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The Swi/Snf Chromatin Remodeling Complex Is Required for Ribosomal DNA and Telomeric Silencing in *Saccharomyces cerevisiae*

Vardit Dror[1,2] and Fred Winston[1]*

Department of Genetics, Harvard Medical School, Boston, Massachusetts,[1] and Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel[2]

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The Swi/Snf chromatin remodeling complex has been previously demonstrated to be required for transcriptional activation and repression of a subset of genes in *Saccharomyces cerevisiae*. In this work we demonstrate that Swi/Snf is also required for repression of RNA polymerase II-dependent transcription in the ribosomal DNA (rDNA) locus (rDNA silencing). This repression appears to be independent of both Sir2 and Set1, two factors known to be required for rDNA silencing. In contrast to many other rDNA silencing mutants that have elevated levels of rDNA recombination, *snf2*Δ mutants have a significantly decreased level of rDNA recombination. Additional studies have demonstrated that Swi/Snf is also required for silencing of genes near telomeres while having no detectable effect on silencing of *HML* or *HMR*.

The *Saccharomyces cerevisiae* Swi/Snf complex is an ATP-dependent chromatin remodeling complex that can activate or repress transcription (see references 3, 34, and 44 for recent reviews). Swi/Snf contains 11 different subunits, including Snf2, a highly conserved ATPase. The Snf2 subunit is the catalytic core of Swi/Snf; single amino acid changes in the DNA-dependent ATPase domain eliminate both the ATPase activity and the chromatin remodeling activity of Swi/Snf (13, 53). While Swi/Snf binds to both DNA and nucleosomes, it does not do so in a site-specific manner (12, 52). Rather, sequence-specific transcriptional activators and repressors have been shown to target Swi/Snf to specific promoters (for examples, see references 16, 46–48, 51, and 72; see reference 23 for a review).

Gene expression microarray analysis has shown that the mRNA levels of a small subset of *S. cerevisiae* genes are significantly affected by the loss of Swi/Snf activity (25, 65). This apparent specificity of Swi/Snf control is likely caused by several factors, including its recruitment by particular transcriptional regulators. In addition, there is strong evidence that Swi/Snf is redundant with other transcription complexes in vivo and may therefore play a wider role than is indicated by microarray analysis (4, 50, 54, 64). A third factor is that Swi/Snf may be required only at promoters with a particular chromatin structure. Indeed, one study has suggested that different chromatin structures can determine the dependency upon Swi/Snf (9).

Since chromosomal context can influence chromatin structure, we wanted to test whether genomic position might affect the Swi/Snf dependence of a gene. To address this issue, we randomly integrated the *SUC2* gene, which is strongly Swi/Snf dependent (69), into the yeast genome to identify locations where *SUC2* expression becomes independent of Swi/Snf. Surprisingly, we discovered that when *SUC2* is integrated into the ribosomal DNA (rDNA) locus (*RDN1*, hereafter referred to as rDNA), its dependence on Swi/Snf is reversed. That is, when *SUC2* is located in the rDNA, *SUC2* transcription is repressed rather than activated by Swi/Snf.

The *S. cerevisiae* rDNA consists of a tandem array of 9.1-kb units repeated 100 to 200 times on chromosome XII (49) (Fig. 1). The rDNA is located in the nucleolus in an arrangement reminiscent of the heterochromatin of higher eukaryotes (reviewed in references 45 and 57). Each rDNA repeat unit includes the 5S rRNA gene, transcribed by RNA polymerase III, and a 35S precursor rRNA gene, transcribed by RNA polymerase I. About half of the tandemly repeated rRNA genes are transcriptionally active; the active rRNA gene copies are randomly distributed along the ribosomal rRNA gene locus (14). Unlike the results seen with rRNA genes, the expression of several different Pol II-transcribed genes, when integrated into various regions of the rDNA, is repressed (7, 18, 60). rDNA silencing also represses recombination, believed to play an important role in preventing rDNA loss (21).

Many *trans*-acting proteins required for rDNA silencing of Pol II transcription have been identified (6–8, 60, 62, 63, 66). These include Sir2, a member of a highly conserved family of NAD-dependent protein and histone deacetylases (30, 40, 61), and Set1, a histone methyltransferase (6, 8). The experiments presented in this paper identify another factor required for rDNA silencing, the Swi/Snf complex. Our results strongly suggest that Swi/Snf-mediated silencing occurs by a mechanism independent of Sir2 and Set1. Additional experiments show that Swi/Snf is also required for silencing at telomeres but not at silent-mating-type cassettes.

