MOLECULAR AND CELLULAR BIOLOGY, Sept. 2004, p. 8312–8321 Vol. 24, No. 18
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.18.8312–8321.2004

Role for Nhp6, Gcn5, and the Swi/Snf Complex in Stimulating Formation of the TATA-Binding Protein–TFIIA–DNA Complex

Debabrata Biswas,[1] Anthony N. Imbalzano,[2] Peter Eriksson,[1]† Yaxin Yu,[1] and David J. Stillman[1]*

Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah,[1] and Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts[2]

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

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

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

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

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

* Corresponding author. Mailing address: Department of Pathology, University of Utah, 30 North 1900 East, Salt Lake City, UT 84132-2501. Phone: (801) 581-5429. Fax: (801) 581-4517. E-mail: david.stillman@path.utah.edu.

† Present address: National Institutes of Health, Bethesda, MD 20892.

TABLE 1. Strains used in this study

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

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

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

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

MATERIALS AND METHODS

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

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

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

TABLE 2. Multicopy plasmids

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

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

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

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

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

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

RESULTS

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

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

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

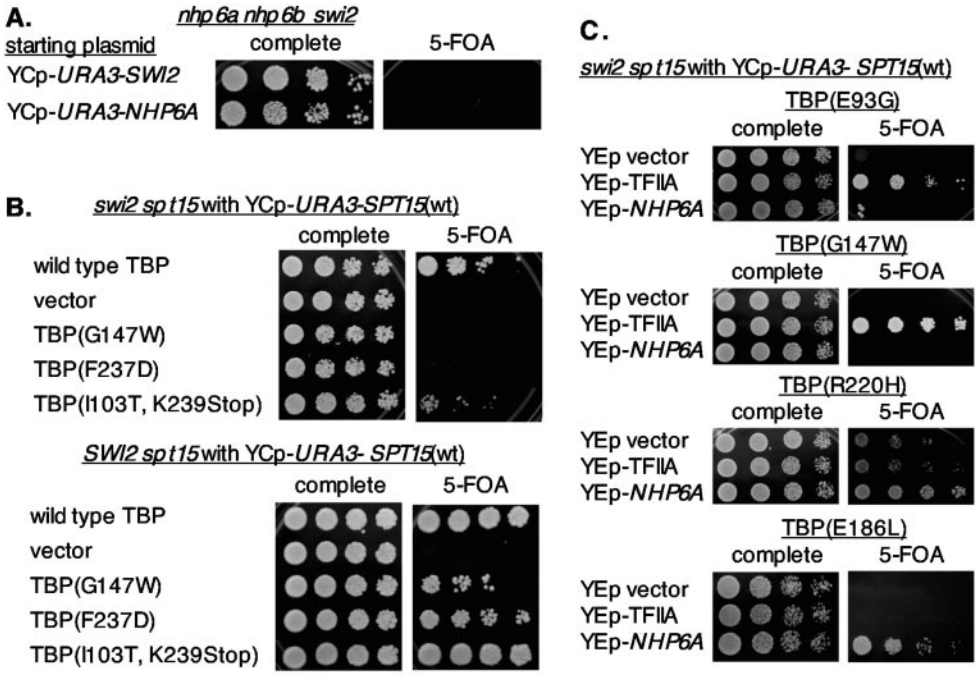

FIG. 1. Genetic interactions among *SWI2*, *NHP6*, TBP, and TFIIA. (A) *nhp6ab* is synthetically lethal with *swi2*. Dilutions of strains DY8660 (*nhp6ab swi2* strain with a YCp-*URA3-SWI2* plasmid) and DY8668 (*nhp6ab swi2* strain with a YCp-*URA3-NHP6A* plasmid) were plated onto complete medium- or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

