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Mediator, TATA-binding Protein, and RNA Polymerase II Contribute to Low Histone Occupancy at Active Gene Promoters in Yeast*

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Background: Promoters of active genes in eukaryotes are typically depleted of histone proteins.
Results: Histone eviction from the induced *CHA1* promoter is compromised by mutations in transcription factors, and higher histone occupancy is also seen at constitutively active promoters in such mutants.
Conclusion: Preinitiation complex formation promotes reduced histone occupancy at active gene promoters.
Significance: Preinitiation complex components participate in chromatin remodeling.

Transcription by RNA polymerase II (Pol II) in eukaryotes requires the Mediator complex, and often involves chromatin remodeling and histone eviction at active promoters. Here we address the role of Mediator in recruitment of the Swi/Snf chromatin remodeling complex and its role, along with components of the preinitiation complex (PIC), in histone eviction at inducible and constitutively active promoters in the budding yeast *Saccharomyces cerevisiae*. We show that recruitment of the Swi/Snf chromatin remodeling complex to the induced *CHA1* promoter, as well as its association with several constitutively active promoters, depends on the Mediator complex but is independent of Mediator at the induced *MET2* and *MET6* genes. Although transcriptional activation and histone eviction at *CHA1* depends on Swi/Snf, Swi/Snf recruitment is not sufficient for histone eviction at the induced *CHA1* promoter. Loss of Swi/Snf activity does not affect histone occupancy of several constitutively active promoters; in contrast, higher histone occupancy is seen at these promoters in Mediator and PIC component mutants. We propose that an initial activator-dependent, nucleosome remodeling step allows PIC components to outcompete histones for occupancy of promoter sequences. We also observe reduced promoter association of Mediator and TATA-binding protein in a Pol II (*rpb1-1*) mutant, indicating mutually cooperative binding of these components of the transcription machinery and indicating that it is the PIC as a whole whose binding results in stable histone eviction.

Transcription of protein-coding genes in eukaryotes depends on both RNA polymerase II and an array of coactivators that do not bind directly to DNA but are recruited, directly or indirectly, by sequence-specific DNA-binding activators. Among other functions, these coactivators mediate cooperative assembly of the preinitiation complex (PIC),[2] comprising the general transcription factors such as TFIID, TFIIB, and so forth, and Pol II. As eukaryotic DNA is packaged by the histone proteins into chromatin, another role for coactivators is to overcome repressive effects of chromatin and to interact with local chromatin features to facilitate transcriptional events such as elongation and PIC assembly. How these coactivators are recruited to gene promoters, and the mechanisms by which they contribute to transcriptional activation, are not well understood.

In this work we address the relationships between the Swi/Snf and Mediator complexes, and their roles in remodeling chromatin during transcriptional activation. The Mediator complex was first described as bridging activators with the general transcription machinery, although it is now clear that it plays additional roles in transcriptional activation and also repression (1). Mediator comprises 25 subunits in the budding yeast *Saccharomyces cerevisiae*, and can be roughly divided on structural and functional criteria into four domains, the tail, middle, head, and cyclin-CDK modules (2). The tail module appears to function mainly if not exclusively as a target for DNA-binding activators to effect recruitment of Mediator at a subset of genes, whereas the middle and head modules interact directly with Pol II and also contain subunits that are targets for specific activators (1). Mediator is needed for transcriptional activation and PIC recruitment at most if not all Pol II transcribed genes in yeast (3, 4), and also functions in activator-independent (basal) transcription *in vitro* and *in vivo*, suggest-

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[2] The abbreviations used are: PIC, pre-initiation complex; Pol II, RNA polymerase II; TBP, TATA-binding protein; CSM, complete synthetic medium; YPD, yeast extract peptone dextrose; MNase, micrococcal nuclease; TF, transcription factor.

TABLE 1
Yeast strains used in this study

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Yeast knockout collection
BY4742	*MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Yeast knockout collection
RMY410	*MATa his3Δ1 leu2Δ0 ura3Δ0 srb4Δ::kanMX GAL11-13MYC::HIS3MX* RY2844 *(CEN LEU2 SRB4) med3Δ::URA3*	37
LS10	*MATα med3Δ::kanMX4 med15Δ::kanMX4 his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0*	45
RMY415	*MATα med3Δ::kanMX4 med15Δ::kanMX4 his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SRB5-13MYC* (*S.k.*)[a]	37
RMY521	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 srb4Δ::KanMX SRB5-13MYC::HIS3*(*S.k.*)[a] RY2844 *(CEN LEU2 SRB4)*	11
RMY522	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 srb4Δ::KanMX SRB5-13MYC::HIS3*(*S.k.*)[a] RY2844 *(CEN LEU2 srb4-138)*	11
RMY525	*MATα med3Δ::kanMX4 med15Δ::kanMX4 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SNF2-TAP::HIS3* (*S.k.*)[a]	This study
Z111	*MATα, ura3-52, his3Δ200, leu2-3,112, rpb1-1, ade2*	49
Z111-Srb5-Myc	*MATα, ura3-52, his3Δ200, leu2-3,112, rpb1-1, ade2 SRB5-13MYC::HIS3 (S.k.)*[a]	This study
Z579	*MATa his3Δ200 leu2-3,112 ura3-52 srb4Δ::HIS3* RY2844 *(CEN LEU2 SRB4)*	4
Z628	*MATa his3Δ200 leu2-3,112 ura3-52 srb4Δ::HIS3* RY2882 *(CEN LEU2 srb4-138)*	4
BYΔ2	*MATa ura3-52 trp1Δ1 leu2-ade2-101C lys2*-YCplac22-TBP (*CEN TRP1 TBP)*	48
BYΔ2-ts1	*MATa ura3-52 trp1Δ1 leu2-ade2-101C lys2*-YCplac22-tbp-ts-1 (*CEN TRP1 tbp ts-1*)	48
BY4705	*MATα ade::higG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0*	29
EPY4706	*MATα ade::higG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0* pRS316	This study
YFR912	*MATα ade::higG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 kin28::kin28-L83G, bur2Δ::LEU2* pSH579 (*ARS CEN URA3 kin28-L83G*)	54
YMH14	*MATα cyc1-5000 cyc7-67 ura3-52 leu2-3,112 cyh2*	
YMH124	*MATα cyc1-5000 cyc7-67 ura3-52 leu2-3,112 cyh2 sua7-1*	54

[a] Designates the *HIS3* allele from *Saccharomyces kluyveri* (18).

ing it may be regarded as part of the general transcription machinery (5–7).

In addition to its role in PIC recruitment, Mediator also plays a role at some promoters in the recruitment of other co-activator complexes. For example, in *S. cerevisiae*, Mediator was reported to be required for recruitment of the Swi/Snf chromatin remodeling complex at several glucose-regulated promoters and at the Gcn4-dependent *ARG1* and *SNZ1* promoters, and for Swi/Snf-dependent remodeling as well as Swi/Snf recruitment at the induced *RNR3* promoter (8–10).

To better understand the functional relationship between Mediator and other coactivator complexes, we previously examined the dependence on Mediator of Swi/Snf recruitment and chromatin remodeling at the induced *CHA1* promoter (11). *CHA1* encodes a serine-threonine dehydratase that enables yeast cells to use serine or threonine as nitrogen sources, and is rapidly induced upon addition of serine to complete synthetic medium (CSM) (12). *CHA1* activation is essentially abolished at the restrictive temperature in yeast harboring a temperature-sensitive mutation in the essential Mediator subunit Srb4/Med17, and recruitment of PIC components TBP, Kin28, and Pol II, is greatly impaired (11). Induction of *CHA1* also results in recruitment of the Swi/Snf complex and in remodeling of a nucleosome that incorporates the TATA element (11, 13–15). Surprisingly, this nucleosome remodeling, which is accompanied by histone eviction, occurs normally in *swi*/*snf* mutant yeast (11, 14). In contrast to the strong effect seen on recruitment of PIC components TBP, Kin28, and Pol II in *srb4*/*med17 ts* yeast, neither recruitment of Swi/Snf nor chromatin remodeling were affected in this Mediator mutant (11). This result suggested that chromatin remodeling and Swi/Snf recruitment at the induced *CHA1* promoter occur independently of Mediator. However, we also found that although head and middle module subunits of Mediator were not recruited in *srb4*/*med17 ts* yeast, tail module subunits were still associated with several active promoters, including that of *CHA1* (3, 11). Thus, we could not rule out a role for the tail module of Mediator in Swi/Snf recruitment or chromatin remodeling, and we report here the results of investigating this possibility.

We also found that *CHA1* activation in wild type yeast, as has been reported for other inducible promoters, was accompanied by reduced histone association at the promoter, as monitored by ChIP against histone H3 (11). Surprisingly, histone eviction was not observed upon *CHA1* induction in *srb4*/*med17 ts* yeast, despite the chromatin remodeling observed by altered cleavage of chromatin by micrococcal nuclease (MNase). To explain this apparent discrepancy, we hypothesized that the altered MNase accessibility measured in both wild type and *srb4/med17 ts* yeast at the induced *CHA1* promoter reflected an alteration in nucleosome dynamics, whereas stable eviction resulting in a diminished ChIP signal occurred in a later step requiring PIC assembly. A prediction of this model was that deficient PIC assembly caused by other means than the *srb4*/*med17 ts* mutation would similarly result in a lack of histone eviction at the induced *CHA1* promoter without compromising remodeling assessed by MNase cleavage. Furthermore, we speculated that other active promoters might also exhibit increased (relative to wild type controls) histone promoter occupancy in mutants that compromised PIC formation. We report here the results of examining the effects of mutations in Mediator and PIC components on histone H3 association with the induced *CHA1* and additional gene promoters, and provide support for PIC-dependent histone eviction at active promoters.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—The *S. cerevisiae* strains used in this study are listed in Table 1. For the experiments of Fig. 1, *B* and *C*, BY4741 was used as wild type except for the kinetic analysis of *snf5*Δ yeast, where the Rpb3-TAP strain from the yeast TAP-tagged collection was used (16); deletion mutants were from the Yeast Deletion Collection (17). *SNF2-TAP* and *SRB5–13MYC* alleles were introduced into yeast (see Table 1) by standard techniques (18). Transformations were done using a standard lithium acetate protocol (19). Yeast cells were grown at 30 °C on CSM lacking appropriate

amino acids (6.7 g/liter Yeast Nitrogen Base without amino acids, 2% glucose, or 1.5% raffinose, CSM (DropOut) powder (Bio101)) or Yeast Extract Peptone dextrose (YPD) (20 g/liter of bactopeptone, 10 g/liter of yeast extract, 2% glucose, 0.15 g/liter of L-tryptophan) media. For temperature shift experiments, samples were grown at 25 °C to an optical density at 600 nm between 0.7 and 1.0, and then shifted rapidly to 37 °C by addition of pre-warmed media and incubated at 37 °C for times as indicated in the figure legends prior to cross-linking for ChIP. For *CHA1* induction, serine was added to a concentration of 1 mg/ml for 2 h prior to measuring transcription (Fig. 1*B*), 30 min before cross-linking for ChIP, and 30 min before nuclei preparation for MNase digestion. For *MET* gene induction, $CdCl_2$ was added to a concentration of 0.5 mM 60–90 min prior to cross-linking. Yeast harboring the *kin28-as* analog-sensitive mutation, and the control strain, were grown in CSM-ura containing 1 mg/ml of serine (to allow *CHA1* activation) and treated for 1 h with 1 μg/ml of NA-PP1 prior to cross-linking. Plasmid p$P_{pho5v33}$-lacZ and galactose induction conditions were as reported previously (20, 21).