MATERIALS AND METHODS

Yeast strains, genetic methods, and plasmids. All *S. cerevisiae* strains used in this study (Table 1) are derivatives of a *GAL2*$^+$ S288C strain (70). Standard strain construction methods and medium recipes were as described previously (55). Deletion of *SUC2* was achieved by replacing the open reading frame with the PCR-amplified *KanMX4* gene from plasmid pRS400 (5). The *snf2*Δ::*LEU2*

* Corresponding author. Mailing address: Department of Genetics, Harvard Medical School, 77 Ave. Louis Pasteur, Boston, MA 02115. Phone: (617) 432-7768. Fax: (617) 432-6506. E-mail: winston@genetics.med.harvard.edu.

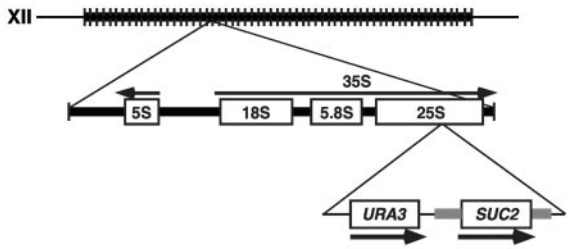

FIG. 1. Diagram of the *SUC2* insertion in the rDNA. The top line represents the rDNA array on the right arm of chromosome XII. A representative rDNA repeat, indicating the position of the integration of the *SUC2 URA3* cassette (see text), is shown below. The thick gray line flanking *SUC2* represents DNA that normally flanks the *SUC2* gene.

(10), *snf2-798* (K-to-A change of amino acid 798) (33), and *snf5Δ2* (64) alleles have been described previously. Strains used for assaying telomeric silencing were previously described (1). Strains with *mURA3-LEU2* integrated in the rDNA and at the *leu2Δ1* locus were generated by a cross to strains previously described, JS215-10 and JS210-1 (60). Plasmids were constructed and isolated from *Escherichia coli* by standard methods (2). Plasmid pVD1 is a derivative of plasmid pRS406 (59) that contains a BglII fragment with *SUC2* sequences from −1187 to +2076 with respect to the *SUC2* ATG.

Isolation of transformants with *SUC2* integrated at random locations and in the rDNA. To randomly integrate the plasmid pVD1 into the yeast genome, the restriction enzyme-mediated transformation method (43, 58) was used. The plasmid pVD1 was linearized with the restriction enzyme SacI, and 10 μg of the plasmid was used to transform the strain FY2310 in the presence of 100 units of the restriction enzyme BglII. Strain FY2310 contains no homology to pVD1 and also contains the *snf5-51* mutation, a temperature-sensitive mutation in *SNF5*, which encodes a component of Swi/Snf. Transformants were selected on synthetic complete (SC) plates lacking uracil. To determine the site of integration of plasmid pVD1, genomic DNA was extracted and digested with BamHI, which digests only once in pVD1, and the DNA was self-ligated under dilute conditions. This DNA was then used to transform *E. coli* strain DH5α. The resulting plasmids, which contained genomic DNA flanking the site of plasmid integration, were then isolated from the bacteria and sequenced. To directly integrate a *URA3-SUC2* cassette into the same position within the rDNA, *URA3* and *SUC2* from plasmid pVD1 were amplified by PCR, using the primers F (5′ GGC TTG GCA GAA TCA GCG GGG AAA GAA GAC CCT GTT GAG GAT GCC GGG AGC AGA CAA GC 3′) and R (5′ ACA CCC TCT ATG TCT CTT CAC AAT GTC AAA CTA GAG TCA CAA AAG CTG GAG CTC CAC CG 3′). This 5.2-kb *URA3-SUC2* PCR fragment was then used to transform strain FY2310 to Ura$^+$.

