show a role of yFACT in regulating TBP binding at the promoter region. How do we reconcile these two facts about the functions of yFACT in regulating transcription at elongation as well as at the initiation step? Is it possible that a defect in transcriptional elongation may have affect transcriptional initiation at the promoter region? Although the experimental evidence presented in this thesis does not rule out this possibility, I favor a model where yFACT has a direct role in regulating transcription at both the initiation and the elongation steps. This dual role of a transcription factor in regulating transcription has been described for other transcription factors as well. For example, Spt2 has been shown earlier to have a

role in transcriptional initiation in yeast (Katcoff et al., 1993; Pollard and Peterson, 1997; Winston et al., 1984; Yu et al., 2000). However, recent evidence has suggested a role for Spt2 in transcriptional elongation also (Nourani et al., 2006). A large amount of evidence suggests that TFIIS has a role in regulation of both transcriptional initiation as well as transcriptional elongation step (Cui and Denis, 2003; Davie and Kane, 2000; Denis et al., 2001; Kaplan et al., 2005; Kettenberger et al., 2004; Krogan et al., 2002; Kulish and Struhl, 2001; Malagon et al., 2004; Prather et al., 2005; Wery et al., 2004). Chromatin immunoprecipitation experiment has shown the presence of yFACT at the promoter region of number of genes (Mason and Struhl, 2003). It could be possible that a subset of genes in yeast require yFACT at both the promoter region as well as the coding region for regulating transcription. A genome wide analysis of presence of the yFACT complex may provide an answer to this question.

Future directions

Results described in my thesis show that yFACT also has a role in TBP binding at the promoter region along with a role in transcriptional elongation. Sequential recruitment of different transcription factors during activation of a gene has been described in several instances (Agalioti et al., 2000; Cosma et al., 1999). As RNA polymerase II makes the transition from an initiating polymerase to an elongating polymerase, there is an exchange of associated factors. Phosphorylation of the CTD of RNA polymerase II at Ser-5 and Ser-2 is an important criterion for recruitment of different transcription factors during elongation. Although yFACT has been implicated in elongation and initiation steps of transcription, the effect of yFACT in recruitment of other transcription factors is

totally unknown. To address this question, the mutant yFACT strains that are described in this thesis and elsewhere (Schlesinger and Formosa, 2000) can be used.

Transcription factors that have a role in both transcriptional initiation and elongation often have a distinct mechanism in regulating different steps. Most frequently it is found that these factors act in several different complexes to regulate different functions *in vivo*. The components of the yFACT complex have also been immunoprecipitated in complex with other transcription factors (Krogan et al., 2002). Functions of some of these factors in yFACT mediated transcription are not known. It would be interesting to test the functional relationship between these factors and the yFACT complex in regulating transcription. It would also be very exciting to test whether the role of yFACT in regulation of both initiation and elongation is independent of any other transcription factors or in association with different transcription factors in different complexes.

In this thesis I have reported several genetic interactions between components of the yFACT complex and transcription factors that have a role in either the initiation or elongation steps of transcription. How these factors coordinate with yFACT in regulating transcription is unknown. Our genetic studies may provide an excellent opportunity to elucidate the functions of these factors in regulating yFACT mediated transcription. I have shown a negative relationship between the histone methylation at H3-K36 and yFACT in regulating TBP and RNA polymerase II binding at promoters. How does the methylated H3-K36 regulate TBP binding at the promoter region? Are there some effects of mislocalization of the Rpd3(S) complex in a *set2* strain at the promoter region? Although genome-wide analysis of histone H3-K36 di- and tri-methylation revealed a preferential localization of these modifications at the 3' end of the genes, histone H3-K36

di-methylation has also been observed at promoter regions of some genes (Xiao et al., 2003b). Based on this observation, we may speculate that histone methylation at H3-K36 at promoters by Set2 helps in recruitment of the Rpd3(S) complex at the promoter region. A deletion of *SET2* would abolish this recruitment of the Rpd3(S) complex at the promoter region. This may lead to an increase in histone acetylation resulting in a disrupted chromatin structure. We have shown that histone acetylation promotes TBP binding *in vivo*. Therefore an increase in histone acetylation may facilitate TBP binding in a *set2* strain in an yFACT dependent manner. Alternatively, a disrupted chromatin structure may expose the TATA site within the nucleosome for TBP binding. We are currently pursuing studies to test these hypotheses.

The data suggest that yFACT positively regulates transcription. We have performed microarray analysis to compare the transcript levels between wild type and *spt16* strains. Expression of several genes is down-regulated in a *spt16* strain compared to a wild type strain. However, we have also observed a set of genes that shows increased expression in the *spt16* mutant (Biswas and Stillman; unpublished data). It is possible that yFACT has a negative role in regulating expression of a subset of genes. In fact, microarray experiments show that most transcriptional coactivators regulate gene expression both positively and negatively. For example, mutations affecting the Swi/Snf chromatin remodeling factor, the Mot1 factor that interacts with TBP, and the Spt3 component of the SAGA complex have both positive and negative effects on transcription (Belotserkovskaya et al., 2000; Dasgupta et al., 2002; Dudley et al., 1999; Muldrow et al., 1999; Sudarsanam et al., 2000; Wang, 2003; Yu et al., 2000). It would be interesting to identify the nature of negative regulation played by yFACT in regulating transcription.