RNA Isolation and Northern Analysis—RNA was isolated from yeast cultures grown to an optical density at 600 nm of 0.6 to 1.0. For kinetic analyses of *CHA1* induction (Fig. 1*D*), an aliquot was taken from each culture to serve as a no serine sample. The *CHA1* promoter was then induced by adding 1 mg/ml of serine to the cultures. At time points ranging from 1 min to 3 h, 10-ml aliquots were transferred to 50 ml of polypropylene tubes containing 50 μl of 10% sodium azide to halt transcription. RNA isolation and Northern analysis were performed as described (22, 23). Probes were prepared by PCR and blots successively probed for *CHA1* and *PYK1* or 26 S rRNA for normalization. For kinetic analyses, the values from each replicate were normalized by setting the sum of the 5-, 15-, 30-, and 60-min samples to 100 prior to calculating mean ± S.D. for each time point. This normalization allows easier visual comparison between kinetics of wild type and mutant, but erases the differences in mRNA levels between the deletions and the wild type, which are therefore not apparent in the plots of Fig. 1*D*.

Chromatin Immunoprecipitation (ChIP)—ChIP was performed essentially as described previously (3, 11, 20) with the following modifications: the samples were sonicated using a Diagenode Bioruptor at high power setting, 30-s "on"/30-s "off" for a total of 15 min to obtain sheared DNA 200–600 bp in length. For induction of the *CHA1* gene, 1 mg/ml of serine was added to the medium 30 min before cross-linking. To induce methionine biosynthesis genes, 0.5 mM $CdCl_2$ was added to the medium for 60–90 min (24) before cross-linking. For ChIP of myc-tagged proteins, whole cell extract was incubated with ~2 μg of anti-myc antibody (Roche Applied Science). For ChIP of TAP-tagged proteins, antibody to protein A (Sigma) was used. Antibodies against TBP, Snf5, Spt3, Gcn5, and Ada2 were a generous gift of Tony Weil (Vanderbilt University). Other antibodies used in ChIP reactions were against Rpb4 from Abcam (5 μl/ChIP reaction) and pan-H3 from Abcam (2.5 μl/reaction). For ChIP against Snf5, initial experiments (Fig. 2*A*) were done using antibody from Abcam; subsequent experiments (performed after this antibody was discontinued for sale) were done using antibody generously provided by Tony Weil (Vanderbilt University). ChIP at the *PHO5v33* locus was performed as described, and normalized relative to *ACT1* (20). A paired *t* test was used to calculate *p* values.

TABLE 2
Primers used in this study

Gene	Primer sequence
Chromosomal loci	
PMA1-prom.F1	5′-ACCCCAGCTAGTTAAAGAAAATCA-3′
PMA1-prom.R1	5′-CGTCATCGTCAGAAGATTCAGATG-3′
ChrV up	5′-GGCTGTCAGAATATGGGGCCGTAGTA-3′
ChrV down	5′-CACCCCGAAGCTGCTTTCACAATAC-3′
RPL12A-prom.F1	5′-CAAGGCTTCTTTGAGTTACAGTTC-3′
RPL12A-prom.R1	5′-GACCAATCTTTGGAGCCAAAGCAG-3′
PHO84-prom.F1	5′-TTCCTCATCTCGTAGATCACCAGG-3′
PHO84-prom.R1	5′-CCAGAGGATCTTCAATATGAGCAA-3′
SPO20-prom.F1	5′-GCTCGCTTTAGCTACTGATAAACC-3′
SPO20-prom.R1	5′-TGCAACTTCAGGTTCTTATGGTGC-3′
SPS19-prom.F1	5′-GTTTGGGAGGTCGTATGTTCGAGT-3′
SPS19-prom.R1	5′-GCCAAGAAGAACCAAGGCTTCTGT-3′
MET6-prom.F1	5′-AAGCAAGCATCTAAGAGCATTGAC-3′
MET6-prom.R1	5′-TGAGTTCTCAAATCCTTACCGACC-3′
MET2-prom.F1	5′-CTACCTGCTATCTTGTTCACGGAT-3′
MET2-prom.R1	5′-CCAGCTCTTGGAGCGTTTTCGATT-3′
CHA1-prom.F1	5′-ATCAAATTGTTTGCGATGAGATAAGATA-3′
CHA1-prom.R1	5′-GAAGCCTTTCCGGGGAAGAATTGACG-3′
PIK1-prom.F1	5′-GTCTCTTTGGTGCTTCCTGGATAT-3′
PIK1-prom.R1	5′-AGGCAGCTCTCTTTCTTGGCAAGA-3′
TCM1-prom.F1	5′-GTCAATCCTCATCTTTCTTTACTC-3′
TCM1-prom.R1	5′-CAACTGGCTTGGATCTCTCATCCT-3′
PDC1-prom.F1	5′-CTCTCCTTGCAATCAGATTTGGGT-3′
PDC1-prom.R1	5′-ACCTGGCAAACCGAAAACGGTGTT-3′
EFB1-prom.F1	5′-CTACCTTTCAGCACTGAAGAGTCC-3′
EFB1-prom.R1	5′-CTTCCCATCATAAACACGGACCAA-3′
ARG4-prom.F1	5′-TGTTCGTTCTTGTGGTGGTTACTC-3′
ARG4-prom.R1	5′-GCGAATATTCTCCCCTAGCTAAAG-3′
MAE1-prom.F1	5′-AAGTCGTACCCGTTACCGGCATGA-3′
MAE1-prom.R1	5′-AGCAACGGAAACGGATAGTCTGGT-3′
SNQ2-prom.F1	5′-AAACGAAGGTATACGCGGACGTACC-3′
SNQ2-prom.R1	5′-GGTTATGCCCGTAAATGATTCTTCTG-3′
PHO5v.33	
UASP2-For	GAATAGGCAATCTCTAAATGAATCGA
UASP2-Rev	GAAAACAGGGACCAGAATCATAAATT
UASP2-TaqMan	ACCTTGGCGGAAGACTCTCCTCCG
ACT1-For	TGGATTCCGGTGATGGTGTT
ACT1-Rev	TCAAAATGGCGTGAGGTAGAGA
ACT1-TaqMan	CTCACGTCGTTCCAATTTACGCTGGTTT

Quantitations were done by real time PCR; $\log_2$ ratios of immunoprecipitation/input are depicted in figures after subtracting the $\log_2$ ratios obtained for a non-transcribed region of ChrV (25). Promoter regions were interrogated using primers from about +100 to −100 relative to the starting ATG (Table 2). For analyses of the plasmid-borne *PHO5v33* promoter, the probe for indirect end labeling and the primers for ChIP analysis were specific for the plasmid-borne *PHO5v33* as opposed to the chromosomal *PHO5* locus.

Chromatin Preparation and Indirect End Label Analysis—Yeast nuclear extract was prepared from yeast grown to mid-log phase, aliquots were digested for 8–10 min at 37 °C with micrococcal nuclease (Worthington Biochemicals), and samples were analyzed by indirect end labeling as previously described (26). ClaI accessibility assays were done as described previously (27, 28).

RESULTS

Dependence of Swi/Snf Complex Recruitment on Mediator—*CHA1* activation is accompanied by remodeling of a nucleosome that incorporates the TATA element (Fig. 1*A*) (14, 15). Previously, it was reported that this remodeling does not