Northern hybridization analysis. For measurement of *SUC2* mRNA levels, strains were grown in yeast extract-peptone-dextrose (YPD) to approximately 10^7 cells/ml, washed in water, resuspended in either YPD (repressed sample) or yeast extract-peptone (YEP) plus 0.05% glucose (derepressed sample), and grown for 2 h 45 min. For measurement of *URA3* mRNA levels to assay telomeric silencing, strains were grown in SC medium containing 100 mg of uracil/liter (1). RNA was prepared and analyzed as described previously (67). The *SUC2* probe was synthesized by PCR amplification of 603 bp of plasmid pRB58 (11) corresponding to positions +949 to +1552 of the *SUC2* open reading frame.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype
FY49	*MAT***a** *ura3-52 lys2-128Δ snf1Δ*
FY78	*MAT***a** *his3Δ200*
FY328	*MATα his3Δ200 his4-917δ lys2-173R2 snf2Δ1::HIS3*
FY1658	*MAT***a** *his3Δ200 ura3-52 lys2-128δ snf5Δ2*
FY1856	*MATα his3Δ200 leu2Δ0 lys2-128Δ ura3Δ0*
FY2084	*MAT***a** *ura3Δ0 snf2-798*
FY2310	*MAT***a** *his3Δ200 ura3Δ0 lys2-128δ suc2Δ::KanMX4 snf5-51*
FY2311	*MATα lys2-128δ leu2Δ0 his3Δ200 ura3Δ0 suc2Δ::KanMX4*
FY2312	*MATα lys2-128δ leu2Δ0 his3Δ200 ura3Δ0 suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2313	*MATα lys2-128δ leu2Δ0 his3Δ200 ura3Δ0 suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2314	*MATα ura3Δ0 lys2-128δ leu2Δ0 snf1Δ suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2315	*MAT***a** *ura3-52 arg4-12 leu2Δ0 snf2Δ::LEU2 suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2316	*MAT***a** *ura3Δ0 lys2-128δ leu2Δ1 snf2Δ::LEU2 suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2317	*MATα ura3Δ0 lys2-128δ leu2Δ0 trp1Δ63 snf2Δ::LEU2 suc2Δ::KanMX4 rDNA::URA3-SUC2*
FY2318	*MAT***a** *leu2Δ0 his3Δ0 ura3-52 his4-912δ lys2-128δ suc2Δ::KanMX4 set1Δ::KanMX4 rDNA::URA3-SUC2*
FY2319	*MAT***a** *leu2Δ0 arg4-12 ura3-52 lys2-128δ snf2Δ::LEU2 suc2Δ::KanMX4 set1Δ::KanMX4 rDNA::URA3-SUC2*
FY2320	*MATα leu2Δ0 ura3Δ0 his3Δ200 lys2-128δ suc2Δ::KanMX4 met15::URA3-SUC2*
L1075	*MAT***a** *leu2Δ0 his3Δ0 orΔ200 ura3Δ0 lys2-128δ suc2Δ::KanMX4 sir2Δ::KanMX4 rDNA::URA3-SUC2*
FY2321	*MAT***a** *leu2Δ0 ura3Δ0 his3Δ200 snf2-798 suc2Δ::KanMX4 rDNA::URA3-SUC2*
L1076	*MAT? his3Δ0 leu2Δ0 ura3-52 suc2Δ::KanMX4 sir2Δ::KanMX4 snf2Δ::LEU2 rDNA::URA3-SUC2* (mating type not known due to *sir2* mutation)
L1077	*MATα arg4-12 his3Δ0 leu2Δ0 ura3-52 suc2Δ::KanMX4 sir2Δ::KanMX4 snf2Δ::LEU2 rDNA::URA3-SUC2*
L1078	*MATα ura3-52 leu2Δ0 snf2Δ::LEU2 suc2Δ::KanMX4 met15::URA3-SUC2*
L1079	*MATα leu2Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 snf2Δ::LEU2 rDNA::mURA3-LEU2*
L1080	*MATα leu2Δ1Δ0 ura3Δ0 his3Δ200 lys2Δ0 snf2Δ::LEU2 rDNA::mURA3-LEU2*
L1081	*MATα leu2Δ0 ura3-52 his3Δ200 lys2Δ0 met15Δ0 trp1Δ63 rDNA::mURA3-LEU2*
L1082	*MAT***a** *leu2Δ1 ura3Δ0 his3Δ200 lys2Δ0 met15Δ0 trp1Δ63 leu2Δ1::mURA3-LEU2*
L1083	*MATα leu2Δ0 ura3Δ0 his3Δ200 met15Δ0 trp1Δ63 leu2Δ1::mURA3-LEU2*
L1084	*MAT***a** *leu2Δ0 ura3Δ0 his3Δ200 rDNA::mURA3-LEU2*
L1085	*MAT***a** *leu2Δ1 ura3Δ0 his3Δ200 met15Δ0 lys2Δ0 snf2Δ::LEU2 leu2Δ1::mURA3-LEU2*
L1086	*MATα leu2Δ1 ura3Δ0 his3Δ200 snf2Δ::LEU2 leu2Δ1::mURA3-LEU2*
L1087	*MATα his3 ura3 leu2Δ1 TEL-VR::URA3*
L1088	*MAT***a** *ura3Δ0 leu2Δ0 TEL-VR::URA3*
L1089	*MAT***a** *ura3-52 leu2Δ0 snf2Δ::LEU2 TEL-VR::URA3*
L1090	*MATα his3 ura3Δ0 leu2Δ1 snf2Δ::LEU2 TEL-VR::URA3*
L1091	*MAT***a** *his3 ura3Δ0 leu2Δ1 met15Δ0 sir2Δ::KanMX4 TEL-VR::URA3*
L1092	*MAT***a** *his3 ura3-52 leu2Δ0 met15Δ0 sir2Δ::KanMX4 TEL-VR::URA3*

