SWI/SNF-dependent chromatin remodeling of *RNR3* requires TAF_{II}s and the general transcription machinery

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Gene expression requires the recruitment of chromatin remodeling activities and general transcription factors (GTFs) to promoters. Whereas the role of activators in recruiting chromatin remodeling activities has been clearly demonstrated, the contributions of the transcription machinery have not been firmly established. Here we demonstrate that the remodeling of the *RNR3* promoter requires a number of GTFs, mediator and RNA polymerase II. We also show that remodeling is dependent upon the SWI/SNF complex, and that TFIID and RNA polymerase II are required for its recruitment to the promoter. In contrast, Gcn5p-dependent histone acetylation occurs independently of TFIID and RNA polymerase II function, and we provide evidence that acetylation increases the extent of nucleosome remodeling, but is not required for SWI/SNF recruitment. Thus, the general transcription machinery can contribute to nucleosome remodeling by mediating the association of SWI/SNF with promoters, thereby revealing a novel pathway for the recruitment of chromatin remodeling activities.

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Transcription by RNA polymerase II requires the coordination of general transcription factors (GTFs), promoter-specific regulatory proteins, and a plethora of chromatin modifying and remodeling activities (Orphanides et al. 1996; Roeder 1998; Lemon and Tjian 2000). Transcriptional activators facilitate preinitiation complex formation by at least two mechanisms. The first mechanism is by their stabilization of GTFs at promoters by direct interactions, or indirectly through coactivators (Roeder 1998; Naar et al. 2001). The second mechanism is by their ability to recruit complexes that regulate chromatin structure and thus increase the accessibility of the DNA to transcription factors (Workman and Kingston 1998; Peterson and Workman 2000; Roth et al. 2001; Berger 2002; Narlikar et al. 2002). It is a widely held belief that activators play a dual role in the simultaneous or sequential recruitment of chromatin remodeling activities and GTFs. A connection between the GTFs and chromatin remodeling has been proposed based on the copurification of both nucleosome remodeling and histone modifying activities with RNA polymerase II and TFIID (Wilson et al. 1996; Grant et al. 1997; Nakajima et al. 1997; Cho et al. 1998; Neish et al. 1998; Lorch et al. 2000; Saurin et al. 2001; Nakamura et al. 2002). Specifically, the ATP-dependent chromatin remodeling complex, SWI/SNF, was reported to copurify with the RNA polymerase II holoenzyme/mediator in yeast (Wilson et al. 1996; Grant et al. 1997) under some conditions, but these claims were disputed by others (Cairns et al. 1996; Myers et al. 1998; Liu et al. 2001). This controversial issue remains unresolved.

The significance of the interactions between GTFs and chromatin remodeling activities has been called into question due to the belief that transcription, RNA polymerase II, and GTFs are dispensable for chromatin remodeling in vivo. This idea arose from studies demonstrating that inhibiting transcription of a number of genes by mutating promoter sequences or inactivating GTF mutants had no effect on nucleosome remodeling (Hirschhorn et al. 1992; Fascher et al. 1993; Patterton and Simpson 1994; Verdone et al. 1996; Moreira and Holmberg 1998). However, only a handful of genes have been analyzed to date; thus, mechanisms derived from the analysis of a few genes may not be applicable to all cases. Not all evidence argues against a role for GTF and RNA polymerase II in regulating chromatin structure. Tethering RNA polymerase II holoenzyme to the *PHO5* promoter by LexA-Gall1p in the absence of the specific

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activator of this gene, Pho4p, resulted in chromatin remodeling at the promoter (Gaudreau et al. 1997), thus arguing that holoenzyme can recruit remodeling activities. In addition, whereas HSP82 transcribed by native RNA polymerase II displayed remodeled chromatin, a version engineered to be transcribed by T7 RNA polymerase did not (Sathyanarayana et al. 1999), suggesting a role for RNA polymerase II-associated factors, and not the act of transcription per se in chromatin remodeling. Finally, the sliding of a nucleosome over the IFN-β promoter requires the TBP-induced bending of DNA (Lomvardas and Thanos 2001), and alteration of the TATA box and DPE of the *Drosophila* hsp26 gene affects the generation of DNAse I-hypersensitive sites upstream of the promoter (Leibovitch et al. 2002).

The genes encoding the enzyme ribonucleotide reductase (RNR) are predominantly regulated by a transcriptional repression mechanism through upstream repression sequences (URS), the damage-responsive elements (DREs) or x-boxes, which serve as binding sites for the sequence-specific DNA binding protein Crt1p (Huang et al. 1998). The Ssn6–Tup1 corepressor complex is recruited to the promoter via the N terminus of Crt1, and activation of DNA damage checkpoints results in phosphorylation of Crt1 and its reduced cross-linking to the promoter (Huang et al. 1998; Li and Reese 2000; Davie et al. 2002). Ssn6–Tup1 recruitment establishes a nucleosomal array over the promoter of *RNR3*, and derepression of *RNR3* correlates with the disruption of nucleosome positioning; thus, chromatin remodeling may be an essential requirement for the expression of this gene (Li and Reese 2001). We showed previously that the derepression of DNA damage-inducible genes requires a subset of yeast TAF_{II}s, and that the DREs confer TAF_{II}-dependent transcription to these promoters (Li and Reese 2000; Reese et al. 2000; Durso et al. 2001). Furthermore, the TAF dependency of the *RNR2* and *RNR4* genes can be alleviated by the deletion of *SSN6* or *CRT1*, suggesting that TAF_{II}s act to antagonize the repressive effects of the Ssn6–Tup1 complex (Li and Reese 2000).

Here we examined the requirement for TAF_{II}s and other GTFs in mediating the remodeling of chromatin over the *RNR3* promoter. Our studies demonstrate for the first time that the remodeling of a gene in vivo requires the function of GTFs and RNA polymerase II. Inactivation of conditional mutants of *TAF1*, *TAF12*, the large subunit of RNA polymerase II (*RPB1*), *SRB4*, and the CTD kinase *KIN28* abolished chromatin remodeling of *RNR3* in response to DNA damage signals. In contrast to nucleosome remodeling, acetylation of histone H3 by the SAGA histone acetyltransferase complex occurs independently of TFIID and RNA polymerase II. Moreover, we provide evidence that GTFs recruit the SWI/SNF complex to the promoter, and are required for the retention of SWI/SNF at the remodeled promoter. Our data indicate that preinitiation complex components contribute to the remodeling of nucleosomes at *RNR3* by recruiting or stabilizing the association of SWI/SNF with the promoter.

Results

The TBP-associated factors, RNA polymerase II, and Kin28 *are required for the remodeling of nucleosomes at* RNR3

Derepression of the DNA damage-inducible *RNR3* gene requires the TBP-associated factors (TAF_{II}s) and correlates with extensive remodeling of the nucleosomal structure over its promoter, prompting us to examine the requirement for TAF_{II}s in the remodeling of this gene. Strains containing temperature-sensitive mutations in *TAF1* and *TAF12* were exposed to the nonpermissive temperature and were treated with the DNA-damaging agent methyl methanesulfonate (MMS). Afterwards, nuclei isolated from these cells were subjected to a nucleosome mapping protocol using micrococcal nuclease (MNase). Figure 1A shows that in the absence of DNA damage, an array of positioned nucleosomes was detected over the *RNR3* promoter in wild-type cells and the mutants. Specifically, the region encompassing the TATA box was protected from digestion, and the internucleosomal DNA displayed hypersensitivity to MNase.

