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# A Novel Mechanism of Antagonism between ATP-Dependent Chromatin Remodeling Complexes Regulates RNR3 Expression▽

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**Gene expression depends upon the antagonistic actions of chromatin remodeling complexes. While this has been studied extensively for the enzymes that covalently modify the tails of histones, the mechanism of how ATP-dependent remodeling complexes antagonize each other to maintain the proper level of gene activity is not known. The gene encoding a large subunit of ribonucleotide reductase, *RNR3*, is regulated by ISW2 and SWI/SNF, complexes that repress and activate transcription, respectively. Here, we studied the functional interactions of these two complexes at *RNR3*. Deletion of *ISW2* causes constitutive recruitment of SWI/SNF, and conditional reexpression of *ISW2* causes the repositioning of nucleosomes and reduced SWI/SNF occupancy at *RNR3*. Thus, ISW2 is required for restriction of access of SWI/SNF to the *RNR3* promoter under the uninduced condition. Interestingly, the binding of sequence-specific DNA binding factors and the general transcription machinery are unaffected by the status of ISW2, suggesting that disruption of nucleosome positioning does not cause a nonspecific increase in cross-linking of all factors to *RNR3*. We provide evidence that ISW2 does not act on SWI/SNF directly but excludes its occupancy by positioning nucleosomes over the promoter. Genetic disruption of nucleosome positioning by other means led to a similar phenotype, linking repressed chromatin structure to SWI/SNF exclusion. Thus, incorporation of promoters into a repressive chromatin structure is essential for prevention of the opportunistic actions of nucleosome-disrupting activities in vivo, providing a novel mechanism for maintaining tight control of gene expression.**

Gene regulation involves a multitude of elaborate steps that culminate in the assembly of large macromolecular complexes over the promoters of genes (41). An early step in the process is the remodeling and modification of chromatin to allow access of DNA binding proteins, coactivators, and the general transcription machinery to the DNA. As for any biological process, the balance of opposing activities dictates the outcome. This is best understood for histone modifications. The first example described was the antagonism between histone acetyltransferases and histone deacetylases in maintaining dynamic histone acetylation levels (2, 23, 33, 34). This was soon followed by the identification of opposing pairs of enzymes that maintain the balance of histone phosphorylation, lysine methylation, arginine methylation, and lysine ubiquitylation (2, 21, 37, 46, 49).

ATP-dependent remodeling complexes are a group of transcriptional regulators that modify histone-DNA contacts within the nucleosome. There are four major families that influence gene activity, which are defined by the structure and degree of homology within the catalytic domains of their ATPase subunits: (i) SWI/SNF-RSC, (ii) ISWI, (iii) Mi2/CHD, and (iv) INO80/SWR (19, 29, 44). The prototype for ATP-dependent remodelers is the SWI/SNF group, which can remodel nucleosomes by multiple mechanisms, including sliding, H2A-H2B dimer disassociation, creation of bulges, and octamer transfer (12, 24, 39). A large body of biochemical and genetic evidence indicates that this family remodels nucleosomes to activate transcription (27, 39). A second group of remodeling complexes is the ISWI family, which slides nucleosomes without permanently disrupting histone-DNA contacts (12, 24). The complexes in the ISWI family are implicated in both gene repression and activation, but they are better known for their roles in repression. A great deal of our knowledge on the function of these complexes comes from studies of *Saccharomyces cerevisiae*. The ISWI class of remodelers in yeast includes ISW1a, ISW1b, and ISW2 (28). ISW2 in particular has been shown to repress transcription by positioning nucleosomes in vivo (11, 14, 18, 53). Its mechanism of action involves the sliding of nucleosomes to specific translational positions rather than eviction of the nucleosome. Thus, these two classes of remodeling enzymes function by distinct mechanisms and have opposing actions on transcription. Despite a great deal of work on the actions of these complexes individually, how they cooperate to maintain a fluid chromatin structure at a particular locus is unclear.

The *RNR3* (ribonucleotide reductase 3) gene of *Saccharomyces cerevisiae* has become a model for dissection of the functions of chromatin remodeling factors. Nucleosomes are positioned across the entire gene when the gene is repressed, and activation of transcription is accompanied by extensive chromatin remodeling and nucleosome eviction (25, 48, 53). Previous work has revealed that ISW2 is responsible for positioning nucleosomes at this locus, and deleting the gene encoding the ATPase subunit of this complex results in a micro-

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TABLE 1. Strains used in this study

| Strain | Genotype |
|---|---|
| BY4705 | *MAT***a** *ade2*Δ::*hisg his3*Δ*200 leu2*Δ*0 lys2*Δ*0 met15*Δ*0 trp1*Δ*63 ura3*Δ*0* |
| JR720 BY4705 | *isw2*Δ::*HIS3* |
| JR730 BY4705 | *isw2*Δ::*HIS3 snf2*Δ::*LEU2* |
| BY4741 | *MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0* |
| JR1242 BY4741 | *crt1*Δ::*URA3* |
| JR1243 BY4741 | crt1Δ::URA3 *isw2*Δ::*KanMx* |
| PH499 | *MAT***a** *ade2-101*$^{ochre}$ *his3-*Δ*200 leu2-*Δ*1 ura3-52 trp1-*Δ*63 lys2-801*$^{amber}$ |
| JR972 PH499 | *RAP1*::*13MYC*::*KanMx* |
| JR978 PH499 | *isw2*Δ::*HIS3 RAP1*::*13MYC*::*KanMx* |
| JR1012 PH499 | *isw2*Δ::*KanMx* |
| JR1097 PH499 | *snf2*Δ::*KanMx* |
| JR1098 PH499 | *snf2*Δ::*KanMx isw2*Δ::*HIS3* |
| W303 | *MAT***a** *ade2-11 his3-11,15 leu2-3,112 ura3-1 trp1-1 can1-100 RAD5*$^{+}$ |
| TTY196 W303 | *isw2*Δ::*LEU2* |
| YTT446 W303 | *GAL1*$_{pro}$*-ISW2-FLAG2*::*URA3* |
| MSY552 | *MAT***a** *ura3-52 lys2-*Δ*201 leu2-3112* Δ(*HHT1 HHF1*) Δ(*HHT2 HHF2*) pMS337[*CEN ARS LEU2 HHT1 HHF1*] |
| MSY344 | *MAT***a** *ura3-52 lys2-*Δ*201 leu2-3112* Δ(*HHT1 HHF1*) Δ(*HHT2 HHF2*) pMS358[*CEN ARS LEU2 hht1-2*(Δ*1-28*)*-HHF1*] |
| MSY590 | *MAT*α *ura3-52 lys2-*Δ*201 leu2-3,112* Δ(*hht2-hhf2*) *HHT1-HHF1* |
| MSY711 | *MAT***a** *ura3-52 lys2-*Δ*201 leu2-3,112* Δ(*hht1-hhf1*) Δ(*hht2-hhf2*) *HHT1-hhf1-8*(Δ*2-26*) |
| MSY1913 | *MAT***a** *his3*Δ*1 ura3*Δ*0 leu2*Δ*0 hht1-hhf1*Δ::*KanMX hhf2-hht2*Δ::*NatMX hta1-htb1*Δ::*HphMX hta2-htb2*Δ::*NatMX* pJH33[*HTA1-HTB1-HHF2-HHT2 URA3*/*CEN*] |
| MSY1975 | *MAT***a** *his3*Δ*1 ura3*Δ*0 leu2*Δ*0 hht1-hhf1*Δ::*KanMX hhf2-hht2*Δ::*NatMX hta1-htb1*Δ::*HphMX hta2-htb2*Δ::*NatMX* pJH53 [*hta1* Δ*1-20-HTB1-HHF2-HHT2 LEU2*/*CEN*] |
| MSY1979 | *MAT***a** *his3*Δ*1 ura3*Δ*0 leu2*Δ*0 hht1-hhf1*Δ::*KanMX hhf2-hht2*Δ::*NatMX hta1-htb1*Δ::*HphMX hta2-htb2*Δ::*NatMX* pJH49 [*HTA1-htb1*(Δ*1-32*)*-HHF2-HHT2 LEU2*/*CEN*] |

coccal nuclease (MNase) digestion pattern similar to that of naked DNA, therefore suggesting that the chromatin structure is fully disrupted (25, 53). Under DNA damage-inducing conditions, nucleosome disruption requires the recruitment of SWI/SNF, and inactivating SWI/SNF blocks the remodeling of the promoter and the activation of transcription (35, 51). SWI/SNF is primarily required for eviction of the core promoter nucleosome, which is suggested by the observation that excluding nucleosome formation over the promoter can suppress the requirement for SWI/SNF (48). The level of activity of *RNR3* is likely to be regulated by the opposing actions of these two chromatin remodeling complexes.

