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*

Debabrata Biswas, Yaxin Yu, Doyel Mitra and David J. Stillman[1]

Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah 84132

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

[1]*Corresponding author:* Department of Pathology, University of Utah, 30 North 1900 E., Room 5C126 SOM, Salt Lake City, UT 84132-2501. E-mail: david.stillman@path.utah.edu

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.

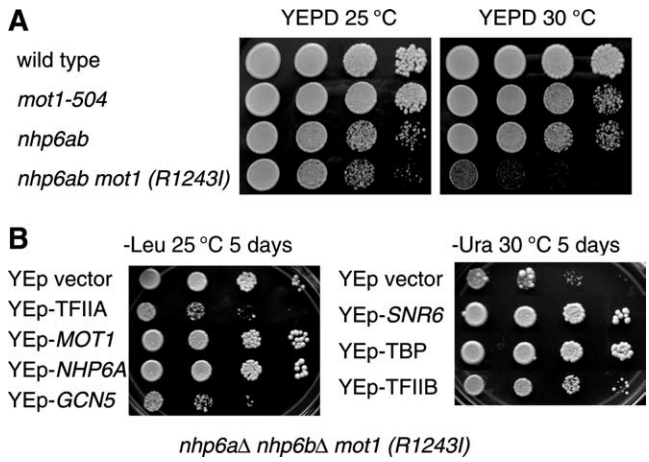

Figure 1.—Genetic interactions of *MOT1* with *NHP6*. (A) *nhp6ab mot1(R1243I)* is lethal at 30°. Strains DY150 (wild type), DY7139 (*nhp6ab*), DY7463 [*mot1(R1243I)*], and DY7847 [*nhp6ab mot1(R1243I)*] were plated on YEPD medium for 4 days at 25° or for 2 days at 30°. (B) Strain DY7847 [*nhp6ab mot1 (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

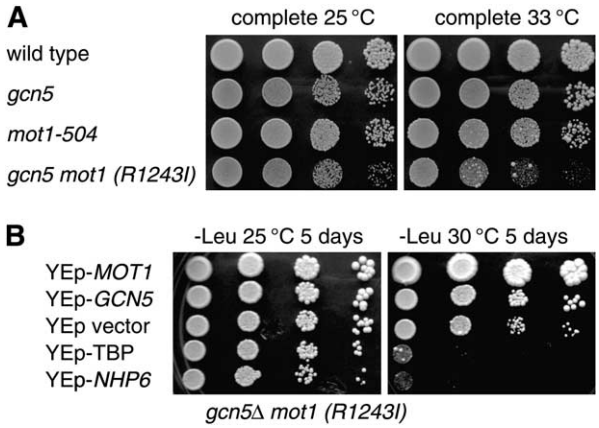

Figure 2.—Genetic interactions of *MOT1* with *GCN5*. (A) *gcn5 mot1(R1243I)* is lethal at 33°. Strains DY150 (wild type), DY5265 (*gcn5*), DY7463 [*mot1(R1243I)*], and DY7841 [*gcn5 mot1(R1243I)*] were plated on complete medium for 3 days at either 25° or 33°. (B) Strain DY7841 [*gcn5 mot1(R1243I)*]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

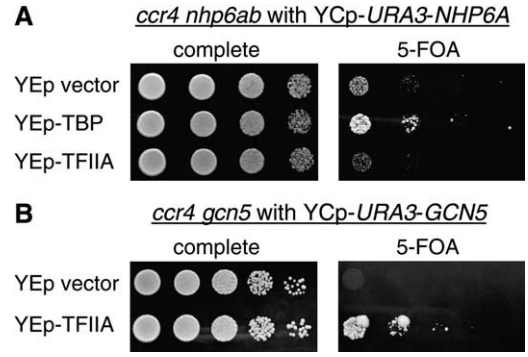

Figure 3.—Genetic interactions of *CCR4* with *GCN5* and *NHP6*. (A) The *nhp6ab ccr4* synthetic lethality is suppressed by TBP overexpression. Strain DY7441 (*nhp6ab ccr4* with a YCp–*URA3–NHP6B* plasmid) was transformed with the indicated *LYS2* multicopy plasmids, and dilutions were plated at 30° 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 *nhp6a*Δ/+ *nhp6b*Δ/+ *ccr4*Δ/+ 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