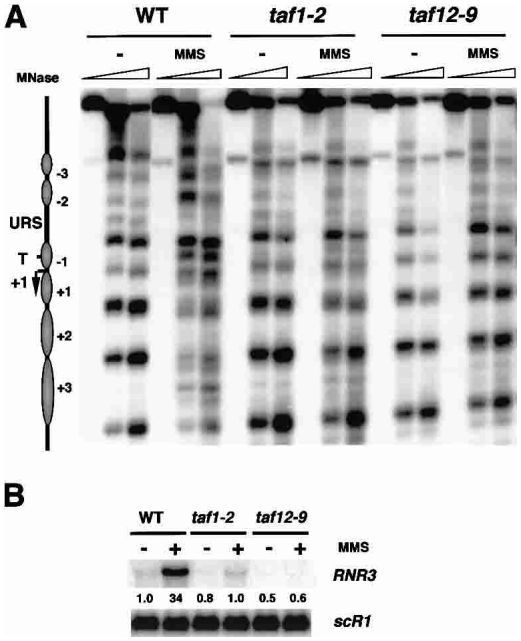


Figure 1. TAF_{II}s are required for MMS-induced chromatin remodeling of *RNR3*. (*A*) Nucleosome mapping in TAF_{II} mutants. Wild type (SW87) and strains containing temperature-sensitive mutations in yeast *taf1-2* (YSW93) and *taf12-9* (YJR21-9) were grown and treated with MMS at 37°C as described in the text. Cells were then harvested to prepare nuclei for chromatin mapping and RNA for Northern blotting. The nucleosome organization of the *RNR3* promoter is illustrated on the *left* side of the panel and is based on previous detailed mapping studies (Li and Reese 2001). (*B*) Northern blot analysis of *RNR3* mRNA levels in repressed and MMS-treated cells. scR1 is a loading control. Quantification of Northern blots was performed using the ImageQuant software, and was normalized to the signal in the –MMS sample of the wild type, which was arbitrarily defined as a unit of 1.

Treating wild-type cells with MMS resulted in a dramatic disruption of the nucleosomal architecture over the promoter, as evidenced by the loss of MNase hypersensitivity of the internucleosomal DNA and increased digestion within the nucleosomal DNA (Fig. 1A, +MMS). In particular, a large increase in the accessibility of the TATA box to MNase was observed, and is indicated by the appearance of a strong doublet within that region of the gel. In contrast, inactivation of conditional mutants of *TAF1* and *TAF12* strongly reduced and abolished chromatin remodeling, respectively, indicated by the preservation of the hypersensitive sites and the continued protection of the TATA region from digestion. The level of derepression corresponded to the reductions in remodeling in each mutant. For example, incubation of the *TAF12* mutant at 37°C completely abolished both the expression of *RNR3* and chromatin remodeling, whereas inactivation of the *TAF1* mutant resulted in a very weak increase in *RNR3* RNA in response to MMS treatment and a very slight amount of chromatin remodeling (Fig. 1B). Thus, these experiments show that TAF_{II}s are required for the chromatin remodeling step in the derepression of *RNR3*.

We next questioned whether the requirement for TAF_{II}s is specific, or if other general transcription factors are essential for the remodeling of the *RNR3* promoter. Strains containing a mutation in the large subunit of RNA polymerase II, *RPB1*, and in the CTD kinase subunit of the TFIIH complex, *KIN28*, were used. These strains were exposed to the nonpermissive temperature, treated with MMS, and processed for nucleosome mapping as described above. Similar to the observations in the TAF_{II} mutants, inactivation of either the *rpb1-1* or the *kin28-16* allele did not affect the nucleosomal structure over the promoter in untreated cells (Fig. 2A), but the MMS-induced chromatin remodeling was abolished in these mutants (Fig. 2A). A Northern blot for *RNR3* RNA confirms that the expression of *RNR3* is abolished in both mutants (Fig. 2B). Thus, both RNA polymerase II and the Kin28p subunit of the TFIIH, two general transcription factors, are required for the nucleosome remodeling step in *RNR3* derepression.

Our results are inconsistent with the traditional view that GTFs and RNA polymerase II are dispensable for chromatin remodeling. This poses the question as to whether *RNR3* is unique, and different types of promoters utilize different strategies to remodel nucleosomes, or whether the results using these mutants are misleading. To address this question we analyzed the chromatin structure of a gene, *SUC2*, remodeled independent of transcription activity (Hirschhorn et al. 1992). Wild-type cells and the RNA polymerase II mutant were incubated at 37°C and transferred to prewarmed media contain-

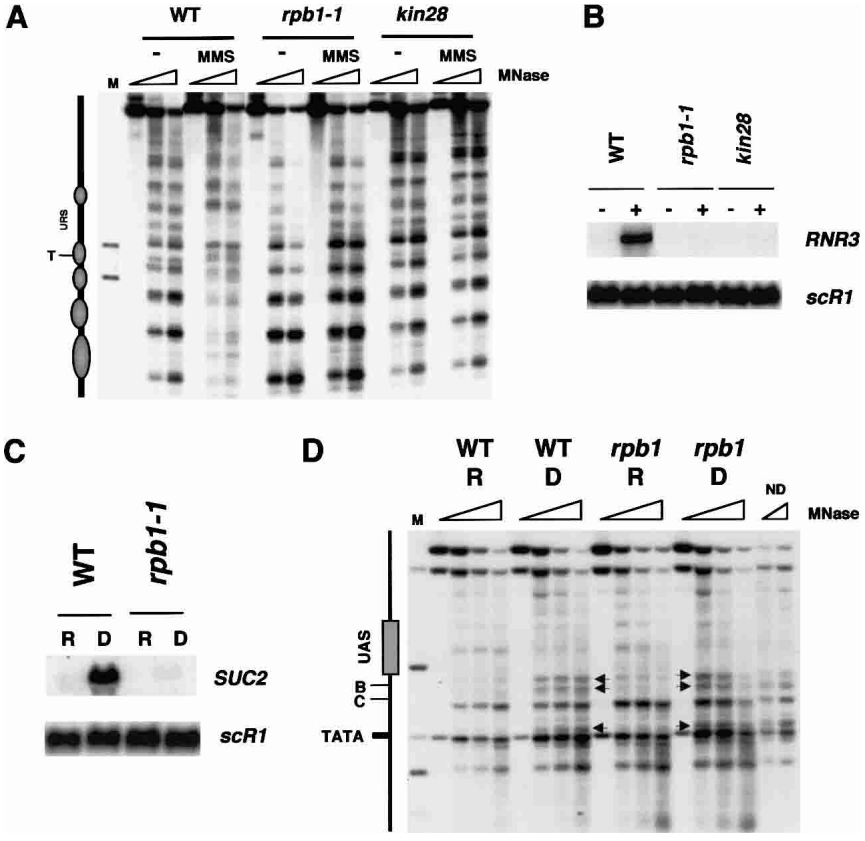


Figure 2. RNA polymerase II and Kin28 are required for MMS-induced chromatin remodeling of *RNR3*. Chromatin mapping and transcriptional analysis were performed in temperature-sensitive *rpb1-1* and *kin28-16* mutants using the same procedure described in the legend for Figure 1. (*A*) MNase mapping, as in Figure 1. The first lane (M) contains a DNA marker. (*B*) Northern blot analysis of *RNR3* mRNA levels. (*C*,*D*). Analysis of the expression and chromatin remodeling of the *SUC2* promoter in the *rpb1-1* mutant. Wild-type cells and a strain containing a temperature-sensitive mutation in *rpb1-1* were grown in YPD (2% dextrose) at the permissive temperature (23°C) and then mixed with an equal volume of prewarmed fresh YPD medium (50°C) to rapidly elevate the temperature of the culture to 37°C. After a 45-min preinactivation, cells were collected by filtration and washed with 37°C YP media. The cell pellets were then resuspended in 37°C low-dextrose (0.05%) YP medium and incubated at 37°C for 2 h. Cells were harvested and subjected to chromatin mapping and Northern blotting. (*C*). Northern blot analysis of *SUC2* and *scR1* mRNA levels. (*D*) MNase mapping. The arrows indicate the regions that are hypersensitive to MNase under the derepressing condition and correspond to the TATA box and the B and C boxes (Hirshhorn et al. 1992). The first lane (M) contains a DNA marker, and digested naked DNA (ND) is shown to the *right*.

ing 0.05% dextrose to activate the glucose-sensing pathway. The Northern blot for *SUC2* mRNA from these cells presented in Figure 2C confirms that inactivation of the *rpb1-1* mutant abolished the derepression of *SUC2*. As expected, transferring wild-type cells to the derepressing conditions resulted in increased nuclease digestion within the *SUC2* promoter, in particular near the TATA box and over the B and C boxes (Fig. 2D). This pattern is nearly identical to digested naked DNA, indicating extensive chromatin remodeling at the promoter. More importantly, the extent of chromatin remodeling in *rpb1-1* was indistinguishable from that observed in the wild-type strain (Fig. 2D). These results suggest that *RNR3* utilizes a different strategy for derepression than *SUC2*, and that different classes of genes utilize different mechanisms to achieve nucleosome remodeling.