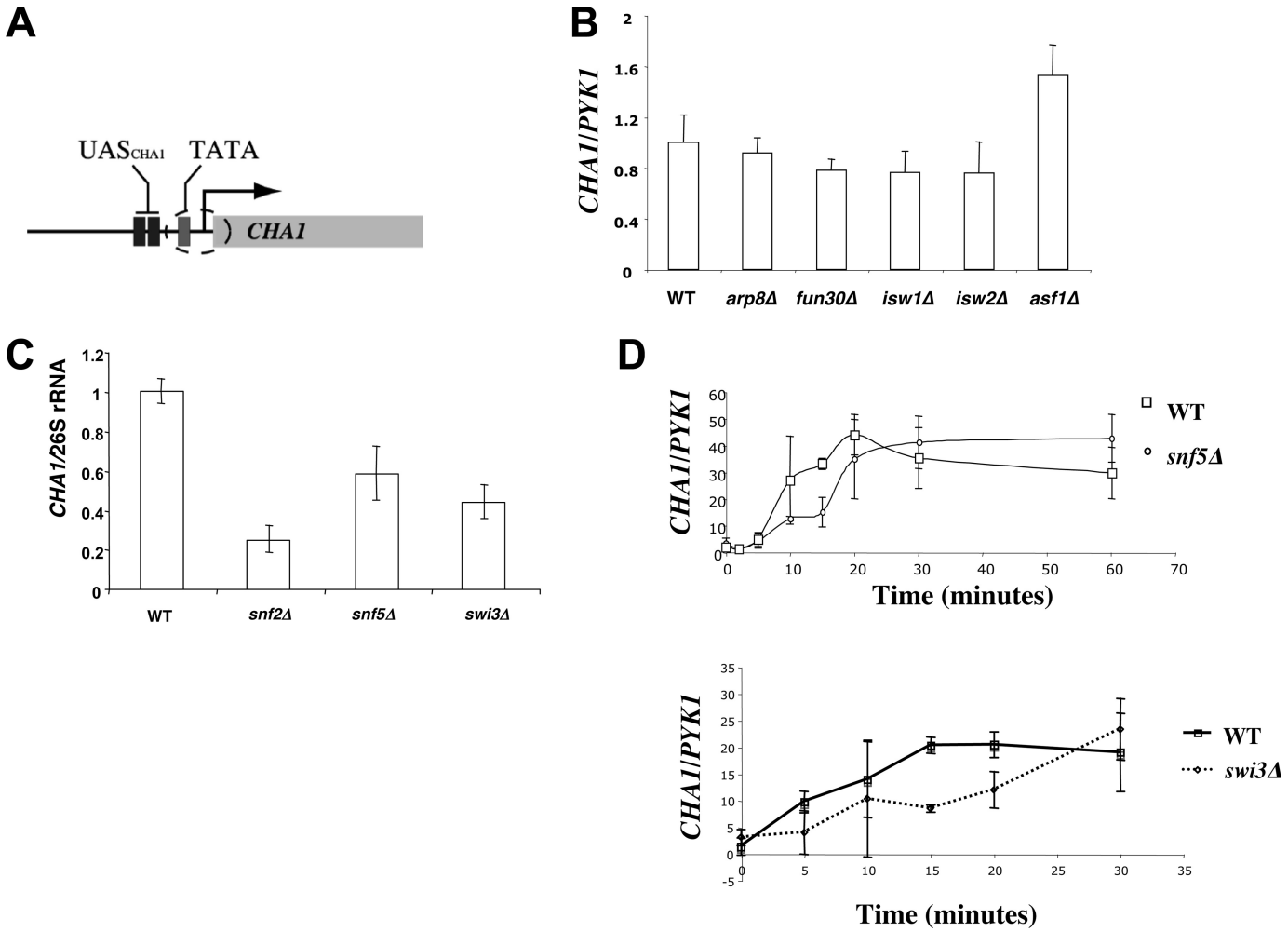

FIGURE 1. **CHA1 induction depends on the Swi/Snf complex.** *A*, schematic diagram of the *CHA1* promoter. The *dashed ellipse* represents the nucleosome that occludes the TATA element in the uninduced promoter and is remodeled upon activation. *B*, *CHA1* expression in CSM containing 1 mg/ml of serine was measured by Northern analysis and normalized to *PYK1* mRNA in wild type (BY4741) and deletion mutants as indicated. *Error bars* represent S.D. ($n = 4$). *C*, *CHA1* expression in CSM containing 1 mg/ml of serine was measured by Northern analysis and normalized to 26 S rRNA in wild type (BY4741) and *snf*/*swi* deletion mutants as indicated. *Error bars* represent S.D. ($n = 4$). *D*, delayed induction kinetics of *CHA1* in *snf5*Δ and *swi3*Δ yeast. Transcription was halted by addition of 10 mM sodium azide at the indicated times following addition of 1 mg/ml of serine to yeast cultures grown in CSM, and RNA analyzed by Northern blotting. *Error bars* represent S.D. ($n = 2$–4; note $n = 4$ for $t = 0, 5, 15, 30$, and 60 min (*left panel*)). For the kinetic analyses in *D*, data were normalized to allow easier visual comparison (see "Experimental Procedures"); this erases the difference in transcription level between wild type and mutant, which was otherwise consistent with that in *C*.

require Gcn5, Swi/Snf, or Rpb1 (11, 14). To gain additional insight into chromatin remodeling during activation of *CHA1*, we examined the effects of the loss of core subunits of known nucleosome remodeling complexes on *CHA1* activation. Little or no change was seen in steady-state-induced expression levels of *CHA1* in yeast strains lacking core members of the ISW and INO80 complexes (30, 31), or Fun30, which has homology to Snf2 and functions in gene silencing in heterochromatin and DNA repair (Fig. 1*B*) (32–34). We also tested the effect on *CHA1* induction in the absence of the histone chaperone Asf1, which contributes to chromatin remodeling and transcriptional activation of the paradigmatic *PHO5* gene in yeast, particularly under conditions of partial phosphate depletion (35, 36), and again found no significant effect (Fig. 1*B*). In contrast, individual deletion of genes encoding three core members of the Swi/Snf complex, *SNF2*, *SWI3*, and *SNF5*, decreased *CHA1* expression by 2–4-fold (14) (Fig. 1*C*). Furthermore, we observed delayed induction of *CHA1* in *snf2*Δ and *swi3*Δ yeast (Fig. 1*D*). Thus, although the Swi/Snf complex is not required for chromatin remodeling at the *CHA1* promoter upon activation (11, 14), it contributes to both the kinetics and steady-state level of induced expression.

Consistent with its playing a role in *CHA1* activation, the Swi/Snf complex is recruited to the *CHA1* promoter upon induction (11, 13). In previous work examining the relationship between the Mediator complex and chromatin remodeling of *CHA1*, we found that Swi/Snf was recruited in an *srb4*/*med17 ts* mutant, in which recruitment of the Mediator middle and head modules was not observed (11). This suggested that Swi/Snf recruitment might occur via a pathway independent of Mediator. However, the tail module of Mediator remains associated with the induced *CHA1* promoter, and with many other promoters, in *srb4*/*med17 ts* yeast (3, 11), leaving open the possibility that the tail module could play a role in recruitment of the Swi/Snf complex. We recently showed that although deletions of individual subunits from the Mediator tail module do not affect *CHA1* activation, a *gal11*/*med15*Δ *med3*Δ mutation abrogates *CHA1* activation and results in a failure to recruit sub-

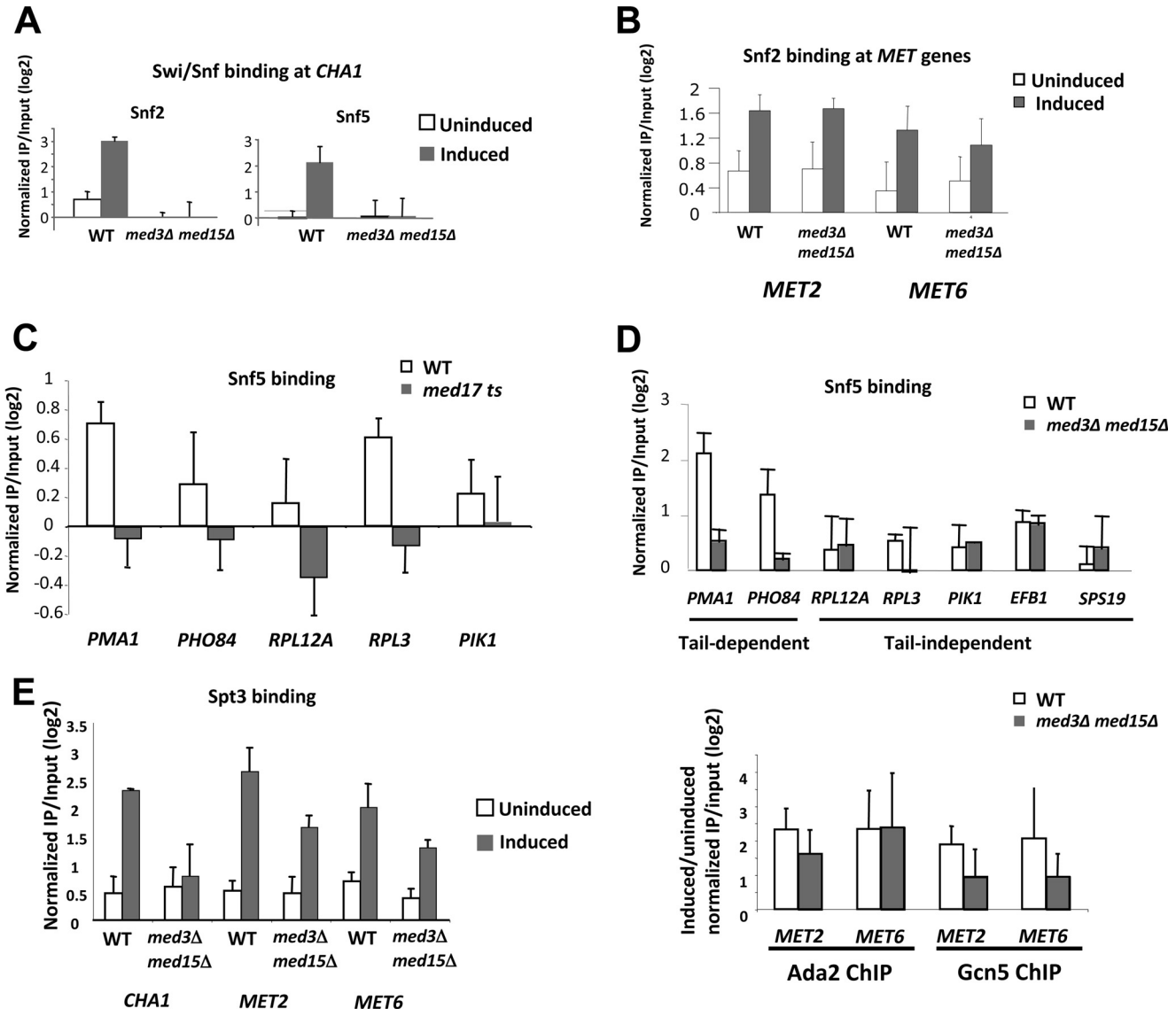

FIGURE 2. **Dependence on Mediator of Swi/Snf and SAGA association with active gene promoters.** *A*, Snf2 and Snf5 association with the *CHA1* promoter in wild type (WT; BY4742 for Snf5 and Research Genetics Snf2-TAP strain for Snf2) and *gal11*/*med15*Δ *med3*Δ (LS10 for Snf5 and RMY525 for Snf2) yeast grown in CSM without (uninduced) or with 1 mg/ml of serine (induced) was measured by ChIP. *B,* Snf2 association with the *MET2* and *MET6* promoters in WT (Snf2-TAP strain, Research Genetics) and *gal11*/*med15*Δ *med3*Δ (RMY525) yeast grown in YPD without (uninduced) or with (induced) 0.5 mM $CdCl_2$. *C,* Snf5 association with the indicated promoters was measured in WT (RMY521) and *srb4*/*med17 ts* (RMY522) yeast grown in YPD after 1 h at 37 °C. *D*, Snf5 association with promoters whose transcription is dependent or independent of the Mediator tail module, as indicated, was measured in WT (Snf2-TAP strain, Research Genetics) and *gal11*/*med15*Δ *med3*Δ (RMY525) yeast grown in YPD. *E*, effect of Mediator tail mutation on SAGA recruitment. *Left panel*: Spt3 association with the *CHA1* promoter was measured by ChIP in wild type (Snf2-TAP strain, Research Genetics) and *gal11*/*med15*Δ *med3*Δ yeast (RMY525) grown in CSM without (uninduced) or with (induced) 1 mg/ml of serine, and association with the *MET2* and *MET6* promoters was measured in wild type (RMY521) and *gal11*/*med15*Δ *med3*Δ yeast (RMY415) grown in YPD without (uninduced) or with (induced) 0.5 mM $CdCl_2$. *Right panel*, Ada2 and Gcn5 association at the uninduced and induced *MET2* and *MET6* promoters were measured by ChIP, and the $\log_2$ ratios of the association at the induced/uninduced promoters in wild type and *gal11*/*med15*Δ *med3*Δ yeast are shown, as indicated. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). *Error bars* represent S.D. ($n = 3–4$).

