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Regulation of TATA-Binding Protein Binding by the SAGA Complex and the Nhp6 High-Mobility Group Protein

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Transcriptional activation of the yeast *HO* gene involves the sequential action of DNA-binding and chromatin-modifying factors. Here we examine the role of the SAGA complex and the Nhp6 architectural transcription factor in *HO* regulation. Our data suggest that these factors regulate binding of the TATA-binding protein (TBP) to the promoter. A *gcn5* mutation, eliminating the histone acetyltransferase present in SAGA, reduces the transcription of *HO*, but expression is restored in a *gcn5 spt3* double mutant. We conclude that the major role of Gcn5 in *HO* activation is to overcome repression by Spt3. Spt3 is also part of SAGA, and thus two proteins in the same regulatory complex can have opposing roles in transcriptional regulation. Chromatin immunoprecipitation experiments show that TBP binding to *HO* is very weak in wild-type cells but markedly increased in an *spt3* mutant, indicating that Spt3 reduces *HO* expression by inhibiting TBP binding. In contrast, it has been shown previously that Spt3 stimulates TBP binding to the *GAL1* promoter as well as *GAL1* expression, and thus, Spt3 regulates these promoters differently. We also find genetic interactions between TBP and either Gcn5 or the high-mobility-group protein Nhp6, including multicopy suppression and synthetic lethality. These results suggest that, while Spt3 acts to inhibit TBP interaction with the *HO* promoter, Gcn5 and Nhp6 act to promote TBP binding. The result of these interactions is to limit TBP binding and *HO* expression to a short period within the cell cycle. Furthermore, the synthetic lethality resulting from combining a *gcn5* mutation with specific TBP point mutations can be suppressed by the overexpression of transcription factor IIA (TFIIA), suggesting that histone acetylation by Gcn5 can stimulate transcription by promoting the formation of a TBP/TFIIA complex.

Binding of the TATA-binding protein (TBP) to promoters is an essential event in transcriptional activation by RNA polymerase II (22, 37). In vitro studies have shown that binding by TBP is followed by that of transcription factor IIA (TFIIA) and TFIIB and that this TBP/TFIIA/TFIIB/DNA complex can then recruit other factors, resulting in the formation of a preinitiation complex. Thus, regulation of DNA binding by TBP could be a critical mechanism for regulating gene expression (41).

The SAGA complex has at least 14 subunits and regulates transcriptional activity by modulating chromatin structure (52, 59). Genetic analysis suggests that SAGA is encoded by three groups of genes. Deletion of the *SPT7* or *SPT20* gene causes severe growth defects. Other SAGA genes in this group (*TRA1* and TBP-associated factors [TAFs]) are essential for viability, but these genes encode proteins that are also present in other transcriptional regulatory complexes. It is believed that Spt7 and Spt20 are part of the core of SAGA, because *spt7* and *spt20* mutations affect the structural integrity of the complex. In contrast, the Gcn5 and Spt3 modules may function on the periphery of SAGA, as mutations in these genes result in an intact SAGA complex. These mutants have modest but distinct phenotypes, suggesting different functions (42, 53). *GCN5* encodes a histone acetyltransferase (8), and it is required for chromatin acetylation at promoters in vivo (28).

Spt3 has been shown to physically interact with TBP, and genetic experiments show allele-specific interactions between *SPT3* and TBP (19). Spt3 is required for expression of the *GAL1* gene, and chromatin immunoprecipitation experiments show that TBP binding to the *GAL1* promoter requires *SPT3* (17). Experiments with specific alleles of Spt3 and TBP show that a specific interaction between these proteins is required for *GAL1* activation (30). While Spt3 stimulates TBP binding to the *GAL1* promoter, other experiments suggest that Spt3 can act oppositely, inhibiting TBP binding to the *HIS3* and *TRP3* promoters (3).

High-mobility-group (HMG) proteins are small, abundant chromatin proteins that bend DNA sharply and modulate gene expression (10). The yeast Nhp6 HMG-like factor is encoded by two redundant genes, *NHP6A* and *NHP6B*. *HO* expression is reduced in an *nhp6a nhp6b* mutant, and genetic analysis suggests that Nhp6 and Gcn5 function in the same pathway of *HO* activation (60). Several experiments by Paull et al. (38) suggest that Nhp6 stimulates transcription by promoting the formation of preinitiation complexes. In vivo studies with chimeric promoter constructs suggest that Nhp6 acts at core promoters, and in vitro binding experiments show that Nhp6 stimulates the formation of a TBP/TFIIA/DNA complex that has an increased affinity for TFIIB. Since formation of a TBP/TFIIA/TFIIB/DNA complex is required for transcriptional initiation, Nhp6 may stimulate transcription by promoting formation of this complex.

The transcriptional activation of the yeast *HO* gene is preceded by the sequential binding of factors (6, 11, 12). First, the Swi5 DNA-binding factor binds far upstream and facilitates binding of the Swi/Snf and Mediator complexes. These factors promote the binding of the SAGA complex containing the Gcn5 histone acetyltransferase, resulting in changes in histone acetylation at the *HO* promoter (26). Finally, the SBF DNA-

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TABLE 1. Yeast strains

Strain	Genotype
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY151	*MAT*α *ade2 can1 his3 leu2 trp1 ura3*
DY1696	*MAT***a** *sin4*::*URA3 ade2 can1 his3 leu2 trp1 ura3*
DY2381	*MAT*α *nhp6a*::*URA3 nhp6b*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY2382	*MAT***a** *nhp6a*::*URA3 nhp6b*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY3398	*MAT***a** *ade2 can1 his3 leu2 trp1*
DY5199	*MAT*α *gcn5*::*TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5454	*MAT*α *ho*(URS2Δ) *ade2 can1 his3 leu2 trp1 ura3*
DY5457	*MAT*α *gcn5*::*TRP1 ho*(URS2Δ) *ade2 can1 his3 leu2 trp1 ura3*
DY5925	*MAT***a** *gcn5*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY5926	*MAT*α *gcn5*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY5929	*MAT***a** *sin4*::*URA3 gcn5*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY6155	*MAT***a** *nhp6a*::*KanMX nhp6b*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY6178	*MAT***a** *spt8*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY6219	*MAT***a** *spt3*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6277	*MAT***a** *gcn5*::*HIS3 spt3*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6404	*MAT***a** *ahc1*::*LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY6422	*MAT***a** *gcn5*::*HIS3 spt8*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY6441	*MAT***a** *nhp6a*::*KanMX nhp6b*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY6603	*MAT***a** *gcn5*::*HIS3 URA3*::*GCN5(E173Q) ade2 can1 his3 leu2 trp1*
DY6707	*MAT***a** *spt20*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY6728	*MAT***a** *nhp6a*::*URA3 nhp6b*::*HIS3 spt8*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY6758	*MAT***a** *swi6*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6760	*MAT*α *swi6*::*TRP1 spt3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY6771	*MAT*α *nhp6a*::*URA3 nhp6b*::*HIS3 gcn5*::*TRP1 spt8*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY6776	*MAT***a** *nhp6a*::*KanMX nhp6b*::*ADE2 gcn5*::*HIS3 spt3*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6778	*MAT***a** *nhp6a*::*KanMX nhp6b*::*ADE2 spt3*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6857	*MAT*α *gcn5*::*HIS3 nhp6a*::*KanMX nhp6b*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY7202	*MAT*α *ho*(URS2Δ) *spt3*::*ADE2 gcn5*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY7206	*MAT*α *ho*(URS2Δ) *spt3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY7242	*MAT***a** *spt15*::*LEU2* + *SPT15*(YCp-*URA3*) *ade2 ade3 can1 his3 leu2 trp1 ura3*
DY7244	*MAT*α *nhp6a*::*KanMX nhp6b*::*HIS3 spt15*::*LEU2* + *SPT15*(YCp-*URA3*) *ade2 ade3 can1 his3 leu2 lys2 trp1 ura3*
DY7247	*MAT***a** *GALp*::*CDC20*::*ADE2 SPT15-HA*::*URA3 spt3*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY7250	*MAT*α *GALp*::*CDC20*::*ADE2 SPT15-HA*::*URA3 ade2 can1 his3 leu2 trp1 ura3*
DY7515	*MAT*α *gcn5*::*HIS3 spt15*::*LEU2* + *SPT15*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7593	*MAT***a** *spt15*::*LEU2* + *spt15-21*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7592	*MAT*α *spt3*::*ADE2 URA3*::*spt3-401 ade2 can1 his3 leu2 trp1*
DY7593	*MAT***a** *spt3*::*ADE2 URA3*::*spt3-401 spt15*::*LEU2* + *spt15-21*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1*
DY7596	*MAT*α *gcn5*::*HIS3 spt3*::*ADE2 URA3*::*spt3-401 ade2 can1 his3 leu2 trp1*
DY7597	*MAT*α *gcn5*::*HIS3 spt15*::*LEU2* + *spt15-21*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7598	*MAT***a** *gcn5*::*HIS3 spt3*::*ADE2 URA3*::*spt3-401 spt15*::*LEU2* + *spt15-21*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1*
DY7604	*MAT*α *gcn5*::*HIS3 spt3*::*ADE2 spt15*::*LEU2* + *spt15-21*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY7723	*MAT***a** *nhp6a*::*KanMX nhp6b*::*HIS3 spt3*::*ADE2 spt15*::*LEU2* + *SPT15*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
DY8158	*MAT***a** *gcn5*::*HIS3 spt15*::*KanMX* + *SPT15*(YCp-*URA3*) *ade2 can1 his3 leu2 trp1 ura3*
FY61	*MAT***a** *his4-917*δ *leu2 ura3*
FY1006	*MAT***a** *spt7*::*LEU2 his4-917*δ *lys2-173R2 leu2*