Whereas the antagonism of histone-modifying enzymes has been well established, very little is known about how nucleosome remodeling activities cooperate to balance gene expression. Here, we use the *RNR3* gene as a model to characterize the functional antagonism between two ATP-dependent chromatin remodeling complexes in vivo. ISW2 maintains chromatin structure by positioning nucleosomes and preventing the binding of SWI/SNF to genes. In addition, we show that disrupting chromatin structure by deleting the H4 tail specifically, leads to a similar phenotype, suggesting that ISW2 does not act directly on SWI/SNF but excludes it from the promoter by regulating chromatin structure. Thus, we describe a novel antagonistic relationship between two ATP-dependent nucleosome remodeling complexes and suggest a mechanism for how opportunistic actions of transcription factors are suppressed to prevent misregulation of genes.

## MATERIALS AND METHODS

**Strains and media.** The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Cells were grown at 30°C in YPD (1% yeast extract, 2% peptone, and 2% dextrose) medium supplemented with 0.05 mg/ml adenine. Where indicated, methyl methanesulfonate (MMS) was added to give a final concentration of 0.03% for 2.5 h. Gene deletion and epitope tagging were carried out by homologous recombination using PCR-generated cassettes (30). For the ISW2 reexpression studies, cells were grown to an optical density at 600 nm ($OD_{600}$) of 1.0 in YP (1% yeast extract, 2% peptone) containing 2.5% raffinose, and then galactose was added to give a final concentration of 3% for the times indicated in Fig. 4.

**Northern blotting and chromatin mapping.** Cells from 10 ml of yeast culture ($OD_{600}$ = 0.7) were harvested for total RNA extraction. Fifteen micrograms of total RNA was separated on 1.2% formaldehyde-containing agarose gels and transferred to a Hybond-XL membrane (GE Biosciences, Piscataway NJ) by capillary blotting. Probes for *RNR3* or *Scr1* were prepared by PCR. The signal of *scR1* (small cytoplasmic RNA) in each sample was used to correct for recovery and loading of RNA. The details of this method have been published elsewhere (32). MNase mapping of nucleosomes by indirect end labeling was carried out using a published protocol (50).

**ChIP.** The chromatin immunoprecipitation (ChIP) assay was performed essentially as described in previous publications (35, 48). One hundred milliliters of yeast culture ($OD_{600}$ = 0.7 to 1.0) was treated with formaldehyde (1% [vol/vol]) for 15 min at room temperature, and cross-linking was quenched by the addition of glycine to give 125 mM. Whole-cell extracts were prepared by glass bead disruption and sheared into fragments averaging 300 to 600 bp in size by using a Bioruptor (Diagenode, Philadelphia PA). Whole-cell extracts were immunoprecipitated with antibodies indicated throughout the text. RNAPII antibody was obtained from commercial sources (8WG16; Covance, Berkeley CA). The following antibodies were raised in rabbits and were described in previous publications (51): TBP (full-length), TAF1 (amino acids 1 to 225), Tup1 (full-length), and Swi2 (amino acids 1 to 851). The protein-immune complexes were recovered using 30 μl of protein A-Sepharose CL-4B beads (GE Biosciences, Piscataway, NJ). The immunoprecipitated DNA and input DNA were analyzed by semiquantitative PCR with primers directed toward *RNR3* (36, 48). The percent immunoprecipitation was calculated, and data are expressed relative to the cross-linking observed in untreated wild-type cells. Data are presented as the means and standard deviations of results from at least three independent experiments. For detecting nucleosome eviction over the *RNR3* promoter, cells were processed as described above except that the chromatin was sheared extensively into fragments averaging 200 base pairs in length. Chromatin was precipitated using an antibody to the core domain of H3 (Abcam, Cambridge, MA). The oligonucleotides used in this study are listed in Table 2.

TABLE 2. Oligonucleotides used in this study

| Description | Sequence |
|---|---|
| RNR3 promoter | 5′-CGTTTTTCGTGTCAGCGTTC-3′<br>5′-ACGTACATTTGTGTGGGAG-3′ |
| RNR3 URS | 5′-AGCAAGCCCTCGTTCTTGGC-3′<br>5′-TCCCCATCTTGTCGTTTGGTCC-3′ |
| RNR3 nucleosome −1 primers | 5′-GTTTTTCGTGTCAGCGTTC-3′<br>5′-GCTGCTATTCTTGCTTGTC-3′ |
| REC104 promoter | 5′-GTCCTTTAGCTAATAGAGTAAGCC-3′<br>5′-ATGGACATGTTGTCCAAGTTGCTG-3′ |
| iYGLWδ6 | 5′-CAGATTTAATGAAGGTGATATGCAAG-3′<br>5′-CGTATAACCAATCATATTCGCGAG-3′ |
| Subtelomeric region (TEL) | 5′-AGCAACGACTTCGTCTCAGAAG-3′<br>5′-GTTAGGATCACGTTCGAATCCT-3′ |

## RESULTS

**Examination of the functional interaction between ISW2 and SWI/SNF.** Two ATP-dependent chromatin remodeling complexes regulate the chromatin structure at *RNR3*. ISW2 is required for placement of nucleosomes into precise translational positions and contributes to the repression of transcription, and SWI/SNF is required for the eviction of nucleosomes and activation of transcription (35, 52, 53). Since these two complexes have opposing actions on chromatin, we examined the functional relationship between these two complexes. Strains containing *isw2*Δ, *swi2*Δ, and double *isw2*Δ *swi2*Δ mutations were constructed. The double mutant grew significantly less well than either of the single mutants, indicating that the combined mutations cause severe "synthetic sickness" (Fig. 1A). Synthetic lethality between *swi2*Δ mutants and components of the RSC complex and *CHD1* has been noted previously, suggesting that functional interactions between chromatin remodeling complexes extend beyond SWI/SNF and ISW2 (5, 43).

Next, we examined the expression of *RNR3* in the mutants under repressing (−MMS) and induced (+MMS) conditions in the mutants. Deleting ISW2 caused a small increase in the level of mRNA under the uninduced condition but had no effect on the induction of *RNR3* by MMS (Fig. 1B, lanes 3 and 4). These results are consistent with its role in repression and nucleosome positioning. In contrast, the activation of *RNR3* was essentially eliminated in the *swi2*Δ strain. Interestingly, the double mutant displayed the same phenotype as the *swi2*Δ mutant, indicating that deleting *ISW2* cannot suppress the requirement for SWI/SNF in the activation of *RNR3*. The slight increase in the level of MMS-induced mRNA in the double mutant, compared to the level in the single *swi2*Δ mutant, is not seen consistently. This is in contrast to the results for the *GAL1* and *INO1* genes, where deleting ISW2 suppressed the transcription defects of a *swi2*Δ mutant (22). Thus, the nature of the functional interaction between ISW2 and SWI/SNF is gene specific.

The chromatin structure over *RNR3* was then examined. MNase mapping and histone H3 cross-linking were used to determine the effects of the mutations on nucleosome positioning and eviction, respectively. MNase mapping cannot distinguish random nucleosome placement from eviction, and examination of remodeling by the ChIP assay using antibodies to core histones cannot definitely detect random positions of nucleosomes, because the overall signal across a region may diminish somewhat but will remain high. Thus, the combined use of MNase mapping and H3 cross-linking provides a clearer picture of chromatin structure. *RNR3* is packaged into precisely positioned nucleosomes in the repressed state, which is obvious from the internucleosomal hypersensitive sites regularly spaced over the promoter and the 5′ end of the open reading frame (Fig. 2A). As described previously (53), deleting *ISW2* caused a digestion pattern that is significantly different from that of wild-type cells; the internucleosomal hypersensitive sites were diminished, indicating that nucleosome positioning was lost. On the other hand, deleting *swi2*Δ had no effect on nucleosome positions in untreated cells (Fig. 2A). We next examined if the loss of nucleosome positioning in the *isw2*Δ mutant is dependent upon SWI/SNF. The double mutant had a digestion pattern indistinguishable from that of *isw2*Δ, indicating that SWI/SNF is not required for depositioning of nucleosomes in the *isw2*Δ cells under normal growth conditions.