units from the head, middle, and tail modules of Mediator to the induced *CHA1* promoter (37). Based on this result, we tested the effect of a *gal11*/*med15*Δ *med3*Δ mutation on Swi/Snf recruitment to the *CHA1* promoter under inducing conditions and found that Swi/Snf recruitment was abolished (Fig. 2*A*). Thus, Mediator, and specifically the tail module, is required for Swi/Snf recruitment to the induced *CHA1* promoter.

To further probe the relationship between Mediator and Swi/Snf recruitment, we examined Swi/Snf recruitment to the Met4-activated genes *MET2* and *MET6*, which can be induced by addition of $CdCl_2$ (24) and require the Mediator tail module for activation (37, 38). We observed a modest but consistent recruitment of both Snf2 and Snf5 to the induced *MET2* and *MET6* promoters (about 1.8-fold ($p < 0.02$); Fig. 2*B* and data not shown) in wild type yeast. However, in contrast to *CHA1*, Snf2 recruitment in *gal11*/*med15*Δ *med3*Δ yeast at *MET2* ($p < 0.02$) was indistinguishable from that seen in wild type yeast. Recruitment of Snf2 also appeared similar at *MET6*, although this latter finding was not statistically significant ($p = 0.15$). Thus, the dependence on Mediator of Swi/Snf recruitment to

active promoters can vary in a promoter/activator-dependent manner.

Swi/Snf-dependent chromatin remodeling has for the most part been investigated during gene induction (39, 40). However, Swi/Snf complex subunits are also found associated with some gene promoters that show constitutive activity in yeast grown in rich medium (41, 42). We examined Snf5 association with several such promoters in wild type and *srb4*/*med17 ts* yeast (Fig. 2*C*). Although only modest Swi/Snf association was observed relative to a non-transcribed region of Chromosome V (25), decreased association was consistently seen in *srb4*/*med17 ts* yeast ($p < 0.05$ for *PMA1*, *PHO84*, and *RPL3*), indicating Mediator-dependent association of Swi/Snf at these constitutively active promoters. To control for possible general effects of this Mediator mutation, we also examined Swi/Snf association in a *gal11*/*med15*Δ *med3*Δ mutant that only affects transcription and Mediator recruitment at a subset of genes. This mutation reduces transcription and Mediator recruitment at *PMA1* and *PHO84*, but not at *RPL12A*, *RPL3*, *PIK1*, *EFB1*, or *SPS19* (37). Correspondingly, the former two genes exhibit reduced Snf5 association in this mutant ($p < 0.06$), whereas the latter five are not affected (Fig. 2*D*). Taken together, these results indicate that the Swi/Snf complex is present at many active gene promoters in yeast, and that its association often depends on the Mediator complex. In contrast, the induced *CHA1* promoter appears to be unusual in that the tail module of Mediator can recruit the Swi/Snf complex independently of the middle and head modules (in *srb4*/*med17 ts* yeast), and Swi/Snf recruitment occurs completely independently of Mediator at the induced *MET* genes.

Dependence of SAGA Recruitment on Mediator Is Promoter-dependent—Induction of *CHA1* and *MET2* and *MET6* genes results in recruitment of the SAGA complex as well as of the Swi/Snf complex (13, 38). We examined recruitment of Spt3 from the SAGA complex and found that Spt3 recruitment is lost in *gal11*/*med15*Δ *med3*Δ yeast at the induced *CHA1* promoter but is still observed in *gal11*/*med15*Δ *med3*Δ yeast at the induced *MET2* and *MET6* promoters (Fig. 2*E*). Because recruitment of Mediator is greatly reduced at these promoters in *gal11*/*med15*Δ *med3*Δ yeast (37), this indicates that Spt3 (and by inference the SAGA complex) can be recruited to *MET2* and *MET6* independently of Mediator, whereas it depends on Mediator at the induced *CHA1* promoter. ChIP experiments also showed recruitment of SAGA subunits Ada2 and Gcn5 to the induced *MET2* and *MET6* promoters in both wild type and *gal11*/*med15*Δ *med3*Δ yeast, although Gcn5 recruitment was somewhat reduced in the mutant (Fig. 2*E*, *right panel*). We also examined Spt3 association with promoters of several constitutively active genes, including both SAGA-dependent genes whose activity is reduced in *gal11*/*med15*Δ *med3*Δ yeast and TFIID-dependent genes whose activity was unchanged in this mutant (37), and did not see any clear dependence on Mediator for Spt3 association.[3] These results indicate that SAGA recruitment depends on Mediator at the active *CHA1* promoter, but occurs independently of Mediator at *MET2* and *MET6*, consistent with previous findings that SAGA and Mediator are recruited independently to genes activated by Met4 (38).

Chromatin Remodeling at the Induced CHA1 Promoter Is Independent of Mediator—Chromatin remodeling of the induced *CHA1* promoter occurs independently of the Swi/Snf complex, and also does not depend on Gcn5 (11, 14). We previously found that chromatin remodeling, assessed by altered MNase accessibility, occurred normally upon *CHA1* induction in *srb4*/*med17 ts* yeast, indicating that remodeling did not depend on the middle or head modules of Mediator (11). However, as mentioned earlier, Mediator tail module subunits are present at normal levels at the induced *CHA1* promoter in *srb4*/*med17 ts* yeast (3, 11). Because Mediator and Swi/Snf association with the *CHA1* promoter is completely abolished in *gal11*/*med15*Δ *med3*Δ yeast (Fig. 2) (37), we examined the chromatin structure under inducing conditions in this mutant to determine whether remodeling occurs independently of the Mediator and Swi/Snf complexes.

MNase cleavage sites in the region of the *CHA1* promoter were mapped by indirect end labeling (43, 44) in nuclei from yeast under non-inducing and serine-induced conditions. Remodeling was clearly seen by increased MNase cleavage in the vicinity of the TATA element (Fig. 3, *arrow*). These alterations in MNase cleavage characteristic of *CHA1* induction occurred indistinguishably in wild type yeast and *gal11*/*med15*Δ *med3*Δ yeast (Fig. 3, *left panel*). We also examined chromatin remodeling in a *med3*Δ *gal11*/*med15-myc* strain; in the *med3*Δ background, the *gal11*/*med15-myc* allele behaves as a hypomorph, with similar but less pronounced effects on transcription and much less effect on growth in CSM than the *gal11*/*med15*Δ *med3*Δ mutant (37, 45). This strain also showed altered MNase cleavage upon *CHA1* induction that was indistinguishable from that seen in the corresponding wild type strain (Fig. 3, *right panel*). These results indicate that the chromatin structure is altered upon induction of the *CHA1* gene via a pathway that is independent of the Mediator and Swi/Snf complexes. We conclude that some other, unknown activator-dependent function is able to effect a change in the chromatin structure of the *CHA1* promoter upon induction.

Dependence of Histone Eviction on Mediator—Previously we showed that in wild type yeast, chromatin remodeling of the induced *CHA1* promoter is accompanied by histone eviction as assessed by ChIP against histone H3. Surprisingly, histone eviction was not observed in *srb4*/*med17 ts* yeast, even though remodeling detected by an altered MNase cleavage pattern still occurs (11). We corroborated and extended this finding by measuring histone H3 association at the uninduced and induced *CHA1* promoter in *gal11*/*med15*Δ *med3*Δ yeast by ChIP; in this mutant, transcriptional activation of *CHA1* and concomitant Mediator recruitment are abolished (37), but chromatin remodeling is still observed upon induction (Fig. 3). Similarly to *srb4*/*med17 ts* yeast, no change was seen in histone H3 association in the *gal11*/*med15*Δ *med3*Δ mutant upon *CHA1* induction, whereas about a 2-fold decrease in H3 association was seen at the induced *CHA1* promoter in wild type cells (Fig. 4*A*). This experiment provides further evidence for

[3] S. A. Ansari, E. Paul, and R. H. Morse, unpublished observations.