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

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

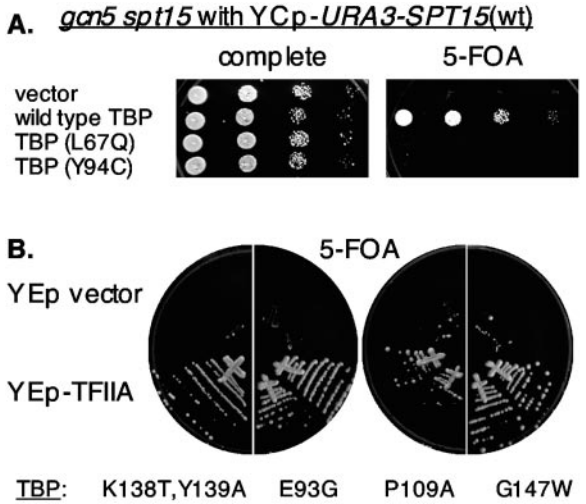

FIG. 2. Genetic interactions among *GCN5*, TBP, and TFIIA. (A) Examples of synthetic lethality of TBP mutants and *gcn5*. Dilutions of strain DY7514 (*gcn5 spt15*) transformed with the indicated TBP mutation plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated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

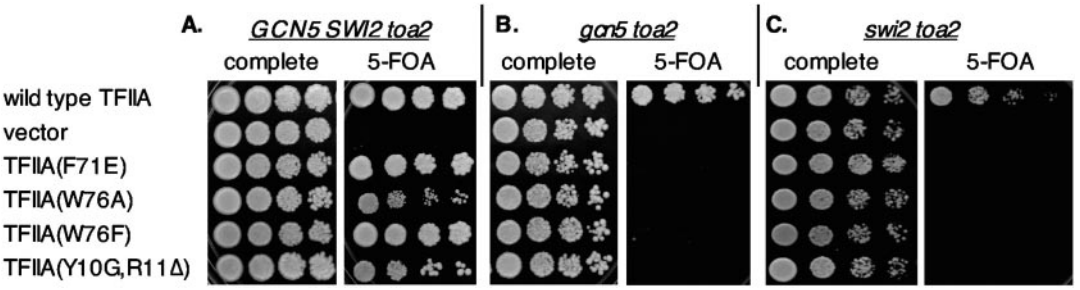

FIG. 3. TFIIA mutants are lethal in *gcn5* or *swi2* mutant strains. (A) Dilutions of strain DY8541 (*toa2*) transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing plates, and the plates were incubated at 33°C for 3 days. (B) Dilutions of strain DY8709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

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

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

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

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

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

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

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

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

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

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

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

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

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

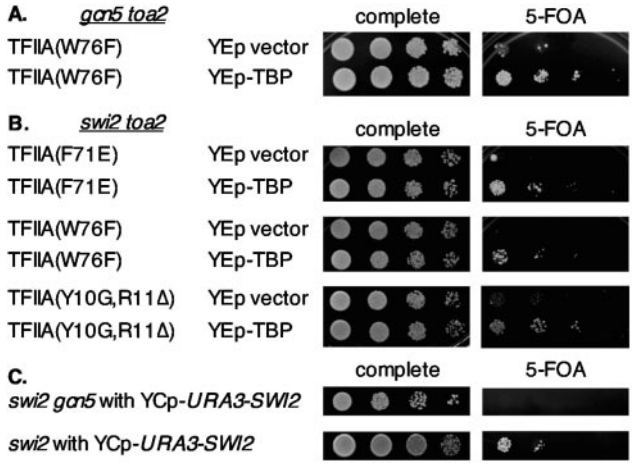

FIG. 4. Overexpression of TBP suppresses *gcn5*-TFIIA and *swi2*-TFIIA lethalities. (A) Strain DY8709 (*gcn5 toa2*) was transformed with two plasmids, a *LEU2* plasmid corresponding to the TFIIA W76F mutant and either pRS327 (YEp-*LYS2* vector) or M4533 (YEp-TBP), dilutions were plated onto complete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