Mediator and SWI/SNF are required for chromatin remodeling of RNR3

RNA polymerase II is reported to copurify with two chromatin modifying activities. One is the mediator that contains a subunit, Nut1p, with intrinsic histone acetyltransferase activity (Lorch et al. 2000), and the other is the SWI/SNF complex that disrupts nucleosomes in an ATP-dependent manner (Wilson et al. 1996). Thus, we explored the requirement for the mediator as a whole, and *NUT1* specifically, by analyzing the expression and chromatin remodeling of *RNR3* in the *srb4-138* temperature-sensitive mutant and in a strain containing a deletion of *NUT1*. Inactivation of the *srb4-138* mutant abolished the derepression of *RNR3* (Fig. 3A), and nucleosome mapping reveals that the loss of transcription is accompanied by reduced chromatin remodeling in this strain (Fig. 3B). Deletion of *NUT1* reduced the DNA damage-dependent expression of *RNR3* (Fig. 3C) to ~50% of the level observed in wild-type cells, but expression in the Δ*nut1* strain was significantly higher than in the *srb4-138* mutant (Fig. 3, cf. A and C). Also, nucleosome mapping of the *RNR3* promoter revealed that deleting *NUT1* had no detectable effect on chromatin remodeling (Fig. 3D). Similar results, in regards to the transcript level and the degree of chromatin remodeling, were also observed in a Δ*srb2* and Δ*srb5* mutant, two other nonessential subunits of the mediator complex (data not

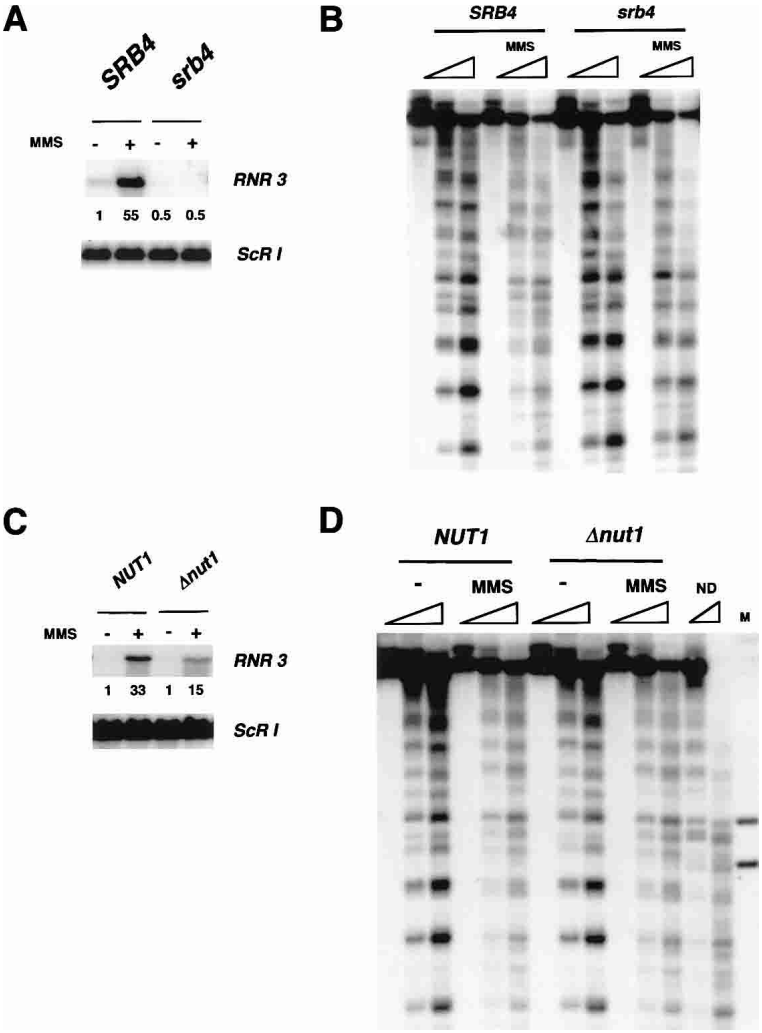


Figure 3. Mediator (*SRB4*), but not *NUT1* specifically, is required for the DNA damage-induced chromatin remodeling of *RNR3*. Wild type (Z579) and temperature-sensitive *srb4-138* (Z628) mutant were grown and processed as in Figure 1. (*A*) Northern blot analysis of *RNR3* mRNA. (*B*) MNase mapping. Analysis of expression and chromatin structure of *RNR3* in the *srb4-138* mutant. (*C*). Northern blot analysis of *RNR3* mRNA levels in wild type and Δ*nut1* mutant. Cells were treated with (+) or without (–) MMS for 2.5 h at 30°C. Quantification of *RNR3* transcripts was performed using the ImageQuant software program. The expression level in wild-type cells without MMS treatment is arbitrarily defined as a unit of 1. *scR1* is a loading control. (*D*) MNase chromatin mapping was performed using wild type (PH499) and the Δ*nut1* (YJR709) mutant treated with or without MMS for 2.5 h at 30°C. ND, digested naked DNA; M, a DNA size marker.

shown). Whereas the mediator as a whole is necessary for high levels of transcription and chromatin remodeling of *RNR3*, these two functions are not strictly dependent upon *NUT1*.

We next examined the requirement for the SWI/SNF complex in the derepression of *RNR3* by using a mutant deleted of the gene encoding the catalytic subunit of the complex, *SNF2*. Deletion of *SNF2* reduced the derepression of *RNR3* to ~20% of the level of wild-type cells (Fig. 4A). The formation of the preinitiation complex at the promoter was examined using the chromatin immunoprecipitation assay (ChIP) with antibodies to TBP and RNA polymerase II. MMS treatment increased the cross-linking of both factors approximately fivefold in wild-type cells, but was strongly reduced in the Δ*snf2* mutant (Fig. 4B). Analysis of the nucleosomal organization of *RNR3* revealed that the extent of chromatin remodeling was likewise greatly reduced in the Δ*snf2* strain, suggesting that the reduced transcription and preinitiation complex (PIC) formation is largely attributed to reduced nucleosome remodeling (Fig. 4C). Similar results were obtained using a strain containing a point mutation in the ATPase motif (K798A) of *SNF2* (data not shown), suggesting that the ATP-dependent nucleosome remodeling activity is required for transcription and remodeling. Next, we sought to verify that SWI/SNF is directly regulating *RNR3* by using the ChIP assay. We analyzed the association of Snf2p with *RNR3* using primer pairs directed to regions upstream and downstream of the promoter, and found that the highest level of cross-linking was observed over the promoter (Fig. 4D). The cross-linking to the promoter in untreated cells was approximately twofold above background levels (anti-HA), and MMS treatment increased its cross-linking an additional 3.2-fold (Fig. 4D). The cross-linking of Snf2 diminished further away from the promoter, and only background levels were detected +1500 base pairs away from the promoter within the open reading frame (ORF). Moreover, no specific cross-linking was observed at the promoter of the SWI/SNF independent *ADH1* (data not shown). Thus, Snf2 is predominantly localized over the promoter, and is increased by DNA damage.