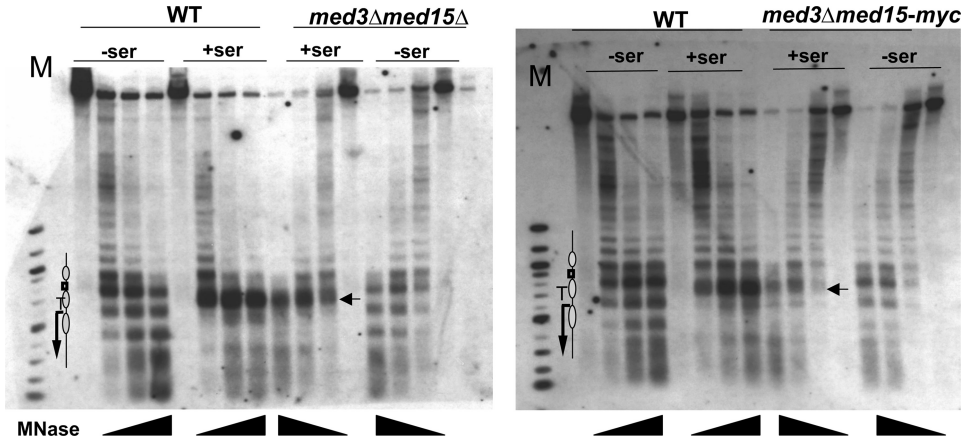

FIGURE 3. **Chromatin remodeling of the induced *CHA1* promoter occurs normally in yeast harboring Mediator tail mutants that are defective for *CHA1* transcriptional activation.** MNase cleavage sites were mapped relative to the BamHI site at +602 in the *CHA1* ORF in chromatin prepared from yeast grown in CSM without or with 1 mg/ml of serine (30 min induction). Increasing amounts of MNase were used for each sample as indicated by the *triangles* at the *bottom*; the lowest level in each group corresponds to no MNase addition, and the highest to 50 units/ml. Marker lanes (*M*) contain 100-bp DNA ladders. The *small arrow* in each panel indicates the strong cleavage induced by serine addition in the vicinity of the *CHA1* TATA element.

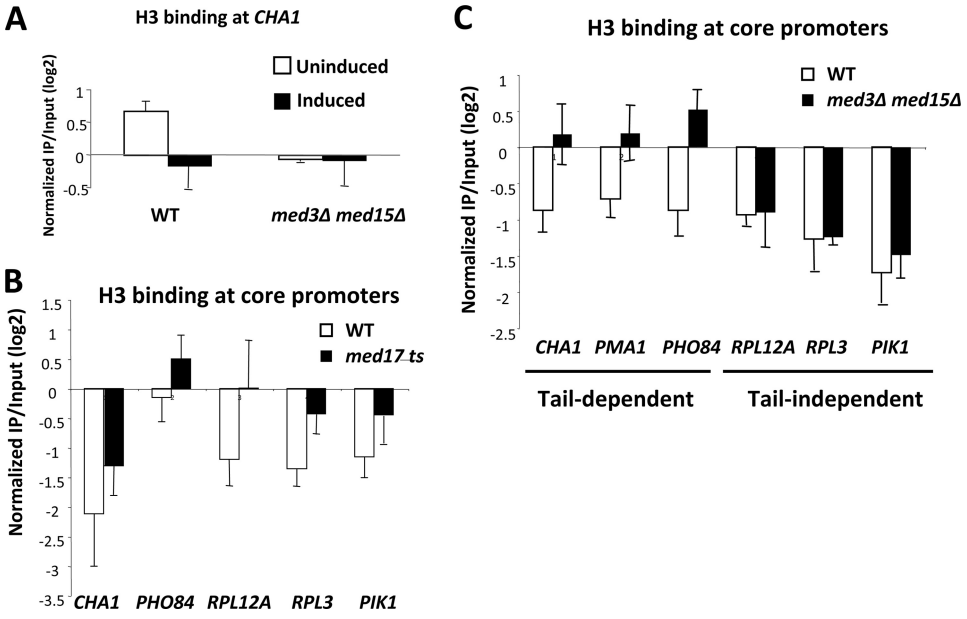

FIGURE 4. **Histone H3 association with active gene promoters is increased in Mediator mutants.** *A*, histone H3 association with the *CHA1* promoter was measured by ChIP using wild type (BY4742) and *gal11*/*med15*Δ *med3*Δ (LS10) yeast grown in CSM without (uninduced) or with (induced) 1 mg/ml of serine. *B,* histone H3 association with the indicated promoters was measured in wild type (RMY521) and *srb4*/*med17 ts* (RMY522) yeast grown in YPD after 1 h growth at 37 °C. *C,* histone H3 association with promoters whose transcription is dependent or independent of the Mediator tail module, as indicated, was measured in wild type (BY4742) and *gal11*/*med15*Δ *med3*Δ (LS10) yeast grown in YPD. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). *Error bars* represent S.D. ($n = 3$).

uncoupling of chromatin remodeling and histone eviction at the induced *CHA1* promoter.

To examine the relationship between Mediator, chromatin remodeling, and histone eviction at another inducible promoter, we used a variant of the classic *PHO5* promoter, *PHO5v33,* in which the two binding sites for the activator Pho4 were replaced by two strong Gal4 binding sites (20, 21). A *lacZ* reporter gene fused to this promoter was rapidly induced upon addition of galactose to yeast grown in raffinose medium, and this induction was greatly reduced in *gal11*/*med15*Δ *med3*Δ yeast (Fig. 5*A*). As seen for *CHA1*, chromatin remodeling, monitored in this case by accessibility to the restriction enzyme ClaI, was similar in wild type and *gal11*/*med15*Δ *med3*Δ yeast (Fig. 5*B*). Histone H3 association with the *PHO5v33* promoter, measured by ChIP, was strongly reduced following galactose induction in wild type yeast (Fig. 5*C*). However, in contrast to *CHA1*, the decrease in H3 association following induction was very similar in wild type and *gal11*/*med15*Δ *med3*Δ yeast (Fig. 5*C*). Thus, the uncoupling of chromatin remodeling, monitored by nuclease accessibility, and histone eviction, monitored by ChIP, seen at *CHA1* in Mediator-deficient yeast is not universal.

To further examine the role of Mediator in determining nucleosome occupancy at promoters, we measured histone H3 occupancy at several additional promoters. All of the promoters we examined showed increased H3 levels in *srb4*/*med17 ts* yeast ($p < 0.01$ for the null hypothesis of no change in H3 association between wild type and *srb4*/*med17 ts* mutant yeast) (Fig. 4*B*). Furthermore, in *gal11*/*med15*Δ *med3*Δ yeast (grown

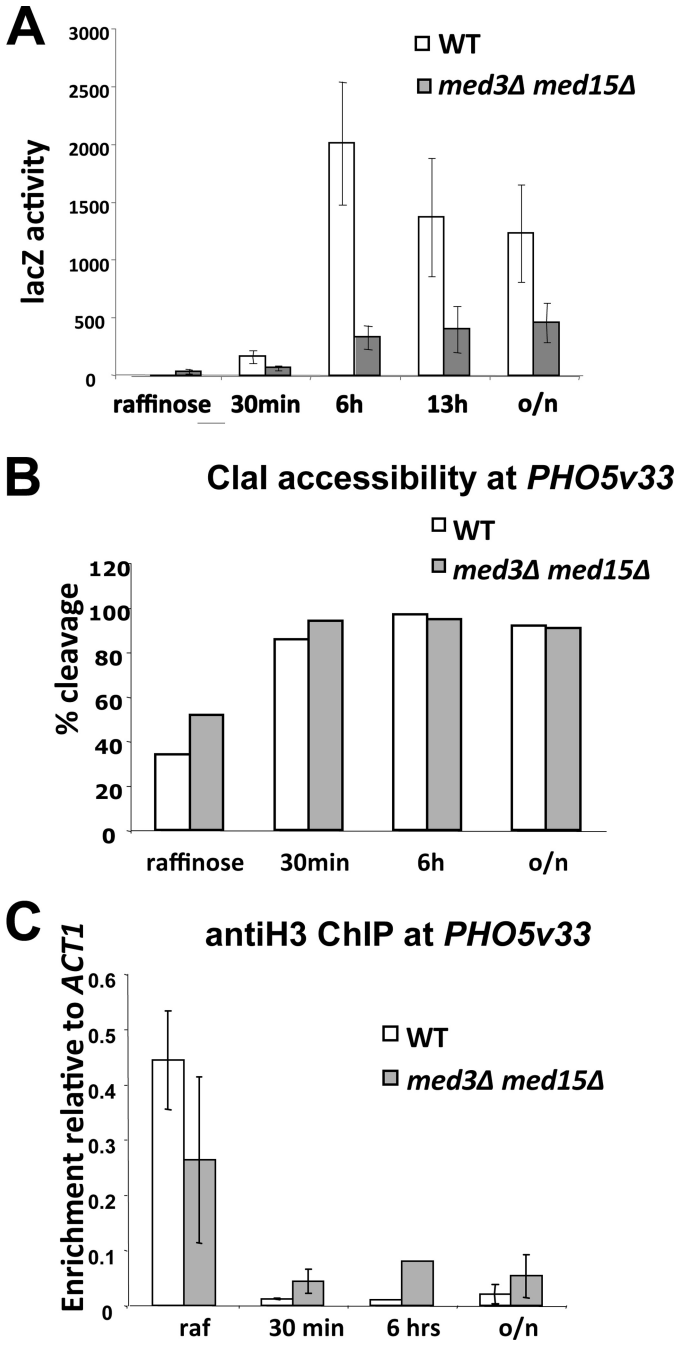

FIGURE 5. **Dependence on Mediator for transcription, chromatin opening, and histone eviction of the *PHO5v33* promoter.** *A,* β-galactosidase activity (Miller units) was measured for wild type (BY4742) and *gal11*/*med15*Δ *med3*Δ (LS10) yeast harboring the plasmid p$P_{pho5v33}$-lacZ and grown in raffinose-containing medium and induced by addition of galactose for the indicated times. *Error bars* represent S.D. ($n = 3$). *B,* ClaI accessibility of the *PHO5v33* promoter was determined in nuclei prepared from strains and using growth conditions as in *A*. Biological replicates for the 30-min induction time point of wild type and mutant gave 88 and 94% ClaI accessibility, respectively. *C,* histone H3 association at the *PHO5v33* promoter was measured by ChIP using strains and growth conditions as in *A*. *Error bars* represent the variation between two biological replicates.