H3 cross-linking to the promoter of *RNR3* was measured using primers directed within nucleosome −1, which resides over the TATA box (48). Activation of gene expression leads to a three- to fourfold reduction in H3 cross-linking in wild-type cells, indicating a loss of the promoter nucleosome (Fig. 2B). Cross-linking was only slightly reduced in the *isw2*Δ mutant in uninduced cells, indicating that even though the MNase digestion pattern indicates that nucleosome positioning is disrupted, no histone eviction occurs under this condition. Treating the mutant with MMS led to nucleosome eviction over the promoter, however. Thus, *ISW2* is required for maintenance of nucleosome positions at *RNR3* but is not involved in eviction. This explains why the level of derepression and preinitiation

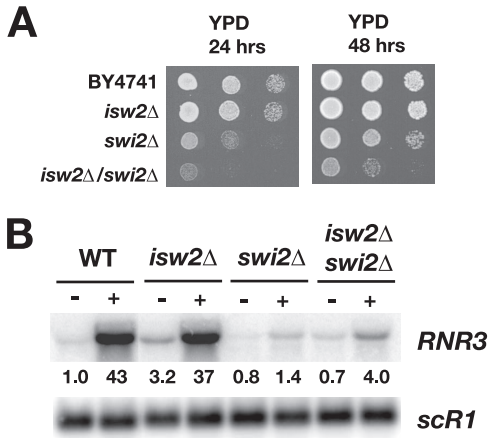


FIG. 1. Interplay between ISW2 and SWI/SNF at *RNR3*. (A) Spot test for strain growth. Cells were spotted and grown at 30°C on YPD plates for 24 and 48 h. (B) Northern blotting for *RNR3* mRNA. Wild-type (WT) and mutant cells were treated (+) or not treated (−) with 0.03% MMS for 2.5 h, and mRNA levels were detected by Northern blotting. *scR1* is a loading control. The level of mRNA is indicated below the panel and is expressed relative to the signal of that from untreated wild-type cells observed after correction for loading.

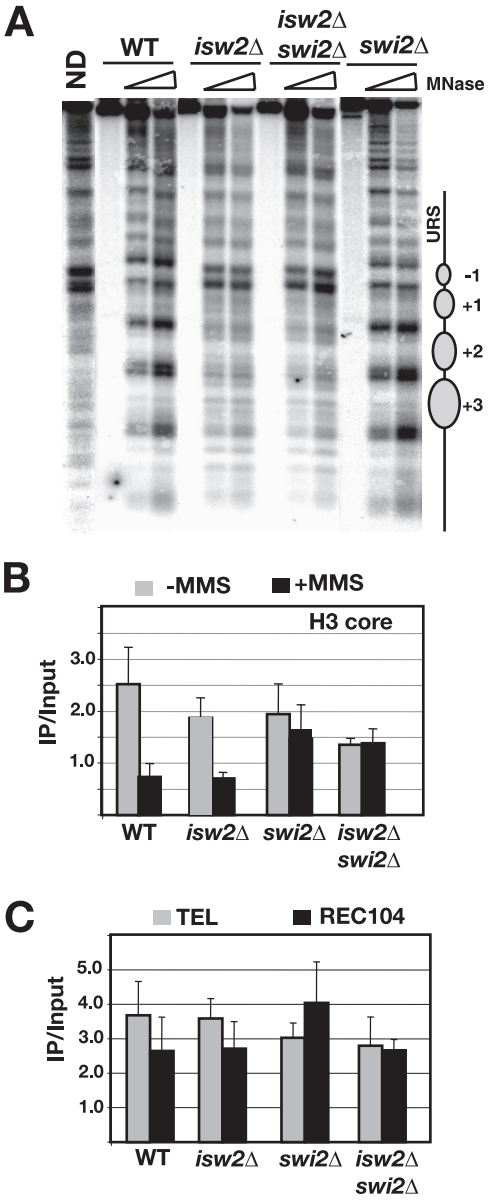


FIG. 2. Chromatin structure and nucleosome density in ATP-dependent chromatin remodeling complex mutants. (A). MNase mapping of nucleosome positions at *RNR3*. The approximate positions of nucleosomes are indicated on the left. The URSs, which contain the binding sites for Crt1, are indicated within the graphic. The TATA box resides within nucleosome −1 and is at position −75 relative to the start site of transcription (25). ND, digested naked DNA; WT, wild type. (B) ChIP analysis of histone H3 cross-linking over the core promoter of *RNR3*. Wild-type and mutant cells were treated (+) or not treated (−) with 0.03% MMS for 2.5 h and then cross-linked with formaldehyde. Data are represented as percentages of immunoprecipitated (IP) signal versus input. Data are presented as the means and standard deviations of results from at least three independent experiments. (C) Same as in panel B, except primers directed to a subtelomeric region (TEL) or the ISW2-regulated gene *REC104* were used to amplify the DNA.

complex (PIC) formation is low in the *isw2*Δ mutant even though the MNase digestion pattern suggests complete disruption of nucleosome positioning. Deletion of *SWI2* likewise led to a slight but not statistically significant decrease in H3 cross-linking in untreated cells, and as expected, MMS-induced nucleosome eviction was impaired. Thus, SWI/SNF is required for the removal of a nucleosome from the core promoter under the activated condition (+MMS). The level of cross-linking of H3 in the double *isw2*Δ *swi2*Δ mutant was essentially the same as that in the *isw2*Δ strain when the cells were untreated. However, deleting *SWI2* in the *isw2*Δ background blocked the MMS-induced nucleosome eviction. Thus, *ISW2* cannot suppress the activation defect of the *swi2*Δ mutant, because the core promoter nucleosome, while adopting a random position, is not evicted in the double mutant. Next, we examined the cross-linking of H3 in the mutants to a subtelomeric region (TEL) and *REC104* as controls (Fig. 2C). *ISW2* is required for the sliding of the promoter nucleosome upstream toward the upstream repression sequence (URS) of *REC104* (11, 13, 14), and the chromatin mapping pattern at this gene indicates that nucleosomes adopt new positions rather than being evicted in the *isw2*Δ mutant (11). ChIP analysis detected no nucleosome eviction over *REC104* (Fig. 2C), which is supported also by recent genome-wide mapping of nucleosome positions in an *isw2*Δ mutant (45). A subtelomeric region was also examined as a control region, and there was not a significant change in the H3 cross-linking over this region of the genome in any of the mutants.

**ISW2 excludes SWI/SNF from *RNR3*.** We next examined the recruitment of Swi2 to *RNR3* in the mutants to determine if ISW2 regulates SWI/SNF binding. ISW2 associates with *RNR3* across the entire gene and regions upstream of the promoter, suggesting that it is broadly associated with the gene in an untargeted manner (13, 45, 53). Interestingly, deleting *ISW2* caused a very significant increase in the cross-linking of Swi2 to *RNR3* in untreated cells (Fig. 3A). The level of cross-linking was very close to that observed in MMS-treated wild-type cells. Treatment of the *isw2*Δ cells with MMS led to a slight increase in Swi2 cross-linking, however. Thus, SWI/SNF is constitutively associated with *RNR3* in the *isw2*Δ mutant. As a control, we examined Swi2 cross-linking to another ISW2 target gene, *REC104*. Swi2 cross-linking was not increased at this ISW2-regulated gene, suggesting that the increase in SWI/SNF association is gene specific (also see Fig. 5).

To determine if the disrupted chromatin structure caused by the deletion of *ISW2* led to a broad and nonspecific increase in the cross-linking of transcription factors known to be recruited to *RNR3*, we expanded our ChIP analysis to monitor the cross-linking of two components of the TFIID complex, Rap1 and Tup1. Deleting ISW2 did not lead to an increase in TBP binding, nor did it cause an increase in the cross-linking of TAF1, a TFIID-specific $TAF_{II}$ (Fig. 3B). Rap1 is a sequence-specific DNA binding protein that we recently identified as a regulator of the RNR genes, and this protein is recruited in a checkpoint-dependent manner to *RNR3* (42). Furthermore, Rap1 is required for SWI/SNF recruitment to *RNR3* (42), and thus, Rap1 may be responsible for the recruitment of SWI/SNF in uninduced *isw2*Δ cells. If this were true, we would expect Rap1 cross-linking to change when *ISW2* is deleted. However, deletion of *ISW2* failed to cause Rap1 binding in untreated cells (Fig. 3C). Therefore, while Rap1 is required for recruitment of SWI/SNF to *RNR3* in wild-type cells, the constitutive recruitment of SWI/SNF in the *isw2*Δ mutant occurs by a different mechanism. Next, we examined the recruitment of