TFIID and RNA polymerase II are required for SWI/SNF and mediator recruitment, but not histone acetylation

To increase our understanding of the TAF_{II}- and GTF-dependent nucleosome remodeling, we analyzed the cross-linking of transcription factors to the *RNR3* promoter in wild-type and mutant cells. First, we examined the cross-linking of TBP, TAF_{II}s, and Rpb1 to the promoter in wild-type cells grown at 30°C. The results in Figure 5A show that even in the absence of DNA damage, a significant level of TBP and TAF_{II} cross-linking was detected at the promoter. The amounts of *RNR3* promoter precipitated using antiserum against five different TAF_{II}s and TBP were approximately two- to three- and sevenfold above that observed using preimmune serum, respectively. Furthermore, the amount of promoter DNA brought down in untreated cells was above that of ORF DNA (Fig. 5A) or telomeric DNA (data not shown). The addition of MMS resulted in an additional approximately threefold, approximately fivefold, and approxi-

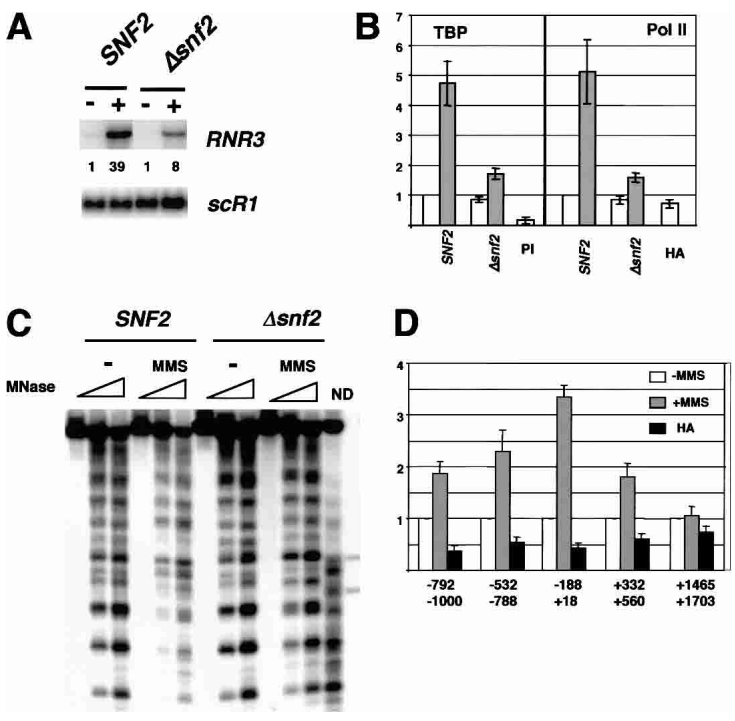


Figure 4. SWI/SNF regulates *RNR3* expression and nucleosome remodeling. (*A*). Northern blot analysis of *RNR3* mRNA levels. (*B*). Chromatin immunoprecipitation assay (ChIP) for TBP and RNA polymerase II cross-linking. *SNF2* and Δ*snf2* strains were grown at 30°C and treated with MMS for 2 h (gray bars) or left untreated (white bars). Immunoprecipitations (IPs) were carried out using a polyclonal antibody against TBP, a monoclonal antibody against RNA polymerase II (8WG16), and preimmune serum (PI) or anti-HA (HA) as controls. Immunoprecipitated and input DNA were amplified by PCR using primers specific for the core promoter regions of *RNR3.* Data are expressed as the means and standard deviations of the cross-linking from three independent experiments. The amount of cross-linking in untreated wild-type cells was arbitrarily set at a value of 1. (*C*) MNase mapping. The lane on the *right* (ND), indicates digestion of naked DNA by MNase. (*D*) ChIP assay for Snf2-myc13 binding. Strain YJR589 (*SNF2-MYC13*) was treated with (2 h) and without MMS prior to cross-linking as indicated. ChIPs were carried out using monoclonal antibodies to the MYC tag and HA tag (as a negative control for background). Similar background was observed in IPs using untagged strains. Immunoprecipitated and input DNA were amplified by PCR using primers directed to the regions of *RNR3* indicated in the panel. For reference, +1 is the major transcription start site; the TATA box is located at –75 and the URS between –330 and –200.

mately sevenfold increase in the cross-linking of TAF_{II}s, TBP, and RNA polymerase II, respectively. The MMS-induced cross-linking of TBP was greater than that of each of the TAF_{II}s examined. What accounts for this difference is not known, but it may result from either the presence of other TBP-containing complexes at the promoter or more efficient cross-linking of TBP to DNA.

Next, we examined the cross-linking of these factors in TAF_{II} and RNA polymerase II mutants at the restrictive temperature. The results in Figure 5B indicate that inactivating *taf1-2*, *taf12-9*, or *rpb1-1* abolished the MMS-induced increases in TBP, TAF_{II}, and RNA polymerase II cross-linking to the promoter, indicating that their association with the promoter is mutually dependent. Also, inactivation of *srb4-138* abolished the cross-linking of RNA polymerase II, and reduced the cross-linking of TAF1p and TAF12p (see Supplemental Material). Surprisingly, we found that the increased cross-linking of TFIID subunits to *RNR3* requires RNA polymerase II. This differs from a previous analysis showing that TBP is recruited to a number of other yeast genes in the *rpb1-1* allele (Kuras and Struhl 1999), suggesting that TBP and TFIID recruitment can occur by multiple mechanisms and display distinct transcription factor dependencies. In addition, since TBP cross-linking was eliminated upon the loss of *TAF1* function, it is likely that the increase in TBP cross-linking is dependent on its association with TAF_{II}s within the TFIID complex.

The requirement for TAF_{II}s and RNA polymerase II for nucleosome remodeling of *RNR3* suggests that they participate in the recruitment of SWI/SNF. This was examined by comparing the cross-linking of Snf2-myc to the *RNR3* promoter in mutants before and after MMS treatment. The cross-linking of Srb4-myc was also examined. The results displayed in Figure 5B reveal that treating wild-type cells with MMS at 37°C resulted in an increase in the cross-linking of both Srb4-myc and Snf2-myc to the promoter by ~1.8-fold and 2.5-fold, respectively. More importantly, we found that inactivation of *taf1-2*, *taf12-9*, or *rpb1-1* eliminated the DNA damage-induced enhancement of Snf2-myc and Srb4-myc cross-linking to the *RNR3* promoter (Fig. 5B), thus indicating that SWI/SNF and mediator recruitment is dependent on RNA polymerase II and TAF_{II}s. Further, inactivation of *srb4-138* reduced SWI/SNF recruitment (see Supplemental Material), which is consistent with the extent of nucleosome remodeling observed in this strain (Fig. 3A).

Derepression of *RNR3* transcription is accompanied by an increase in histone H3 acetylation caused by the release of Ssn6/Tup1 and HDACs from the promoter (Davie et al. 2002; V. Sharma and J. Reese, unpubl.). Given the interdependence of nucleosome remodeling and histone acetylation (Belotserkovskaya and Berger 1999; Hassan et al. 2001a,b; Narlikar et al. 2002), we examined the acetylation state of histone H3 at the promoter in the TAF_{II} and RNA polymerase II mutants. Consistent with our previous observations, treating cells with MMS resulted in an ~2.7-fold increase in histone H3 acetylation in wild-type cells (Fig. 5B). Interestingly, the level of acetylation of histone H3 in both the *taf1-2* and *rpb1-1* mutants was very similar to that observed in wild-type cells, increasing by 2.4- and 2.2-fold (89% and 80% of wild type), respectively. Thus, whereas both nucleosome remodeling and SWI/SNF recruitment are strictly dependent on *TAF1* and RNA polymerase II, histone H3 acetylation occurs independently of these factors. This observation strongly suggests that these mutants are specifically blocked at the nucleosome remodeling step, and that the phenotypes we observe are unlikely to result from trivial defects in the DNA-damage sensing and response pathways. In contrast to what was observed in the *taf1-2* mutant, inactivation of *taf12-9* eliminated the MMS-induced histone H3 acetylation (Fig. 5B). TAF12p is a component of TFIID and SAGA, and is essential for SAGA's nucleosomal HAT activity in vitro (Grant et al. 1998). Thus, SAGA may be the HAT complex responsible for the MMS-induced histone H3 acetylation. To test this, we examined the derepression of *RNR3* and its histone acetylation levels in Δ*gcn5* and Δ*ada2* mutants. Gcn5 is the catalytic subunit of SAGA, and Ada2 is required for the acetylation of nucleosomal substrates (Balasubramanian et al. 2002). Deletion of either *GCN5* or *ADA2* reduced the level of derepression of *RNR3* to ~50%–60% of that observed in wild-type cells (Fig. 6A), and abolished the DNA damage-induced increase in histone H3 acetylation (Fig. 6B). Since Gcn5 is an integral subunit of both the SAGA and ADA complexes (Grant et al. 1997), we confirmed the SAGA dependence by examining the histone acetylation in a SAGA-specific (Δ*spt7*) and ADA-specific (Δ*ahc1*) mutant. Consistent with our results using a TAF12 ts mutant, disrupting SAGA by deleting *SPT7* eliminated histone acetylation, whereas histone acetylation occurred normally in the Δ*ahc1* strain (Fig. 6B).

