Mediator Requirement Downstream of Chromatin Remodeling during Transcriptional Activation of CHA1 in Yeast*

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Mediator complex is essential for transcription by RNA polymerase II in eukaryotes. Although chromatin remodeling is an integral part of transcriptional activation at many promoters, whether Mediator is required for this function has not been determined. Here we have used the yeast CHA1 gene to study the role of Mediator in chromatin remodeling and recruitment of the transcription machinery. We show by chromatin immunoprecipitation that Mediator subunits are recruited to the induced CHA1 promoter. Inactivation of Mediator at 37 °C in yeast harboring the srb4-138 (med17) ts mutation severely reduces CHA1 activation and prevents recruitment to the induced CHA1 promoter of Med18/Srb5, from the head module of Mediator, and Med14/Rgr1, which bridges the middle and tail modules. In contrast, recruitment of Med15/Gal11 from the tail module is unaffected in med17 ts yeast at 37 °C. Recruitment of TATA-binding protein (TBP) is severely compromised in the absence of functional Mediator, whereas Kin28 and polymerase II recruitment are reduced but to a lesser extent. Induced levels of histone H3K4me3 at the CHA1 promoter are not diminished by inactivation of Mediator, whereas recruitment of Paf1 and of Ser2- and Ser5-phosphorylated forms of Rbp1 are reduced but not eliminated. Loss of histone H3 from the induced CHA1 promoter is seen in wild type yeast but is greatly reduced by loss of intact Mediator. In contrast, Swi/Snf recruitment and nucleosome remodeling are unaffected by loss of Mediator function. Thus, Mediator is required for recruitment of the transcription machinery subsequent to chromatin remodeling during CHA1 induction.

The Mediator complex functions as an intermediary between activators and the general transcriptional machinery in eukaryotes, and its importance in messenger RNA transcription has been demonstrated both for individual genes and genome-wide (1–5). Mediator is structurally and functionally conserved across eukaryotes, comprising 25–30 subunits (6, 7). In the budding yeast Saccharomyces cerevisiae, 25 subunits have been identified, 21 of which form an extended structure with three distinct modules termed the head, middle, and tail (8, 9). Mediator is important for both basal and activated transcription in vitro and stimulates the phosphorylation of the carboxyl-terminal domain (CTD)9 of the Rpb1 subunit of the RNA polymerase II (pol II) core enzyme by TFIIH-associated protein kinase (10–13). In vivo, Mediator appears to be a direct target of activators and is recruited to gene promoters at the preinitiation stage of transcription (14–20). Mediator in turn facilitates recruitment of the preinitiation complex (PIC) and Spt-Ada-Gcn5-acetyltransferase (SAGA) complex in a promoter-dependent fashion (21–24). Recruitment of pol II is probably achieved in part by direct contacts with the CTD domain of the Rpb1 subunit via the middle and head modules of Mediator, as visualized by electron microscopy (25, 26). Genetic interactions between Mediator and pol II have also been shown by the identification of the SRB genes, which encode Mediator subunits, as suppressors of a truncated rpb1 mutant (27, 28). In addition, the Mediator complex was found to be associated with pol II in biochemical experiments (11, 27, 29).

Several studies have examined the relationship between Mediator complex and the recruitment of chromatin remodeling activities during gene activation. Stable association between Mediator and Swi/Snf was reported but also disputed (10, 30, 31). Direct recruitment of Mediator was shown to suffice for PHO5 remodeling and activation in “activator bypass” experiments (32). Activation of the GAL10 promoter by direct recruitment of Mediator was found to depend strongly on Swi/Snf, suggesting an intimate relationship between the two complexes (33). More recently, it was shown that such direct recruitment of Mediator was indeed accompanied by Swi/Snf recruitment and that recruitment of Swi/Snf to the activated GAL1 promoter depends on functional Mediator complex (34). Recruitment of Swi/Snf by Gcn4 also depends strongly, but not completely, on Mediator integrity (35). Thus, several studies have reported a connection between Mediator and Swi/Snf recruitment. However, apart from a single report showing that Swi/Snf-dependent chromatin remodeling of the activated RNR3 promoter depends on Mediator, the requirement of Mediator for remodeling per se has not been examined (36). Furthermore, the relationship between Mediator and chromatin remodeling at genes at which such remodeling does not depend on Swi/Snf has remained unexplored.

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To address this issue and to further our understanding of the role of the Mediator complex in gene activation, we have investigated the induction of the *S. cerevisiae* CHA1 gene. CHA1 encodes 3-seryl/threonyl dehydratase, and its expression can be induced by serine or threonine (37, 38). The CHA1 promoter has an upstream activating sequence about 230 bp upstream of its ORF, which is constitutively bound by the activator, Cha4 (39, 40), and a TATA box at −90 bp (Fig. 1A); both are required for CHA1 transcription (40, 41). The TATA element is occupied by a positioned nucleosome when the CHA1 gene is uninduced, and this nucleosome is remodeled upon activation in a Cha4–dependent manner, exposing the TATA element for TBP to bind (42). The mechanism by which the TATA-occupying nucleosome is remodeled during CHA1 activation is currently unknown. Remodeling does not require the Swi/Snf complex, although the complex is recruited to the CHA1 promoter during its activation (39, 42).