binding factor, composed of the Swi4 and Swi6 factors, binds to the promoter and it is believed that SBF ultimately activates *HO* transcription.

In this report, we provide evidence that Gcn5 and Nhp6 promote expression of the yeast *HO* gene via TBP. We also show that Spt3 acts to inhibit *HO* expression by blocking TBP binding to the *HO* promoter. Interactions among these factors are important for regulating other yeast genes, as indicated by the observation of multiple genetic interactions between TBP and both Gcn5 and Nhp6 and the suppression of mutant growth defects by either an *spt3* mutation or by TFIIA overexpression.

MATERIALS AND METHODS

All strains listed in Table 1 are isogenic in the W303 background (55), except for FY61 and FY1006, which are S288c strains kindly provided by Fred Winston (20). Standard genetic methods were used for strain construction (43, 46). W303 strains with disruptions in *ahc1*, *gcn5*, *nhp6a*, *nhp6b*, *sin4*, *spt20*, *spt3*, *spt8*, and *swi6* have been described previously (16, 18, 58, 60), and these were crossed to other W303 strains to produce the strains used in this study.

The integrated *GALp*::*CDC20* allele marked with *ADE2* has been described previously (6), as has the *SPT15*-hemagglutinin (HA)-epitope-tagged allele marked with *URA3* (29), provided by Kevin Struhl. A "marker swap" strategy (14), using plasmids M3926 (*leu2*::*KanMX*), M3927 (*ura3*::*KanMX*), M2371 (*his3*::*ADE2*), and M3938 (*trp1*::*ADE2*), was used to change disruption markers. By using this approach, *spt15*::*LEU2* was converted to *spt15*::*KanMX*, *nhp6a*::*URA3* was converted to *nhp6a*::*KanMX*, *nhp6b*::*HIS3* was converted to *nhp6b*::*ADE2*, and *spt3*::*TRP1* was converted to *spt3*::*ADE2*.

A strain with the *ho*(URS2Δ) promoter deletion (removing nucleotides [nt] −929 to −129 from ATG) was made in two steps by first inserting *URA3* into the promoter and then replacing *URA3* with the *ho*(URS2Δ) construct by selection on 5-fluoroorotic acid (5-FOA). Plasmid pDE124-1, provided by Fred Winston, was cleaved with *Eco*RV to integrate the *spt3-401* allele at the *URA3* locus. Plasmid *gcn5*(E173Q)-pRS306, provided by Shelley Berger, was cleaved with *Nsi*I to integrate the *gcn5*(E173Q) allele at the *URA3* locus. The *SPT15* gene was disrupted with plasmid pKA23, provided by Karen Arndt. All gene disruptions and promoter replacements were confirmed by Southern blot analysis.

Plasmids are described in Table 2. Cells were grown in yeast extract-peptone-

TABLE 2. Plasmid list

Plasmid	Description	Source or reference
YEp351	YEp-*LEU2* vector	23
pRS314	YCp-*TRP1* vector	48
YEplac195	YEp-*LEU2* vector	21
gcn5(E173Q)	*gcn5*(E173Q) in pRS306	56
pSH223	TBP (wild type) in YEp-*LEU2* plasmid	Steve Hahn
pDE28-6	TBP (wild type) in YCp-*URA3* plasmid	19
pTM8	TBP (wild type) in YCp-*TRP1* plasmid	25
pDE58-1	TBP(G174E) (*spt15-21*) in YCp-*TRP1* plasmid	19
M4492	TBP (F237D) in YCp-*TRP1* plasmid	49
M4493	TBP (K138T,Y139A) in YCp-*TRP1* plasmid	50
M4495	TBP (E188A) in YCp-*TRP1* plasmid	31
pSH346	TFIIA in YEp351	Steve Hahn
pKA23	*spt15*::*LEU2* disruptor	2
M2371	*his3*::*ADE2* marker swap converter	Manuscript in preparation
M3926	*leu2*::*KanMX* marker swap converter	Manuscript in preparation
M3927	*ura3*::*KanMX* marker swap converter	Manuscript in preparation
M3938	*trp1*::*ADE2* marker swap converter	Manuscript in preparation

dextrose (YEPD) medium (46) at 30°C, except where the use of higher 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. 5-FOA medium was prepared as described previously (7).

RNA levels were determined by S1 nuclease protection assays with the *HO* and *CMD1* probes as described previously (5). Strains with the *GAL1*:*CDC20* allele were synchronized by removing galactose to arrest cells in mitosis, and then galactose was added to release from the arrest, as described previously (6). Chromatin immunoprecipitations were performed as described previously (6) by using 12CA5 monoclonal antibody to the HA epitope.

RESULTS

Gcn5 is required for *HO* activation to counteract Spt3 repression. The sequential recruitment of multiple transcription factors is required for activation of the yeast *HO* gene (12). SAGA is one of these factors, and a mutation in the *GCN5* histone acetyltransferase subunit reduces *HO* expression (39). This defect in *HO* activation can be suppressed by mutations in *SIN3*, *RPD3*, or *SIN4* (60). Sin3 and Rpd3 are part of a histone deacetylase complex, while Sin4 is present in two distinct transcriptional regulatory complexes, SAGA itself and the Mediator (32; P. Grant and J. Workman, personal communication). To address the mechanism by which a *sin4* mutation suppresses the Gcn5 requirement for *HO* expression, we asked whether mutations in other Mediator or SAGA components had similar effects. Mutations in Mediator genes *GAL11*, *MED2*, and *HRS1*, which are in the Sin4 subcomplex of the Mediator, result in reduced *HO* expression, which is opposite to the effect of a *sin4* mutation (6). In contrast, mutations in the genes encoding SAGA components Spt3 and Spt8 did not reduce *HO* expression (Fig. 1A). Importantly, these *spt* mutations suppressed the *gcn5* defect and *HO* expression in the *gcn5 spt3* and *gcn5 spt8* strains resembled that of *gcn5 sin4* mutants (Fig. 1A through C). This suggests that Sin4 affects *HO* regulation through its role as a member of SAGA.