The SAGA dependence was unexpected given that we failed to observe increases in TAF12p cross-linking in the *taf1-2* or *rpb1-1* mutant even though acetylation increased upon MMS treatment (Fig. 5B). Furthermore, we failed to detect specific cross-linking of multiple SAGA-specific subunits to *RNR3* in the absence or presence of MMS anywhere around the promoter (data not shown). Precipitations were carried out using polyclonal antibodies to ADA1p, ADA3p, and Spt7p, and strains containing Gcn5-myc, Spt3-myc, or Ada2-myc were examined; all produced negative results (data not shown). All of the subunits indicated above cross-linked to the *GAL1-GAL10* promoter by two groups (Bhaumik and Green 2001; Larschan and Winston 2001) and thus are capable of being cross-linked at that locus. Either SAGA is associated with the *RNR3* promoter in a form that is refractory to cross-linking, or perhaps more likely, it may not be directly targeted to the promoter by an activator and acetylation occurs via a global, nontargeted mechanism (Vogelauer et al. 2000; Katan-Khaykovich and Struhl 2002).

Histone acetylation and nucleosome remodeling cooperate to regulate the expression of many genes; thus, we examined whether deletion of *GCN5* affects the remodeling of nucleosomes at *RNR3*. The results in Figure

6C show that the extent of nucleosome remodeling in the Δ*gcn5* mutant was less than that observed in wild type. Digestion within the TATA-box region and the diminished intensity of the bands marking the internucleosomal hypersensitive sites were observed, but to a lesser degree than the wild-type strain. Thus, acetylation by Gcn5 is not essential for nucleosome remodeling, but rather increases the extent of remodeling or the fraction of promoters (cells) remodeled in the steady state.

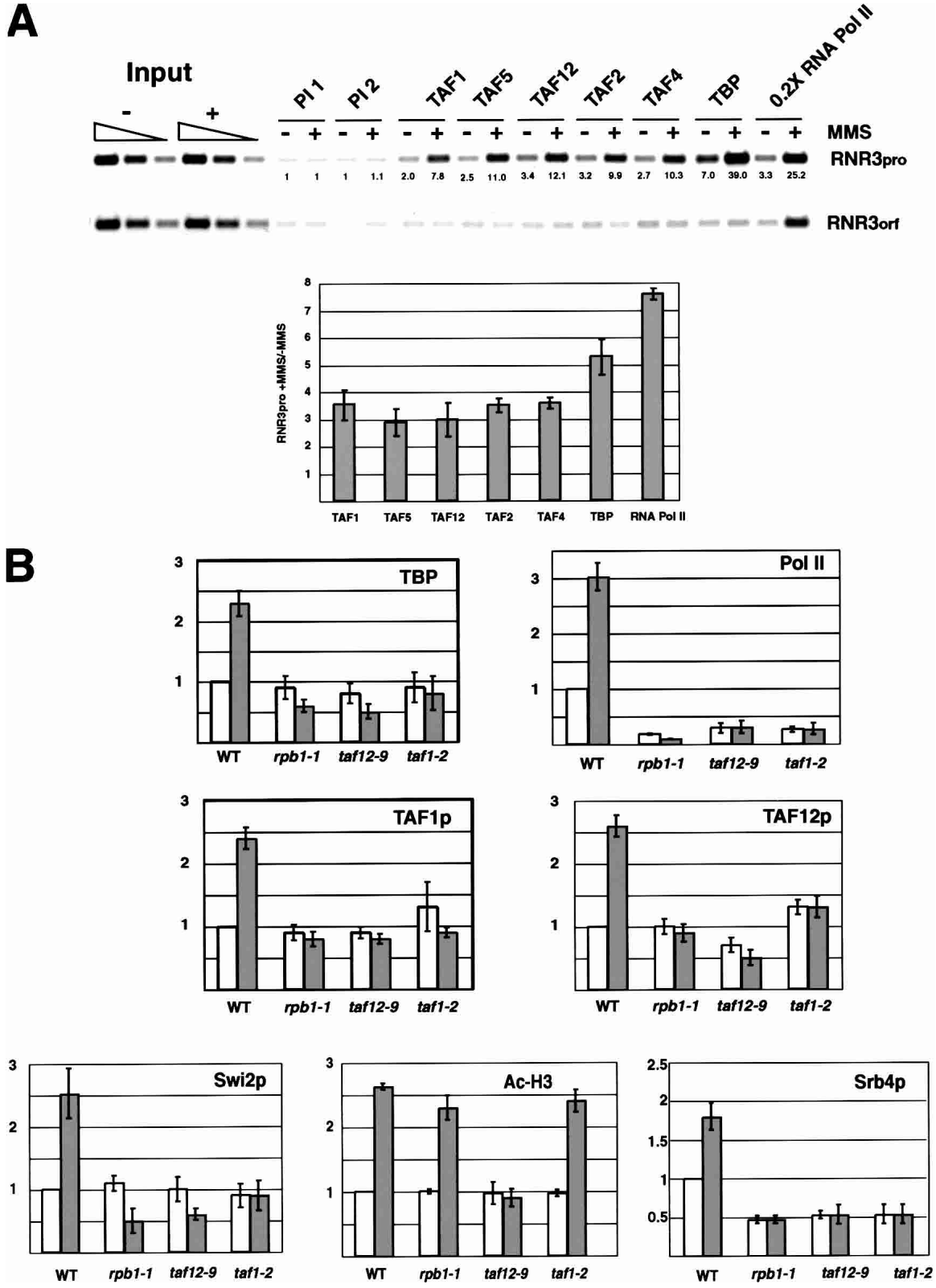
Next, we investigated whether the reduced remodeling results from impaired SWI/SNF recruitment, and whether acetylation affects a postrecruitment step. Deleting *GCN5*, in fact, slightly increased the cross-linking to *RNR3* in the presence and absence of MMS (Fig. 6D), 3.8-fold versus 2.7-fold and 1.7-fold versus 1-fold, respectively. The cause of this is unknown. Nonetheless, Snf2-myc was cross-linked at least as well as in a wild-type strain. Thus, the recruitment of SWI/SNF appears not to require *GCN5*-dependent acetylation. Deleting *GCN5* did, however, reduce the MMS-induced cross-linking of RNA polymerase II to *RNR3* (Fig. 6D), to a level ~70% of that observed in a wild-type strain. Taken together, these results suggest that acetylation facilitates, but is not essential for, SWI/SNF-induced nucleosome remodeling and is not required for SWI/SNF recruitment.

TAFs and RNA polymerase II are required to retain SWI/SNF at the promoter

The experiments conducted thus far analyzed the requirement for the PIC to recruit SWI/SNF and initiate chromatin remodeling on a repressed promoter, as the temperature-sensitive mutants were inactivated shortly before the addition of DNA damaging agent. We next examined the effects of losing PIC assembly at the constitutively remodeled *RNR3* promoter. The strategy employed involves deleting *CRT1*, the gene encoding the DNA binding protein that recruits Ssn6–Tup1 to the promoter. Deletion of *CRT1* derepresses *RNR3* transcription and results in chromatin remodeling indistinguishable from cells treated with DNA-damaging agents (Li and Reese 2001). Deleting *CRT1* in an otherwise wild-type background resulted in the constitutive cross-linking of TAFs, TBP, Pol II, and SWI/SNF to the promoter at 37°C (Fig. 7A), consistent with the fully derepressed/remodeled state of the promoter (Li and Reese 2001; see below). Importantly, the cross-linking of these factors was strongly reduced or abolished in the *rpb1-1*/Δ*crt1*, *taf12-9*/Δ*crt1*, and *taf1-2*/Δ*crt1* cells (Fig. 7A), thus arguing that the maintenance of the PIC is required to retain these factors at the promoter.