In essence, studies on the yFACT complex described in my thesis provide several functional links between yFACT and other regulators of transcription. This knowledge can be further used to elucidate the functions of the yFACT complex in greater detail in future.

References

Agalioti, T., Lomvardas, S., Parekh, B., Yie, J., Maniatis, T., and Thanos, D. (2000). Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. Cell *103*, 667-678.

Alexander, D. E., Kaczorowski, D. J., Jackson-Fisher, A. J., Lowery, D. M., Zanton, S. J., and Pugh, B. F. (2004). Inhibition of TATA binding protein dimerization by RNA polymerase III transcription initiation factor Brf1. J Biol Chem *279*, 32401-32406.

Allard, S., Utley, R. T., Savard, J., Clarke, A., Grant, P., Brandl, C. J., Pillus, L., Workman, J. L., and Cote, J. (1999). NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *Embo Journal 18*, 5108-5119.

Arndt, K. M., Wobbe, C. R., Ricupero-Hovasse, S., Struhl, K., and Winston, F. (1994). Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. Mol Cell Biol *14*, 3719-3728.

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

Auble, D. T., Wang, D., Post, K. W., and Hahn, S. (1997). Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP-driven enzyme that dissociates TATA-binding protein from DNA. Mol Cell Biol *17*, 4842-4851.

Badarinarayana, V., Chiang, Y. C., and Denis, C. L. (2000). Functional interaction of CCR4-NOT proteins with TATAA-binding protein (TBP) and its associated factors in yeast. Genetics *155*, 1045-1054.

Bannister, A. J., and Kouzarides, T. (2005). Reversing histone methylation. Nature *436*, 1103-1106.

Basehoar, A. D., Zanton, S. J., and Pugh, B. F. (2004). Identification and distinct regulation of yeast TATA box-containing genes. Cell *116*, 699-709.

Becker, P. B., and Horz, W. (2002). ATP-dependent nucleosome remodeling. Annu Rev Biochem *71*, 247-273.

Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M., and Reinberg, D. (2003). FACT facilitates transcription-dependent nucleosome alteration. Science *301*, 1090-1093.

Belotserkovskaya, R., Sterner, D. E., Deng, M., Sayre, M. H., Lieberman, P. M., and Berger, S. L. (2000). Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol Cell Biol *20*, 634-647.

Bernstein, B. E., Humphrey, E. L., Erlich, R. L., Schneider, R., Bouman, P., Liu, J. S., Kouzarides, T., and Schreiber, S. L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci U S A *99*, 8695-8700.

Bianchi, M. E., and Agresti, A. (2005). HMG proteins: dynamic players in gene regulation and differentiation. Curr Opin Genet Dev *15*, 496-506.

Biswas, D., Imbalzano, A. N., Eriksson, P., Yu, Y., and Stillman, D. J. (2004). Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TATA-binding protein-TFIIA-DNA complex. Mol Cell Biol *24*, 8312-8321.

Biswas, D., Yu, Y., Prall, M., Formosa, T., and Stillman, D. J. (2005). The yeast FACT complex has a role in transcriptional initiation. Mol Cell Biol *25*, 5812-5822.

Boa, S., Coert, C., and Patterton, H. G. (2003). *Saccharomyces cerevisiae* Set1p is a methyltransferase specific for lysine 4 of histone H3 and is required for efficient gene expression. Yeast *20*, 827-835.

Botstein, D., Falco, S. C., Stewart, S. E., Brennan, M., Scherer, S., Stinchcomb, D. T., Struhl, K., and Davis, R. W. (1979). Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene *8*, 17-24.

Bottomley, M. J. (2004). Structures of protein domains that create or recognize histone modifications. EMBO Rep *5*, 464-469.

Brehm, A., Tufteland, K. R., Aasland, R., and Becker, P. B. (2004). The many colours of chromodomains. Bioessays *26*, 133-140.

Brewster, N. K., Johnston, G. C., and Singer, R. A. (2001). A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol Cell Biol *21*, 3491-3502.

Briggs, S. D., Bryk, M., Strahl, B. D., Cheung, W. L., Davie, J. K., Dent, S. Y., Winston, F., and Allis, C. D. (2001). Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. Genes Dev *15*, 3286-3295.

Brownell, J. E., and Allis, C. D. (1995). An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in Tetrahymena macronuclei. Proc Natl Acad Sci U S A *92*, 6364-6368.

Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843-851.

Bryk, M., Briggs, S. D., Strahl, B. D., Curcio, M. J., Allis, C. D., and Winston, F. (2002). Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in S. cerevisiae by a Sir2-independent mechanism. Curr Biol *12*, 165-170.