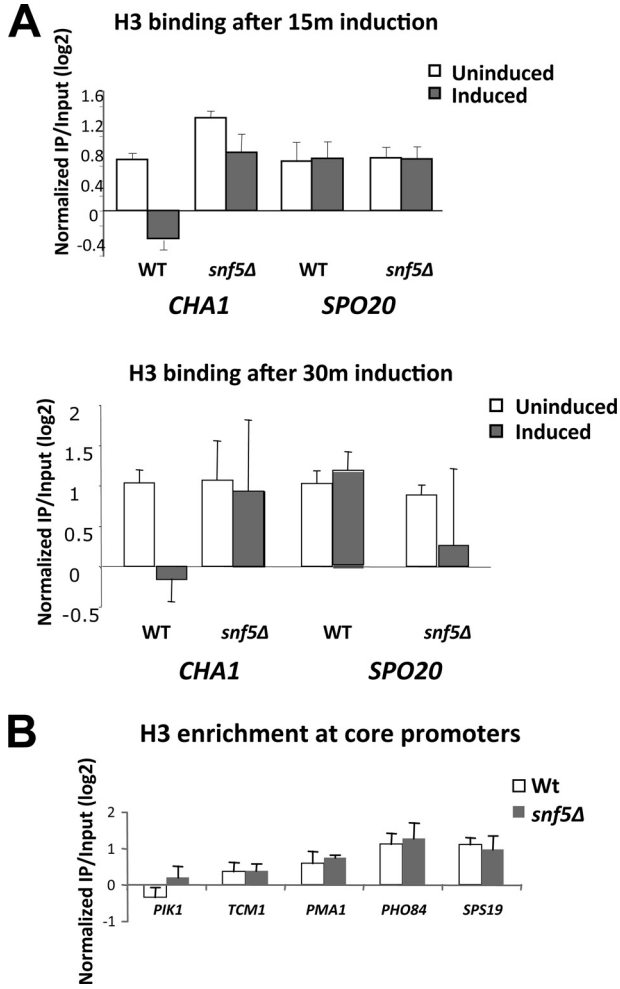

FIGURE 6. **Dependence of histone H3 association on the Swi/Snf complex.** *A,* association of histone H3 with the *CHA1* and *SPO20* promoters was measured by ChIP in wild type (BY4741) or *snf5*Δ (yeast deletion collection) yeast grown in CSM under non-inducing conditions (no serine) or after induction for 15 or 30 min by addition of 1 mg/ml of serine. *B,* histone H3 association at the indicated promoters was measured using the samples from *A* obtained from yeast grown under non-inducing conditions for 30 min. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). *Error bars* represent S.D. ($n = 3$).

in YPD), we observed increased H3 occupancy at promoters of genes showing dependence on the Mediator tail module (*CHA1*, *PMA1*, and *PHO84*) ($p < 0.02$) but not at genes that do not show such dependence (*RPL12A*, *RPL3*, and *PIK1*) (Fig. 4*C*). (*CHA1* is partially induced in YPD medium, which contains serine.) Thus, promoters showing decreased Mediator association also showed increased histone H3 occupancy.

The Swi/Snf complex is required for histone eviction at the induced *SUC2*, *DAN1*, *PHO5*, and *PHO8* promoters (20, 41, 46, 47), and Swi/Snf association is decreased at several promoters when Mediator recruitment is deficient (Fig. 2). Furthermore, *CHA1* induction is partially inhibited and is delayed in *swi/snf* mutant yeast (Fig. 1). These observations suggest that the effect of impaired Mediator function on histone H3 eviction could be caused by impaired Swi/Snf recruitment. To test this idea, we examined H3 association at the *CHA1* promoter 15 and 30 min after serine induction in wild type and *snf5*Δ yeast (Fig. 6*A*). A significant decrease ($p < 0.05$) in histone H3 association was observed in wild type yeast 15 and 30 min after *CHA1* induction, consistent with its transcriptional induction (Fig. 1). In contrast, histone H3 association was essentially unchanged 15 or 30 min after serine induction in *snf5*Δ yeast. These results

indicate that the Swi/Snf complex contributes to histone eviction during *CHA1* induction.

Swi/Snf is also present at constitutively active genes such as *PMA1* and *PHO84* (Fig. 2), and these promoters, like the active *CHA1* promoter, show increased H3 association in Mediator-defective yeast (Fig. 4). We therefore asked whether H3 association at such genes increased in *snf5*Δ yeast. In contrast to the increased H3 occupancy seen during *CHA1* induction in *snf5*Δ yeast (Fig. 6*A*), we did not observe significant changes in H3 association at these genes in *snf5*Δ compared with wild type yeast (Fig. 6*B*). This suggests that the role of Swi/Snf in assisting histone eviction may be specific to gene induction and that Swi/Snf is not needed to maintain low levels of histone association at constitutively expressed promoters. Furthermore, it implies that the increased H3 occupancy seen in Mediator mutants (Fig. 4) is not directly due to failure to recruit Swi/Snf. Rather, some other feature of Mediator recruitment is important for low histone occupancy at active gene promoters.

Dependence of Histone H3 Eviction on PIC Components—Histone eviction is observed at the induced *CHA1* promoter in wild type yeast but not in *srb4*/*med17 ts* and *gal11*/*med15*Δ *med3*Δ mutants, whereas chromatin remodeling, seen by altered MNase accessibility, occurs in both wild type and mutant strains. To explain this uncoupling, we hypothesized a two-stage mechanism leading to histone eviction. In this scenario, gene activation leads to recruitment of a factor or factors that alter nucleosome conformation or positioning without causing histone displacement. This alteration increases accessibility to the associated DNA by MNase, thus exposing cleavage sites that are diagnostic of chromatin remodeling (Fig. 3), and renders the DNA of the proximal promoter region accessible to the general transcription machinery. In wild type cells, activation also leads to recruitment of Mediator and other coactivators, such as Swi/Snf and SAGA, and formation of a functional PIC. We hypothesized that association of the PIC with the promoter occurs in competition with histone binding, leading to histone eviction. According to this scenario, in yeast defective for Mediator recruitment, defective PIC recruitment allows histones to remain associated with activated promoters as in the uninduced state, albeit in a state different from canonical nucleosomes.

To test this model, we used ChIP to measure histone H3 association in the temperature-sensitive mutants *rpb1-1* and *tbp ts-1* (48, 49), which show defective association of Pol II and TBP, respectively, with target genes at 37 °C (8, 50, 51). Wild type and mutant strains were grown at 24 °C, shifted to 37 °C for 30 min, and *CHA1* induced by addition of serine for an additional 30 min while maintaining cultures at 37 °C prior to ChIP (11). We found that in both *rpb1-1 ts* and *tbp ts-1* yeast, histone H3 association with the *CHA1* promoter was unchanged upon induction, in contrast to the ~2-fold decrease seen in wild type yeast (Fig. 7*A*). Thus, Pol II is required for stable histone eviction upon *CHA1* induction. We also examined several other promoters in *rpb1-1 ts* and found increased H3 occupancy compared with wild type ($p < 0.01$, not including the data for *CHA1*, for the null hypothesis of no change in H3 association between wild type and *rpb1-1* mutant yeast) (Fig. 7*B*). Because these latter promoters are, unlike *CHA1*, constitutively active, this result indicates that Pol II is needed for the maintenance of low histone occupancy at these active promoters.

In *tpb ts*-1 yeast, histone H3 levels increased at some promoters (*PDC1*, *RPL12A*, and *RPL3*; Fig. 7*C*), but some promoters (*MAE1*, *ARG4*, and *SNQ2*) did not show increased H3 occupancy.[3] However, the latter promoters also did not show decreased TBP binding in the *tbp ts-1* mutant, for unknown reasons, whereas those promoters showing decreased TBP binding also showed increased H3 levels in the *tbp ts-1* mutant[3] (Fig. 7*E*). All of the promoters examined in the *rpb1-1* mutant showed decreased binding of Pol II (Fig. 7*D*). Thus, defective PIC formation correlated well with increased histone H3 occupancy.

To test whether processes related to or depending on PIC recruitment are important for histone H3 eviction, we compared H3 occupancy at active gene promoters in two additional mutant strains. The *sua7-1* mutation encodes a point mutation in TFIIB (TFIIB-E62K) that affects start site selection and confers a strong cold-sensitive phenotype (which we verified), but does not impair interactions with Pol II or TBP *in vitro*, and has little effect on transcription levels *in vivo* (52–54). TFIIB is also important in bringing 5′ and 3′ regions of transcribed genes into close proximity (gene looping), and this looping is strongly impaired in *sua7-1* mutant yeast, including at the active *CHA1* promoter (53, 55). We observed a modest (about 2-fold) increase in induced levels of *CHA1* expression in the *sua7-1* mutant,[3] whereas ChIP experiments revealed decreased H3 occupancy upon *CHA1* induction that was indistinguishable in *sua7-1* and wild type yeast (Fig. 8*A*). We also monitored H3 occupancy in *sua7-1* yeast at several of the same promoters that exhibited increased H3 occupancy in *srb4*/*med17 ts* and *rpb1-1 ts* yeast and saw no significant differences in histone H3 occupancy between the *sua7-1* mutant and wild type yeast (Fig. 8*B*). The *sua7-1* mutation did not cause any defects in Pol II or TBP association with the induced *CHA1* promoter or with the constitutively active promoters that we tested, consistent with *in vitro* results (52) (Fig. 8, *A* and *B*).

We also compared histone H3 occupancy at promoters of active genes in wild type and *bur2*Δ *kin28-as* mutant yeast. Kin28 contributes to phosphorylation of Ser-5 of the carboxyl-terminal domain of the large subunit (Rpb1) of Pol II, which is important for recruitment of various factors involved in transcriptional elongation and associated processes, including mRNA capping enzyme, the Paf1 complex, Bur1, and the Rpd3(S) complex (56–60). The *kin28-as* mutation allows inactivation of *kin28* kinase activity by the chemical inhibitor NA-PP1 (61). However, although this treatment results in reduced recruitment of Paf1C and the Rpd3(S) complex, compensatory Ser-5 phosphorylation occurs via the Bur1/2 kinase complex (62). We therefore examined histone H3 occupancy in the *kin28-as bur2*Δ double mutant after 15 min of NA-PP1 treatment (58, 60). No significant difference was seen in histone H3 occupancy at any of the promoters examined, although a modest trend toward decreased, not increased (as in PIC mutants) H3 occupancy may be present (Fig. 8*B*). Thus, our examination of mutations affecting events occurring post-PIC recruitment do not indicate any contribution to promoter occupancy by histone H3.