We next examined the nucleosomal structure over the *RNR3* promoter in cells grown at the permissive temperature and after a 2-h exposure to 37°C (Fig. 7B). The extent of chromatin remodeling in the *rpb1-1*/Δ*crt1*, *kin28-16*/Δ*crt1*, and *taf12-9*/*crt1* double mutants was very similar to the Δ*crt1* strain at the permissive temperature, consistent with the high levels of transcription under this condition (see Supplemental Material). Remarkably, thermoinactivation of the transcription factors in the Δ*crt1* background resulted in the reversion of the chromatin back towards the repressed state. The reappearance of the internucleosomal hypersensitive sites (Fig. 7B, arrowhead) and the decreased digestion within the TATA region (filled circle) is consistent with the repositioning of nucleosomes over the promoter. Inactivation of *taf12-9* appeared to have a stronger effect than *rpb1-1*, and in this experiment it appears as though the strongest repositioning of nucleosomes occurred near the promoter and start site (nuc –1, +1, and +2) in the *rpb1-1* and *kin28-16* mutants within the 2-h period. The positioning of nucleosomes under these conditions is not as sharp as in the repressed state, which might be expected since Crt1 is no longer available to recruit Ssn6/Tup1 and other repressors required to fully reset the promoter to the repressed state. Moreover, the repositioning of nucleosomes accompanied the loss of PIC formation (Fig. 7A) and reduced transcription (see Supplemental Material). Interestingly, deleting *CRT1* in the *taf12-9* allele could not overcome the TAF_{II} dependence of *RNR3* as observed at *RNR2* and *RNR4* in other

Figure 5. Cross-linking of transcription factors to the *RNR3* promoter in the presence and absence of DNA damage. (*A*) YJR589 was grown at 30°C and treated with or without MMS where indicated. IPs were carried out using polyclonal antibodies against TBP, TAF_{II}s, and a monoclonal antibody against RNA polymerase II (8WG16). (*Upper* panel) Results from one experiment. The IPs using preimmune sera shown correspond to the rabbits used to raise antiserum against TAF1p and TBP. Similar levels of background were observed using three other preimmune sera or HA monoclonal antibody. IP and input DNA were amplified by PCR using primers specific for the promoter of *RNR3* or +1500 bp from the start site of transcription (ORF). Lanes *1–6* show PCR reactions using 3.3-fold dilutions of input DNA. The DNA from the RNA polymerase II IP was diluted fivefold prior to amplification to assure linearity. The ChIP results are expressed as signal above preimmune sera, which was arbitrarily set at 1.0. (*Lower* panel) Graphical representation of the MMS-induced increase in cross-linking from three to five experiments, using at least three independently prepared chromatin preparations. (*B*) Chromatin immunoprecipitation analysis of transcription factor occupancy at the *RNR3* promoter in temperature-sensitive mutants. Wild-type (YJR589) and *taf1-2* (YJR595), *taf12-9* (YJR592), and *rpb1-1* (YJR598) strains, all containing *SNF2-13MYC*, were grown at 25°C and then shifted to 37°C for 45 min to inactivate the transcription factors. Cells were then treated with 0.02% MMS for 2 h (gray bars) or left untreated (white bars). IPs were carried out with antibodies against TBP, TAF1p, TAF12p, Pol II (8WG16), Ac-H3 (Di-acetyl Lys 9 and Lys 14), and (9E10) c-myc. Cross-linking of Srb4-myc was performed the same as the others except strains contained *SRB4-13MYC* (YJR588, YJR594, YJR591, and YJR597). The data are presented as a ratio of the amount of PCR product in wild-type cells, –MMS. Data are from at least three independent chromatin preparations and experiments. The increase in TAF1p cross-linking in the *taf1-2* mutant (–MMS) is the result of a single aberrant value, and is not truly indicative of increased cross-linking in all repetitions.



(*Figure 5 legend on facing page*)

TAF_{II} mutants (Li and Reese 2000). Both *RNR2* and *RNR4* have significantly higher levels of transcription under the uninduced condition, and may be less dependent on SWI/SNF and nucleosome positioning. These results show that GTFs are required to maintain remodeling and SWI/SNF association at *RNR3*. In addition, our data indicate that the remodeling defect is downstream of *CRT1* and the DNA-damage response pathway, thus arguing that GTF mutants directly affect *RNR3* remodeling.

Discussion

Our analysis of the regulation of *RNR3* has revealed a number of novel observations regarding the mechanisms of chromatin remodeling and the cooperation between nucleosome mobilization and histone-modifying complexes. The first is that the remodeling of nucleosomes at *RNR3* requires contributions from preinitiation complex (PIC) components, thereby indicating that GTFs do play a role in regulating chromatin structure at some

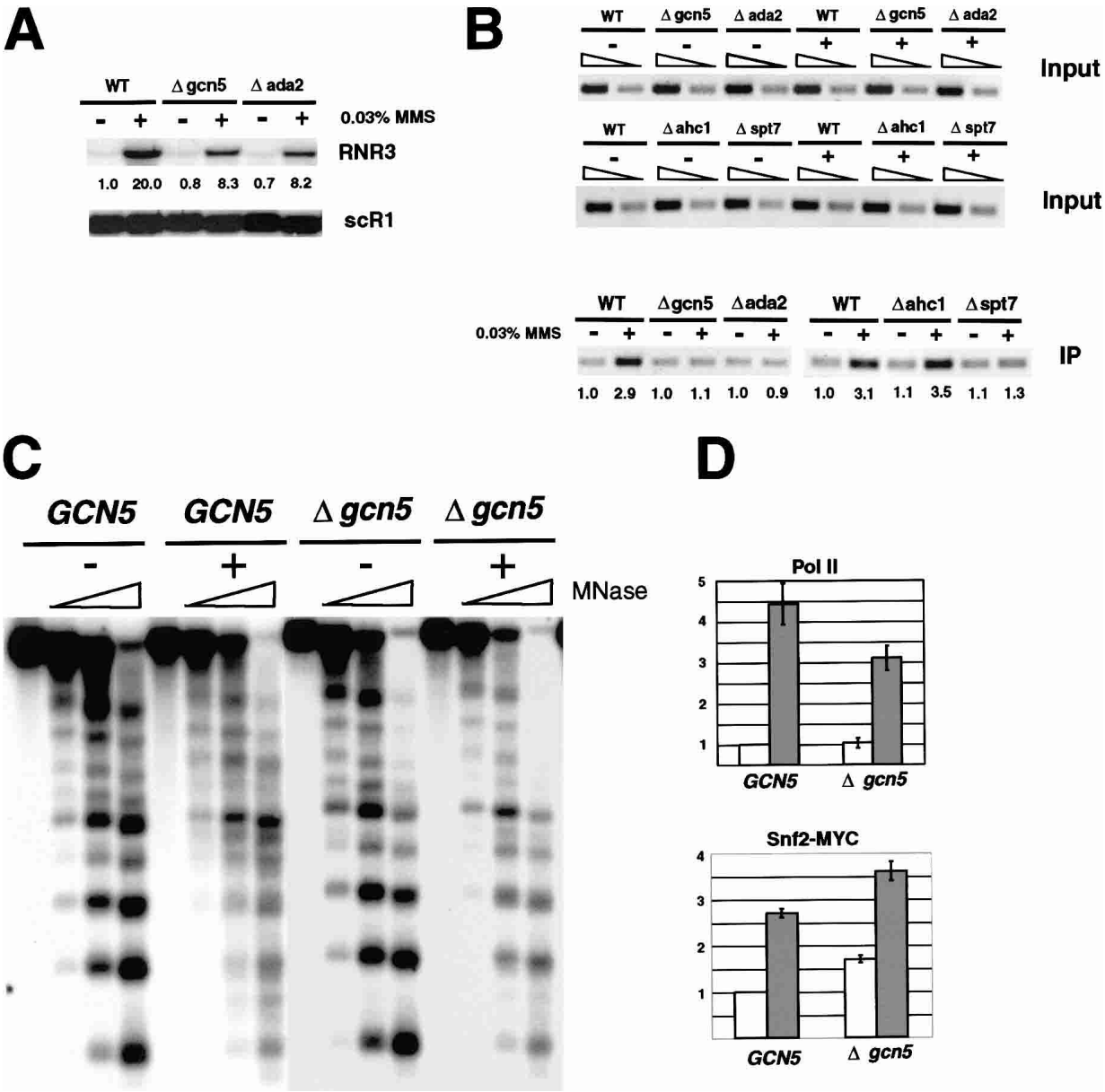


Figure 6. SAGA facilitates SWI/SNF remodeling at *RNR3*. (*A*) Northern blot for *RNR3* in Δ*gcn5* and Δ*ada2* mutants. PSY316, PSY316Δ*gcn5*, and PSY316Δ*ada2* were grown in YPAD at 30°C and treated with MMS for 2 h. *scR1* is a loading control. (*B*) Analysis of histone H3 acetylation in HAT complex mutants. A single experiment is shown. Results from three independent chromatin preparations and experiments yielded similar results with errors of 10%. (*C*) Chromatin mapping in wild-type and Δ*gcn5* cells. (*D*) SWI/SNF and polymerase II recruitment. As in Figure 5, except strains YJR589 (*SNF2-MYC13*) and YJR715 (Δ*gcn5*; *SNF2-MYC13*) were used. The data are presented relative to the amount of PCR product in IPs from untreated wild-type cells (–MMS), and is the result of at least three independent chromatin preparations and experiments. White bars, –MMS; gray bars, +MMS.