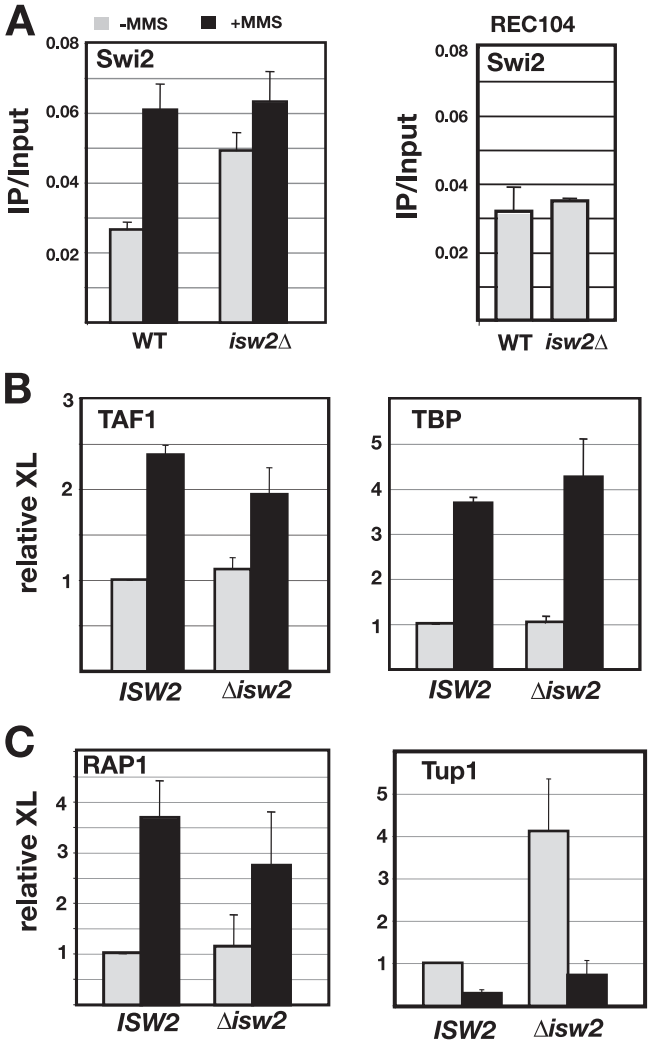


FIG. 3. Analysis of SWI/SNF and transcription factor cross-linking to *RNR3*. (A) ChIP assays were conducted as described for Fig. 2B. Antibodies to the N terminus of Swi2 were used to immunoprecipitate (IP) chromatin, and primers directed to *RNR3* (left) and *REC104* (right) were used to amplify DNA. Data are presented as percentages of IP. WT, wild type. (B) ChIP assay for TFIID components. Cross-linking data are presented relative to levels for untreated wild-type cells. XL, cross-linking. (C). ChIP analysis for the activator Rap1 and the repressor Tup1. Rap1 was immunoprecipitated using antibodies to the myc epitope that was incorporated into its C terminus.

Tup1. We previously showed that deleting *ISW2* led to an increase in Tup1 cross-linking over the URS of *RNR3* (53), and this was observed again here (Fig. 3C). Thus, while deletion of ISW2 caused increased recruitment of SWI/SNF and Tup1, it does not result in the nonspecific association of all transcription factors to *RNR3*. Surprisingly, the ChIP data indicate that SWI/SNF is recruited in the *isw2*Δ mutant in untreated cells, yet nucleosomes are not evicted efficiently (Fig. 2B and 3A). This suggests either that SWI/SNF is more effective at evicting nucleosomes from promoters configured in the repressed state or that eviction requires the recruitment of Rap1. We believe that the latter is more likely because the DNA binding domain of Rap1 is required for the eviction of nucleosomes at the *RNR3* and *HIS4* promoters, preventing the reassembly of nucleosomes back to the repressed state (42, 47).

**Restoration of ISW2 suppresses SWI/SNF recruitment.** The constitutive recruitment of SWI/SNF in the Δ*isw2* mutant was unexpected. To provide a more direct correlation between ISW2 occupancy and the suppression of SWI/SNF recruitment, we used a strategy developed by Fazzio and Tsukiyama (11). A strain containing ISW2 under the control of the *GAL1* promoter was grown in raffinose to reduce ISW2 levels, and expression was restored upon the addition of galactose to the medium. Switching the cells from raffinose to galactose led to an increase in the level of Isw2p within 45 min, and this increase leveled off by 90 min (Fig. 4A). The increase in Isw2p in the cells correlated with its cross-linking to *RNR3* (Fig. 4B) and the reestablishment of nucleosome positioning over the 5′ end of the gene (Fig. 4C). The restoration of the repressive chromatin structure is indicated by the reappearance of the internucleosomal hypersensitive sites. Importantly, the levels of Swi2 and Swi3 do not change over the time course of ISW2 reexpression.

Next, we used this strain to monitor Swi2 recruitment to *RNR3* after the cells were shifted to galactose to correlate the association of ISW2 and the return of nucleosome positioning to changes in the level of SWI/SNF recruitment. Results for a representative experiment are shown in Fig. 5A, left panel, indicating that the cross-linking of Swi2 to *RNR3* was reduced within 45 min after the addition of galactose and that the effect was complete by 90 min. A graph showing the averages and standard errors of results from multiple experiments is shown in the right panel, indicating that the results are highly reproducible. The kinetics of the reduction in Swi2 cross-linking matches that of ISW2 association and the reestablishment of nucleosome positions at *RNR3* (Fig. 4). Since the changes are observed rapidly and correlate with the appearance of ISW2 at *RNR3*, this suggests that the effects that we observe are direct. Furthermore, the level of Swi2 remained constant throughout (Fig. 4A), so the effect is not due to changes in protein levels. On the other hand, Swi2 cross-linking was not decreased at *REC104* or *iYGLWδ6*, two other ISW2-regulated regions, or at subtelomeric DNA (Fig. 5A, B, and C). It should be noted that the level of cross-linking of Swi2 to *REC104* and *iYGLWδ6* represents background levels, but the data nonetheless indicate that restoring ISW2 expression does not lead to a change in the cross-linking of SWI/SNF at these ISW2-regulated loci. Thus, disruption of nucleosome positioning at other ISW2-dependent genes does not lead to SWI/SNF recruitment. As additional controls, we examined the cross-linking of RNAPII and Tup1 to the promoter. As noted before, cells lacking ISW2 have elevated Tup1 recruitment (Fig. 3C) (53), and reexpression of ISW2 leads to reduced cross-linking of this factor (Fig. 5C). There was no change in RNAPII cross-linking, as expected (Fig. 5C). These results indicate that restoring ISW2 to the cell specifically suppresses the elevated level of SWI/SNF recruitment to *RNR3*. Thus, we provide solid evidence that ISW2 antagonizes the recruitment of SWI/SNF to *RNR3*.

**The N-terminal tail of histone H4 restricts SWI/SNF recruitment.** ISW2 can antagonize the recruitment of SWI/SNF by removing it from the promoter directly or by regulating the chromatin structure over *RNR3*. We addressed these two possibilities by altering the chromatin over *RNR3* without chang-

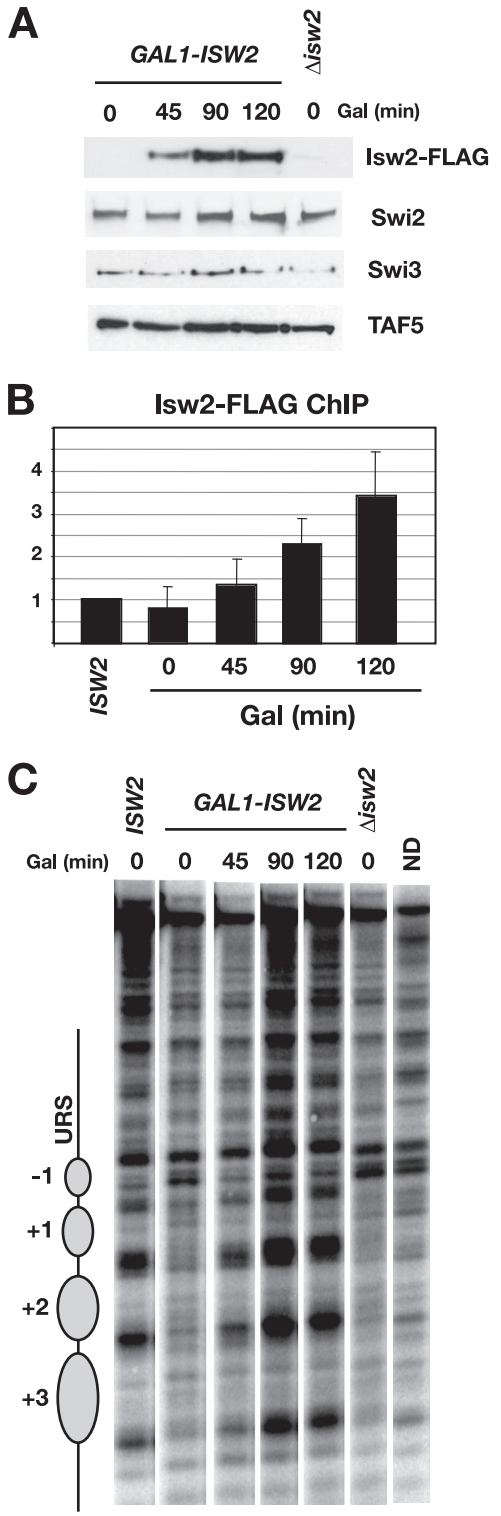


FIG. 4. Conditional reexpression of Isw2 causes nucleosome positioning. A strain containing ISW2-FLAG under the control of the *GAL1* promoter (11) was grown in raffinose to deplete Isw2 and then supplemented with galactose. Aliquots were removed prior to galactose addition (0) and 45, 90, or 120 min afterwards. (A) Western blotting of extracts from cells. Anti-Flag antibodies were used to detect Isw2, and polyclonal antibodies to SWI/SNF and TFIID subunits were used for loading controls. (B) ChIP assay using anti-Flag antibodies to detect Isw2 cross-linking over the *RNR3* promoter. The amount of DNA in immunoprecipitates from an untagged strain (ISW2) was set to 1.0, and cross-linking relative to that value is presented as the means and standard deviations of results from three experiments. (C) MNase mapping of the *RNR3* promoter was carried out as described for Fig. 2A. Multiple concentrations of MNase were used in the mapping experiment, but a panel of one concentration is shown to allow a better side-by-side comparison of the patterns at each time point. ND, digested naked DNA.