These experiments have been done using strains with a *gcn5* gene disruption. It is possible that the histone acetyltransferase activity of Gcn5 is not required for *HO* activation but that the mere presence of the Gcn5 polypeptide as a component of SAGA would be sufficient for *HO* expression. To address this question, we constructed an isogenic strain with a Gcn5 (E173Q) mutation that eliminates the histone acetyltransferase catalytic activity (56). The experiment depicted in Fig. 1D shows that the Gcn5 catalytic mutant does not express *HO* and thus the histone acetyltransferase activity is required for *HO* expression.

The fact that *spt3* and *spt8* mutations allow *HO* to be expressed in the absence of the Gcn5 histone acetyltransferase suggests that Gcn5's major role is to overcome the repression caused by Spt3 and Spt8. If this were true, then *spt7* or *spt20* mutations, which disrupt the structural integrity of SAGA (53), should not affect *HO* expression, since it would result in the loss of both a repressor and the factor responsible for overcoming the repression. In fact, *HO* expression was not affected by *spt7* or *spt20* mutations (Fig. 1E and F) and thus, eliminating

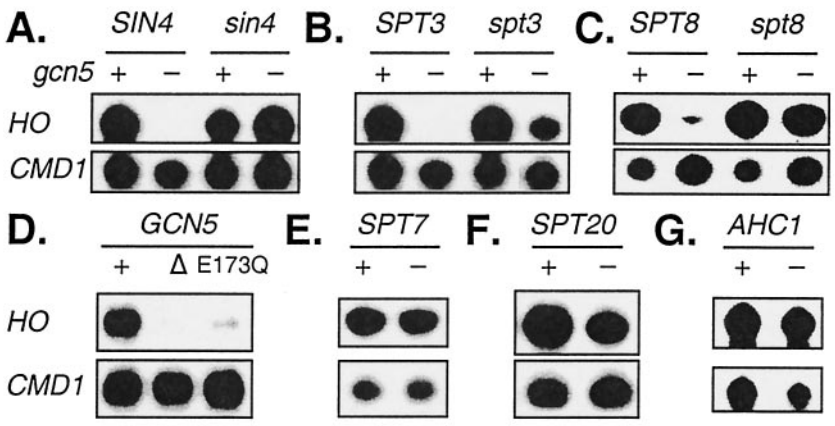

FIG. 1. *spt* mutations allow *HO* expression in the absence of Gcn5. S1 nuclease protection assays were performed with probes specific for *HO* and *CMD1* (internal control). RNAs were prepared from the following strains: (A) DY150, DY5925, DY1696, and DY5929; (B) DY150, DY5925, DY6219, and DY6277; (C) DY150, DY5925, DY6178, and DY6422; (D) DY151, DY5926, and DY6603; (E) FY61 and FY1006; (F) DY150 and DY6707; (G) DY150 and DY6404.

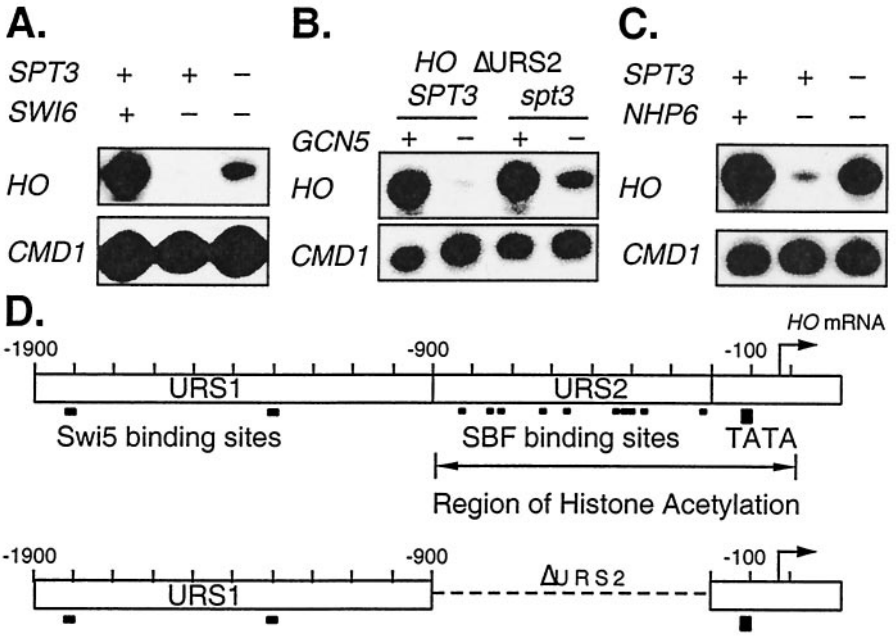

FIG. 2. *spt3* effect is independent of SBF. S1 nuclease protection assays were performed with probes specific for *HO* and *CMD1* (internal control). RNAs were prepared from the following strains: (A) DY150, DY6758, and DY6760; (B) DY5454, DY5457, DY7206, and DY7202; (C) DY150, DY2382, and DY6778. (D) The upper map shows the positions of the Swi5-binding sites in URS1, the SBF binding sites in URS2, and the TATA element of the *HO* promoter, as well as the region that is subject to *GCN5*-dependent histone acetylation (26). The lower map shows the ΔURS2 version of the *HO* promoter.

SAGA entirely does not affect *HO* expression. In addition to being a component of SAGA, Gcn5 is also present in a second complex, ADA, and an *ahc1* mutation disrupts the integrity of the ADA complex (18). *HO* expression was unaffected in an *ahc1* mutant (Fig. 1G), indicating that the ADA complex is not required for *HO* expression. These experiments support a model in which Gcn5 functions within SAGA to overcome the repression meditated by SAGA components such as Sin4, Spt3, and Spt8.

***spt3* effect is independent of SBF.** The SBF DNA-binding factor, composed of the Swi4 and Swi6 subunits, is the last factor recruited to the *HO* promoter, but SBF does not bind to *HO* in a *gcn5* mutant (12). *HO* is expressed in a *swi6 sin4* double mutant (60), and thus, *sin4* suppresses the *swi6* defect. The experiment depicted in Fig. 2A shows that an *spt3* mutation also partially suppressed *swi6*. These results indicate that a major function of the SBF activator is to overcome the repression caused by Sin4 and Spt3 in SAGA.

The *HO* promoter has been divided into three regions: the URS1 region (nt −1900 to −1000), where the Swi5 factor binds; the URS2 region (nt −900 to −200), where the SBF factor binds; and the TATA region (36). Histones in the URS2 and TATA regions of the *HO* promoter, but not in URS1, are acetylated in a Gcn5-dependent manner (26). To address which promoter regions confer Gcn5 dependence on the *HO* promoter, we deleted the URS2 region of the promoter and found that the *HO* ΔURS2 promoter required Gcn5 for activation (Fig. 2B). Importantly, an *spt3* mutation could suppress the requirement for Gcn5 for activation of the *HO* ΔURS2 promoter to a similar extent as the native promoter (compare Fig. 2B and 1B). The *HO* ΔURS2 promoter contains the URS1 and TATA regions, but of these, only the TATA region is acetylated by Gcn5 (26). Additionally, while an *spt20* mutation did not affect *HO* (Fig. 1E), expression of a *HO-lacZ* reporter is reduced in an *spt20* (*ada5*) mutant (34) although this reporter contains the *CYC1* TATA region instead of the *HO* TATA region. These results suggest that it is the *HO* TATA region that is the target of regulation by Gcn5 and Spt3.