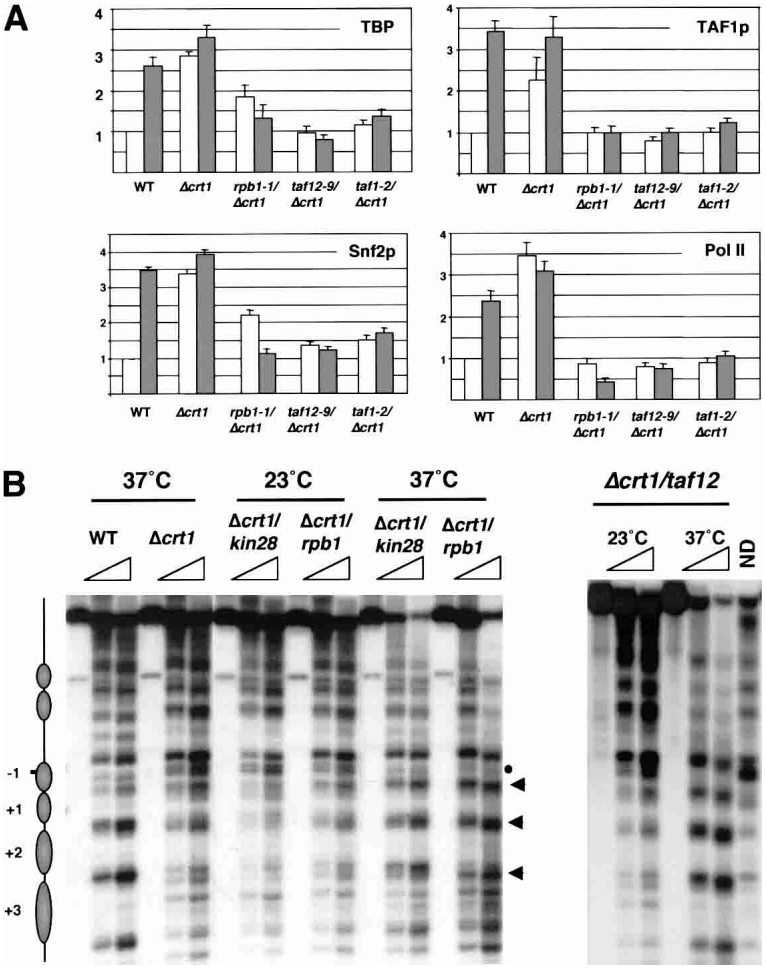


Figure 7. PIC formation is required to retain SWI/SNF at the promoter. (*A*) ChIP assay. Wild-type (YJR589), Δ*crt1* (YJR732), *taf1-2*/Δ*crt1* (YJR734), *taf12-9*/Δ*crt1* (YJR733), and *rpb1-1*/Δ*crt1* (YJR735) strains, all containing *SNF2-13MYC,* were grown at 25°C and then shifted to 37°C for 45 min to inactivate the transcription factors (white bars), treated with 0.02% MMS for 2 h at 37°C (gray bars), cross-linked with formaldehyde and processed. (*B*) RNA polymerase II and Kin28 are required for maintaining the fully remodeled state at the *RNR3* promoter. Wild-type (SW87), Δ*crt1* (YJR352), *rpb1-1*/Δ*crt1* (YJR658), *kin28-16*/Δ*crt1* (YJR660) and *taf12-9*/Δ*crt1* (YJR733) strains were grown at the permissive temperature (23°C) and then rapidly shifted to 37°C by mixing with an equal volume of prewarmed fresh YPD medium (50°C). Cultures were maintained at 37°C for 2 h prior to harvesting for chromatin mapping. Note: The lane on the far left in the *taf12-9*/*crt1* panel on the *right* is digested naked DNA (ND).

promoters. We demonstrate that inactivation of a number of general transcription factors, each predicted to serve distinct functions in transcription initiation, greatly reduces or abolishes nucleosome remodeling at *RNR3*. Secondly, our data argue that TFIID and RNA polymerase II play a specific role in mediating nucleosome remodeling, the recruitment (or stabilization) of SWI/SNF to the promoter. Inactivating these factors had a minimal effect on histone acetylation, but greatly reduced or abolished the disruption of nucleosome positioning. To our knowledge, this is the first description of this mechanism. Finally, both SAGA and SWI/SNF regulate *RNR3*, and *GCN5*-dependent acetylation is dispensable for SWI/SNF recruitment, but is required for full remodeling of the promoter.

Why does nucleosome remodeling at RNR3 *require the PIC?*

Models of the mechanisms of chromatin remodeling have largely focused on the role of sequence-specific DNA binding proteins in recruiting nucleosome-remodeling machines and histone-modifying activities (Peterson and Workman 2000; Hassan et al. 2001b; Narlikar et al. 2002). Clearly, this mechanism is likely to be essential for the remodeling of chromatin at the promoters of a large number of genes in vivo. Our present results provide some convincing evidence that chromatin remodeling of at least some promoters requires components of the general transcription machinery, defining another path towards chromatin remodeling. The results described here are in contrast to what has been described for a number of other yeast genes, including SWI/SNF-dependent *SUC2* (Hirschhorn et al. 1992; Gavin and Simpson 1997). We even verified the RNA polymerase II independence of the remodeling at *SUC2* in our assays, which argues against differences in methodologies. This leads to the question of why *RNR3* requires contributions from GTFs, whereas genes such as *CHA1*, *PHO5*, and *SUC2* do not? One possibility may be the types or strengths of the regulatory proteins that bind to the promoters of these genes. It is known that certain types of activation domains, for example, glutamine- or proline-rich, cannot recruit chromatin remodeling activities to promoters on their own, yet they can direct high levels of transcription (Utley et al. 1998; Neely et al. 1999; Yudkovsky et al. 1999; Hassan et al. 2001a). It is clear from our data in Figures 5B and 7A that the activator(s), if any, that bind to the *RNR3* promoter are not sufficient to recruit or retain SWI/SNF without contributions from

PIC components. Given that strong activators can recruit chromatin remodeling activities directly (for review, see Peterson and Workman 2000; Hassan et al. 2001b; Narlikar et al. 2002), and can even do so at sites lacking core promoter sequences (Kuo et al. 2000; Park et al. 2001), it is likely that certain activators can bypass the contributions from PIC components. Perhaps GTFs play a subtle role in remodeling even at genes such as *SUC2*, but their contributions are not detectable under the experimental conditions employed. Evidence suggesting that factors other than the activator can affect the recruitment mechanisms of chromatin remodeling activities is available. For example, the DNA-binding protein Pho4 drives the expression and chromatin remodeling of *PHO5* and *PHO8*, but only *PHO8* shows dependence on the SWI/SNF complex (Gregory et al. 1999). Furthermore, the codependence and order of recruitment of coactivators by the enhanceosome of the IFN-β promoter are regulated by core promoter architecture (Agalioti et al. 2000; Lomvardas and Thanos 2002). The core promoter dependence suggests that the composition of the PIC may be important.

How do GTFs and RNA polymerase II contribute to remodeling?

The identification of chromatin-modifying activities within yTAF1/dTAF1 (Mizzen et al. 1996; Pham and Sauer 2000) and mediator/RNA polymerase II holoenzyme (Myer and Young 1998; Lorch et al. 2000; Malik and Roeder 2000; Myers and Kornberg 2000) might suggest that remodeling is dependent on these activities. However, we believe this is not the case at *RNR3*. First, it is unlikely that the putative intrinsic HAT activity of TAF1p or HAT activity associated with RNA polymerase II is involved in nucleosome modification, because inactivating the *taf1-2* mutant or the *rpb1-1* mutant had a minimal to no effect on histone acetylation. Furthermore, Gcn5 accounts for the entirety of the DNA damage-induced histone H3 acetylation at the *RNR3* promoter. Thus, it is unlikely that histone-modifying activities of TFIID (TAF1p), mediator, or RNA polymerase II are directly involved in remodeling nucleosomes at *RNR3*.