Buratowski, S. (2005). Connections between mRNA 3' end processing and transcription termination. Curr Opin Cell Biol *17*, 257-261.

Buratowski, S., and Zhou, H. (1992). Transcription factor IID mutants defective for interaction with transcription factor IIA. Science *255*, 1130-1132.

Cairns, B. R. (2005). Chromatin remodeling complexes: strength in diversity, precision through specialization. Curr Opin Genet Dev *15*, 185-190.

Cang, Y., Auble, D. T., and Prelich, G. (1999). A new regulatory domain on the TATA-binding protein. *Embo Journal 18*, 6662-6671.

Carrozza, M. J., Florens, L., Swanson, S. K., Shia, W. J., Anderson, S., Yates, J., Washburn, M. P., and Workman, J. L. (2005). Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. Biochim Biophys Acta *1731*, 77-87; discussion 75-76.

Cheng, X., Collins, R. E., and Zhang, X. (2005). Structural and sequence motifs of protein (histone) methylation enzymes. Annu Rev Biophys Biomol Struct *34*, 267-294.

Coleman, R. A., Taggart, A. K., Benjamin, L. R., and Pugh, B. F. (1995). Dimerization of the TATA binding protein. J Biol Chem *270*, 13842-13849.

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

Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. Cell *97*, 299-311.

Costa, P. J., and Arndt, K. M. (2000). Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. Genetics *156*, 535-547.

Cui, Y., and Denis, C. L. (2003). In vivo evidence that defects in the transcriptional elongation factors RPB2, TFIIS, and SPT5 enhance upstream poly(A) site utilization. Mol Cell Biol *23*, 7887-7901.

Cuthbert, G. L., Daujat, S., Snowden, A. W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P. D., Tempst, P., Bannister, A. J., and Kouzarides, T. (2004). Histone deimination antagonizes arginine methylation. Cell *118*, 545-553.

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

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

Davie, J. K., and Kane, C. M. (2000). Genetic interactions between TFIIS and the Swi-Snf chromatin-remodeling complex. Mol Cell Biol *20*, 5960-5973.

de la Cruz, X., Lois, S., Sanchez-Molina, S., and Martinez-Balbas, M. A. (2005). Do protein motifs read the histone code? Bioessays *27*, 164-175.

Dehe, P. M., Pamblanco, M., Luciano, P., Lebrun, R., Moinier, D., Sendra, R., Verreault, A., Tordera, V., and Geli, V. (2005). Histone H3 lysine 4 mono-methylation does not require ubiquitination of histone H2B. J Mol Biol *353*, 477-484.

Deluen, C., James, N., Maillet, L., Molinete, M., Theiler, G., Lemaire, M., Paquet, N., and Collart, M. A. (2002). The Ccr4-not complex and yTAF1 (yTaf(II)130p/yTaf(II)145p) show physical and functional interactions. Mol Cell Biol *22*, 6735-6749.

Denis, C. L., and Chen, J. (2003). The CCR4-NOT complex plays diverse roles in mRNA metabolism. Prog Nucleic Acid Res Mol Biol *73*, 221-250.

Denis, C. L., Chiang, Y. C., Cui, Y., and Chen, J. (2001). Genetic evidence supports a role for the yeast CCR4-NOT complex in transcriptional elongation. Genetics *158*, 627-634.

Doyon, Y., and Cote, J. (2004). The highly conserved and multifunctional NuA4 HAT complex. Curr Opin Genet Dev *14*, 147-154.

Dryhurst, D., Thambirajah, A. A., and Ausio, J. (2004). New twists on H2A.Z: a histone variant with a controversial structural and functional past. Biochem Cell Biol *82*, 490-497.

Dudley, A. M., Rougeulle, C., and Winston, F. (1999). The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev *13*, 2940-2945.

Eisenmann, D. M., Arndt, K. M., Ricupero, S. L., Rooney, J. W., and Winston, F. (1992). SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev *6*, 1319-1331.

Eriksson, P., Biswas, D., Yu, Y., Stewart, J. M., and Stillman, D. J. (2004). TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol Cell Biol *24*, 6419-6429.

Exinger, F., and Lacroute, F. (1992). 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr Genet *22*, 9-11.
Fazzio, T. G., Kooperberg, C., Goldmark, J. P., Neal, C., Basom, R., Delrow, J., and

Tsukiyama, T. (2001). Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. Mol Cell Biol *21*, 6450-6460.

Formosa, T. (2003). Changing the DNA landscape: putting a SPN on chromatin. Curr Top Microbiol Immunol *274*, 171-201.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y., and Stillman, D. J. (2001a). Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome- binding factor SPN. *Embo Journal 20*, 3506-3517.

Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y., and Stillman, D. J. (2001b). Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *Embo Journal 20*, 3506-3517.