***spt3* mutation allows *HO* expression in an *nhp6* mutant.** Nhp6 is a small HMG-like protein encoded by two redundant genes, *NHP6A* and *NHP6B*. Nhp6 is required for *HO* expression, as *HO* is not expressed in an *nhp6a nhp6b* mutant (60). Because a *sin4* mutation allows *HO* to be expressed in the absence of either Gcn5 or Nhp6, we tested whether an *spt3* mutation also suppresses *nhp6. HO* was expressed in both the *nhp6a nhp6b spt3* (Fig. 2C) and *nhp6a nhp6b spt8* (data not shown) strains, and thus, *spt3* or *spt8* can suppress the requirement for either Gcn5 or Nhp6 in *HO* activation, similar to *sin4*. Furthermore, overexpression of Nhp6B partially suppresses the *gcn5* defect in *HO* expression (60), extending the genetic interactions between *GCN5* and *NHP6*.

Suppression of growth phenotypes by *spt3* and *spt8*. Mutations in *SPT3* and *SPT8* allow *HO* to be expressed in the absence of either Gcn5 or Nhp6, but is this suppression a general phenomenon? A *nhp6a nhp6b* double-mutant strain is defective for growth at 37°C (13), but this temperature sensitivity was suppressed by *spt3* or *spt8* mutations (Fig. 3A). Furthermore, combining a *gcn5* mutation with *nhp6a nhp6b* resulted in a severe growth defect in the *gcn5 nhp6a nhp6b* strain (Fig. 3B) (60), suggesting that Gcn5 and Nhp6 have functional targets in common. However, the *gcn5 nhp6a nhp6b* growth defect could be suppressed by *spt3* and *spt8* mutations (Fig. 3B) and also by a *sin4* mutation (60). These results again suggest

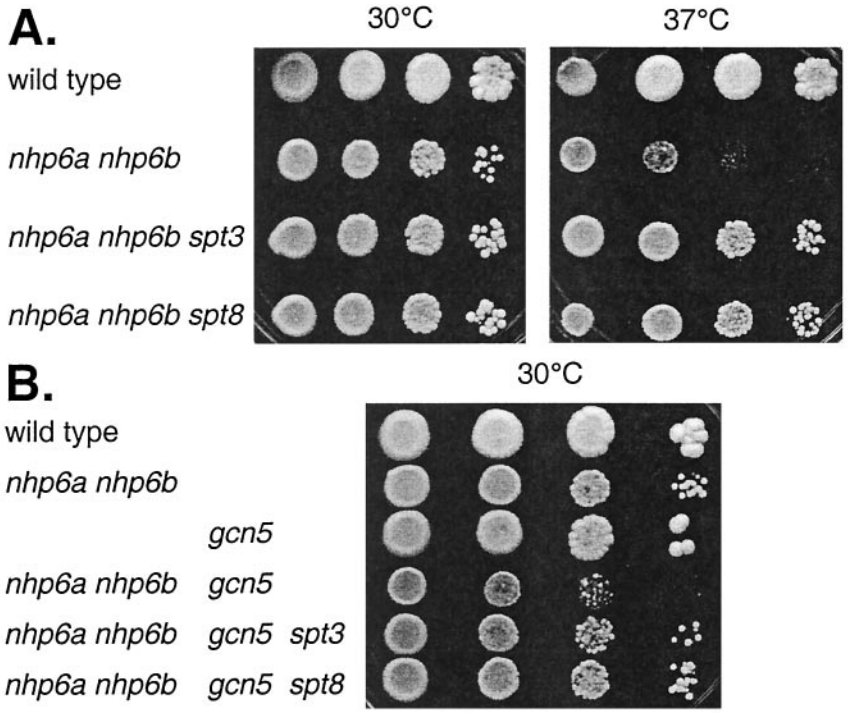

FIG. 3. Suppression of growth phenotypes by *spt3*. (A) The following strains were plated on YEPD medium and grown for 5 days at 37°C: DY2381, DY6778, and DY6728. (B) The following strains were plated on YEPD medium and grown for 4 days at 30°C: DY6857, DY6776, and DY6771.

that Spt3 and Spt8 act in opposition to Gcn5 and Nhp6 at certain critical promoters.

TBP as the critical target of regulation. Several lines of evidence support a model in which TBP is the critical target of this regulation by Spt3. Spt3 interacts with TBP in both biochemical and genetic experiments (17, 19), and it has been suggested that Spt3 inhibits TBP binding at some promoters (3). We found that overexpression of TBP from a multicopy plasmid allowed an *nhp6a nhp6b* mutant to grow at 38°C (Fig. 4A). This restoration of growth in the absence of Nhp6 by high-copy-number TBP was consistent with the hypothesis that Nhp6 normally promotes TBP binding. TBP overexpression also partially restored *HO* expression in both *gcn5* and *nhp6a nhp6b* mutants (Fig. 4B), and similarly, Nhp6B overexpression increases *HO* expression in a *gcn5* mutant (60).

Recent work has shown that the temperature sensitivity of *nhp6a nhp6b* mutants is due in part to defects in the transcription of RNA polymerase III genes (27, 33, 35). The suppression of *nhp6a nhhp6b* mutants by YEp-TBP is consistent with the fact that TBP is also part of the RNA polymerase III factor TFIIIB (24). The fact that TBP overexpression partially suppresses the *nhp6* defect in *HO* expression (Fig. 4B) suggests that the genetic interaction between Nhp6 and TBP also affects RNA polymerase II transcription.

To analyze Nhp6's role in regulating TBP binding to the TATA element by the chromatin immunoprecipitation method, we attempted to construct an *nhp6a nhp6b* mutant bearing an HA-epitope-tagged version of TBP. However, we found that this *nhp6a nhp6b* HA-TBP strain was inviable, suggesting that the HA epitope tag at the N terminus diminishes TBP function in a way not tolerated in the absence of Nhp6. We also tagged TBP at the C terminus, and this TBP-HA allele was also synthetically lethal with *nhp6*. To further analyze these allele-specific effects, we determined whether other previously characterized viable TBP mutations were synthetically lethal with *nhp6*. We constructed a strain with the *SPT15* (encoding TBP), *NHP6A*, and *NHP6B* genes deleted; TBP is essential for via-

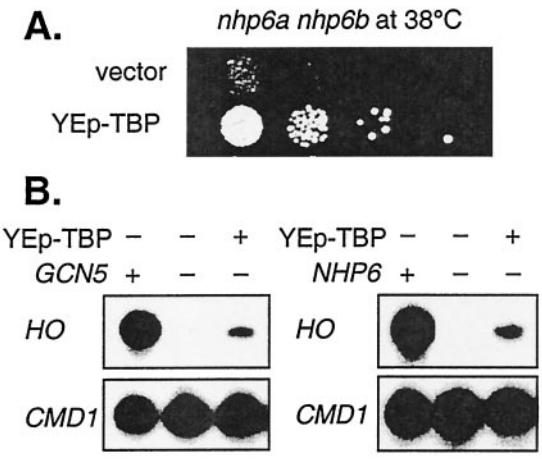

FIG. 4. Suppression by overexpression of TBP. (A) Strain DY6155 (*nhp6a nhp6b*) was transformed with plasmids YEp351 (YEp-*LEU2* vector) or pSH223 (YEp-*LEU2* with TBP), and dilutions were plated on YEPD and grown for 2 days at 38°C. (B) RNA was prepared from cells grown without uracil to select for plasmids and used for S1 nuclease protection assays. RNAs were prepared from the following strains: DY3398 (wild type) with YEplac195 vector, DY5199 (*gcn5*) with YEplac195 vector, DY5199 (*gcn5*) with pJG18-2 (YEp-*URA3* with TBP), DY3398 (wild type) with YEplac195 vector, DY6441 (*nhp6a nhp6b*) with YEplac195 vector, and DY6441 (*nhp6a nhp6b*) with pJG18-2 (YEp-*URA3* with TBP).