Here, we report the role of the Mediator complex in the recruitment of both PIC components and chromatin remodeling activities to the induced CHA1 promoter. We find that intact Mediator is required for recruitment of TBP and, to a somewhat lesser extent, pol II. Intact Mediator is required for histone H3 loss from the induced CHA1 promoter but, surprisingly, not for recruitment of Swi/Snf or for chromatin remodeling, the latter of which occurs independently of both Mediator and Swi/Snf. Furthermore, although loss of functional Mediator complex results in decreased levels of Pafl and Ser5-phosphorylated CTD of pol II, both of which are required for H3 Lys4 trimethylation (H3K4me3) (43, 44), induction of H3K4me3 is not diminished.

### EXPERIMENTAL PROCEDURES

**Yeast Strains and Growth**—Yeast cultures were grown on complete synthetic dropout media. Transformation of yeast was performed as previously described (45). Yeast cultures were rapidly shifted to 37 °C by adding an equal volume of medium prewarmed to 50 °C to cultures grown at 25 °C. The CHA1 gene was induced by the addition of 1 mg/ml serine to growth medium.

Strains used in this study are listed in Table 1. Strains harboring the *srb4-138* allele and their wild type counterparts were created by first introducing the plasmid pRY2844 or pRY2882 (harboring *SRB4* or *srb4-138*) (1) and then replacing the chromosomal *SRB4* ORF with the KanMX cassette. Strain RMY503 was derived from Z628 (1). Strains RMY504 and RMY505 were derived from FY2103 (46). Strains RMY511 and RMY512 were derived from H3028 (47). Strains RMY521 and RMY522 were derived from MSY120 (48). Strains RMY541 and RMY542 were derived from HQY389 (49). Strains RMY551 and RMY552 were derived from BY4741 using EcoRV-digested pHQ1300 (a generous gift of Hongfang Qiu and Alan Hinnebusch) to introduce a 10× Myc tag to Rpb3 prior to replacement of the endogenous *SRB4* with plasmid-borne *SRB4* or *srb4-138*. Strains RMY513-516, RMY531, RMY532, RMY561, and RMY562 were derived from the yeast TAP-tagged collection (50).

**Chromatin Immunoprecipitation (ChIP)**—ChIP was performed essentially as described, except that cross-linking was done at 37 °C and with the following modifications (40, 51, 52). For ChIP against Ser5- and Ser2-phosphorylated forms of the Rpb1 CTD, whole cell extract was incubated with ~20 μg of H14 and H5 antibody (Covance), respectively, at 4 °C for 3 h, after which 0.5 μg of anti-mouse IgM antibody (Invitrogen) was added, and incubation continued overnight. Antigens were precipitated by protein A-agarose beads, and DNA was extracted. Antibodies against Myc and HA tags were purchased from Roche Applied Science; antibody against H3K4me3 was purchased from Upstate Biotechnology, Inc., and antibody against the COOH terminus of H3 (pan-H3 antibody) was from Lake Placid Biologicals. For ChIP of TAP-tagged proteins, antibody to protein A was used (Sigma). Quantitations were done either by PCR and Southern blot, using PCR conditions determined to be in the exponential phase of amplification (40), or quantitative real time PCR and normalized as indicated in the figure legends. ChIP was repeated three or four times for each experiment, and S.D. values are shown in the figures. Primer sequences are available upon request.

**Chromatin Preparation and Indirect End Labeling**— Chromatin structure was analyzed as described (40, 53), with all
steps through MNase digestion performed at 37 °C to ensure the inactivation of Srb4 throughout the experiment. All chromatin mapping experiments were done at least twice and with a range of micrococcal nuclease concentrations.

**RNA and Protein Analysis**—For analysis of CHA1 mRNA, RNA was prepared by standard methods, and Northern blotting was conducted as described previously (54, 55). CHA1 mRNA was normalized to 25S rRNA.

For Western blotting, 10 optical density at 600 nm (A$_{600}$) units of cells (A$_{600}$ = 1.0) were precipitated and added to 3 ml of 50 mM Tris (pH 7.5), 10 mM NaN$_3$ on ice, spun down, resuspended in 100 µl of ESB (2% SDS, 80 mM Tris (pH 6.8), 10% glycerol, 1.5% dithiothreitol, 0.1 mg/ml bromphenol blue), and quickly transferred to microcentrifuge tubes for a 3-min incubation at 100 °C. Glass beads were added to reach the meniscus, and the samples were vortexed at top speed for 2 min. An additional 100 µl of ESB was added, and the samples were heated to 100 °C for 1 min. Following standard SDS-polyacrylamide gel electrophoresis, the proteins were electroblotted to Millipore polyvinylidene difluoride membrane. The membrane was blocked by blotto buffer (5% nonfat milk in phosphate-buffered saline) and incubated for about 3 h at 4 °C. For Western blot against hyperphosphorylated Rpb1-CTD, the membrane was incubated at 4 °C overnight with antibodies H14 (1:3,000; Covance) and H5 (1:1,200; Covance) mixed with rabbit anti-IgM antibody (1:30,000; Invitrogen). For Western blot against histone H3, the membrane was incubated at 4 °C overnight with antibody directed against the carboxyl terminus of histone H3 (1:15,000; Upstate Biotechnology). Following washing by PBST (phosphate-buffered saline plus 0.05% Tween 20), the blots were incubated for about 3 h at room temperature with horseradish peroxidase-linked anti-rabbit antibody. After washing, ECL (GE Healthcare) was used to illuminate the reactive band.