We argue that the function of TFIID and polymerase is to assist in the recruitment of the SWI/SNF complex to the promoter. In other words, the PIC can perform recruitment functions normally attributed to activator proteins. Significantly, a complex composed of the mediator and TFIID undergo reciprocal, cooperative interactions at promoters that can bypass the requirement for activators in PIC assembly by forming a platform on immobilized templates (Johnson et al. 2002). Likewise, TFIID and other PIC components may form a platform and recruit the SWI/SNF complex to *RNR3* in vivo. Alternatively, TFIID and PIC components may stabilize SWI/SNF association after it has been directed to the promoter by a gene-specific factor (activator). We found that deletion of *CRT1* causes the constitutive remodeling and recruitment of the transcription factors and remodeling machineries in wild-type cells, and disrupting the PIC under this situation causes the loss of SWI/SNF recruitment and maintenance of the remodeled state. This supports two conclusions: (1) It is unlikely that Crt1 itself or Ssn6/Tup1 is required for recruiting the remodeling machinery. This was a possibility, given recent analyses of galactose and stress-regulated genes (Papamichos-Chronakis et al. 2002; Proft and Struhl 2002). (2) Even if an unidentified gene regulatory protein binds to the *RNR3* promoter, it is insufficient to maintain the presence of SWI/SNF and PIC components at the promoter. It seems logical that there is some contribution from a gene-specific factor(s) because, otherwise, TFIID could independently recruit chromatin remodeling activities to most promoters. Specifically, the core promoter architecture of the IFN-β promoter specifies SWI/SNF recruitment (Lomvardas and Thanos 2002). However, since the TAF_{II} dependence of *RNR3* is specified by the upstream repression sequences (URSs), and not the core promoter (Li and Reese 2000), contributions from the core promoter alone cannot explain our results, but they cannot be ruled out as a contributing factor. Studies identifying this factor(s) are beyond the scope of this study.

Compared to other genes that have been characterized to date, the recruitment of transcription factors and coactivators to *RNR3* appears to display a higher degree of cooperativity. For example, the recruitment (as measured by increased cross-linking) of TFIID, mediator, and SWI/SNF is dependent on RNA polymerase II. In contrast, recruitment of TBP to many yeast genes does not require Pol II (Kuras and Struhl 1999; Li et al. 1999), nor does the recruitment of mediator or SWI/SNF (Bhoite et al. 2001; Cosma et al. 2001; Park et al. 2001). Our data could be interpreted as if the recruitment of transcription factors occurs as part of a holoenzyme or larger complex, but such conclusions are premature. Our results do indicate that RNA polymerase II is required for the association of mediator and SWI/SNF with the *RNR3* promoter; however, our results cannot distinguish whether or not these two complexes are corecruited with RNA polymerase as a holoenzyme, or recruited separately, or whether polymerase is required to stabilize their association after they have been recruited. Regardless of whether or not SWI/SNF and mediator are stable or stoichiometric components of an RNA polymerase II holoenzyme, the copurification of chromatin-remodeling activities with certain PIC components (Wilson et al. 1996; Grant et al. 1997; Nakajima et al. 1997; Cho et al. 1998; Neish et al. 1998), the genetic interactions between subunits of these complexes (Carlson 1997; Roberts and Winston 1997; Myer and Young 1998; Hampsey and Reinberg 1999), and the results described here indicate that an interaction among mediator, SWI/SNF, and RNA polymerase II may be essential for the regulation of gene expression.

The role of SAGA and histone acetylation at RNR3

TAF_{II}s contribute to *RNR3* regulation as components of the TFIID and SAGA histone acetyltransferase complexes. They cooperate with other GTFs as components

of TFIID to recruit SWI/SNF, and within SAGA to increase histone H3 acetylation. These two functions appear to be independent of each other, as acetylation occurs after inactivation of TFIID or RNA polymerase II, and SWI/SNF recruitment does not require acetylation; however, the coordination of these two steps is required for the expression of *RNR3* as noted previously at other loci, specifically *HO* and *PHO8* (for review, see Belotserkovskaya and Berger 1999; Hassan et al. 2001b; Fry and Peterson 2001; Narlikar et al. 2002). *RNR3* may be more similar to *PHO8* than the *HO* gene. In the case of *PHO8*, a transient burst of acetylation is required for marking a single nucleosome for remodeling (Gregory et al. 1999; Reinke et al. 2001). However, *RNR3* is different from *PHO8* in that acetylation affects the extent of remodeling of many nucleosomes over the promoter, and acetylation is sustained after remodeling. Moreover, the aforementioned study did not distinguish between effects on SWI/SNF recruitment versus remodeling activity; therefore, our study provides additional insights into the mechanisms of how chromatin remodeling and modifying activities cooperate to regulate gene expression. Our data suggests that acetylation affects remodeling without affecting SWI/SNF recruitment in vivo. However, even though SWI/SNF is cross-linked to the promoter as efficiently in a Δ*gcn5* mutant, acetylation could subtly affect the ability of SWI/SNF to remain bound to a single nucleosome through the multiple rounds of ATP hydrolysis that are required to generate the multiple remodeled states or to catalytically remodel multiple nucleosomes on an array (Hassan et al. 2001a; Narlikar et al. 2001, 2002; Peterson 2002). Finally, acetylation could facilitate remodeling indirectly by preventing the reassociation of repressor complexes onto the promoter. SWI/SNF is continuously required to maintain the remodeled state of genes in vivo (Fig. 7; Biggar and Crabtree 1999; Sudarsanam et al. 1999); thus, SWI/SNF is certainly in constant competition with various repressors in vivo. Acetylation may be unfavorable for the reassociation of Ssn6/Tup1 with *RNR3*, and in its absence, Tup1 can compete with the actions of SWI/SNF more effectively. This model is consistent with Tup1's ability to bind to hypoacetylated histones preferentially (Edmondson et al. 1996) and SWI/SNF's ability to antagonize Tup1-mediated repression on *RNR3*, *SUC2* and *FLO1* (this study; Gavin and Simpson 1997; Fleming and Pennings 2001; Li and Reese 2001).

Materials and methods

Yeast strains and genetic manipulations

A list of strains is provided as Supplemental Material. The myc-tagged strains were generated using the PCR-directed method with a 13MYC-KanMx cassette (Longtine et al. 1998).

MNase mapping

Temperature-shift experiments were typically performed as follows: 0.5 L of cell culture was grown to log-phase (OD_{600} = 1.0 to 1.2) at 23°C, and then an equal volume of prewarmed YPD medium (50°C) was added. Following a 45-min preincubation, MMS was added to the final concentration of 0.015%, and the culture was maintained at 37°C for 2–2.5 h. Nuclei preparation was carried out essentially as described (Li and Reese 2001). For low-resolution mapping of nucleosome by indirect end labeling, the specific DNA sequences were detected by hybridization with a probe directed towards the end of the PstI site +468 to +725 relative to the major start site of RNR3, and to the HinfI site of *SUC2* (Hirschhorn et al. 1992)

ChIP assay

The ChIP assay was performed essentially as described in two previous publications (Hecht and Grunstein 1999; Li et al. 1999). Cultures were treated for 2 or 2.5 h with 0.02% MMS prior to cross-linking, where indicated. Briefly, a 200-mL culture of yeast grown in YPAD to an OD_{600} = 1.0 was treated with formaldehyde (1% v/v) for 15 min at 23°C, followed by an additional 15 min in 125 mM glycine. For temperature-shift experiments, an equal volume of YPD medium prechilled to approximately 4°C was added to the culture prior to the addition of formaldehyde. Immunoprecipitated and input DNAs were analyzed by semiquantitative PCR analysis with promoter-specific primers spanning the core promoter region of each gene and the coding region of *RNR3*. Only one amount of immunoprecipitated DNA and two or three titrations of input DNA are shown in the figures to conserve space, but multiple dilutions were analyzed in other gels. The PCR products were detected by illumination of ethidium bromide-stained 2% agarose gels, and images were captured and quantified using the Typhoon system (Molecular Dynamics) and IimageQuant (Molecular Dynamics) software.

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