bility, and this strain is kept alive with wild-type TBP on a YCp-*URA3* plasmid. Since strains with a wild-type *URA3* gene cannot grow on media containing 5-FOA (7) and the plasmid supplies the essential TBP protein, this strain cannot grow on 5-FOA (Fig. 5A, line 1). Introducing a *TRP1* plasmid with the wild-type TBP gene allows for loss of the *URA3* plasmid and growth on 5-FOA (Fig. 5A, line 2). The TBP(K138T, Y139A) and TBP(F237D) mutations eliminate interaction with TFIIA in vitro (49, 50), and plasmids with these TBP mutations did not permit growth of DY7244 on 5-FOA (Fig. 5A, lines 3 and 4). Importantly, these TBP mutations did support viability in an *NHP6*$^+$ strain, as evidenced by growth on 5-FOA plates (Fig. 5B). Thus TBP(K138T, Y139A) and TBP(F237D) cannot support growth in a cell lacking Nhp6, consistent with the suggestion that Nhp6 stimulates TBP-TFIIA interaction (38). In contrast, the TBP(E188A) mutation, which affects interaction with TFIIB (31), was viable in the absence of Nhp6 (Fig. 5A). Finally, the TBP(G174E) mutation, which affects interaction with Spt3 (19), was also lethal without Nhp6 (Fig. 5A).

We have found that an *spt3* mutation can suppress growth defects in both the *nhp6a nhp6b* and *gcn5 nhp6a nhp6b* strains (Fig. 3). We were therefore interested in determining whether an *spt3* mutation could suppress the synthetic lethality observed between *nhp6* and mutant TBP alleles. We constructed an *spt15 nhp6a nhp6b spt3* strain with wild-type TBP on a YCp-*URA3* plasmid. This strain was transformed with *TRP1* plasmids carrying either wild-type TBP, TBP(K138T, Y139A), or no insert, and these transformed strains were then plated on 5-FOA medium to determine whether the cells were viable after loss of the YCp-*URA3* plasmid with wild-type TBP. The TBP(K138T, Y139A) mutation was viable and reasonably healthy in the *nhp6a nhp6b spt3* strain (Fig. 5C), while this same mutation was lethal in the *nhp6a nhp6b SPT3* strain (Fig. 5A). This was not true of all TBP alleles, as *spt3* suppressed the *nhp6a nhp6b* TBP(G174E) lethality only weakly and *spt3* did not suppress the *nhp6a nhp6b* TBP(F237D) lethality at all (data not shown). This demonstrates that the lethality of the TBP(K138T, Y139A) mutation in the absence of Nhp6 could be suppressed by an *spt3*-null mutation and suggests that Nhp6 and Spt3 act in opposition on TBP.

We have seen that the absence of either Gcn5 or Nhp6 reduces *HO* expression, and these defects can be suppressed by *sin4*, *spt3*, or *spt8*. Additionally, there are synergistic effects of combining *gcn5* with *nhp6a nhp6b* mutations. With this similarity in mind, we wanted to determine whether these TBP point mutations were lethal in the absence of the Gcn5 histone acetyltransferase. We constructed a *gcn5 spt15* yeast strain, kept alive with wild-type TBP on a YCp-*URA3* plasmid. This strain was transformed with the *TRP1* plasmids containing the TBP alleles, and growth on 5-FOA in the absence of wild-type TBP on YCp-*URA3* was assessed. The results showed that TBP (K138T, Y139A) is lethal in the *gcn5* mutant (Fig. 5D), while the TBP(F237D) mutation showed no growth defect in absence of Gcn5 (data not shown). The TBP(G174E) allele showed significant genetic interactions with *gcn5* and *spt3*, as described below.

As the TBP(K138T, Y139A) mutation eliminates interaction with TFIIA in vitro (50), it seemed possible that overexpression of TFIIA might suppress the synthetic lethality observed with *nhp6* or *gcn5* mutations. A multicopy plasmid with *TOA1* and *TOA2*, the two genes encoding the TFIIA subunits, was transformed into the *nhp6 spt15* and *gcn5 spt15* strains that had two TBP plasmids, a YCp-*URA3* plasmid with wild-type TBP and a YCp-*TRP1* plasmid with TBP(K138T, Y139A). The *nhp6* strain with TBP(K138T, Y139A) and the YEp-TFIIA plasmid was unable to grow on 5-FOA medium (data not shown), and thus, overexpression of TFIIA cannot suppress this synthetic lethality. However, this YEp-TFIIA plasmid did allow the *gcn5 spt15* strain with TBP(K138T, Y139A) to grow on 5-FOA (Fig. 6), and thus, TFIIA overexpression can suppress the lethality caused by the TBP(K138T, Y139A) mutation in the *gcn5* mutant. TFIIA is required for this effect, as the YEp vector, without any gene insert, does not allow this strain to grow on 5-FOA. A similar experiment was done with the TBP(G174E) allele, and the results showed that TFIIA overexpression suppresses the synthetic growth defect in the *gcn5* TBP(G174E) double mutant (Fig. 6).

These experiments show important genetic interactions between TBP and both Nhp6 and Gcn5. The TBP(K138T, Y139A) point mutation, which is viable in a *NHP6A NHP6B GCN5* strain, is lethal in the absence of either Nhp6 or Gcn5. Additionally, TBP has been shown to interact with Spt3 (19), and an *spt3* mutation suppresses the synthetic lethality of the TBP (K138T, Y139A) *nhp6a nhp6b* genotype, suggesting that Spt3 and Nhp6 function in opposing directions. Finally, overexpression of TFIIA overcomes the lethality caused by combining the *gcn5* mutation with either TBP(K138T, Y139A) or TBP (G174E), suggesting that Gcn5 functions in vivo to promote the formation of a TBP/TFIIA complex.

***spt3* mutations affect TBP binding to *HO*.** TBP and Spt3 physically interact, and genetic interactions between TBP and *SPT3* mutations are highly allele specific, suggesting that this interaction is functionally important (19, 30). Spt3 promotes expression of the *GAL1* gene (17), and thus, Spt3 functions as an activator at *GAL1*, although it inhibits *HO* expression. It should be noted that the experiments from the Winston lab (17, 30) used a strain background different from those used here. They examined TBP binding to the *GAL1* promoter in strains with specific TBP and *SPT3* mutations (30). TBP (G174E), expressed from the *spt15-21* allele, appears to be defective for interaction with Spt3, as *spt15-21* phenotypes can be suppressed by *spt3-401* [Spt3(E240K)]. TBP binds to the *GAL1* promoter under inducing conditions, and this binding requires Spt3 (17). The TBP(G174E) mutant does not bind to *GAL1*, but this defect is suppressed by Spt3(E240K), suggesting that TBP(G174E) and Spt3(E240K) interact like the two wild-type proteins (30).