Formosa, T., Ruone, S., Adams, M. D., Olsen, A. E., Eriksson, P., Yu, Y., Rhoades, A. R., Kaufman, P. D., and Stillman, D. J. (2002). Defects in SPT16 or POB3 (yFACT) in *Saccharomyces cerevisiae* cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. Genetics *162*, 1557-1571.

Fragiadakis, G. S., Tzamarias, D., and Alexandraki, D. (2004). Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for FRE2 transcriptional activation. *Embo Journal 23*, 333-342.

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

Gietz, R. D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527-534.

Goldmark, J. P., Fazzio, T. G., Estep, P. W., Church, G. M., and Tsukiyama, T. (2000). The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. Cell *103*, 423-433.

Goppelt, A., and Meisterernst, M. (1996). Characterization of the basal inhibitor of class II transcription NC2 from *Saccharomyces cerevisiae*. Nucleic Acids Res *24*, 4450-4455.

Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., *et al.* (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev *11*, 1640-1650.

Grewal, S. I., and Moazed, D. (2003). Heterochromatin and epigenetic control of gene expression. Science *301*, 798-802.

Hassan, A. H., Prochasson, P., Neely, K. E., Galasinski, S. C., Chandy, M., Carrozza, M. J., and Workman, J. L. (2002). Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell *111*, 369-379.

Havas, K., Whitehouse, I., and Owen-Hughes, T. (2001). ATP-dependent chromatin remodeling activities. Cell Mol Life Sci *58*, 673-682.

Hebbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988). A direct link between core histone acetylation and transcriptionally active chromatin. *Embo Journal 7*, 1395-1402.

Hiragami, K., and Festenstein, R. (2005). Heterochromatin protein 1: a pervasive controlling influence. Cell Mol Life Sci *62*, 2711-2726.

Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell *95*, 717-728.

Huisinga, K. L., and Pugh, B. F. (2004). A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. Mol Cell *13*, 573-585.

Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481-485.

Kadosh, D., and Struhl, K. (1997). Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. Cell *89*, 365-371.

Kadosh, D., and Struhl, K. (1998). Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol Cell Biol *18*, 5121-5127.

Kamada, K., Shu, F., Chen, H., Malik, S., Stelzer, G., Roeder, R. G., Meisterernst, M., and Burley, S. K. (2001). Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex. Cell *106*, 71-81.

Kao, C. F., Hillyer, C., Tsukuda, T., Henry, K., Berger, S., and Osley, M. A. (2004). Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. Genes Dev *18*, 184-195.

Kaplan, C. D., Holland, M. J., and Winston, F. (2005). Interaction between transcription elongation factors and mRNA 3'-end formation at the *Saccharomyces cerevisiae* GAL10-GAL7 locus. J Biol Chem *280*, 913-922.

Kaplan, C. D., Laprade, L., and Winston, F. (2003). Transcription elongation factors repress transcription initiation from cryptic sites. Science *301*, 1096-1099.

Kassavetis, G. A., and Steiner, D. F. (2006a). NHP6 is a transcriptional initiation fidelity factor for RNA polymerase III transcription in vitro and in vivo. J Biol Chem.

Kassavetis, G. A., and Steiner, D. F. (2006b). Nhp6 Is a Transcriptional Initiation Fidelity Factor for RNA Polymerase III Transcription in Vitro and in Vivo. J Biol Chem *281*, 7445-7451.

Kasten, M. M., Dorland, S., and Stillman, D. J. (1997). A large protein complex containing the yeast Sin3p and Rpd3p transcriptional regulators. Mol Cell Biol *17*, 4852-4858.

Katcoff, D. J., Yona, E., Hershkovits, G., Friedman, H., Cohen, Y., and Dgany, O. (1993). SIN1 interacts with a protein that binds the URS1 region of the yeast HO gene. Nucleic Acids Res *21*, 5101-5109.

Kelley, D. E., Stokes, D. G., and Perry, R. P. (1999). CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. Chromosoma *108*, 10-25.

Kent, N. A., Eibert, S. M., and Mellor, J. (2004). Cbf1p is required for chromatin remodeling at promoter-proximal CACGTG motifs in yeast. J Biol Chem *279*, 27116-27123.

Kettenberger, H., Armache, K. J., and Cramer, P. (2004). Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS. Mol Cell *16*, 955-965.

Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F., and Buratowski, S. (2004). Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. *Embo Journal 23*, 354-364.

Kim, T. K., Zhao, Y., Ge, H., Bernstein, R., and Roeder, R. G. (1995). TATA-binding protein residues implicated in a functional interplay between negative cofactor NC2 (Dr1) and general factors TFIIA and TFIIB. J Biol Chem *270*, 10976-10981.

Kimura, A., and Horikoshi, M. (2004). Partition of distinct chromosomal regions: negotiable border and fixed border. Genes Cells *9*, 499-508.
Kimura, A., Matsubara, K., and Horikoshi, M. (2005). A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. J Biochem (Tokyo) *138*, 647-662.