**RESULTS**

**Mediator Is Essential for CHA1 Activation**—Mediator has been suggested to be required for all transcription mediated by pol II on the basis of *in vivo* and *in vitro* results (1, 13). However, a recent study found no association of Mediator above background levels with a number of yeast genes, calling this general requirement into question (3). To test whether Mediator is required for CHA1 induction, we used a ts mutant in the MED17/SRB4 gene, which encodes a subunit of the head module of Mediator and is essential in yeast. In yeast harboring the temperature-sensitive mutant *srb4-138* (med17 ts), the head module of Mediator is disrupted at 37 °C, and mRNA transcription is decreased to less than 10% of wild type levels (1, 2, 13). We monitored CHA1 expression in *srb4* ts yeast and a corresponding wild type strain after rapidly shifting both strains from 25 to 37 °C prior to the addition of serine or inducing at 25 °C as a control and normalized to the 25 S rRNA message, which is transcribed by pol I and does not require Mediator. Results show that although CHA1 is induced to comparable levels at 25 °C over the first 2 h of induction in wild type and *srb4-138* yeast, CHA1 expression in the *srb4-138* mutant is greatly reduced at 37 °C compared with wild type (Fig. 1B; see also Fig. 2A). Thus, MED17/SRB4, and therefore intact Mediator, is essential for CHA1 activation.

**Effect of Loss of Med17/Srb4 Function on Mediator Recruitment**—To test whether loss of CHA1 activation in *srb4-138* ts yeast at 37 °C is caused by a direct effect, we used ChIP to assess Mediator recruitment to the induced CHA1 promoter, using the protocol depicted in Fig. 2A. Isogenic wild type and *srb4-138* strains were generated from yeast expressing Myc-tagged Med18/Srb5 or Med15/Gal11 or TAP-tagged Med14/Rgr1 by deleting the endogenous MEDI17/SRB4 gene in the presence of an episomal copy of either wild type MEDI17/SRB4 or the *srb4-138* ts mutant allele. The wild type and the mutant strains were grown at 25 °C, rapidly shifted to 37 °C, and kept at 37 °C for 30 min to inactivate the *srb4* ts mutant. Half of each culture was then induced by 1 mg/ml serine for 30 min at 37 °C, whereas the other half was used as the uninduced control. RNA was extracted from an aliquot of the culture to monitor for *CHA1* mRNA, and the remainder was used for ChIP (Fig. 2A).

Results of ChIP assays showed that upon induction, Med18/Srb5, Med14/Rgr1, and Med15/Gal11 are all recruited to the CHA1 promoter in wild type yeast, consistent with Mediator being recruited to the active gene promoter (Fig. 2, B–D). In the *srb4* ts mutant strain, Med18/Srb5 and Med14/Rgr1 were no longer recruited to the activated CHA1 promoter (Fig. 2, B–C).
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Med18/Srb5 is a component of the head module of Mediator, which is disrupted at the restrictive temperature in srb4-138 ts yeast (12), whereas Med14/Rgr1 bridges the middle and tail modules (9). We therefore infer that the head and middle modules of Mediator are not recruited to the induced CHA1 promoter in srb4-138 ts yeast at 37 °C. Similarly, association of the head and middle modules of Mediator with the activated GAL1 promoter is reduced in srb4-138 ts yeast at 37 °C (12, 56). In contrast, Med15/Gal11 recruitment was not affected in srb4-138 ts yeast after the temperature shift, since essentially the same increased level of Med15/Gal11 was measured at the CHA1 promoter upon induction in both the induced wild type and srb4-ts mutant strain (Fig. 2D). Western blotting indicates that levels of Med18/Srb5 and Med15/Gal11 are comparable in wild type and mutant yeast at 37 °C (data not shown), so that the differential recruitment observed cannot be attributed to altered protein levels. Together these results indicate a partial disruption of Mediator complex caused by the loss of Med17/Srb4 function; recruitment of the head and middle modules of the Mediator complex to the induced CHA1 promoter depends on functional Med17/Srb4, whereas recruitment of the tail module does not. This finding is consistent with a report that the tail module can be recruited to promoters activated by Gcn4 independently of the rest of Mediator (48).