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

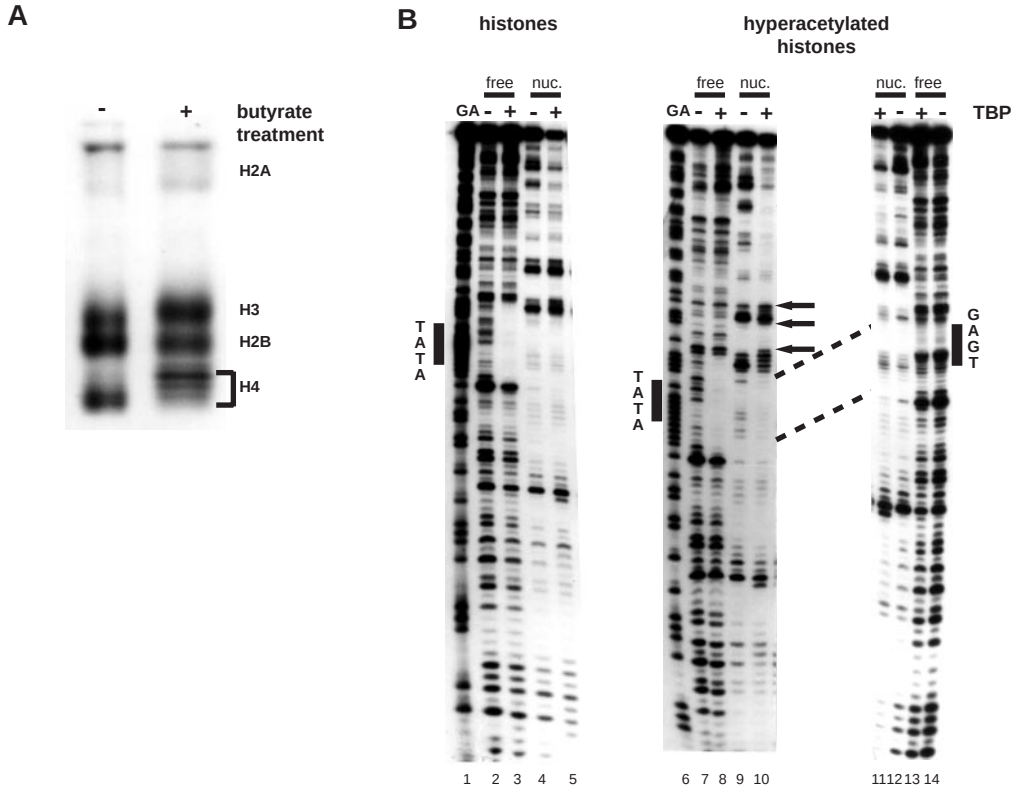

FIG. 5. TBP binds to acetylated nucleosomes. (A) Twenty micrograms of HeLa histones or hyperacetylated HeLa histones were loaded onto a 15% TAU gel and stained with Coomassie brilliant blue following electrophoresis. (B) TBP binding was assessed by DNase I digestions by using free DNA (lanes 2, 3, 7,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

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

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

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

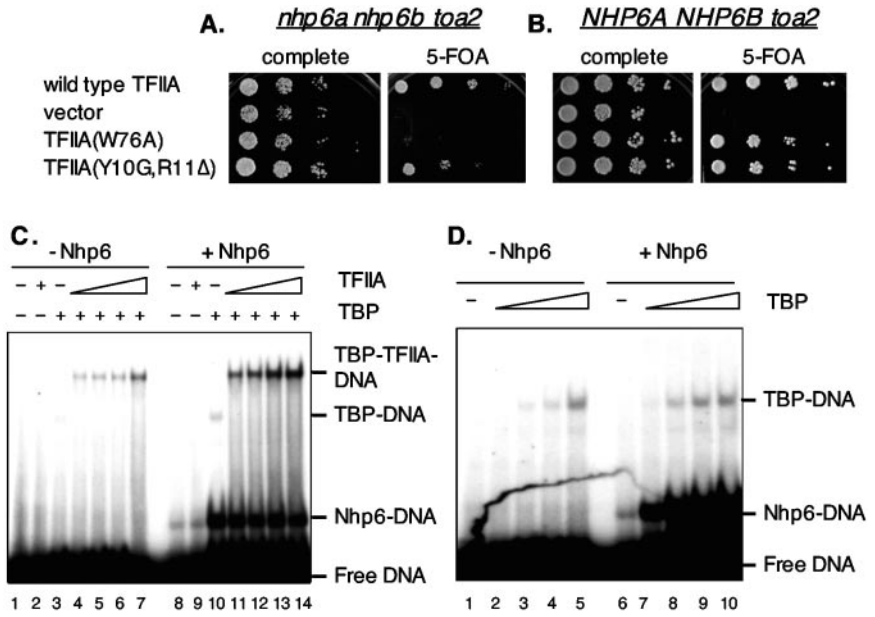

FIG. 6. Nhp6 interacts with TBP and TFIIA. (A) *nhp6ab* is synthetically lethal with TFIIA mutants. Dilutions of strain DY8510 (*nhp6ab toa2*) carrying the YCp-*URA3*-TFIIA (wild type) plasmid and transformed with the indicated TFIIA mutant plasmids were plated onto complete medium- or 5-FOA-containing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