The *ACT1* probe was synthesized by PCR amplification of 190 bases from +532 to +722 of the *ACT1* open reading frame. The *URA3* probe was synthesized by PCR amplification of 474 bases extended from +206 to +680. The α1 probe was synthesized by PCR amplification of 328 bases extended from +40 to +369. All probes were radiolabeled with [α-^{32}P]dATP by random priming (2). Quantitation of relative levels of mRNA was performed by using a PhosphorImager (Molecular Dynamics).

Analysis of chromatin structure by MNase. *S. cerevisiae* strains were grown in YPD medium to 10^7 cells/ml and then shifted to derepressing conditions as described for Northern blot analyses. Spheroplasts were isolated and subjected to micrococcal nuclease (MNase) digestion as adapted from previously described methods (31, 32). Approximately 1.2×10^9 cells were incubated with 2 mg of Zymolyase (ICN)/ml (100,000 units/g) for 2 min. Spheroplasts from 2×10^8 cells were aliquoted and digested with 0, 0.625, 1.25, 2.5, or 5 units of MNase at 37°C for 4 min. Purified genomic DNA from an equivalent amount of cells was digested using either 0.5 or 0.75 units of MNase at 37°C for 1 min to serve as naked DNA controls. The DNA from the MNase-treated chromatin samples was purified and then digested completely with HinfI, separated on a 1% agarose gel, and analyzed by indirect end labeling (24). A 156-bp PCR product corresponding to base pairs +140 to +296 (+1 = ATG) of the *SUC2* open reading frame was synthesized by PCR, radiolabeled by random priming (2), and used as the probe to detect *SUC2* DNA. A 1-kb DNA ladder was used as a size standard to calculate positions of MNase cleavage.

ChIP. The procedure for chromatin immunoprecipitation (ChIP) was adapted from previously described methods (17, 39). Briefly, cells from 200-ml YPD cultures were cross-linked by adding formaldehyde to achieve a final concentration of 1%. Chromatin was prepared in fluorescent-antibody lysis buffer containing 140 mM NaCl and no sodium dodecyl sulfate. Cross-linked chromatin was sonicated to an average length of 500 bp, with a size range from 200 to 1,200 bp. Sir2 was immunoprecipitated from 1/10 of the cross-linked chromatin by a two-step method (22) using rabbit polyclonal anti-Sir2 antibody (26) followed by immunoglobulin G-Sepharose beads (Pharmacia). Dilutions of input DNA (1/200 and 1/400) and immunoprecipitated DNA (1/10 and 1/20) were subjected to quantitative radioactive PCR as described previously (41), and the products were separated on a 7.5% nondenaturing polyacrylamide gel. Primers of 20-nucleotide oligonucleotides for amplification of products of 250 bp for the rDNA were as previously described (28). Specific binding of Sir2 to DNA amplified by each primer set was evaluated by calculating the ratio of the percentage of immunoprecipitation (IP) of the primer set to the percentage of IP of a control region of the genome (36). The control region used amplifies bp 9716 to 9863 of chromosome V, a region devoid of transcription by RNA polymerase II (36). Levels of H3 K4 methylation were measured as previously described (8) by the use of the same control region used for the Sir2 ChIP experiments.