We investigated whether these specific alleles that affect the interaction between TBP and Spt3 would affect the regulation of *HO* expression by constructing isogenic strains differing at the *GCN5*, *SPT3*, and *SPT15* (TBP) loci. Strains with a *gcn5* or a *spt15-21* [TBP(G174E)] single mutation grew well, but the *gcn5 spt15-21* double mutant was extremely sick (Fig. 7A). This suggests that TBP(G174E), which shows no defect in binding DNA in vitro (19), has severe defects when chromatin is underacetylated due to the *gcn5* mutation. We found that the *spt3-401* mutation, but not a *spt3*-null mutation, suppresses this defect. One explanation for these results is that Spt3 stimulates TBP binding to certain promoters such as *GAL1* (17), but wild-type Spt3 cannot interact with TBP(G174E) and thus

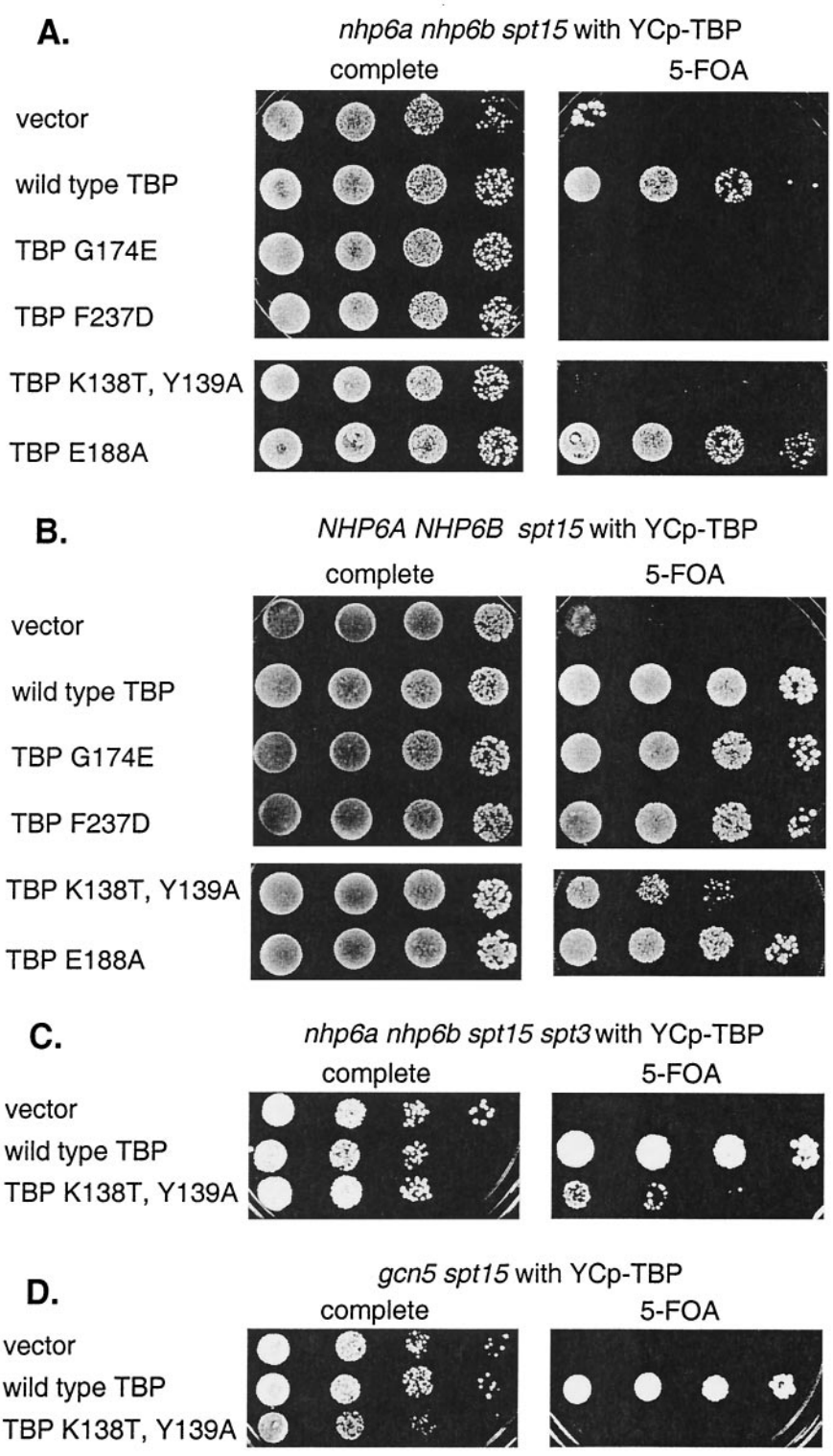

FIG. 5. Genetic interactions with TBP. (A) Strain DY7244 (*nhp6a nhp6b spt15* with wild-type TBP on a YCp-*URA3* plasmid) was transformed with the indicated YCp-*TRP1* plasmid, and dilutions were plated on either synthetic complete or 5-FOA plates and grown for 3 days at 30°C. (B) Strain DY7242 (*spt15* with wild-type TBP on a YCp-*URA3* plasmid) was transformed with the indicated YCp-*TRP1* plasmid, and dilutions were plated on either synthetic complete or 5-FOA plates and grown for 3 days at 30°C. (C) Strain DY7723 (*nhp6a nhp6b spt15 spt3* with wild-type TBP on a YCp-*URA3* plasmid) was transformed with the indicated YCp-*TRP1* plasmid, and dilutions were grown at 30°C on either YEPD plates for 3 days or 5-FOA plates for 4 days. (D) Strain DY7515 (*gcn5 spt15* with wild-type TBP on a YCp-*URA3* plasmid) was transformed with the indicated YCp-*TRP1* plasmid, and dilutions were grown at 30°C on either YEPD plates for 3 days or 5-FOA plates for 4 days.

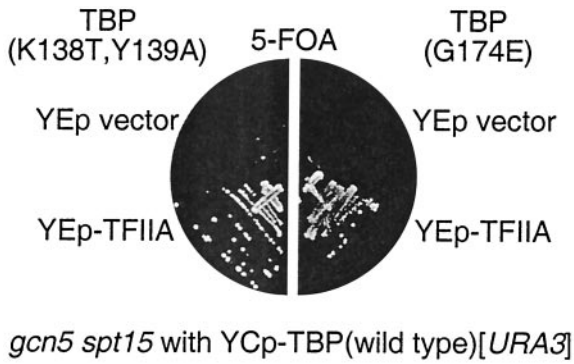

FIG. 6. TFIIA overexpression suppresses the *gcn5* TBP(K138T, Y139A) synthetic lethality. Strain DY8158 (*gcn5 spt15* with wild-type TBP on a YCp-*URA3* plasmid) was transformed with either M4493 [TBP(K138T, Y139A) in YCp-*TRP1*] or pDE58-1 [TBP(G174E) in YCp-*TRP1*] and either YEp351 or pSH346 (LEU2), selecting for the *TRP1* and *LEU2* plasmids. Transformants were grown on 5-FOA plates for either 7 days at 25°C (left half) or 3 days at 30°C (right half).

cannot stimulate TBP binding. This stimulation of TBP binding by Spt3 becomes critical in a *gcn5* mutant, and the ability of Spt3(E240K) to interact with TBP(G174E) restores healthy growth.

HO expression was reduced in a *gcn5* mutant (Fig. 7B, lane 5), but *HO* expression was restored largely in the *gcn5* TBP (G174E) double mutant (lane 6). This result is consistent with the suggestion that TBP(G174E) does not interact with Spt3, and thus, TBP(G174E) is insensitive to repression by Spt3. Combining the TBP(G174E) and Spt3(E240K) mutations in the *gcn5* background, where Spt3(E240K) can interact with and inhibit TBP(G174E), resulted in the loss of *HO* expression (lane 8). This allele-specific interaction provides strong support for the hypothesis that Spt3 inhibits TBP binding to *HO*. These results are similar to those of experiments with these alleles showing that Spt3 inhibits the basal expression of *TRP3* and *HIS3* (3).

Although Spt3 promotes the binding of TBP to the *GAL1* promoter (17), our data suggest that Spt3 inhibits TBP binding to *HO* TATA. Cosma et al. (12) reported that they were unable to detect TBP binding to *HO* by chromatin immunoprecipitation, a result that would be seen if TBP binds only very briefly to this cell-cycle-regulated promoter. We used chromatin immunoprecipitation assays to examine the binding of HA-epitope-tagged TBP to the *HO* promoter in *SPT3* and *spt3* strains that had been synchronized in the cell cycle by a *CDC20* arrest-and-release protocol (6). At various time intervals following release from the cell cycle arrest, samples were taken for *HO* RNA measurement and for chromatin immunoprecipitation. RNA measurements showed a large increase in *HO* mRNA levels in the *spt3* mutant compared to that in the wild type, particularly at the later time points (Fig. 8A). For the chromatin immunoprecipitation experiment, sheared chromatin was prepared, TBP-HA was immunoprecipitated, and the DNA present in the immunoprecipitated material was analyzed by PCR (Fig. 8B). HA-TBP binding to the *PGK1* promoter and the *TRA1* open reading frame were assessed as positive and negative controls, respectively. While only minimal binding of TBP-HA to *HO* was seen in the wild-type strain, consistent with a previous report (12), strong TBP-HA binding to *HO* was seen in the *spt3* mutant. This experiment demonstrates that Spt3 inhibits the binding of TBP to the *HO* promoter.