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

Kobor, M. S., and Greenblatt, J. (2002). Regulation of transcription elongation by phosphorylation. Biochim Biophys Acta *1577*, 261-275.

Kolodrubetz, D., and Burgum, A. (1990). Duplicated NHP6 genes of *Saccharomyces cerevisiae* encode proteins homologous to bovine high mobility group protein 1. J Biol Chem *265*, 3234-3239.

Krogan, N. J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M. A., Dean, K., Ryan, O. W., Golshani, A., Johnston, M., *et al.* (2003a). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol Cell *11*, 721-729.

Krogan, N. J., Keogh, M. C., Datta, N., Sawa, C., Ryan, O. W., Ding, H., Haw, R. A., Pootoolal, J., Tong, A., Canadien, V., *et al.* (2003b). A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell *12*, 1565-1576.

Krogan, N. J., Kim, M., Ahn, S. H., Zhong, G., Kobor, M. S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., and Greenblatt, J. F. (2002). RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. Mol Cell Biol *22*, 6979-6992.

Kruppa, M., Moir, R. D., Kolodrubetz, D., and Willis, I. M. (2001). Nhp6, an HMG1 protein, functions in SNR6 transcription by RNA polymerase III in S. cerevisiae. Mol Cell *7*, 309-318.

Kulish, D., and Struhl, K. (2001). TFIIS enhances transcriptional elongation through an artificial arrest site in vivo. Mol Cell Biol *21*, 4162-4168.

Kuo, M. H., vom Baur, E., Struhl, K., and Allis, C. D. (2000). Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. Mol Cell *6*, 1309-1320.

Kurdistani, S. K., and Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. Nat Rev Mol Cell Biol *4*, 276-284.

Kurdistani, S. K., Tavazoie, S., and Grunstein, M. (2004). Mapping global histone acetylation patterns to gene expression. Cell *117*, 721-733.

Laribee, R. N., Krogan, N. J., Xiao, T., Shibata, Y., Hughes, T. R., Greenblatt, J. F., and Strahl, B. D. (2005). BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. Curr Biol *15*, 1487-1493.

Lee, D. Y., Teyssier, C., Strahl, B. D., and Stallcup, M. R. (2005). Role of protein methylation in regulation of transcription. Endocr Rev *26*, 147-170.

Lindstrom, D. L., Squazzo, S. L., Muster, N., Burckin, T. A., Wachter, K. C., Emigh, C. A., McCleery, J. A., Yates, J. R., 3rd, and Hartzog, G. A. (2003). Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol Cell Biol *23*, 1368-1378.

Liu, H. Y., Badarinarayana, V., Audino, D. C., Rappsilber, J., Mann, M., and Denis, C. L. (1998). The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. *Embo Journal 17*, 1096-1106.

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature *389*, 251-260.

Lusser, A., Urwin, D. L., and Kadonaga, J. T. (2005). Distinct activities of CHD1 and ACF in ATP-dependent chromatin assembly. Nat Struct Mol Biol *12*, 160-166.

Malagon, F., Tong, A. H., Shafer, B. K., and Strathern, J. N. (2004). Genetic interactions of DST1 in *Saccharomyces cerevisiae* suggest a role of TFIIS in the initiation-elongation transition. Genetics *166*, 1215-1227.

Malone, E. A., Clark, C. D., Chiang, A., and Winston, F. (1991). Mutations in SPT16/CDC68 suppress cis- and trans-acting mutations that affect promoter function in *Saccharomyces cerevisiae*. Mol Cell Biol *11*, 5710-5717.

Margueron, R., Trojer, P., and Reinberg, D. (2005). The key to development: interpreting the histone code? Curr Opin Genet Dev *15*, 163-176.

Marmorstein, R. (2003). Structure of SET domain proteins: a new twist on histone methylation. Trends Biochem Sci *28*, 59-62.

Martin, C., and Zhang, Y. (2005). The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol *6*, 838-849.

Martinez-Campa, C., Politis, P., Moreau, J. L., Kent, N., Goodall, J., Mellor, J., and Goding, C. R. (2004). Precise nucleosome positioning and the TATA box dictate requirements for the histone H4 tail and the bromodomain factor Bdf1. Mol Cell *15*, 69-81.

Mason, P. B., and Struhl, K. (2003). The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol Cell Biol *23*, 8323-8333.

Matangkasombut, O., Buratowski, R. M., Swilling, N. W., and Buratowski, S. (2000). Bromodomain factor 1 corresponds to a missing piece of yeast TFIID. Genes Dev *14*, 951-962.

McBride, H. J., Brazas, R. M., Yu, Y., Nasmyth, K., and Stillman, D. J. (1997). Long-range interactions at the HO promoter. Mol Cell Biol *17*, 2669-2678.

Mellor, J., and Morillon, A. (2004). ISWI complexes in *Saccharomyces cerevisiae*. Biochim Biophys Acta *1677*, 100-112.