ing the status of ISW2 within the cell. The H4 tail is required for ISW2 function in vivo and in vitro (7, 10). Furthermore, the tails of histones, in particular that of H4, have been implicated in the folding of nucleosomes into higher-order structures (8, 9, 38). We therefore examined if removing the histone tails individually results in constitutive recruitment of SWI/SNF. Treating wild-type cells with MMS caused a 2- to 2.5-fold increase in Swi2 cross-linking, and the level of Swi2 binding under the uninduced and induced conditions in the H2A, H2B, and H3 N-terminal tail deletion mutants were essentially equal (Fig. 6A). However, there was an obvious increase in the cross-linking of Swi2 to *RNR3* in the untreated histone H4 tail mutant, and the level of cross-linking was not increased significantly by MMS treatment (Fig. 6A). The constitutive SWI/SNF recruitment in the H4 tail mutant was specific for *RNR3*, as SWI/SNF association was not significantly increased at *REC104* (Fig. 6B). Also similar to the *isw2*Δ mutant, cross-linking of TBP or RNAPII was not elevated in untreated H4 tail mutant cells (Fig. 6C); however, the MMS-induced levels of recruitment were reduced somewhat.

We further characterized the changes in chromatin structure at *RNR3* in the H4 tail mutant. First, we examined the positioning of nucleosomes over *RNR3* in the H4 tail mutant. Deleting the tail of H4 caused a partial disruption of chromatin structure, particularly in the region downstream of the promoter (nucleosome +1). The hypersensitive site downstream of nucleosome +1 is altered (Fig. 6D). Specifically, the hypersensitive site between nucleosomes +1 and +2 broadens in appearance and is reduced in intensity, indicating a weakening of positioning in that region. A similar, although more pronounced, change occurs when the promoter nucleosomes are remodeled. Surprisingly, changes in the positioning of nucleosome −1 in the H4 tail mutant are subtle. A slight increase in digestion over the TATA box is observed (doublet within nucleosome −1), but clearly, it is not as pronounced as what occurs when cells are treated with MMS or when *isw2*Δ is deleted. However, treating the H4 mutant with MMS led to a pattern resembling that of the fully remodeled state observed in wild-type cells, suggesting an additional loss of nucleosome positioning. Then, we examine the density of nucleosomes at the core promoter by using an antibody to the core domain of H3 in ChIP assays. In the uninduced state, the density of nucleosomes is not affected by the H4 tail mutation (Fig. 6E). Thus, similar to that in the *isw2*Δ mutant, the disrupted nucleosome positioning detected by MNase mapping in the H4 tail mutant is caused by a disruption in positioning rather than an eviction of nucleosomes. However, there was a reduction in the MMS-induced eviction of the promoter nucleosome in this mutant. The reduction in MMS-induced histone density loss correlated with reduced PIC formation (Fig. 6C) and Tup1

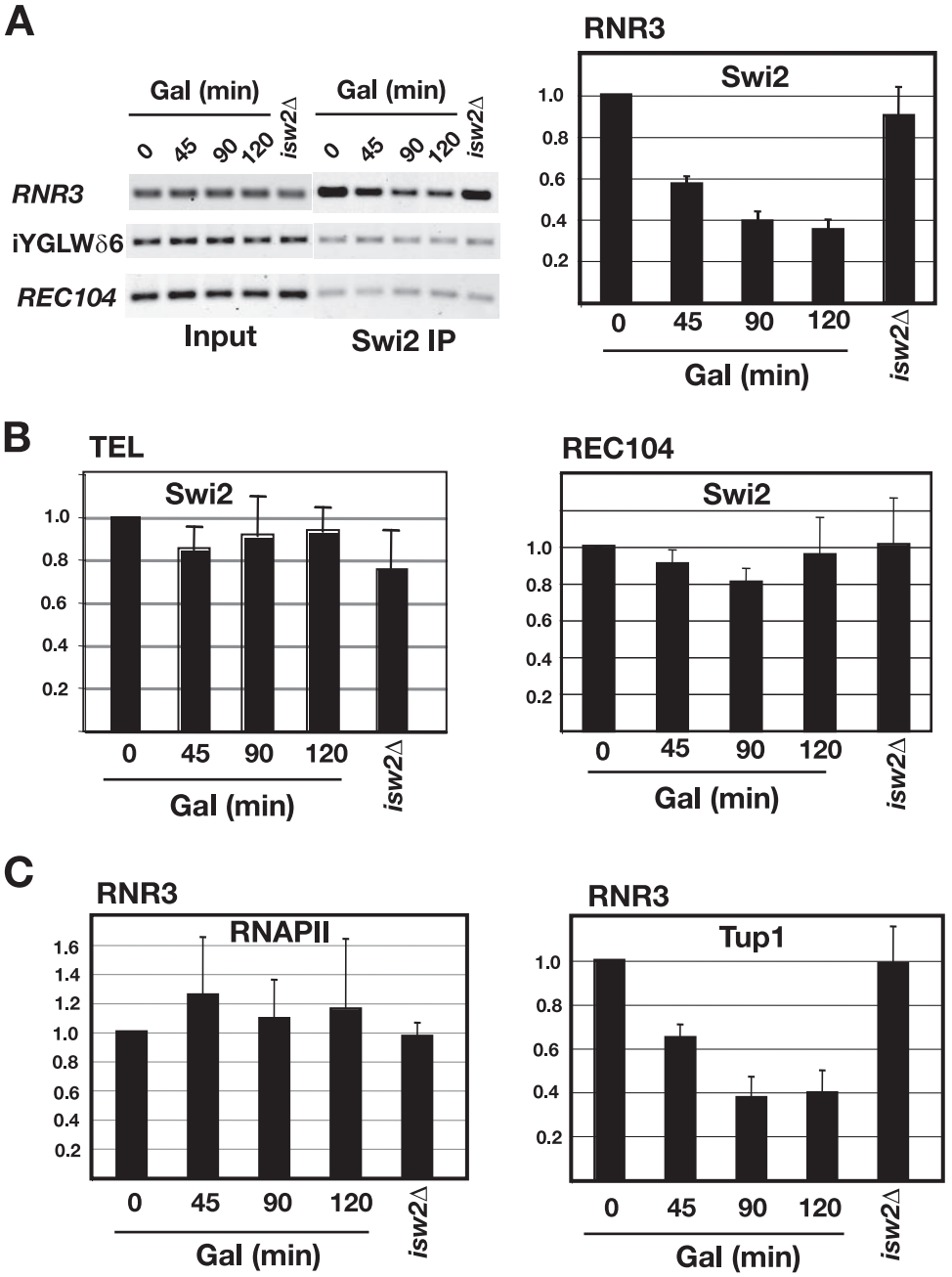


FIG. 5. ISW2-dependent nucleosome positioning excludes SWI/SNF from *RNR3*. Results are shown for a ChIP assay performed after reexpression of Isw2. (A) Cross-linking of Swi2. A representative gel from a single experiment is shown on the left. Averages and standard deviations of results from three experiments measuring the cross-linking of Swi2 to *RNR3* are shown on the right. (B) Cross-linking of Swi2 at a subtelomeric region (TEL) and *REC104*. (C) Cross-linking of RNAPII (8WG16) and Tup1 over *RNR3*.

release (see below). It was a surprise that no single histone tail is required for SWI/SNF recruitment. Thus, if the histone tails play a role in SWI/SNF recruitment, there is redundancy among the tails for this function. In addition, even though the levels of SWI/SNF recruitment in the H4 mutant in the absence of DNA damage are similar to those in induced wild-type cells, the extents and natures of the remodeling under the two conditions are not equal. Stimulated wild-type cells show complete disruption of positioning and nucleosome eviction. In contrast, deleting the H4 tail leads to partial loss of positioning but no eviction. This suggests that DNA damage signals regulate steps in the activation process in addition to SWI/SNF recruitment, such as Rap1 recruitment and PIC formation, which are required for the remodeling of chromatin at *RNR3* (42). Interestingly, the data also suggest that complete disruption of nucleosome positioning is not required for the constitutive association of SWI/SNF with *RNR3*. Deleting the tail of H4 had a significantly lesser effect on nucleosome po-