**TBP Recruitment to the Induced CHA1 Promoter Requires Functional Mediator Complex**—Early studies showed that the Mediator complex interacts physically with TFIID (57), and at promoters such as ADH1 and GAL1, as well as several Gcn4-regulated promoters, loss of Med17/Srb4 function causes loss or decrease of TBP recruitment and subsequent decreased transcription (21, 22, 24). To test the role of Mediator in TBP recruitment to the induced CHA1 promoter, we used a plasmid-borne HA3-tagged version of TBP in ChIP assays, using the protocol of Fig. 2A. Real time PCR was used to quantitate TBP recruitment; for normalization, we used the tQ(UUG)C gene, a tRNA gene whose transcription requires RNA polymerase III and TBP but not Mediator (58). TBP association with the CHA1 promoter was increased by about 4-fold upon induction in wild type yeast, as measured by ChIP. However, in the srb4-ts strain, recruitment of TBP to the induced CHA1 promoter was essentially abrogated at 37 °C (Fig. 3A), consistent with the observed loss of expression (Fig. 1). We conclude that Mediator complex is essential for TBP recruitment to the CHA1 promoter.

We similarly analyzed recruitment of Kin28, a component of TFIIH, which is recruited late during PIC assembly (59). Kin28 shows strong recruitment to the induced CHA1 promoter, and this recruitment is greatly reduced in srb4 ts yeast at 37 °C (Fig. 3B). Thus, two components of the PIC, TBP and Kin28/TFIIH, depend strongly on intact Mediator for their recruitment to the induced CHA1 promoter.

**Recruitment of RNA Polymerase II to the Induced CHA1 Gene Strongly Depends on Mediator Function**—Based on previous findings of direct contacts between the Mediator complex and the DNA pol II core enzyme (25, 26) and the decreased transcription and loss of TBP recruitment accompanying CHA1 activation in srb4 ts yeast, it seemed likely that loss of Med17/Srb4 was disrupted at the restrictive temperature in Med18/Srb5 is a component of the head module of Mediator, which is disrupted at the restrictive temperature in srb4-138 ts yeast (12), whereas Med14/Rgr1 bridges the middle and tail modules (9). We therefore infer that the head and middle modules of Mediator are not recruited to the induced CHA1 promoter in srb4-138 ts mutant in all experiments; a representative Northern blot, along with the ethidium staining showing 25 S rRNA, is shown. Chromatin preparations were done at 37 °C to maintain inactivation of the srb4-138 allele. B, ChIP was performed on yeast expressing Srb5-Myc in wild type (WT) (RMY521) and srb4-138 ts mutant (RMY522) strains, using the protocol shown in A. CHA1 promoter abundance in input and immunoprecipitated samples was measured using primers for amplicon A (Fig. 1A) and normalized to immunoprecipitation (IP)/input ratios for a nontranscribed region of chromosome V. C, Rgr1-TAP recruitment at the CHA1 promoter in wild type (RMY513) and srb4-138 ts mutant (RMY514) strains was determined as in B. D, Gal11-Myc recruitment at the CHA1 promoter in wild type (RMY511) and srb4-138 ts mutant (RMY512) strains was determined as in B. Error bars, S.D. values for three independent ChIP experiments.

**FIGURE 2.** Srb4 is required for recruitment of the head module of the Mediator complex to the active CHA1 promoter but not for recruitment of the tail module. A, experimental strategy. RNA samples were extracted and analyzed to ensure loss of CHA1 expression in the srb4 ts mutant in all experiments; a representative Northern blot, along with the ethidium staining showing 25 S rRNA, is shown. Chromatin preparations were done at 37 °C to maintain inactivation of the srb4-138 allele. B, ChIP was performed on yeast expressing Srb5-Myc in wild type (WT) (RMY521) and srb4-138 ts mutant (RMY522) strains, using the protocol shown in A. CHA1 promoter abundance in input and immunoprecipitated samples was measured using primers for amplicon A (Fig. 1A) and normalized to immunoprecipitation (IP)/input ratios for a nontranscribed region of chromosome V. C, Rgr1-TAP recruitment at the CHA1 promoter in wild type (RMY513) and srb4-138 ts mutant (RMY514) strains was determined as in B. D, Gal11-Myc recruitment at the CHA1 promoter in wild type (RMY511) and srb4-138 ts mutant (RMY512) strains was determined as in B. Error bars, S.D. values for three independent ChIP experiments.

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Thus, association of the RNA pol II core enzyme with the Mediator complex.

Induced CHA1 Promoter

Srb4 function would have a detrimental affect on the association of the pol II core enzyme with the induced CHA1 gene. To address this issue, we used ChIP to examine the association of Myc-tagged Rpb3 to the 5'- and 3'-ends of the CHA1 ORF, using the protocol of Fig. 2A. In the wild type strain, the association of Rpb3 with both the 5'- and 3'-ends of the CHA1 ORF is stronger than that observed in the srb4-138 mutant strain, as predicted, Ser2 and Ser5 phosphorylation decreased throughout the gene region upon loss of Med17/Srb4 function (Figs. 2C and 3C). In contrast, in the induced srb4-138 mutant strain, the association of Rpb3 with the 5'-end of the CHA1 ORF is reduced to about 25% of the level observed in wild type yeast and to about 30% of the level observed in the srb4-138 mutant strain. Thus, association of the RNA pol II core enzyme with the induced CHA1 gene depends strongly, but not completely, on the Mediator complex.