Mermelstein, F., Yeung, K., Cao, J., Inostroza, J. A., Erdjument-Bromage, H., Eagelson, K., Landsman, D., Levitt, P., Tempst, P., and Reinberg, D. (1996). Requirement of a corepressor for Dr1-mediated repression of transcription. Genes Dev *10*, 1033-1048.

Miller, T., Krogan, N. J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., Greenblatt, J. F., and Shilatifard, A. (2001). COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. Proc Natl Acad Sci U S A *98*, 12902-12907.

Mohrmann, L., and Verrijzer, C. P. (2005). Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim Biophys Acta *1681*, 59-73.

Moreau, J. L., Lee, M., Mahachi, N., Vary, J., Mellor, J., Tsukiyama, T., and Goding, C. R. (2003). Regulated displacement of TBP from the PHO8 promoter in vivo requires Cbf1 and the Isw1 chromatin remodeling complex. Mol Cell *11*, 1609-1620.

Morillon, A., Karabetsou, N., Nair, A., and Mellor, J. (2005). Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. Mol Cell *18*, 723-734.

Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N., and Mellor, J. (2003). Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell *115*, 425-435.

Muldrow, T. A., Campbell, A. M., Weil, P. A., and Auble, D. T. (1999). MOT1 can activate basal transcription in vitro by regulating the distribution of TATA binding protein between promoter and nonpromoter sites. Mol Cell Biol *19*, 2835-2845.

Naar, A. M., Lemon, B. D., and Tjian, R. (2001). Transcriptional coactivator complexes. Annu Rev Biochem *70*, 475-501.

Ng, H. H., Dole, S., and Struhl, K. (2003a). The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. J Biol Chem *278*, 33625-33628.

Ng, H. H., Robert, F., Young, R. A., and Struhl, K. (2003b). Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol Cell *11*, 709-719.

Nislow, C., Ray, E., and Pillus, L. (1997). SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol Biol Cell *8*, 2421-2436.

Noma, K., and Grewal, S. I. (2002). Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. Proc Natl Acad Sci U S A *99 Suppl 4*, 16438-16445.

Nourani, A., Robert, F., and Winston, F. (2006). Evidence that Spt2/Sin1, an HMG-like factor, plays roles in transcription elongation, chromatin structure, and genome stability in *Saccharomyces cerevisiae*. Mol Cell Biol *26*, 1496-1509.

Nourani, A., Utley, R. T., Allard, S., and Cote, J. (2004). Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *Embo Journal 23*, 2597-2607.

Oberholzer, U., and Collart, M. A. (1999). In vitro transcription of a TATA-less promoter: negative regulation by the Not1 protein. Biol Chem *380*, 1365-1370.

Okuhara, K., Ohta, K., Seo, H., Shioda, M., Yamada, T., Tanaka, Y., Dohmae, N., Seyama, Y., Shibata, T., and Murofushi, H. (1999). A DNA unwinding factor involved in DNA replication in cell-free extracts of Xenopus eggs. Curr Biol *9*, 341-350.

Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S., and Reinberg, D. (1998). FACT, a factor that facilitates transcript elongation through nucleosomes. Cell *92*, 105-116.

Orphanides, G., and Reinberg, D. (2000). RNA polymerase II elongation through chromatin. Nature *407*, 471-475.

Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature *400*, 284-288.

Ozer, J., Lezina, L. E., Ewing, J., Audi, S., and Lieberman, P. M. (1998). Association of transcription factor IIA with TATA binding protein is required for transcriptional

activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. Mol Cell Biol *18*, 2559-2570.

Palancade, B., and Bensaude, O. (2003). Investigating RNA polymerase II carboxyl-terminal domain (CTD) phosphorylation. Eur J Biochem *270*, 3859-3870.

Pokholok, D. K., Hannett, N. M., and Young, R. A. (2002). Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol Cell *9*, 799-809.

Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., Bell, G. W., Walker, K., Rolfe, P. A., Herbolsheimer, E., *et al.* (2005). Genome-wide map of nucleosome acetylation and methylation in yeast. Cell *122*, 517-527.

Pollard, K. J., and Peterson, C. L. (1997). Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol Cell Biol *17*, 6212-6222.

Prather, D. M., Larschan, E., and Winston, F. (2005). Evidence that the elongation factor TFIIS plays a role in transcription initiation at GAL1 in *Saccharomyces cerevisiae*. Mol Cell Biol *25*, 2650-2659.

Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates, J. R., 3rd, and Grant, P. A. (2005). Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature *433*, 434-438.

Pugh, B. F. (2000). Control of gene expression through regulation of the TATA-binding protein. Gene *255*, 1-14.

Raisner, R. M., Hartley, P. D., Meneghini, M. D., Bao, M. Z., Liu, C. L., Schreiber, S. L., Rando, O. J., and Madhani, H. D. (2005). Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell *123*, 233-248.