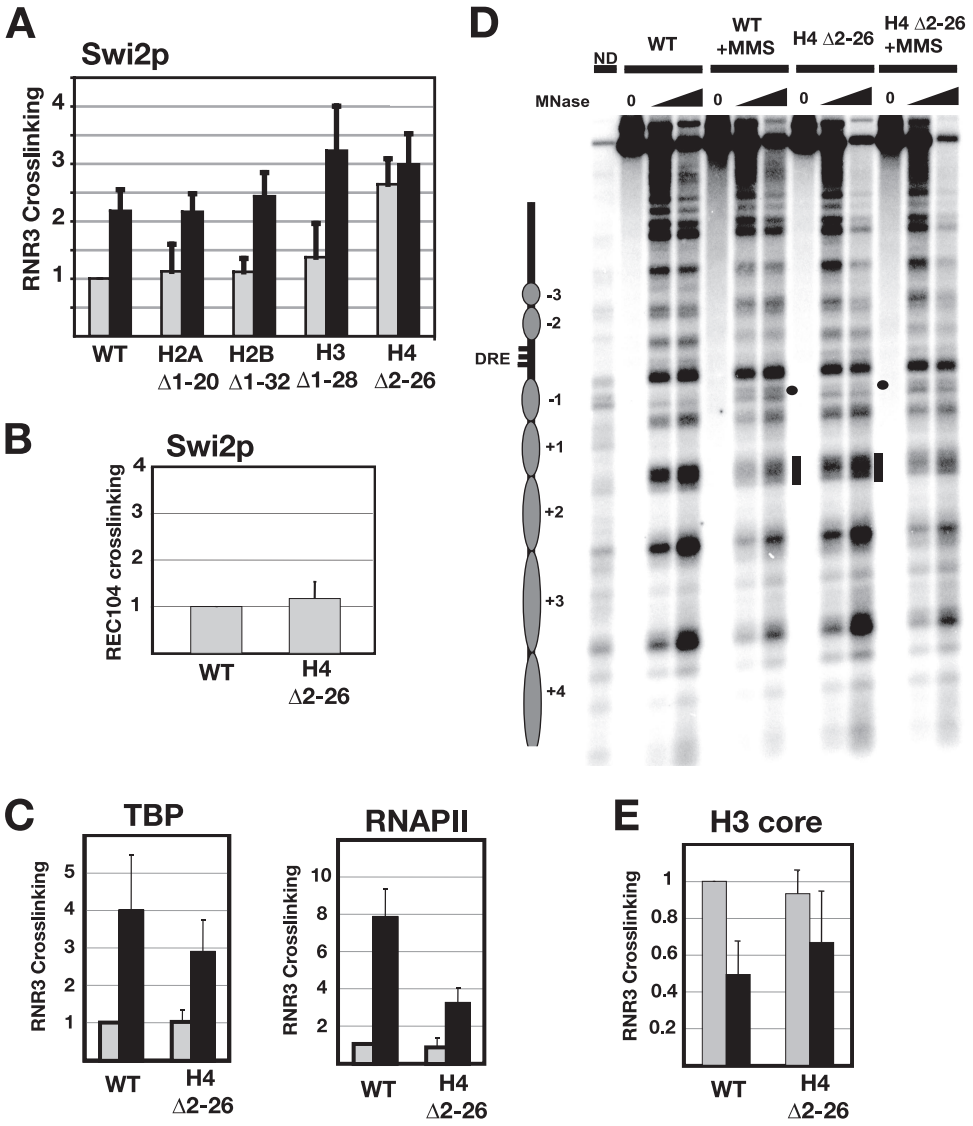


FIG. 6. N-terminal tails of H4 specifically are required for SWI/SNF exclusion. (A) Swi2p cross-linking to *RNR3* in histone tail mutants was examined. Assays were conducted as described for Fig. 3A. Gray bars and black bars show data from untreated and MMS-treated cells, respectively. WT, wild type. (B) Cross-linking of SWI/SNF to *REC104* in untreated cells. (C) As in panel A, except the cross-linking of TBP (left) and RNAPII (right) to *RNR3* was examined. (D) MNase mapping of nucleosome positioning in the histone H4 tail mutant. The circle marks the doublet that appears upon exposure of the TATA box to nuclease. The bar highlights the broadening of the hypersensitive site between nucleosomes +1 and +2 that occurs upon activation of the gene. (E) Cross-linking of histone H3 to the promoter of *RNR3* in the H4 tail mutant. ND, digested naked DNA; DRE, damage response element.

sitioning than deleting *ISW2*, but SWI/SNF recruitment levels were very similar under these conditions. Collectively, the results suggest that disrupting chromatin structure by deleting ISW2 or deleting the tail of H4 leads to the constitutive association of SWI/SNF with *RNR3*.

**Constitutive SWI/SNF recruitment is not dependent on Crt1 or Tup1.** Crt1 and Tup1 are the dominant regulators of *RNR3* (25). Although they were once thought to function only in repression, a growing body of evidence suggests that they have roles in activation as well (26). For example, Tup1 has been implicated in the recruitment of SWI/SNF to stress-induced genes (31), and we recently found that Crt1 plays a transient role in recruiting SWI/SNF to *RNR3* to open up the promoter and can physically interact with SWI/SNF in vitro (51). Although both Crt1 and Tup1 leave the *RNR3* promoter in activated cells, it is possible they are responsible for the constitutive recruitment of SWI/SNF in the *isw2*Δ or H4 tail mutant. First, we examined Tup1 cross-linking in the H4 tail mutant. Tup1 cross-linking is increased in the *isw2*Δ mutant (Fig. 3C), and if the increase in Tup1 association in these cells is responsible for SWI/SNF recruitment, it would be expected that Tup1 would likewise be elevated in the histone H4 mutant. This is not the case. The level of Tup1 cross-linking in this mutant is equivalent to that in wild-type cells, yet this mutant still displayed elevated SWI/SNF levels (Fig. 7A). Second, we examined the recruitment of SWI/SNF to *RNR3* in a Δ*tup1* mutant.

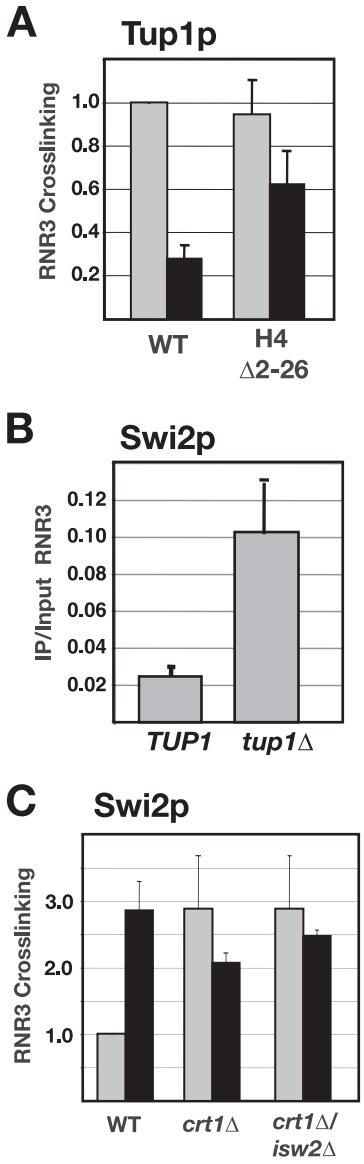


FIG. 7. Crt1 and Tup1 are not responsible for constitutive SWI/SNF recruitment to RNR3. (A) Tup1 cross-linking to RNR3 in the wild type (WT) and the histone H4 mutant. Assays were conducted as described for Fig. 3A. Gray bars and black bars show data from untreated and MMS-treated cells, respectively. (B) SWI/SNF recruitment in a *tup1*Δ mutant. Cells were not treated with MMS. IP, immunoprecipitation. (C) SWI/SNF recruitment in wild-type, *crt1*Δ, and *crt1*Δ *isw2*Δ cells.

Deleting *TUP1* causes chromatin remodeling and PIC formation (25, 52), although the status of SWI/SNF at the promoter is not known. Here, we found that deleting *TUP1* actually led to a very robust SWI/SNF recruitment in the absence of DNA damage signals (Fig. 7B). This result, together with the analysis of Tup1 recruitment in the H4 tail mutant, suggests that Tup1 is not responsible for recruiting SWI/SNF in the *isw2*Δ and histone H4 mutant and also provides another line of evidence that organizing *RNR3* into a repressive chromatin structure suppresses SWI/SNF recruitment.