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

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

DISCUSSION

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

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

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

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

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

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

ACKNOWLEDGMENTS

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

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

REFERENCES

1. **Agalioti, T., S. Lomvardas, B. Parekh, J. Yie, T. Maniatis, and D. Thanos.** 2000. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. Cell **103:**667–678.
2. **Arndt, K. M., S. Ricupero-Hovasse, and F. Winston.** 1995. TBP mutants defective in activated transcription in vivo. EMBO J. **14:**1490–1497.
3. **Arndt, K. M., C. R. Wobbe, S. Ricupero-Hovasse, K. Struhl, and F. Winston.** 1994. Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA recognition specificities. Mol. Cell. Biol. **14:**3719–3728.
4. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.
5. **Brewster, N. K., G. C. Johnston, and R. A. Singer.** 2001. A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol. Cell. Biol. **21:**3491–3502.
6. **Buratowski, S., and H. Zhou.** 1992. Transcription factor IID mutants defective for interaction with transcription factor IIA. Science **255:**1130–1132.
7. **Costigan, C., D. Kolodrubetz, and M. Snyder.** 1994. *NHP6A* and *NHP6B*, which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. Mol. Cell. Biol. **14:**2391–2403.
8. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265:**53–60.
9. **Das, D., and W. M. Scovell.** 2001. The binding interaction of HMG-1 with the TATA-binding protein/TATA complex. J. Biol. Chem. **276:**32597–32605.
10. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. **6:**1319–1331.
11. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58:**1183–1191.
12. **Emerson, B. M.** 2002. Specificity of gene regulation. Cell **109:**267–270.
13. **Eriksson, P., D. Biswas, Y. Yu, J. M. Stewart, and D. J. Stillman.** 2004. TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. Mol. Cell. Biol. **24:**6419–6429.
14. **Eriksson, P., L. R. Thomas, A. Thorburn, and D. J. Stillman.** 2004. pRS yeast vectors with a *LYS2* marker. BioTechniques **36:**212–213.
15. **Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman.** 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. **20:**3506–3517.
16. **Fragiadakis, G. S., D. Tzamarias, and D. Alexandraki.** 2004. Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for *FRE2* transcriptional activation. EMBO J. **23:**333–342.
17. **Geiger, J. H., S. Hahn, S. Lee, and P. B. Sigler.** 1996. Crystal structure of the yeast TFIIA/TBP/DNA complex. Science **272:**830–836.
18. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.