Reinberg, D., Orphanides, G., Ebright, R., Akoulitchev, S., Carcamo, J., Cho, H., Cortes, P., Drapkin, R., Flores, O., Ha, I., *et al.* (1998). The RNA polymerase II general transcription factors: past, present, and future. Cold Spring Harb Symp Quant Biol *63*, 83-103.

Rhoades, A. R., Ruone, S., and Formosa, T. (2004). Structural features of nucleosomes reorganized by yeast FACT and its HMG box component, Nhp6. Mol Cell Biol *24*, 3907-3917.

Robinson, K. M., and Schultz, M. C. (2003). Replication-independent assembly of nucleosome arrays in a novel yeast chromatin reconstitution system involves antisilencing factor Asf1p and chromodomain protein Chd1p. Mol Cell Biol *23*, 7937-7946.

Roth, S. Y., Denu, J. M., and Allis, C. D. (2001). Histone acetyltransferases. Annu Rev Biochem *70*, 81-120.

Rundlett, S. E., Carmen, A. A., Suka, N., Turner, B. M., and Grunstein, M. (1998). Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. Nature *392*, 831-835.

Ruone, S., Rhoades, A. R., and Formosa, T. (2003). Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. J Biol Chem *278*, 45288-45295.

Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., Schreiber, S. L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. Nature *419*, 407-411.

Santos-Rosa, H., Schneider, R., Bernstein, B. E., Karabetsou, N., Morillon, A., Weise, C., Schreiber, S. L., Mellor, J., and Kouzarides, T. (2003). Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. Mol Cell *12*, 1325-1332.

Saunders, A., Werner, J., Andrulis, E. D., Nakayama, T., Hirose, S., Reinberg, D., and Lis, J. T. (2003). Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. Science *301*, 1094-1096.

Schlesinger, M. B., and Formosa, T. (2000). POB3 is required for both transcription and replication in the yeast *Saccharomyces cerevisiae*. Genetics *155*, 1593-1606.

Schneider, J., Wood, A., Lee, J. S., Schuster, R., Dueker, J., Maguire, C., Swanson, S. K., Florens, L., Washburn, M. P., and Shilatifard, A. (2005). Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. Mol Cell *19*, 849-856.

Shahbazian, M. D., Zhang, K., and Grunstein, M. (2005). Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. Mol Cell *19*, 271-277.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., and Casero, R. A. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell *119*, 941-953.

Shi, Y. J., Matson, C., Lan, F., Iwase, S., Baba, T., and Shi, Y. (2005). Regulation of LSD1 histone demethylase activity by its associated factors. Mol Cell *19*, 857-864.

Shimojima, T., Okada, M., Nakayama, T., Ueda, H., Okawa, K., Iwamatsu, A., Handa, H., and Hirose, S. (2003). Drosophila FACT contributes to Hox gene expression through physical and functional interactions with GAGA factor. Genes Dev *17*, 1605-1616.

Sikorski, R. S., and Hieter, P. (1989a). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19-27.

Sikorski, R. S., and Hieter, P. (1989b). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19-27.

Simchen, G., Winston, F., Styles, C. A., and Fink, G. R. (1984). Ty-mediated gene expression of the LYS2 and HIS4 genes of *Saccharomyces cerevisiae* is controlled by the same SPT genes. Proc Natl Acad Sci U S A *81*, 2431-2434.

Simic, R., Lindstrom, D. L., Tran, H. G., Roinick, K. L., Costa, P. J., Johnson, A. D., Hartzog, G. A., and Arndt, K. M. (2003). Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo Journal 22*, 1846-1856.

Sims, R. J., 3rd, Belotserkovskaya, R., and Reinberg, D. (2004). Elongation by RNA polymerase II: the short and long of it. Genes Dev *18*, 2437-2468.

Sims, R. J., 3rd, Chen, C. F., Santos-Rosa, H., Kouzarides, T., Patel, S. S., and Reinberg, D. (2005). Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J Biol Chem *280*, 41789-41792.

Stargell, L. A., and Struhl, K. (1995). The TBP-TFIIA interaction in the response to acidic activators in vivo. Science *269*, 75-78.

Strahl, B. D., and Allis, C. D. (2000). The language of covalent histone modifications. Nature *403*, 41-45.

Sudarsanam, P., Iyer, V. R., Brown, P. O., and Winston, F. (2000). Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A *97*, 3364-3369.

Suka, N., Suka, Y., Carmen, A. A., Wu, J., and Grunstein, M. (2001). Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. Mol Cell *8*, 473-479.

Syntichaki, P., Topalidou, I., and Thireos, G. (2000). The Gcn5 bromodomain co-ordinates nucleosome remodelling. Nature *404*, 414-417.

Szerlong, H., Saha, A., and Cairns, B. R. (2003). The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *Embo Journal 22*, 3175-3187.

Tai, H. H., Geisterfer, M., Bell, J. C., Moniwa, M., Davie, J. R., Boucher, L., and McBurney, M. W. (2003). CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins. Biochem Biophys Res Commun *308*, 170-176.