DISCUSSION

The Gcn5 histone acetyltransferase and the Nhp6 HMG protein are required for expression of the yeast *HO* gene, but a mutation in the *SPT3* gene allows *HO* to be expressed in the absence of either Gcn5 or Nhp6. Spt3 interacts with the TBP, and our data suggest that Gcn5 and Nhp6 stimulate *HO* transcription by promoting TBP binding.

HO activation involves the sequential binding of transcription factors to the promoter (12). We suggest that TBP may be the last factor to bind to *HO* and that TBP binding may trigger *HO* transcription. In vitro binding experiments suggest that TBP is the first factor to bind DNA in the formation of a preinitiation complex (9). However, this is not necessarily the case at specific promoters in vivo, as TBP is the last factor to bind to the beta interferon promoter, after Mediator and RNA polymerase II (1). Mediator binds to the *HO* promoter very early, and binding of RNA polymerase to *HO* as assayed by chromatin immunoprecipitation is much more robust than that for TBP (6, 11). Further work is needed to determine the order of RNA polymerase II and TBP binding to the *HO* promoter. As cells approach the commitment point in the cell cycle (START), the Swi6 component of the SBF factor is activated, directly or indirectly, by the Cdc28 cyclin-dependent kinase (57). SBF already bound to the TATA proximal region of the *HO* promoter is now activated, and we suggest that it promotes binding of TBP, as Swi6 has been shown to interact with TBP-containing complexes (44).

In vitro studies show that some factors remain bound at the promoter after transcriptional initiation, including Mediator and TBP, and that these factors promote subsequent rounds of reinitiation (61). Mediator binds stably to *HO* (6, 11), and thus, continued TBP binding after initiation could promote rapid transcriptional reinitiation. However, TBP binds to *HO* only very transiently, with increased binding seen in an *spt3* mutant (Fig. 8B). Thus Spt3 may function at *HO* to actively displace TBP from the promoter following transcriptional initiation. *HO* encodes an endonuclease, and it may be advantageous to limit expression of the endonuclease gene product by actively blocking the reinitiation pathway.

Our genetic data suggest that TBP binding can be stimulated by the Nhp6 HMG protein and the Gcn5 histone acetyltransferase (Fig. 9). These results include the suppression of defects caused by *nhp6* and *gcn5* mutations by overexpression of TBP (Fig. 4) and synthetic lethality caused by combining TBP mutations with either *nhp6* or *gcn5* (Fig. 5). How do these factors regulate TBP binding? Zhao and Herr (62) have recently shown that TBP binding to DNA is a two-step process, starting with an unstable complex containing unbent DNA that slowly isomerizes into a stable complex with bent DNA. HMG proteins bend DNA, and by doing so, an HMG protein could promote formation of the stable TBP/bent DNA complex. In vitro experiments show that mammalian HMG proteins stimulate TBP binding to DNA (15, 47, 54). Moreover, TFIIB stimulates the formation of a stable TBP/DNA complex (62),

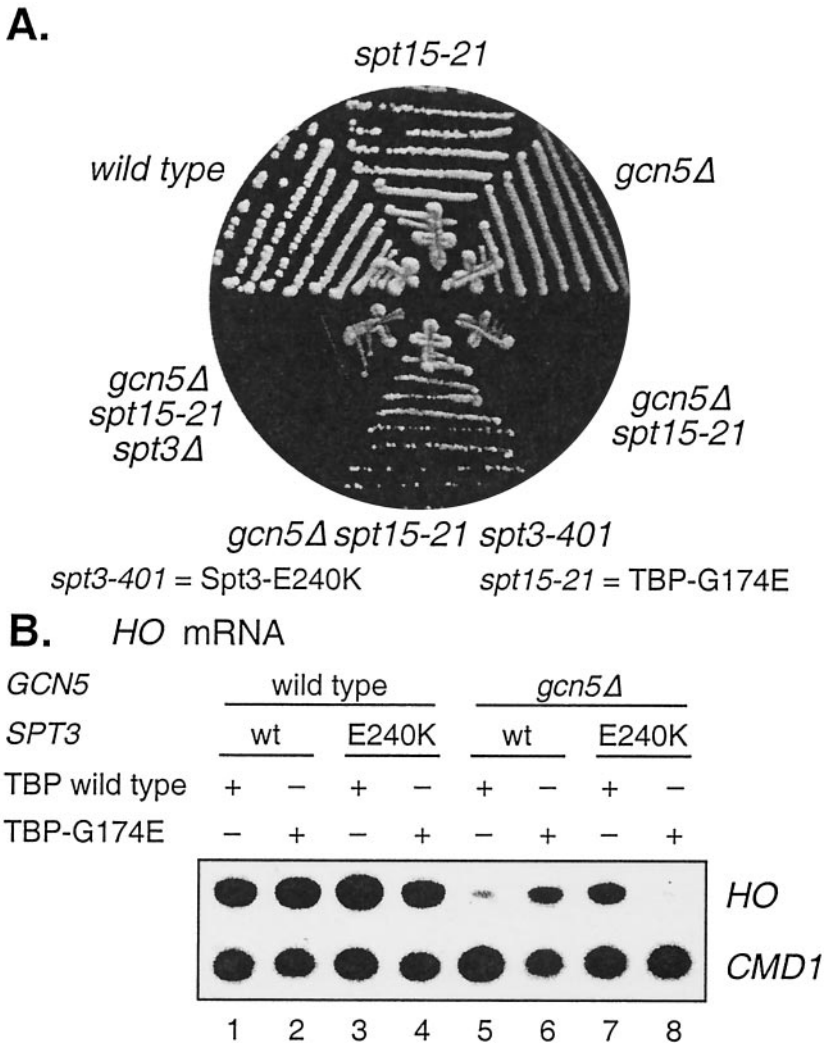

FIG. 7. *spt3-401* suppresses *spt15-21* for growth and *HO* expression. (A) Isogenic strains were constructed, differing at the *GCN5*, *SPT15* and *SPT3* loci. The *gcn5 spt15-21* double mutant had a severe growth defect, but this was suppressed specifically by the *spt3-401* allele. The following strains were grown on YEPD medium for 2 days at 30°C: DY151, DY7593, DY5926, DY7597, DY7598, and DY7604. (B) RNA was prepared from the following strains and used for S1 nuclease protection assays: DY151, DY7593, DY7592, DY7594, DY5926, DY7597, DY7596, and DY7598.

suggesting that association of TFIIB with the complex promotes the formation of the stable bent DNA form. Paull et al. (38) have shown that TFIIB has a higher affinity for a TBP/TFIIA/Nhp6 complex than for the complex lacking Nhp6. Taken together, these results suggest that Nhp6, by bending DNA, promotes the formation of the multiprotein complex of TBP, TFIIA, and TFIIB with DNA that is critical for transcriptional initiation (37).