19. **Godde, J. S., Y. Nakatani, and A. P. Wolffe.** 1995. The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. Nucleic Acids Res. **23:**4557–4564.
20. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the *SPT15* suppressor of *Ty* element insertions. Cell **58:**1173–1181.
21. **Hampsey, M.** 1998. Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. **62:**465–503.
22. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2:**163–167.
23. **Hoopes, B. C., J. F. LeBlanc, and D. K. Hawley.** 1992. Kinetic analysis of yeast TFIID-TATA box complex formation suggests a multi-step pathway. J. Biol. Chem. **267:**11539–11547.
24. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature **370:** 481–485.
25. **Imbalzano, A. N., K. S. Zaret, and R. E. Kingston.** 1994. Transcription factor (TF) IIB and TFIIA can independently increase the affinity of the TATA-binding protein for DNA. J. Biol. Chem. **269:**8280–8286.
26. **Jayaraman, L., N. C. Moorthy, K. G. Murthy, J. L. Manley, M. Bustin, and C. Prives.** 1998. High mobility group protein-1 (HMG-1) is a unique activator of p53. Genes Dev. **12:**462–472.
27. **Kobayashi, A., T. Miyake, Y. Ohyama, M. Kawaichi, and T. Kokubo.** 2001. Mutations in the TATA-binding protein, affecting transcriptional activation, show synthetic lethality with the TAF145 gene lacking the TAF N-terminal domain in *Saccharomyces cerevisiae*. J. Biol. Chem. **276:**395–405.
28. **Kruppa, M., R. D. Moir, D. Kolodrubetz, and I. M. Willis.** 2001. Nhp6, an HMG1 protein, functions in *SNR6* transcription by RNA polymerase III in *S. cerevisiae*. Mol. Cell **7:**309–318.
29. **Kuddus, R., and M. C. Schmidt.** 1993. Effect of the non-conserved N-terminus on the DNA binding activity of the yeast TATA binding protein. Nucleic Acids Res. **21:**1789–1796.
30. **Kuras, L., and K. Struhl.** 1999. Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. Nature **399:**609–613.
31. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature **370:**477–481.
32. **Larschan, E., and F. Winston.** 2001. The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. **15:**1946–1956.
33. **Lee, D. Y., J. L. Hayes, D. Pruss, and A. P. Wolffe.** 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell **72:**73–84.
34. **Lee, M., and K. Struhl.** 1997. A severely defective TATA-binding protein–TFIIB interaction does not preclude transcriptional activation in vivo. Mol. Cell. Biol. **17:**1336–1345.
35. **Lee, T. I., H. C. Causton, F. C. Holstege, W. C. Shen, N. Hannett, E. G. Jennings, F. Winston, M. R. Green, and R. A. Young.** 2000. Redundant roles for the TFIID and SAGA complexes in global transcription. Nature **405:** 701–704.
36. **Lee, T. I., and R. A. Young.** 2000. Transcription of eukaryotic protein-coding genes. Annu. Rev. Genet. **34:**77–137.
37. **Lemon, B., and R. Tjian.** 2000. Orchestrated response: a symphony of transcription factors for gene control. Genes Dev. **14:**2551–2569.
38. **Li, X. Y., A. Virbasius, X. Zhu, and M. R. Green.** 1999. Enhancement of TBP binding by activators and general transcription factors. Nature **399:**605–609.
39. **Lieberman, P. M., M. C. Schmidt, C. C. Kao, and A. J. Berk.** 1991. Two distinct domains in the yeast transcription factor IID and evidence for a TATA box-induced conformational change. Mol. Cell. Biol. **11:**63–74.
40. **Liu, Q., S. E. Gabriel, K. L. Roinick, R. D. Ward, and K. M. Arndt.** 1999. Analysis of TFIIA function in vivo: evidence for a role in TATA-binding protein recruitment and gene-specific activation. Mol. Cell. Biol. **19:**8673–8685.
41. **Lomvardas, S., and D. Thanos.** 2001. Nucleosome sliding via TBP DNA binding in vivo. Cell **106:**685–696.
42. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:**953–961.
43. **Lopez, S., M. Livingstone-Zatchej, S. Jourdain, F. Thoma, A. Sentenac, and M. C. Marsolier.** 2001. High-mobility-group proteins NHP6A and NHP6B participate in activation of the RNA polymerase III SNR6 gene. Mol. Cell. Biol. **21:**3096–3104.
44. **Martens, J. A., and F. Winston.** 2002. Evidence that Swi/Snf directly represses transcription in S. cerevisiae. Genes Dev. **16:**2231–2236.
45. **Martens, J. A., and F. Winston.** 2003. Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr. Opin. Genet. Dev. **13:** 136–142.
46. **Martin, M. P., V. L. Gerlach, and D. A. Brow.** 2001. A novel upstream RNA polymerase III promoter element becomes essential when the chromatin structure of the yeast U6 RNA gene is altered. Mol. Cell. Biol. **21:**6429–6439.
47. **Moreira, J. M., and S. Holmberg.** 2000. Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6A and NHP6B. EMBO J. **19:**6804–6813.