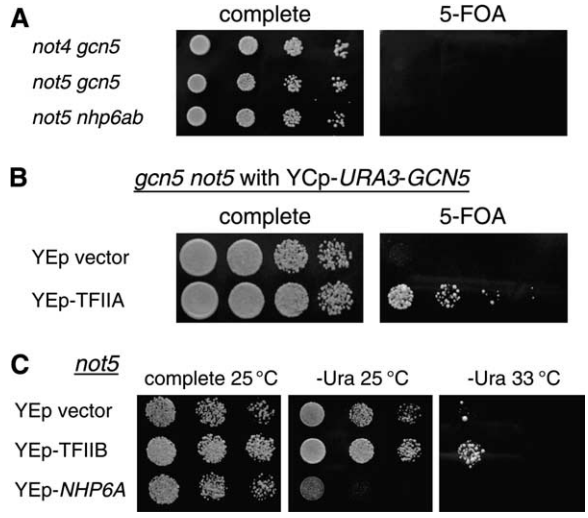

FIGURE 4.—Genetic interactions of *NOT* genes with *GCN5* and *NHP6*. (A) A *not5* mutation is synthetic lethal with *gcn5* and with *nhp6*, and *not4* is synthetic lethal with *gcn5*. Dilutions of strains DY8618 (*not4 gcn5*), DY8628 (*not5 gcn5*), and DY8625 (*not5 nhp6ab*), each carrying a YCp–URA3 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

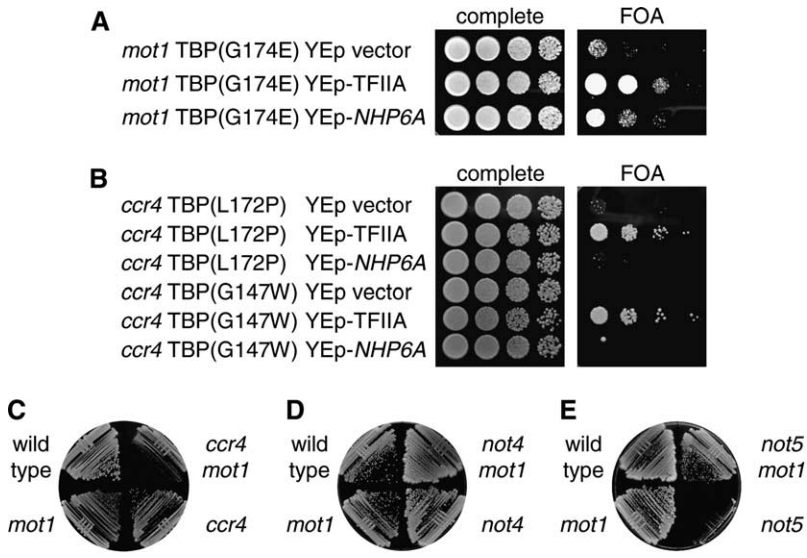

FIGURE 5.—Suppression of *mot1 spt15* and *ccr4 spt15* synthetic lethality. (A) The *mot1(R1243I) spt15*(G174E) [TBP(G174E)] synthetic lethality is suppressed by TFIIA or Nhp6 overexpression. Strain DY9383 [*mot1(R1243I) spt15Δ* with a YCp–*URA3*–*SPT15*(wild-type) plasmid] was transformed with the YCp–*TRP1*–TBP(G174E) 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.

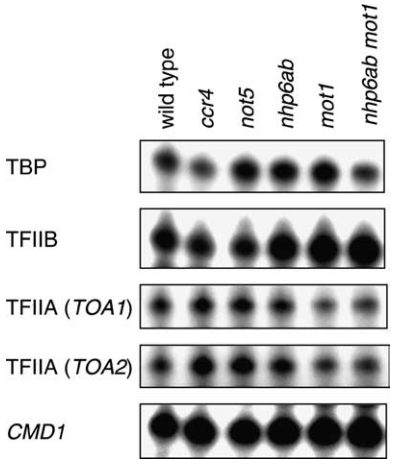

FIGURE 6.—Basal factor expression is not affected by mutants. RNA was prepared from strains DY150 (wild type), DY7176 (*ccr4*), DY8626 (*not5*), DY7139 (*nhp6a*), DY7463 [*mot1 (R1243I)*], and DY7847 [*nhp6ab mot1(R1243I)*] grown at 25° and used for S1 nuclease protection assays to measure TBP (*SPT15*),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

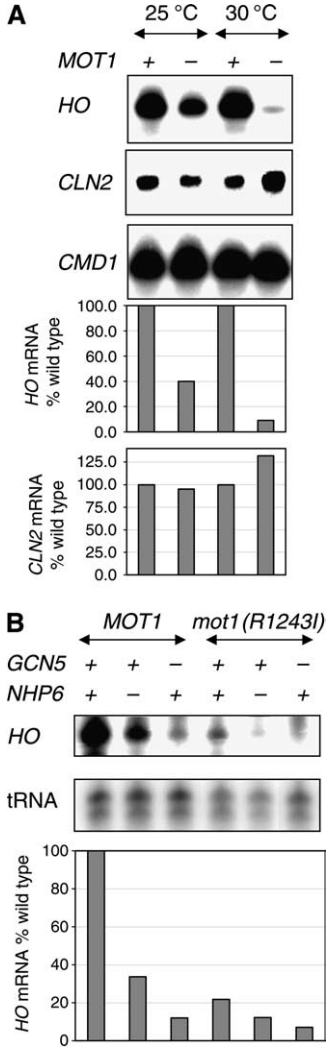

FIGURE 7.—*HO* expression is reduced in mutant strains. (A) RNA was prepared from strains DY150 (wild type) and DY7462 [*mot1(R1243I)*] grown at either 25° or 30° and used for S1 nuclease protection assays to measure *HO*, *CLN2*, and *CMD1* (internal control) RNA levels. (B) RNA was prepared from strains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