Next, we examined the requirement for Crt1 in constitutive SWI/SNF recruitment. We examined the recruitment of SWI/SNF in a *crt1*Δ mutant and a *crt1*Δ *isw2*Δ double mutant. Similar to the phenotype caused by the deletion of *TUP1*, deleting *CRT1* caused constitutive SWI/SNF recruitment (Fig. 7C), as we noted previously (35). In addition, SWI/SNF recruitment was constitutively high in the *crt1*Δ *isw2*Δ double mutant (Fig. 7C). Since the SWI/SNF recruitment was high and constitutive in the absence of Crt1, the data indicate that Crt1 is not required for maintenance of SWI/SNF at the promoter under these conditions.

## DISCUSSION

Chromatin remodeling complexes associate with regions of the genome by targeted and untargeted mechanisms (15, 27, 29, 40). The targeted mechanism involves physical interactions with DNA binding proteins, which directs their action to specific loci in response to cellular signals. The untargeted mechanism, also referred to as the global mechanism, is not well defined. The concept of global association of chromatin remodeling complexes seems straightforward; however, it presents a number of challenges to regulating gene expression. At best, unregulated global association of these complexes on its own cannot provide specific regulation of the genome, and at its worst, this association has the potential to cause interference or short-circuiting of complex regulatory mechanisms. For instance, disrupting normal chromatin structure by genetic ablation of factors such as ISW2, Tup1, Spt6, or Rpd3(S) leads to cryptic transcripts and low specificity of binding of gene-specific regulators in vivo (4, 6, 17, 45). One mechanism for maintaining precise regulation is to counteract the constitutively or globally localized enzyme with an opposing activity whose localization can be regulated by physiological signals. The gain or loss of function of the opposing activity controls the outcome.

Here, we describe a novel mechanism of antagonism between two ATP-dependent remodeling complexes, ISW2 and SWI/SNF. The data strongly suggest that ISW2 suppresses SWI/SNF recruitment to *RNR3* by establishing a repressive chromatin structure, specifically, nucleosome positioning. The results obtained from reexpressing ISW2 from the *GAL1* promoter are particularly strong, as we correlated the timing of the appearance of Isw2 at *RNR3* with the reestablishment of nucleosome positioning and the exclusion of SWI/SNF from the promoter (Fig. 4 and 5). It is unlikely that ISW2 directly antagonizes SWI/SNF by physically excluding it from the promoter or removing it using its ATPase activity because three other genetic strategies used to disrupt nucleosome positioning led to the same phenotype. We have shown previously that ISW2 requires the Crt1-Ssn6-Tup1 complex to position nucleosomes in vivo (25, 53); therefore, it is predictable that we would observe constitutive SWI/SNF recruitment in *crt1*Δ and *tup1*Δ mutants (Fig. 7). While this result supports our hypothesis, there is the caveat that deleting these two repressors also results in constitutive PIC formation and transcription as well. Since SWI/SNF retention at *RNR3* requires components of the PIC, it is difficult to determine if SWI/SNF recruitment is caused by PIC formation or the disruption of chromatin structure (35). Here is the importance of the results obtained with the histone tail mutants. Mutating the H4 tail also increased

SWI/SNF recruitment yet does not result in PIC formation, transcription, or histone eviction. The H4 tail mutation phenocopies the *isw2*Δ mutation in a number of ways. The specificity of the phenotype for the H4 tail deletion mutant is very striking and provides further evidence that the exclusion of SWI/SNF is mediated through ISW2, as this tail is specifically required for the function of *ISW2* in vivo and for the remodeling of nucleosomes in vitro (7, 10). However, since deletion of the H4 tail does not disrupt nucleosome positioning to the same extent as deletion of ISW2 or the repressors of *RNR3*, we cannot rule out the possibility that the H4 tail plays roles in suppressing SWI/SNF recruitment in addition to regulating nucleosome positioning. But even under this alternative scenario, nucleosome positioning may involve the regulation of a higher-order chromatin structure and would be dependent upon ISW2 because the tail is intact in the *isw2*Δ mutant.

It should be noted, however, that disruption of nucleosome positioning is insufficient for targeting SWI/SNF to other *ISW2*-regulated genes, suggesting that the specificity of the constitutive SWI/SNF association may be attributable to a gene-specific transcription factor or a feature of the chromatin structure at *RNR3* and the consequences of the loss of *ISW2* on its organization. We provide solid evidence that neither Crt1 nor Tup1 is required for the constitutive recruitment of SWI/SNF. We cannot rule out that another sequence-specific DNA binding protein plays a role in constitutive SWI/SNF recruitment (20). However, even in this case, the binding of this factor would be prevented by the repressive chromatin structure established by ISW2. Arguing for the second scenario, there are clear differences in the mechanisms of action at and the recruitments of ISW2 to different loci throughout the genome. It has been proposed that ISW2 associates with chromatin by a direct targeting mechanism and by an untargeted mechanism (10). *REC104*, *iYGLWδ6*, and *POT1* utilize the targeted mechanism where ISW2 positions nucleosomes adjacent to the binding sites for gene regulatory proteins (11, 14, 18). As a consequence, deletion of *ISW2* causes the repositioning of nucleosomes to novel stable translational positions within the promoters of *REC104* and *POT1*. In contrast, ISW2 positions nucleosomes across the entire *RNR3* gene and is broadly associated with the gene (53), and an *isw2*Δ mutant shows a complete loss of positioning across the entire *RNR3* gene. Therefore, the mechanisms of action at these loci are quite different. Since ISW2 localization and nucleosome positioning occur across a large region (~3 kb) of *RNR3*, they may regulate long-range chromatin interactions at this locus. The suppression of SWI/SNF recruitment by ISW2 may involve forming higher-order chromatin interactions, which block the determinants of SWI/SNF recruitment. Consistent with this idea is the data showing that deletion of the H4 tail, specifically, leads to constitutive SWI/SNF recruitment. The basic patch on the histone H4 tail is required for the function of *ISW2* in vivo and for higher-order folding of nucleosomes in vitro (9, 10, 38).

Interestingly, we show that deleting *ISW2* increases the recruitment of SWI/SNF but not TBP, RNAPII, or Rap1 (Fig. 3). A key difference between these classes of transcription factors is that Rap1, TBP, and RNAPII need to make direct contact with DNA, which would be inhibited by the randomly positioned nucleosomes present over *RNR3* in the *isw2*Δ mutant. On the other hand, SWI/SNF has subunits with domains that can recognize features of chromatin exposed by unfolding of higher-order structures (3, 16). We can rule out acetylation specifically because deletion of *ISW2* actually results in a slight decrease in histone acetylation (reference 52 and data not shown), consistent with a high level of Tup1 at the promoter (Fig. 3). It has been suggested, although not proven, that ISW2 regulates higher-order structure in vivo. The possibility that the chromatin structure of *RNR3* is regulated beyond the nucleosomal level is suggested by a number of observations. First, Tup1-dependent nucleosome positioning extends well beyond its site of recruitment (53). This suggests long-range interactions across the gene. Second, as described above, ISW2 is required for the positioning of nucleosomes across the entire gene. Finally, SWI/SNF recruitment to the promoter disrupts chromatin across the whole open reading frame, although remodeling in the coding regions could be caused by transcription (35).

Another interesting observation from our work is that no individual histone tail is required for SWI/SNF recruitment under DNA damage conditions. This argues against a specific histone code for SWI/SNF recruitment in yeast (1) and is consistent with the ability of nucleosomes acetylated by either SAGA (H3/H2B) or NuA4 (H4/H2A) to retain SWI/SNF on templates in vitro (16). In fact, redundancy in the tails fits our model well because deletion of the H4 tails could open chromatin and the other tails in the nucleosome are available to retain SWI/SNF.

Our study describes a novel mechanism of antagonism between ATP-dependent chromatin remodeling complexes in vivo. Thus, similar to what is observed for histone-modifying enzymes, a dynamic chromatin structure depends on a balance of activities of opposing ATP-dependent chromatin remodeling factors. It is expected that this form of regulation applies to higher eukaryotes as well, as multiple, functionally distinct remodeling activities are found in organisms across the eukaryotic kingdom.