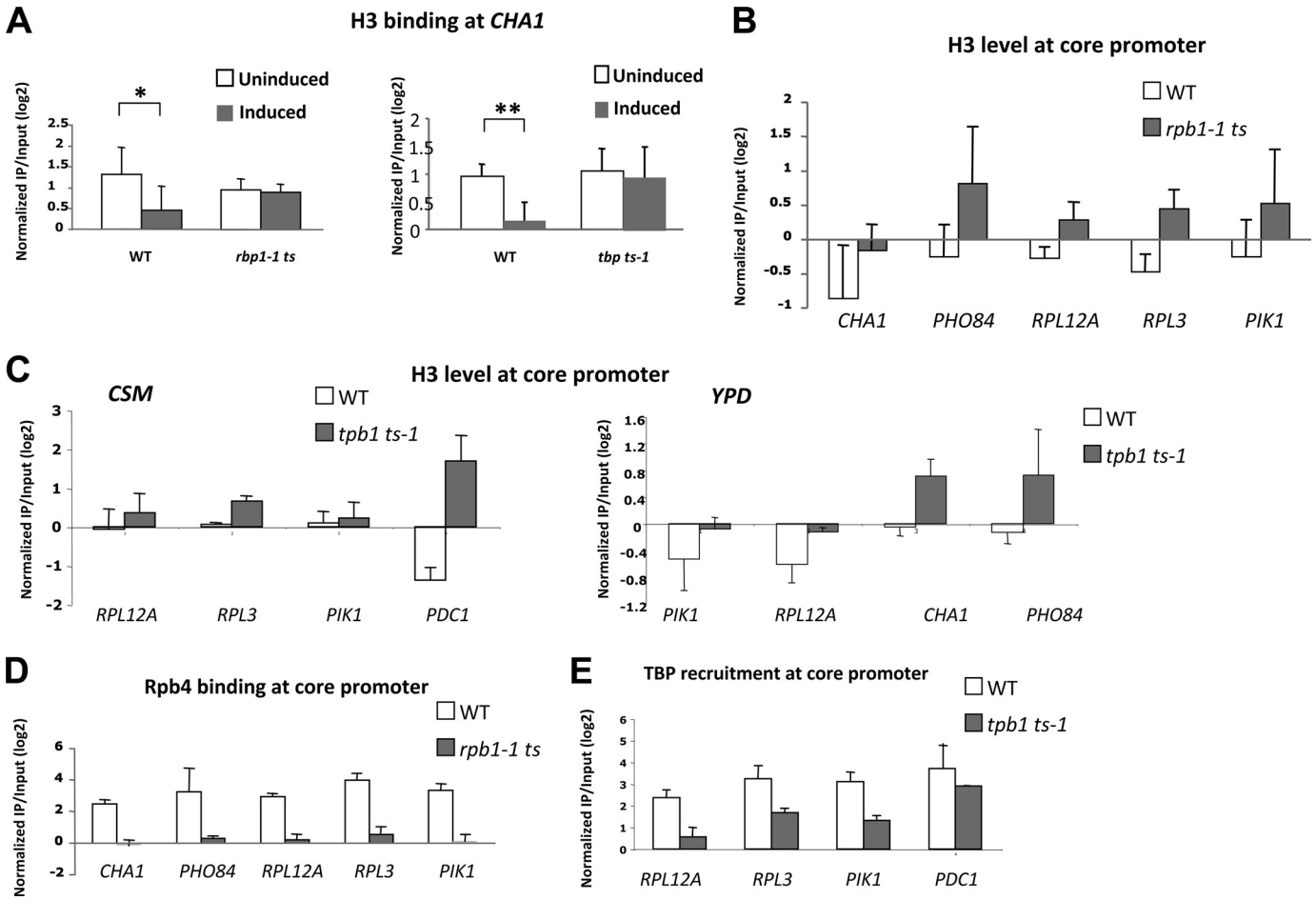

FIGURE 7. **Dependence of histone H3 association on Pol II and TBP.** *A,* association of histone H3 with the *CHA1* promoter was measured by ChIP in yeast grown in CSM without (uninduced) or with (induced) 1 mg/ml of serine and shifted to 37 °C for 1 h. Strains used were RMY521 (wild type) and Z111-Srb5-Myc (*left panel*), and BYΔ2 (wild type) and BYΔ2-ts1 (*tbp ts-1*). *B,* association of histone H3 with the indicated promoters was measured by ChIP for yeast grown in YPD after a 1-h shift to 37 °C in wild type (RMY521) and *rpb1-1 ts* (Z111-Srb5-Myc) yeast. *CHA1* is partially induced in YPD medium. *C,* association of histone H3 with the indicated promoters was measured by ChIP in wild type (BYΔ2) and *tbp ts-1* (BYΔ2-ts1) yeast grown in CSM (*left panel*) or YPD (*right panel*) medium after a 1-h shift to 37 °C. *D*, association of Rpb4 with the core promoter regions of the indicated genes was measured by ChIP in wild type (RMY521) and *rpb1-1 ts* (Z111-Srb5-Myc) yeast grown in YPD after 1 h at 37 °C. *E*, association of TBP with core promoter regions of the indicated genes was measured by ChIP in wild type (BYΔ2) and *tbp ts-1* (BYΔ2 ts-1) yeast grown in CSM after 1 h at 37 °C. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). All *error bars* represent S.D. ($n = 3$ except for *C*, *left panel*, and *E*, *PDC1*, for which $n = 2$); *asterisk in A* indicates $p < 0.05$ and the *double asterisk* indicates $p < 0.01$.

Mediator contributes to recruitment of TBP and Pol II, and TBP to binding of Pol II (11, 51, 63, 64). Thus, the reduced histone H3 eviction seen in yeast harboring defective Mediator complex could reflect a direct role of Mediator or could be due to defective PIC recruitment, as we have postulated. To investigate this further, we measured association of TBP and the Mediator head module subunit Srb5/Med18 by ChIP in wild type and *rpb1-1 ts* yeast (Fig. 9). We found reduced binding of both TBP and Srb5/Med18 at all five of the core promoters that we examined in *rpb1-1 ts* yeast compared with wild type. These findings indicate mutual cooperative interactions among Mediator, TBP, and Pol II. We conclude that all three types of mutants examined here, in Mediator, Pol II, and TBP, affect PIC and Mediator association, and that the effects measured for histone H3 association reflect the reduced association of all of these components. Thus, histone eviction that accompanies *CHA1* activation depends on Mediator recruitment and PIC formation, and reduced histone association with constitutively active promoters (65, 66) also depends in part on binding of these same components.

DISCUSSION

The work reported here had two principal objectives. The first was to test the hypothesis that histone eviction accompanying transcriptional activation depends on stable PIC formation. The second major objective was to gain additional insight into the interdependencies of coactivator complex recruitment during transcriptional activation in yeast.

Previously we showed that during induction of the *CHA1* promoter, histone eviction, as monitored by ChIP, could occur in a step distinct from chromatin remodeling, as monitored by MNase cleavage (11). Here, we confirm and extend these results by showing that whereas histone eviction depends on the integrity of the Mediator complex, the initial chromatin remodeling step occurs completely independently of Mediator. However, dependence on Mediator for histone eviction is not universal,

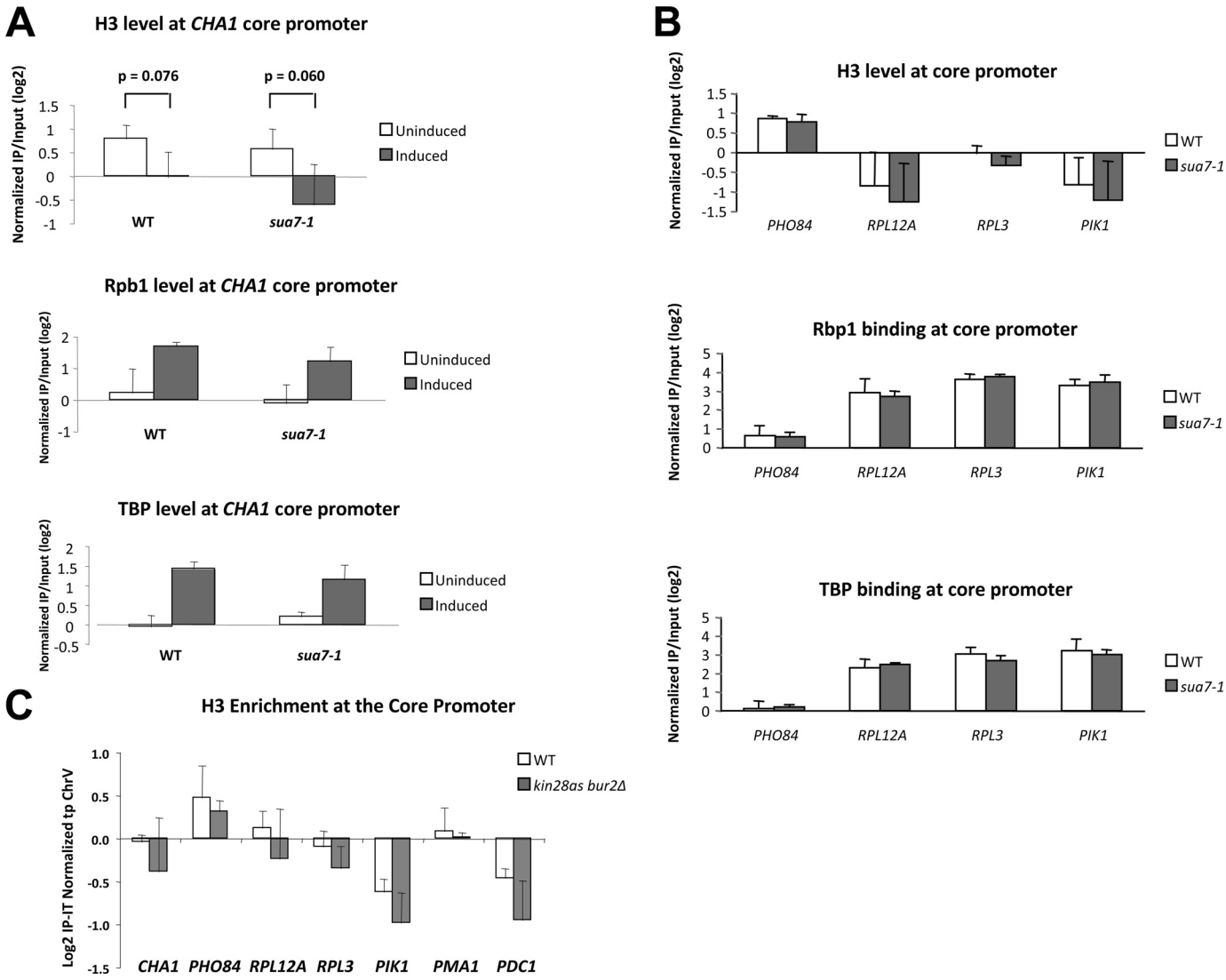

FIGURE 8. **Mutations that affect DNA looping or Ser-5 phosphorylation of the Pol II carboxyl-terminal domain do not alter histone H3 occupancy at active promoters.** *A,* core promoter occupancy by histone H3, Rpb1, and TBP was measured by ChIP at the *CHA1* promoter in wild type and *sua7-1* yeast grown in CSM without (uninduced) and with (induced) 1 mg/ml of serine. *B,* core promoter occupancy by histone H3, Rpb1, and TBP was measured by ChIP at the indicated promoters in wild type and *sua7-1* yeast grown in CSM. *C,* core promoter occupancy by histone H3 was measured by ChIP at the indicated promoters in wild type and *bur2Δ kin28-as* yeast grown in CSM-ura supplemented with 1 mg/ml of serine 60 min after addition of 1 mM NA-PP1. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). *Error bars* represent S.D. ($n = 3$), and *p* values shown in *B* are from a paired *t* test.