Loss of Functional Mediator Complex Decreases Association of Ser2- and Ser5-phosphorylated Forms of Pol II with the Induced CHA1 Promoter—The yeast CHA1 Promoter contains 26 heptapeptide repeats (YSPTSPS). Rpb1 is initially recruited to gene promoters in its unphosphorylated form (60, 61). As elongation begins, Rpb1 is phosphorylated at the Ser5 residue of the CTD domain by the kinase Kin28, a subunit of TFIIH. Ser5 phosphorylation is essential for the association of elongation factors, such as COMPASS, FACT, the Paf1 complex, and mRNA capping enzyme, to the elongating pol II (62, 63). As elongation progresses, Ser5 is dephosphorylated, and the Ctk1 kinase phosphorylates the Ser2 residue of the CTD domain (63). Thus, the level of Ser5 phosphorylation of the CTD peaks at the promoter-proximal region of the gene ORF and decreases further downstream; conversely, Ser2 phosphorylation on the CTD increases from the promoter-proximal region of the ORF toward the 3'-end. Based on the observed decreased association of Rpb3 and Kin28 with the induced CHA1 gene, the level of Ser5- and Ser2-phosphorylated CTD, respectively. Primer sets corresponding to amplicons C and D (Fig. 1A) were used in real time PCR to monitor the level of both types of CTD phosphorylation at the 5'- and 3'-end of the CHA1 gene, respectively. A nontranscribed region of S. cerevisiae chromosome V was used as internal control. Error bars, S.D. values for three independent ChIP experiments.

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Figure 5. H3 Lys4 trimethylation and Paf1 recruitment at the activated CHA1 promoter in wild type and srb4 ts yeast. Histone H3 association (A) and H3 Lys4 trimethylation (B) at the CHA1 promoter in wild type (RMY531) and srb4-138 ts mutant yeast (RMY532) was monitored by ChIP using the protocol of Fig. 2A, using primers for amplicon A (Fig. 1A). C, data from A and B was used to calculate the ratio of H3K4me3 to total H3. D, Paf1 recruitment at the CHA1 promoter in wild type (RMY531) and srb4-138 ts mutant strains (RMY542) was monitored by ChIP, as above. Primers for amplicons C and D (Fig. 1A) were used in real time PCR to monitor the level of Paf1 recruitment at the 5’- and 3’-end of the CHA1 gene, respectively. A nontranscribed region of S. cerevisiae chromosome V was used as internal control.

to Rpb3, indicating that the modifying enzymes Ctk1 and Kin28 do not depend on functional Mediator complex for their recruitment or activity or, alternatively, that stable association of pol II in the absence of functional Mediator depends on CTD phosphorylation.

Effect of Loss of Med17/Srb4 Function on Histone Eviction, H3 Lys4 Trimethylation, and Recruitment of the Paf1 Complex to the Induced CHA1 Gene—Loss of histones from promoter regions upon transcriptional induction has been observed at a number of yeast genes, including PHO5, SUC2, HSP12, and DAN1 (65–69). This loss of histones may reflect eviction of a single promoter-associated nucleosome or may reflect the loss of differing numbers of promoter-associated nucleosomes over a population of cells (70). We used an antibody that recognizes histone H3 independent of its modification state in ChIP assays to monitor histone loss at the CHA1 promoter and found depletion of histone H3 and, by inference, nucleosomes upon induction in wild type yeast at 37 °C (Fig. 5A). Interestingly, histone loss is markedly diminished in srb4-138 yeast (Fig. 5A), indicating that intact Mediator is important for nucleosome eviction during transcriptional activation.

We next examined the effect of loss of Srb4 function on H3 Lys4 trimethylation. Trimethylation of the Lys4 residue of histone H3 is a hallmark of actively transcribed genes in eukaryotic organisms; in yeast, H3K4me3 has been found to peak at the promoter region and the 5’-end of the coding region of transcribed genes genome-wide (44, 71, 72). H3 Lys4 trimethylation is performed by the Set1 methyltransferase in the COMPASS complex and depends on the Paf1 complex (43, 44). Paf1 associates with pol II in the PIC via Spt4, and Set1 recruitment depends on Ser5 phosphorylation of Rpb1 (49, 73). Based on the results discussed thus far, we therefore expected reduced H3K4me3 at the induced CHA1 promoter in srb4 ts yeast at 37 °C.

Using an antibody specific to H3K4me3 in a ChIP assay, we observed a modest but significant increase in the level of H3 Lys4 trimethylation in wild type yeast at the CHA1 promoter upon activation (Fig. 5B). Surprisingly, induction of H3K4me3 was considerably higher in srb4 ts yeast under inducing conditions, indicating that intact Mediator complex is dispensable for H3 Lys4 trimethylation during CHA1 activation. Since levels of H3K4me3 at a given region will obviously depend on the amount of H3 that is available to be trimethylated, we normalized H3K4me3 levels to total H3 (Fig. 5C); the results indicate that H3 Lys4 trimethylation relative to total H3 increases about 4-fold in wild type yeast upon CHA1 induction and about 7-fold in srb4 ts yeast at 37 °C.