48. **Narlikar, G. J., H. Y. Fan, and R. E. Kingston.** 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:**475–487.
49. **Onate, S. A., P. Prendergast, J. P. Wagner, M. Nissen, R. Reeves, D. E. Pettijohn, and D. P. Edwards.** 1994. The DNA-bending protein HMG-1 enhances progesterone receptor binding to its target DNA sequences. Mol. Cell. Biol. **14:**3376–3391.
50. **Orphanides, G., T. Lagrange, and D. Reinberg.** 1996. The general transcription factors of RNA polymerase II. Genes Dev. **10:**2657–2683.
51. **Ozer, J., L. E. Lezina, J. Ewing, S. Audi, and P. M. Lieberman.** 1998. Association of transcription factor IIA with TATA binding protein is required for transcriptional activation of a subset of promoters and cell cycle progression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:**2559–2570.
52. **Paull, T. T., M. Carey, and R. C. Johnson.** 1996. Yeast HMG proteins *NHP6A/B* potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev. **10:**2769–2781.
53. **Pollard, K. J., and C. L. Peterson.** 1997. Role for *ADA/GCN5* products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell. Biol. **17:**6212–6222.
54. **Prelich, G.** 1999. Suppression mechanisms: themes from variations. Trends Genet. **15:**261–266.
55. **Pugh, B. F., and R. Tjian.** 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. Genes Dev. **5:**1935–1945.
56. **Ranish, J. A., W. S. Lane, and S. Hahn.** 1992. Isolation of two genes that encode subunits of the yeast transcription factor IIA. Science **255:**1127–1129.
57. **Roberts, S. M., and F. Winston.** 1997. Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. Genetics **147:**451–465.
58. **Ross, E. D., P. R. Hardwidge, and L. J. Maher III.** 2001. HMG proteins and DNA flexibility in transcription activation. Mol. Cell. Biol. **21:**6598–6605.
59. **Sewack, G. F., T. W. Ellis, and U. Hansen.** 2001. Binding of TATA binding protein to a naturally positioned nucleosome is facilitated by histone acetylation. Mol. Cell. Biol. **21:**1404–1415.
60. **Sherman, F.** 1991. Getting started with yeast. Methods Enzymol. **194:**1–21.
61. **Stargell, L. A., and K. Struhl.** 1996. A new class of activation-defective TATA-binding protein mutants: evidence for two steps of transcriptional activation in vivo. Mol. Cell. Biol. **16:**4456–4464.
62. **Stargell, L. A., and K. Struhl.** 1995. The TBP-TFIIA interaction in the response to acidic activators in vivo. Science **269:**75–78.
63. **Sterner, D. E., and S. L. Berger.** 2000. Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:**435–459.
64. **Sudarsanam, P., V. R. Iyer, P. O. Brown, and F. Winston.** 2000. Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **97:**3364–3369.
65. **Tan, S., Y. Hunziker, D. F. Sargent, and T. J. Richmond.** 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature **381:**127–151.
66. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56:**619–630.
67. **Travers, A. A.** 2003. Priming the nucleosome: a role for HMGB proteins? EMBO Rep. **4:**131–136.
68. **Vettese-Dadey, M., P. A. Grant, T. R. Hebbes, C. Crane-Robinson, C. D. Allis, and J. L. Workman.** 1996. Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J. **15:**2508–2518.
69. **von Schwedler, U. K., M. Stuchell, B. Muller, D. M. Ward, H. Y. Chung, E. Morita, H. E. Wang, T. Davis, G. P. He, D. M. Cimbora, A. Scott, H. G. Krausslich, J. Kaplan, S. G. Morham, and W. I. Sundquist.** 2003. The protein network of HIV budding. Cell **114:**701–713.
70. **Workman, J. L., I. C. Taylor, R. E. Kingston, and R. G. Roeder.** 1991. Control of class II gene transcription during in vitro nucleosome assembly. Methods Cell Biol. **35:**419–447.
71. **Yu, Y., P. Eriksson, L. T. Bhoite, and D. J. Stillman.** 2003. Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. Mol. Cell. Biol. **23:**1910–1921.
72. **Yu, Y., P. Eriksson, and D. J. Stillman.** 2000. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol. Cell. Biol. **20:**2350–2357.
73. **Zappavigna, V., L. Falciola, M. Helmer-Citterich, F. Mavilio, and M. E. Bianchi.** 1996. HMG1 interacts with HOX proteins and enhances their DNA binding and transcriptional activation. EMBO J. **15:**4981–4991.
74. **Zhao, X., and W. Herr.** 2002. A regulated two-step mechanism of TBP binding to DNA: a solvent-exposed surface of TBP inhibits TATA box recognition. Cell **108:**615–627.
75. **Zweidler, A.** 1978. Resolution of histones by polyacrylamide gel electrophoresis in presence of nonionic detergents. Methods Cell Biol. **17:**223–233.
76. **Zwilling, S., H. Konig, and T. Wirth.** 1995. High mobility group protein 2 functionally interacts with the POU domains of octamer transcription factors. EMBO J. **14:**1198–1208.