as we show for a variant *PHO5* promoter driven by the Gal4 activator. Possibly the Gal4 activator recruits additional factors not present at the induced *CHA1* promoter that contribute to histone eviction.

Other examples of uncoupling of chromatin remodeling from histone eviction, in addition to that shown here and previously for *CHA1* (11), have also been reported. The *PHO5* promoter is constitutively active in the absence of the cyclin Pho80. In *pho80 gcn5* yeast, activity of *PHO5* was greatly reduced compared with the *pho80* mutant and a novel chromatin state was observed (67). This chromatin state was characterized by altered accessibility to DNase I and restriction enzymes compared with the repressed or fully active *PHO5* promoter, and thus represented a remodeled state compared with the repressed promoter (67). However, a later study showed that the number of nucleosomes at the *PHO5* promoter in *pho80 gcn5* yeast was unchanged relative to the repressed state in wild type yeast, implying that histone eviction did not occur (68). Similarly, at the yeast *PHO84* promoter, activation is accompanied by increased accessibility to the restriction enzyme HpaI and histone eviction in wild type yeast, whereas in *gcn5* mutant yeast, a delay in transcriptional induction is accompanied by a corresponding delay in histone eviction, with no alteration in the kinetics of increased HpaI accessibility (69). In another example, activation of the yeast *RNR3* promoter in response to DNA damage results in altered MNase cleavage and histone eviction in wild type yeast. In contrast, loss of SAGA complex components in *spt3*Δ or *spt7*Δ yeast strongly inhibits *RNR3* induction and histone eviction, while still allowing essentially normal chromatin remodeling as monitored by MNase cleavage (70). These examples, together with our studies on *CHA1* induction, suggest that during normal transcriptional activa-

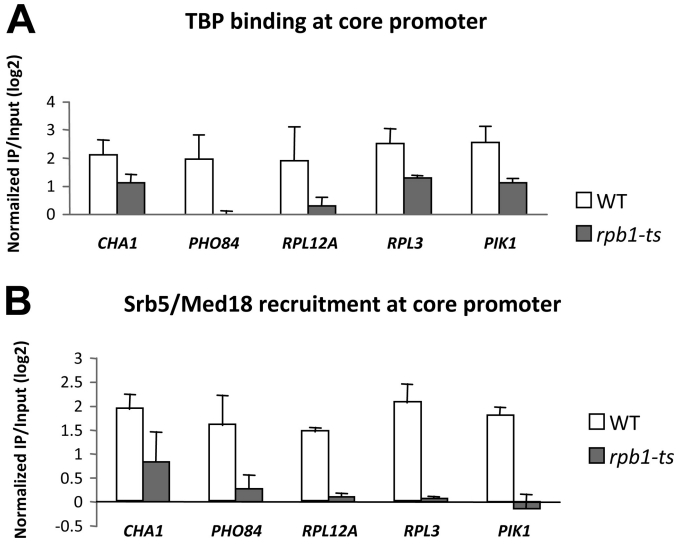

FIGURE 9. **Dependence of TBP and Srb5/Med18 binding on Pol II.** TBP (*A*) and Srb5/Med18 (*B*) association with the indicated core promoters was measured by ChIP in wild type (RMY521) and *rpb1-1 ts* (Z111-Srb5-Myc) yeast grown in YPD after 1 h at 37 °C. Relative association in all cases was determined by quantitative PCR analysis of input and immunoprecipitated (*IP*) samples, and normalized to a non-transcribed region of ChrV (25). *Error bars* represent S.D. ($n = 3$).

tion, nucleosome mobilization and histone eviction are generally tightly coupled, with uncoupling being observed when recruitment of coactivators or the general transcriptional machinery is defective.

We hypothesized that in this two-step model for chromatin remodeling and histone eviction, the latter step might require stable binding of the PIC to compete against histones for promoter occupancy. Others have made similar suggestions; for example, paused Pol II has been argued to compete with histones for occupancy of highly regulated promoters in mammalian cells (71). To test the role of the PIC in histone eviction, we examined the effect of mutations in PIC components TBP and Pol II on histone association at various promoters. We found that in both *rbp1-1* and *tbp ts-1* mutants, histone eviction was impaired at the induced *CHA1* promoter (Fig. 7). Furthermore, higher histone levels were observed at a number of constitutively active promoters in both mutants, consistent with a previous genome-wide study that also found a modest increase in promoter nucleosome occupancy in *rpb1-1* yeast (72). This latter finding implies that the PIC is important for maintenance of low histone occupancy at active promoters, and not just for eviction during activation. These results favor the idea that PIC components assist histone eviction by competing for occupancy at promoters.

An alternative interpretation for the dependence of low promoter histone occupancy on components of the PIC is that histone eviction depends on some other chromatin remodeler whose recruitment depends on the PIC. We did not observe altered H3 occupancy in a *sua7*-1 mutant that impairs DNA looping, or in a *bur2*Δ *kin28-as* mutant that affects Ser-5 phosphorylation of the Pol II CTD and associated processes, and it seems less likely that events occurring post-PIC formation would affect promoter chromatin structure. However, we cannot formally exclude this possibility, nor the possibility that other chromatin remodelers might be recruited in a PIC-dependent fashion. Our examination of *CHA1* activation in yeast lacking several chromatin remodelers and histone chaperones that are known to contribute to chromatin remodeling and histone eviction at specific promoters (35, 36, 73), and are thus also good candidates for contributing to the outcome between competition between histones and PIC components, did not reveal any candidates for such a role (Fig. 1*B*).

PIC components contribute to Swi/Snf recruitment to the induced *RNR3* promoter (8), suggesting that the increased H3 occupancy seen in Mediator, Pol II, and TBP mutants could occur indirectly via deficient Swi/Snf recruitment. Two arguments can be made against this scenario: first, Swi/Snf recruitment occurs normally at the *CHA1* promoter under inducing conditions in *srb4*/*med17 ts* yeast, but histone H3 eviction does not (11); thus, Swi/Snf is not sufficient for histone eviction. Second, no change was seen in histone occupancy at several constitutively active promoters in *snf5*Δ yeast (Fig. 6), but H3 occupancy was increased in *srb4*/*med17*, *rpb1-1*, and *tbp ts* mutant yeast at such promoters (Figs. 4 and 7).

Which PIC components are most important in contributing to histone eviction may not only be difficult to answer, it may be the wrong question to ask. The dependence on Mediator for recruitment of TBP and Pol II is well established, as is the requirement for TBP for Pol II binding at most promoters. We also observed reduced binding of TBP and Mediator in *rbp1-1 ts* yeast, suggesting mutual cooperativity in binding of all of these components at many promoters (Fig. 9). Mutual cooperativity between Mediator and TFIID binding has been observed *in vitro* (74), and reduced association of TBP and Mediator was observed at the activated *RNR3* promoter in *rpb1-1* yeast (8). Furthermore, Mediator can be recruited to a promoter that is activated by direct recruitment of TBP or TFIIB (5). Whether these cooperative interactions depend on promoter structure or activator type remains uncertain, but these observations indicate that it is the PIC as a whole, including Mediator, that needs to be considered as participating in histone eviction at active gene promoters.

Our second major objective was to gain new insight into the interdependencies of coactivator recruitment during transcriptional activation in yeast. We found variable dependence on Mediator for recruitment of Swi/Snf and the SAGA component Spt3 at specific gene promoters, including an unexpected dependence for Swi/Snf recruitment on the Mediator tail module at the induced *CHA1* promoter. Association of Swi/Snf to several constitutively active promoters was reduced in Mediator-defective yeast, suggesting a widespread dependence on Mediator for association of Swi/Snf with transcriptionally active genes. Curiously, despite its presence at constitutively active promoters, Swi/Snf loss did not affect histone occupancy. Future genome-wide studies should provide insight into commonalities and promoter-specific features governing interdependence of coactivator complex association.

In summary, our results support a model in which numerous mutually cooperative interactions govern association of coactivators and the transcriptional apparatus with active gene promoters. Specifically, recruitment of the Swi/Snf complex depends on Mediator not only at inducible promoters such as

CHA1, but also at constitutively active genes such as *PMA1* and *PIK1*. Previous work has also reported complex interdependencies for coactivator recruitment that can exhibit promoter specificity even for the same activator (9, 75). Similarly, we report dependence on Pol II for association of Mediator and TBP with several active gene promoters, indicating mutual cooperativity among PIC components and Mediator. We also provide evidence for distinct steps for nucleosome remodeling and histone eviction, and for PIC recruitment contributing to the second of these steps but not the first. This model suggests that it is not actually histone eviction that is important for allowing PIC access, but rather an altered (remodeled) nucleosome structure that allows effective competition against the histones for binding. Based on this idea, it might also be expected that histone eviction during replication and repair of chromatin-associated sequences also occurs in two stages, with binding of relevant proteins following a chromatin remodeling step that is a necessary prelude to these processes leading to stable loss of histones.

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