Since H3 Lys4 trimethylation depends on the Paf1 complex, we next examined recruitment of Paf1 in wild type and srb4 ts yeast by ChIP. Using Myc-tagged Paf1, we found that in wild type yeast, Paf1 is recruited to the active CHA1 gene region, with strong enrichment compared with the inactive gene
observed at both 5' and 3' regions (Fig. 5D). In contrast, Paf1 recruitment was reduced by 50–70% in the corresponding srb4 mutant strain (Fig. 5D). These results are reasonably consistent with our ChIP assays examining association of Rpb3 and Ser^2- and Ser^3-phosphorylated Rpb1 with the active CHA1 gene region, which showed a similar level of decrease at both the promoter and 3'-end of the CHA1 ORF. We conclude that although functional Mediator is required for association of normal levels of pol II with the active CHA1 gene, the reduced levels seen in srb4 ts yeast are not sufficiently limiting to decrease trimethylation of H3 Lys4 at the induced promoter.

Recruitment of Swi/Snf Complex to the CHA1 Promoter Does Not Depend on Intact Mediator Complex—The failure to recruit TBP to the induced CHA1 promoter in srb4 ts yeast at 37 °C could reflect a direct role of Mediator in TBP recruitment, but it could also be caused by a failure to remodel chromatin, resulting in nucleosomal occlusion of the TATA element (74, 75). We tested this first by using ChIP to examine Swi/Snf recruitment to the induced CHA1 promoter. Although the Swi/Snf nucleosome remodeling complex is not required for chromatin remodeling during CHA1 induction, it is recruited to the induced CHA1 promoter, suggesting that it might function redundantly with other chromatin remodeling activities (39). To test whether its recruitment depends on Mediator function, Myc-tagged Snf2 was used in a ChIP assay following the protocol of Fig. 2A. The results show equivalent recruitment of Snf2 to the induced CHA1 promoter in wild type and srb4 ts mutant yeast (Fig. 6A). Thus, recruitment of the Swi/Snf complex to the induced CHA1 promoter is independent of intact Mediator.

Nucleosome Remodeling during CHA1 Activation Is Independent of the Mediator Complex—We next sought to examine directly the effect of loss of Mediator function on nucleosome remodeling during CHA1 activation. Chromatin structure of the uninduced and serine-induced CHA1 promoter in wild type and srb4 ts mutant yeast (Fig. 6A). Thus, recruitment of the Swi/Snf complex to the induced CHA1 promoter is independent of intact Mediator.
nucleosome-depleted region upstream of this nucleosome and an array of strongly positioned nucleosomes extending downstream into the coding sequence (40, 42, 76), and this configuration is essentially unaltered at 37 °C (Fig. 6B, lanes 2–5). The same chromatin structure is seen in the uninduced CHAI gene at 37 °C in the srb4 ts mutant (data not shown; see Fig. 6C). Upon induction of the CHAI gene in wild type yeast, a strong MNase cleavage site is observed in the region of the TATA-containing nucleosome, and additional new cleavage sites are generated in the downstream nucleosome, indicating substantial chromatin remodeling upon activation, whether at 30 or 37 °C (40, 42) (Fig. 6B, lanes 6–9). Analysis of the induced CHAI promoter in srb4 ts yeast at 37 °C demonstrates that remodeling is indistinguishable from that seen in the wild type strain (Fig. 6B, lanes 11–14). We conclude that nucleosome remodeling during CHAI activation occurs independently of intact Mediator.

Chromatin remodeling and activation of CHAI does not depend on Swi/Snf (42), and Fig. 6B shows that remodeling also does not require intact Mediator. However, since Swi/Snf is recruited to the induced CHAI promoter in the absence of functional Mediator complex (Fig. 6A), it is possible that Mediator and Swi/Snf provide independent pathways to chromatin remodeling during CHAI activation. To test this possibility, we deleted SNF5 in srb4 ts yeast and monitored chromatin structure of CHAI at 37 °C with and without serine induction (Fig. 6C). The results show that chromatin remodeling of induced CHAI indistinguishable from that seen in wild type yeast occurs even in the absence of both Swi/Snf and Mediator function. Thus, Swi/Snf is recruited to the induced CHAI promoter even in the absence of functional Mediator, and neither is required for activator-dependent chromatin remodeling.

**DISCUSSION**

The Mediator complex is well established as an integral component in eukaryotic transcription; however, a detailed description of its mechanistic role in transcriptional activation remains to be elucidated (5). Here we report investigations on the role of the Mediator complex in transcriptional activation of the CHAI gene in yeast. Using the srb4-138 temperature-sensitive mutant of the essential head module subunit Med17/Srb4, we show that Mediator is essential for CHAI activation and TBP recruitment and is important for recruitment of RNA polymerase II to the activated CHAI gene. Consistent with the reduced level of pol II recruitment observed in the absence of functional Mediator, Paf1 recruitment was also significantly reduced; in contrast, induced levels of H3K4me3 were somewhat higher in the absence of intact Mediator than in wild type cells, even after correcting for differences in H3 loss. Interestingly, although Mediator was required for loss of histone H3 at the induced CHAI promoter, it was not needed for recruitment of Swi/Snf or for chromatin remodeling of the CHAI promoter, as assayed by MNase accessibility. Overall, these results define an essential role for Mediator at a step subsequent to chromatin remodeling during transcriptional activation.