Tran, H. G., Steger, D. J., Iyer, V. R., and Johnson, A. D. (2000). The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *Embo Journal 19*, 2323-2331.

Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999). Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. Genes Dev *13*, 686-697.

Utley, R. T., Ikeda, K., Grant, P. A., Cote, J., Steger, D. J., Eberharter, A., John, S., and Workman, J. L. (1998). Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature *394*, 498-502.

Vignali, M., Hassan, A. H., Neely, K. E., and Workman, J. L. (2000). ATP-dependent chromatin-remodeling complexes. Mol Cell Biol *20*, 1899-1910.

Wang, A., Kurdistani, S. K., and Grunstein, M. (2002). Requirement of Hos2 histone deacetylase for gene activity in yeast. Science *298*, 1412-1414.

Wang, W. (2003). The SWI/SNF family of ATP-dependent chromatin remodelers: similar mechanisms for diverse functions. Curr Top Microbiol Immunol *274*, 143-169.

Wang, Y., Wysocka, J., Sayegh, J., Lee, Y. H., Perlin, J. R., Leonelli, L., Sonbuchner, L. S., McDonald, C. H., Cook, R. G., Dou, Y., *et al.* (2004). Human PAD4 regulates histone arginine methylation levels via demethylimination. Science *306*, 279-283.

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

Wery, M., Shematorova, E., Van Driessche, B., Vandenhaute, J., Thuriaux, P., and Van Mullem, V. (2004). Members of the SAGA and Mediator complexes are partners of the transcription elongation factor TFIIS. *Embo Journal 23*, 4232-4242.

Winkler, G. S., Kristjuhan, A., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J. Q. (2002). Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo. Proc Natl Acad Sci U S A *99*, 3517-3522.

Winston, F., Chaleff, D. T., Valent, B., and Fink, G. R. (1984). Mutations affecting Ty-mediated expression of the HIS4 gene of *Saccharomyces cerevisiae*. Genetics *107*, 179-197.

Wittmeyer, J., and Formosa, T. (1997). The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. Mol Cell Biol *17*, 4178-4190.

Wittmeyer, J., Joss, L., and Formosa, T. (1999). Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. Biochemistry *38*, 8961-8971.

Wittschieben, B. O., Fellows, J., Du, W., Stillman, D. J., and Svejstrup, J. Q. (2000). Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo. *Embo Journal 19*, 3060-3068.

Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J. F., *et al.* (2003a). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell *11*, 267-274.

Wood, A., Schneider, J., Dover, J., Johnston, M., and Shilatifard, A. (2003b). The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J Biol Chem *278*, 34739-34742.

Woodage, T., Basrai, M. A., Baxevanis, A. D., Hieter, P., and Collins, F. S. (1997). Characterization of the CHD family of proteins. Proc Natl Acad Sci U S A *94*, 11472-11477.

Wu, J., Suka, N., Carlson, M., and Grunstein, M. (2001). TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. Mol Cell *7*, 117-126.

Xiao, B., Wilson, J. R., and Gamblin, S. J. (2003a). SET domains and histone methylation. Curr Opin Struct Biol *13*, 699-705.

Xiao, T., Hall, H., Kizer, K. O., Shibata, Y., Hall, M. C., Borchers, C. H., and Strahl, B. D. (2003b). Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. Genes Dev *17*, 654-663.

Xiao, T., Kao, C. F., Krogan, N. J., Sun, Z. W., Greenblatt, J. F., Osley, M. A., and Strahl, B. D. (2005). Histone H2B ubiquitylation is associated with elongating RNA polymerase II. Mol Cell Biol *25*, 637-651.

Xie, J., Collart, M., Lemaire, M., Stelzer, G., and Meisterernst, M. (2000). A single point mutation in TFIIA suppresses NC2 requirement in vivo. *Embo Journal 19*, 672-682.

Xu, F., Zhang, K., and Grunstein, M. (2005). Acetylation in histone H3 globular domain regulates gene expression in yeast. Cell *121*, 375-385.

Yang, X. J. (2004). Lysine acetylation and the bromodomain: a new partnership for signaling. Bioessays *26*, 1076-1087.

Yu, Y., Eriksson, P., Bhoite, L. T., and Stillman, D. J. (2003). Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. Mol Cell Biol *23*, 1910-1921.

Yu, Y., Eriksson, P., and Stillman, D. J. (2000). Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol Cell Biol *20*, 2350-2357.

Zhang, H., Roberts, D. N., and Cairns, B. R. (2005a). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell *123*, 219-231.

Zhang, L., Schroeder, S., Fong, N., and Bentley, D. L. (2005b). Altered nucleosome occupancy and histone H3K4 methylation in response to 'transcriptional stress'. *Embo Journal 24*, 2379-2390.

Zhang, Y., and Reinberg, D. (2001). Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev *15*, 2343-2360.

Zhao, X., and Herr, W. (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.