Our data suggest that Gcn5 promotes DNA binding by TBP. In support of this idea, it has been shown that histone acetylation stimulates TBP binding in vivo (45). The TBP(K138T, Y139A) and TBP(G174E) mutations, which are healthy on their own, showed marked growth defects in a *gcn5* mutant (Fig. 5D and 7A). These mutant forms of TBP show no defect in DNA binding in vitro (19, 50), but these defects become apparent when chromatin is underacetylated in the *gcn5* mutant. Importantly, this synthetic growth defect can be suppressed by overexpression of TFIIA (Fig. 6), suggesting that TFIIA overexpression suppresses this defect by promoting formation of the TBP/TFIIA/TFIIB/DNA complex. The TBP (G174E) mutation affects interaction with Spt3, and the TBP (G174E) *gcn5* defect can also be suppressed by the compensatory Spt3(E240K) mutation, which restores interaction with TBP(G174E) (Fig. 7A). Spt3 stimulates TBP binding to the *GAL1* promoter (17), and the defect caused by TBP(G174E) can be suppressed by Spt3(E240K) (30). In summary, we suggest that some TBP mutants have difficulty binding DNA in a *gcn5* mutant when the template is underacetylated, but this can be suppressed by increased TFIIA levels or by restoration of the TBP-Spt3 interaction, each of which may promote formation of the TBP/TFIIA/TFIIB/DNA complex at certain promoters.

Certain TBP mutations show a strong synthetic growth defect in the absence of either Gcn5 or Nhp6 (Fig. 5). We suggest that TBP binding at some critical promoters can be stimulated by either Gcn5 or Nhp6 (Fig. 9), and thus, the defect in TBP

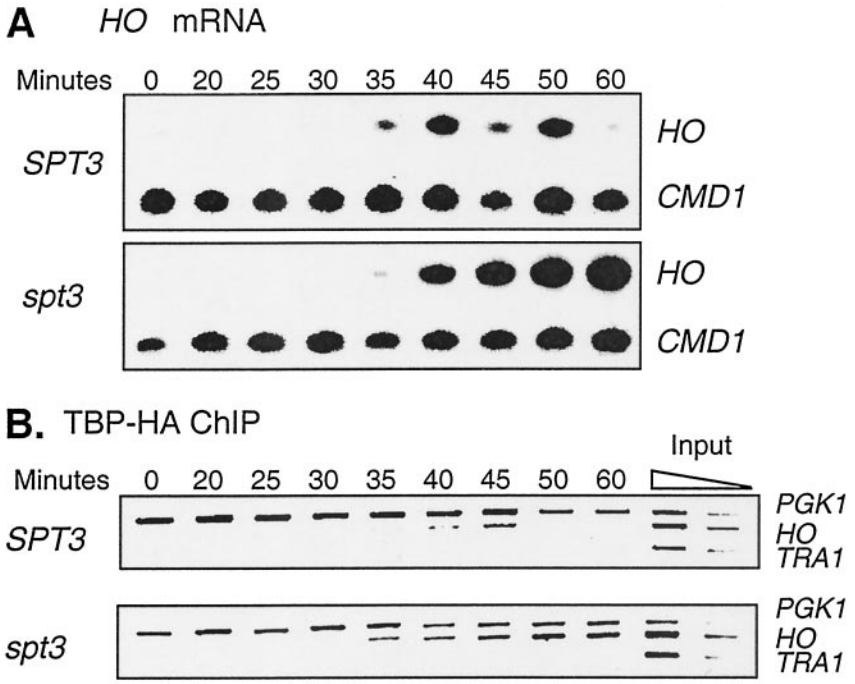

FIG. 8. Spt3 inhibits TBP binding to *HO*. *SPT3* (DY7247) and *spt3* (DY7250) strains with a TBP-HA tag were synchronized, and at timed intervals, samples were taken for RNA analysis and chromatin immunoprecipitation. (A) S1 nuclease protection assays showed that *HO* RNA levels were higher in the *spt3* mutant. (B) Chromatin immunoprecipitation (ChIP) analysis showed increased TBP-HA binding to the *HO* TATA in *spt3* mutants. *PGK1* TATA and the *TRA1* open reading frame served as positive and negative controls for the chromatin immunoprecipitation.

binding in the *gcn5 nhp6a nhp6b* mutant results in a severe growth defect. This growth defect can be suppressed by mutations in *sin4* (60), *spt3*, or *spt8* (Fig. 2B), consistent with our hypothesis that TBP binding at some promoters can be inhibited by Spt3 and/or Spt8 in SAGA. Importantly, SAGA inhibits TBP binding to the *HIS3* promoter in vitro, but this inhibition is not seen with SAGA lacking Spt3 or Spt8 (3). It is noteworthy that the Gcn5 and Spt3 proteins are both part of the same complex, SAGA, but they have opposing roles in the regulation of *HO* expression.

Promoter structure apparently plays a role in how Spt3 regulates transcription, because, in contrast to our results at *HO*, Spt3 stimulates TBP binding to the *GAL1* promoter (17). These results suggest that Spt3 functions differently at distinct promoters (Fig. 9). *HO* is expressed briefly in the cell cycle, and Spt3 may function to limit *HO* expression. *GAL1*, when induced by a nonpreferred carbon source, is expressed at very high levels, and Spt3 may function to stabilize TBP binding and thereby promote reinitiation. A variant form of SAGA, called either SALSA or SLIK (40, 51), which lacks Spt8 and has a

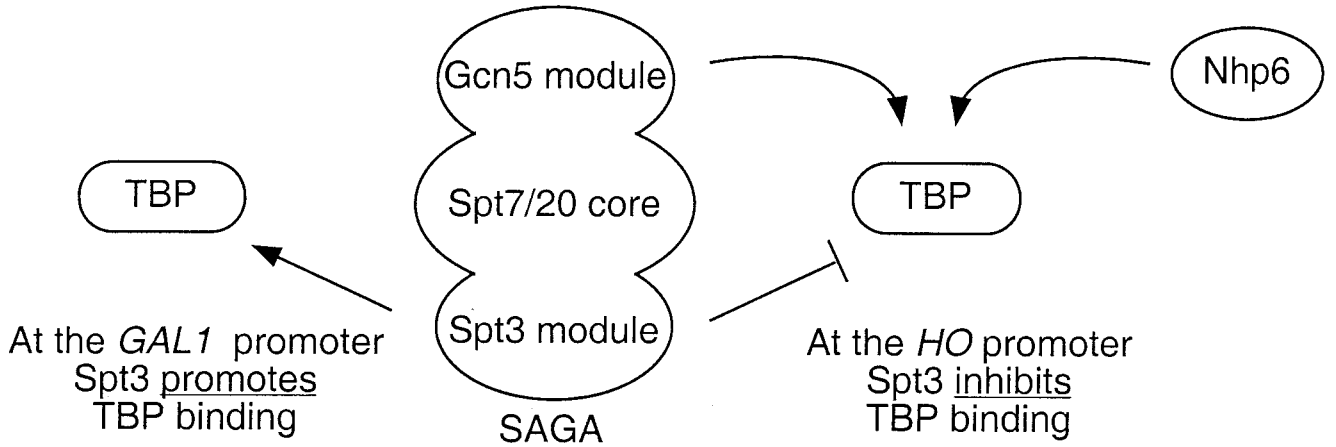

FIG. 9. Model for the differential regulation of TBP binding by Spt3. The Gcn5 module also contained Ada2 and Ada3. In addition to Spt7 and Spt20, the SAGA core also contained Ada1, TAF90, TAF61, TAF60, TAF25, TAF17, and Tra1. The Spt3 module also contained Spt8. At *GAL1* and other promoters, Spt3 promoted TBP binding and Gcn5 was not required (4, 17). At *HO*, Gcn5 and Nhp6 stimulated TBP binding while Spt3 inhibited binding.

truncated Spt7 subunit, has been recently identified. It appears that SALSA/SLIK promotes transcriptional activation, and it may be involved in the activation of *GAL1*, because *SPT8* is not required for *GAL1* expression (4). However, there are genes that require both Spt3 and Spt8 for TBP to bind, suggesting that it is SAGA that functions at these promoters (4). Interestingly, some of these genes are still expressed in a *gcn5* mutant. Further work is needed to decipher how promoter structure determines the requirements for Spt3 and Gcn5 for regulation at any given promoter.

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