**Independent Recruitment of the Tail Module of Mediator—** Consistent with its general role in transcription (1, 13), Mediator is essential for CHAI activation, since CHAI expression is essentially eliminated in either med17/srb4 or med10 ts mutants (Fig. 1) (data not shown). Other inducible promoters, including promoters activated by Gal4, Gcn4, and Met4, require Mediator for their activation (24, 77, 78), and it seems likely that this will hold true for the majority of inducible promoters, although exceptions exist (77, 79). The srb4-138 mutant is probably defective in folding at 37 °C, leading to disruption of the head module of Mediator (13, 56). Consistent with this view, association of subunits from the tail, middle, and head module with the active GAL1–10 promoter is reduced in srb4-138 ts yeast at 37 °C (12, 56). We also observe loss of recruitment of Med18/Srb5 and Med14/Rgr1 to the induced CHAI promoter in srb4-138 ts yeast at 37 °C. In contrast, the tail module subunit Med15/Gal11 is recruited to the active CHAI promoter equally well in wild type and srb4-138 ts yeast (Fig. 2). These findings are consistent with previous work showing that Mediator subunits Gal11/Med2/Pgd1 can be recruited as a free-standing module by Gcn4 in sin4Δ yeast (48).

**Mediator and Recruitment of the General Transcription Machinery to the CHAI Promoter—** Inactivation of the Mediator complex in srb4-138 ts yeast most notably caused decreased recruitment of TBP, Kin28, and RNA pol II to the induced CHAI promoter (Fig. 3). TBP recruitment was essentially abolished, consistent with previous studies (21, 22). However, our results cannot rule out a very low level of TBP recruitment that permits the low level of CHAI mRNA production and pol II recruitment (see below) observed in srb4 ts yeast at 37 °C.

Recruitment of pol II is also substantially impaired in srb4 ts yeast, although apparently not as severely as TBP recruitment. The partial retention of pol II recruitment is supported by ChIP using antibodies against Rpb3 and Paf1, which associates with the elongating form of pol II, as well as antibodies against the Ser2- and Ser5-phosphorylated forms of the CTD of Rpb1. Although the residual amount of pol II that is still observed associated with CHAI is in reasonable agreement with the small amount of transcription observed at 37 °C in srb4 ts yeast (Fig. 1), there is precedent for the idea that pol II association may not always result in productive transcription. First, although the TFIIH-associated kinase Kin28 is required for Ser5-phosphorylation of the pol II CTD and apparently for most mRNA transcription in yeast (79–81), considerable association of pol II with the ARG1 ORF was observed even after inactivation of Kin28 (49, 82). Second, a recent study found that pol II occupies many apparently inactive promoters in human cells (83). Third, a study of mouse ES cells lacking the Med23 subunit, for which there is no apparent yeast homolog, found that the difference in binding of PIC components and pol II between wild type and med23Δ cells was smaller than the difference in transcription (although replicate ChIP experiments were not reported in this study, casting some doubt on quantitative conclusions) (84). These results indicated that Mediator was needed for a step occurring subsequent to PIC formation. Our data hint at a similar possible role for Mediator in yeast, but additional investigations, including examination of other induced genes, will be needed to confirm or refute this possibility.
Lack of Effect on H3 Lys4 Trimethylation upon Loss of Mediator Function—We observed a partial loss of Paf1, which associates with the elongating form of pol II, from the CHA1 ORF in srb4 ts yeast at 37 °C and saw no reduction in induced levels of trimethylation of H3 Lys4, which requires recruitment of the Paf1 complex to elongating pol II (Fig. 5) (62). A possible explanation is that although pol II recruitment and subsequent recruitment of the machinery needed for H3 Lys4 trimethylation is reduced, the accumulation of this stable mark over time is sufficient to achieve normal levels during the time course of the experiment (44). Alternatively, it may be that srb4 ts yeasts are defective in a postinitiation step in transcription, as discussed above, so that a prolonged interaction of pol II with the promoter region compensates for reduced overall levels of pol II recruitment, resulting in approximately normal levels of induced H3 Lys4 trimethylation. Interestingly, pol II and trimethylated H3 Lys4 were recently reported to be present at many promoters that do not produce detectable full-length transcripts in human ES cells (83). It would be interesting to determine whether these genes differ from those producing full-length transcripts by the absence or presence of Mediator.