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## REFERENCES

1. **Agalioti, T., G. Chen, and D. Thanos.** 2002. Deciphering the transcriptional histone acetylation code for a human gene. Cell **111:**381–392.
2. **Berger, S. L.** 2007. The complex language of chromatin regulation during transcription. Nature **447:**407–412.
3. **Boyer, L. A., M. R. Langer, K. A. Crowley, S. Tan, J. M. Denu, and C. L. Peterson.** 2002. Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. Mol. Cell **10:**935–942.
4. **Buck, M. J., and J. D. Lieb.** 2006. A chromatin-mediated mechanism for specification of conditional transcription factor targets. Nat. Genet. **38:**1446–1451.
5. **Cairns, B. R., A. Schlichter, H. Erdjument-Bromage, P. Tempst, R. D. Kornberg, and F. Winston.** 1999. Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol. Cell **4:**715–723.
6. **Carrozza, M. J., B. Li, L. Florens, T. Suganuma, S. K. Swanson, K. K. Lee, W. J. Shia, S. Anderson, J. Yates, M. P. Washburn, and J. L. Workman.**

2005. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell **123:**581–592.
7. **Dang, W. W., M. N. Kagalwala, and B. Bartholomew.** 2006. Regulation of ISW2 by concerted action of histone H4 tail and extranucleosomal DNA. Mol. Cell. Biol. **26:**7388–7396.
8. **Dorigo, B., T. Schalch, K. Bystricky, and T. J. Richmond.** 2003. Chromatin fiber folding: requirement for the histone H4 N-terminal tail. J. Mol. Biol. **327:**85–96.
9. **Dorigo, B., T. Schalch, A. Kulangara, S. Duda, R. R. Schroeder, and T. J. Richmond.** 2004. Nucleosome arrays reveal the two-start organization of the chromatin fiber. Science **306:**1571–1573.
10. **Fazzio, T. G., M. E. Gelbart, and T. Tsukiyama.** 2005. Two distinct mechanisms of chromatin interaction by the Isw2 chromatin remodeling complex in vivo. Mol. Cell. Biol. **25:**9165–9174.
11. **Fazzio, T. G., and T. Tsukiyama.** 2003. Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. Mol. Cell **12:**1333–1340.
12. **Gangaraju, V. K., and B. Bartholomew.** 2007. Mechanisms of ATP dependent chromatin remodeling. Mutat. Res. **618:**3–17.
13. **Gelbart, M. E., N. Bachman, J. Delrow, J. D. Boeke, and T. Tsukiyama.** 2005. Genome-wide identification of Isw2 chromatin-remodeling targets by localization of a catalytically inactive mutant. Genes Dev. **19:**942–954.
14. **Goldmark, J. P., T. G. Fazzio, P. W. Estep, G. M. Church, and T. Tsukiyama.** 2000. The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. Cell **103:**423–433.
15. **Hassan, A. H., K. E. Neely, M. Vignali, J. C. Reese, and J. L. Workman.** 2001. Promoter targeting of chromatin-modifying complexes. Front. Biosci. **6:**D1054–D1064.
16. **Hassan, A. H., P. Prochasson, K. E. Neely, S. C. Galasinski, M. Chandy, M. J. Carrozza, and J. L. Workman.** 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell **111:**369–379.
17. **Kaplan, C. D., L. Laprade, and F. Winston.** 2003. Transcription elongation factors repress transcription initiation from cryptic sites. Science **301:**1096–1099.
18. **Kent, N. A., N. Karabetsou, P. K. Politis, and J. Mellor.** 2001. In vivo chromatin remodeling by yeast ISWI homologs Isw1p and Isw2p. Genes Dev. **15:**619–626.
19. **Kingston, R. E., and G. J. Narlikar.** 1999. ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev. **13:**2339–2352.
20. **Klinkenberg, L. G., T. Webb, and R. S. Zitomer.** 2006. Synergy among differentially regulated repressors of the ribonucleotide diphosphate reductase genes of *Saccharomyces cerevisiae*. Eukaryot. Cell **5:**1007–1017.
21. **Klose, R. J., and Y. Zhang.** 2007. Regulation of histone methylation by demethylimination and demethylation. Nat. Rev. Mol. Cell Biol. **8:**307–318.
22. **Kundu, S., P. J. Horn, and C. L. Peterson.** 2007. SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster. Genes Dev. **21:**997–1004.
23. **Kurdistani, S. K., and M. Grunstein.** 2003. Histone acetylation and deacetylation in yeast. Nat. Rev. Mol. Cell Biol. **4:**276–284.
24. **Langst, G., and P. B. Becker.** 2004. Nucleosome remodeling: one mechanism, many phenomena? Biochim. Biophys. Acta **1677:**58–63.
25. **Li, B., and J. C. Reese.** 2001. Ssn6-Tup1 regulates RNR3 by positioning nucleosomes and affecting the chromatin structure at the upstream repression sequence. J. Biol. Chem. **276:**33788–33797.
26. **Malave, T. M., and S. Y. Dent.** 2006. Transcriptional repression by Tup1-Ssn6. Biochem. Cell Biol. **84:**437–443.
27. **Martens, J. A., and F. Winston.** 2003. Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr. Opin. Genet. Dev. **13:**136–142.
28. **Mellor, J., and A. Morillon.** 2004. ISWI complexes in Saccharomyces cerevisiae. Biochim. Biophys. Acta **1677:**100–112.
29. **Narlikar, G. J., H. Y. Fan, and R. E. Kingston.** 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:**475–487.
30. **Petracek, M. E., and M. S. Longtine.** 2002. PCR-based engineering of yeast genome. Methods Enzymol. **350:**445–469.
31. **Proft, M., and K. Struhl.** 2002. Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. Mol. Cell **9:**1307–1317.
32. **Reese, J. C., and M. R. Green.** 2003. Functional analysis of TFIID components using conditional mutants. Methods Enzymol. **370:**415–430.
33. **Roth, S. Y., J. M. Denu, and C. D. Allis.** 2001. Histone acetyltransferases. Annu. Rev. Biochem. **70:**81–120.
34. **Shahbazian, M. D., and M. Grunstein.** 2007. Functions of site-specific histone acetylation and deacetylation. Annu. Rev. Biochem. **76:**75–100.
35. **Sharma, V. M., B. Li, and J. C. Reese.** 2003. SWI/SNF-dependent chromatin remodeling of RNR3 requires TAF(II)s and the general transcription machinery. Genes Dev. **17:**502–515.
36. **Sharma, V. M., R. S. Tomar, A. E. Dempsey, and J. C. Reese.** 2007. Histone deacetylases RPD3 and HOS2 regulate the transcriptional activation of DNA damage-inducible genes. Mol. Cell. Biol. **27:**3199–3210.
37. **Shi, Y.** 2007. Histone lysine demethylases: emerging roles in development, physiology and disease. Nat. Rev. Genet. **8:**829–833.
38. **Shogren-Knaak, M., H. Ishii, J. M. Sun, M. J. Pazin, J. R. Davie, and C. L. Peterson.** 2006. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science **311:**844–847.
39. **Smith, C. L., and C. L. Peterson.** 2005. ATP-dependent chromatin remodeling. Curr. Top. Dev. Biol. **65:**115–148.
40. **Taverna, S. D., H. Li, A. J. Ruthenburg, C. D. Allis, and D. J. Patel.** 2007. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat. Struct. Mol. Biol. **14:**1025–1040.
41. **Thomas, M. C., and C. M. Chiang.** 2006. The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. **41:**105–178.
42. **Tomar, R. S., S. Zheng, D. Brunke-Reese, H. N. Wolcott, and J. C. Reese.** 2008. Yeast Rap1 contributes to genomic integrity by activating DNA damage repair genes. EMBO J. **27:**1575–1584.
43. **Tran, H. G., D. J. Steger, V. R. Iyer, and A. D. Johnson.** 2000. The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. EMBO J. **19:**2323–2331.
44. **Vignali, M., A. H. Hassan, K. E. Neely, and J. L. Workman.** 2000. ATP-dependent chromatin-remodeling complexes. Mol. Cell. Biol. **20:**1899–1910.
45. **Whitehouse, I., O. J. Rando, J. Delrow, and T. Tsukiyama.** 2007. Chromatin remodelling at promoters suppresses antisense transcription. Nature **450:**1031–1035.
46. **Wysocka, J., C. D. Allis, and S. Coonrod.** 2006. Histone arginine methylation and its dynamic regulation. Front. Biosci. **11:**344–355.
47. **Yu, L., and R. H. Morse.** 1999. Chromatin opening and transactivator potentiation by RAP1 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:**5279–5288.
48. **Zhang, H. S., and J. C. Reese.** 2007. Exposing the core promoter is sufficient to activate transcription and alter coactivator requirement at RNR3. Proc. Natl. Acad. Sci. USA **104:**8833–8838.
49. **Zhang, Y.** 2003. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev. **17:**2733–2740.
50. **Zhang, Z., and J. C. Reese.** 2006. Isolation of yeast nuclei and micrococcal nuclease mapping of nucleosome positioning. Methods Mol. Biol. **313:**245–255.
51. **Zhang, Z., and J. C. Reese.** 2005. Molecular genetic analysis of the yeast repressor Rfx1/Crt1 reveals a novel two-step regulatory mechanism. Mol. Cell. Biol. **25:**7399–7411.
52. **Zhang, Z., and J. C. Reese.** 2004. Redundant mechanisms are used by Ssn6-Tup1 in repressing chromosomal gene transcription in Saccharomyces cerevisiae. J. Biol. Chem. **279:**39240–39250.
53. **Zhang, Z., and J. C. Reese.** 2004. Ssn6-Tup1 requires the ISW2 complex to position nucleosomes in Saccharomyces cerevisiae. EMBO J. **23:**2246–2257.