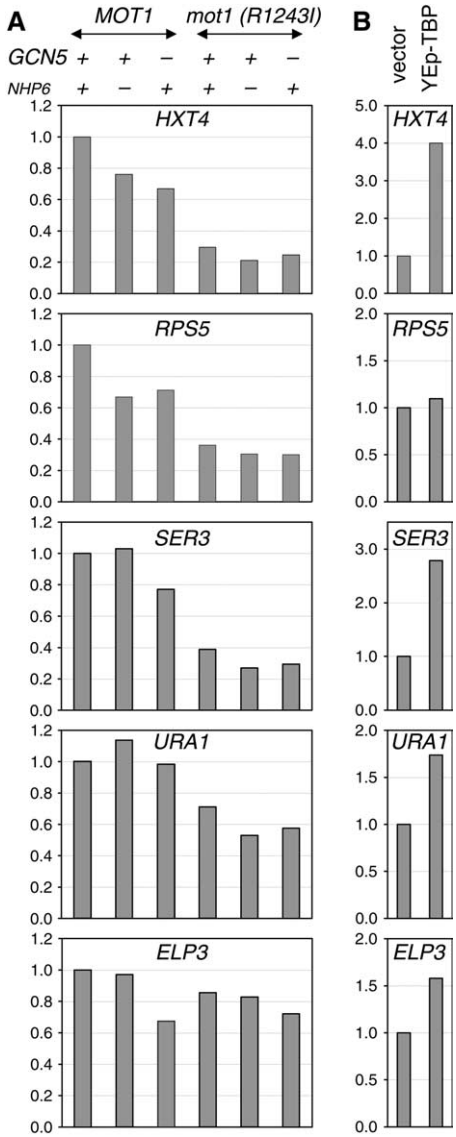

Figure 8.—Mutations affect TBP binding to promoters. TBP occupancy at the indicated promoters was determined by chromatin immunoprecipitation with polyclonal anti-TBP antisera and quantitative PCR, using cells that had been grown at 25° and then shifted to 37° for 3 hr. Relative binding is shown, after 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 Mot1 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 mot1(R1243I)* double mutant shows a growth defect, and more significantly, *mot1(R1243I)* suppresses *not5* growth defects. The *not5* mutant is unable to grow at 30°, but the *not5 mot1(R1243I)* double mutant does grow (Figure 5E). This suggests that the *not5* mutant is defective in some aspect of transcriptional activation and that the *mot1(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 Mot1 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 *mot1* are synthetically lethal, and this synthetic lethality can be suppressed by overexpression of TFIIA (Madison and Winston 1997). Interestingly, both *spt3* and *mot1* are synthetic lethal with substitutions in the Toa1 subunit of TFIIA (Madison and Winston 1997). Both Spt3 and Mot1 are required for nucleosome remodeling at Gal4-dependent promoters (Topalidou *et al.* 2004). Moreover, Spt3 is required for Mot1 to bind to the *GAL1* promoter under inducing conditions, and Mot1 is similarly required for Spt3 binding (Topalidou *et al.* 2004). We note a number of synthetic lethalities or growth defects among these genes: *mot1 spt3* (Madison and Winston 1997), *ccr4 spt3*, and *ccr4 mot1*. We attribute these additive genetic defects to a common target, TBP.

mot1 mutations reduce TBP binding to certain promoters (Andrau *et al.* 2002), while TBP binding to the *INO1* promoter was unaffected by a *mot1* mutation (Dasgupta *et al.* 2005). We chose to study TBP binding in strains with a *mot1* mutation alone or in combination with *gcn5* and *nhp6ab* mutations. Our results show that TBP binding at selected promoters is significantly reduced in a *mot1* mutant (Figure 8). TBP binding is further reduced, although modestly, when *mot1* is combined with either *gcn5* or *nhp6ab*. The *mot1 nhp6ab* strain shows reduced binding of basal transcription factors, and suppression of this defect by overexpression of TBP further supports our hypothesis that the *mot1* and *nhp6ab* mutations cause defects in TBP binding. RNA analysis shows that *HO* expression is reduced in a *mot1* 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, Mot1, 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 Mot1p 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 Mot1, 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