Mediator, Swi/Snf Recruitment, and Chromatin Remodeling at the CHA1 Promoter—Previous work has pointed to a connection between Mediator recruitment and chromatin remodeling during transcriptional activation. First, a direct association between Mediator and Swi/Snf was reported, although the stability of this association was questioned (10, 30, 31). Second, chromatin remodeling at the PHOS5 promoter was observed in experiments using a Pho4-Gal11 fusion, implying that recruitment of Mediator was sufficient for chromatin remodeling (32). Third, activation of the GAL10 promoter by a Gal4-Gal11 fusion depended more strongly on Swi/Snf than did activation by Gal4, suggesting an intimate connection between recruitment of Mediator and Swi/Snf (33). More recently, it was shown by ChIP that a Gal4-Gal11 fusion protein does indeed recruit Mediator and Swi/Snf to the GAL1–10 promoter (34).

We find that histone H3 eviction and, by inference, nucleosome loss accompanies induction of CHA1 in wild type yeast at 37 °C but is greatly diminished in srb4 ts yeast (Fig. 5A). In contrast, chromatin remodeling at the induced CHA1 promoter occurs in the absence of intact Mediator (Fig. 6). How can these apparently discordant results be reconciled? One possibility is that components of the PIC that are present in wild type yeast and whose recruitment in srb4 ts yeast is decreased may occlude the H3 epitope in wild type but not srb4 ts yeast. This seems unlikely, since histone eviction measured at other active promoters has been confirmed by utilization of multiple epitopes on different histones in ChIP experiments and confirmed by independent approaches as well (65, 67, 69). Alternatively, nucleosome remodeling and histone eviction may be distinct events. Thus, remodeling may reflect an altered nucleosome structure that allows enhanced nucleosome accessibility without actual loss of histone-DNA contacts. A third possibility is that both assays (histone H3 association as monitored by ChIP and remodeling as monitored by MNase accessibility) reflect the same event, but the dynamics of histone loss are altered by the presence of intact Mediator. The idea that nucleosome loss/chromatin remodeling reflects a dynamic equilibrium has been proposed before (67, 85) and could account for the observed results if histone H3 association with the induced CHA1 promoter increases during the time scale of cross-linking in the ChIP protocol in srb4 ts yeast compared with wild type while still being evicted at a rate that allows increased accessibility to MNase during the 5-min digestion period. Perhaps TBP recruitment prevents histone reassociation at the CHA1 promoter, so that in the absence of TBP recruitment in srb4 ts yeast, histone association is still observed. It would be interesting to examine histone association at other promoters under conditions where chromatin remodeling correlates poorly with transcriptional activation (86).

We also found that remodeling of the induced CHA1 promoter can occur in the absence of both functional Swi/Snf and Mediator complexes (Fig. 6C) and also does not depend on remodeling pathways involving Asfl, Ino80, Isw1, Isw2, Gcn5, or the Rsc complex2 (42, 87). Although it is possible that redundancy among some of these potential pathways may exist, in some cases promoter-bound nucleosomes may be sufficiently unstable that recruitment of the general transcription machinery suffices to alter local chromatin structure (88). Arguing against this possibility for CHA1, however, is the finding that artificial recruitment of TBP to this promoter does not activate transcription or remodel local chromatin structure (89). More work will be needed to unravel mechanisms for chromatin remodeling at promoters, such as PHO5, ADH2, and CHA1, for which essential chromatin remodeling activities remain unidentified (85).

In contrast to the lack of dependence on intact Mediator for chromatin remodeling or Swi/Snf recruitment to the induced CHA1 promoter reported here, Mediator was found to be essential for remodeling of the RNR3 gene promoter and for Swi/Snf recruitment to the GAL1–10 promoter in yeast (34, 36). These latter experiments employed the same srb4-138 ts mutant used here; however, inactivation of Med17/Srb4 results in reduced association of the tail module of Mediator with the GAL1–10 promoter (12), whereas we have found recruitment of the tail module to the induced CHA1 promoter in srb4-138 ts yeast to be unimpaired (Fig. 2). (The effect of Med17/Srb4 inactivation on recruitment of the Mediator tail module to RNR3 was not examined (36).) Thus, it is possible that the tail module contributes to Swi/Snf recruitment and/or chromatin remodeling at all three of these promoters but that it remains associated only at CHA1 in srb4 ts yeast at 37 °C. Further experiments will be needed to test this possibility.

TBP-associated factors are also needed for Swi/Snf recruitment to the active RNR3 and GAL1–10 promoters and for remodeling of RNR3 (34, 36). (Transcriptional activation and presumably chromatin remodeling of GAL1–10 does not require Swi/Snf (90).) Since CHA1 undergoes chromatin remodeling in srb4-138 ts yeast, whereas TBP recruitment is virtually eliminated, it seems likely that TBP-associated factors are not needed for remodeling CHA1. This is also consistent with previous work showing that chromatin remodeling of the induced CHA1 promoter occurs in yeast expressing nonre-
crucial mutants as their only source of TBP, which prevents CHAI transcription (42). Although the RNR3 gene differs in being under the control of the Ssn6/Tup1 global repressor complex, it is surprising that GAL1-10 and CHAI, which are both controlled by classical activators, should differ in these regards. More work will be needed to clarify the basis for the apparent mechanistic distinctions at these promoters and to determine whether other activated promoters follow these examples or display additional differences.

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