Spot tests to assay expression of *mURA3-LEU2*. *SNF2* and *snf2*Δ strains, containing the *mURA3-LEU2* marker in the rDNA or at the *leu2Δ1* locus, were grown in 10 ml of YPD cultures to saturation at 30°C. Tenfold serial dilutions of each culture were made in sterile water, and 5 μl of each dilution was spotted onto YPD and 5-FOA solid medium. Plates were photographed after 2 days of incubation at 30°C.

Mitotic stability of the *URA3* gene in the rDNA. The mitotic stability of the *URA3* gene was assayed as described previously (7). Briefly, single Ura$^+$ colonies were inoculated into 10 ml of YPD medium and grown overnight at 30°C. Cultures were diluted 1:10,000 in fresh YPD and grown to saturation. Appropriate dilutions of the ninth serial culture were spread on YPD solid medium to obtain 50 to 200 cells per plate. After growth, colonies were counted and the plates were replica plated to SC medium lacking uracil. Recombination frequencies were calculated by counting the number of colonies that failed to grow on medium lacking uracil and dividing that number by the total number of colonies that grew on YPD. This procedure was performed three times for each strain.

RESULTS

Integration of *SUC2* at random locations in the *S. cerevisiae* genome. Our studies began with the goal of testing whether the genomic position of a gene might affect its control by the Swi/Snf chromatin remodeling complex. To do this, we integrated the Swi/Snf-dependent gene *SUC2* at several random locations in the *S. cerevisiae* genome and then tested whether its expression was still dependent upon Swi/Snf. Random integrants were obtained under conditions in which there is no homology between the transforming DNA and the genome (58) (see Materials and Methods). Briefly, a linearized plasmid (pVD1) containing *SUC2* and *URA3* was used to transform *S. cerevisiae* strain FY2310 (Table 1), which contains a temperature-sensitive allele of *SNF5* (*snf5-51*) (20) and lacks the complete *SUC2* and *URA3* genes. We selected for Ura$^+$ transformants and then screened them for the dependence of *SUC2* expression on Swi/Snf at both permissive (30°C) and nonpermissive (37°C) temperatures for *snf5-51*. This was done by screening the transformants for growth on YEP raffinose medium, which is dependent upon *SUC2* expression.

Of 18 Ura$^+$ transformants, we identified 3 candidates that were Raf$^+$ at 37°C. For each of the three candidates, the site of integration was determined as described in Materials and Methods. For the first two cases, the apparent Swi/Snf independence was likely due to multiple copies of *SUC2* DNA. In one, the plasmid had integrated into the 2μm circle plasmid; in the other, several tandem copies had integrated into the *NUM1* gene, which contains tandem repeats (37). In the third transformant, however, a single copy of *SUC2* DNA had integrated into the 35S region of rDNA. As this was our only Raf$^+$ isolate in which *SUC2* was present in single copy, we focused our studies on expression of *SUC2* in this genomic position. To retest the phenotype and to simplify our subsequent analyses, we first constructed a new *URA3-SUC2* cassette with fewer plasmid sequences and integrated it into the same position within the 35S of the rDNA repeat as was identified for the original isolate (Fig. 1) (see Materials and Methods). By pulsed-field gel electrophoresis and Southern blot analyses, we verified that seven of eight *URA3-SUC2* cassette transformants were present in single copy in the rDNA. Three of these were mapped to different repeats within the array; however, all behaved similarly with respect to *SUC2* transcription in wild-type and *snf2*Δ mutants (described below and data not shown). Two of these transformants were used for the remainder of the experiments described in later sections.

***SUC2* transcription is repressed by the Swi/Snf complex when *SUC2* is in the rDNA.** Our initial characterization had suggested that when *SUC2* is located in the rDNA its expression is Swi/Snf independent. To characterize the effect of Swi/Snf on expression of *SUC2* in the rDNA in greater detail, we measured *SUC2* mRNA levels in a wild-type (*SNF2*) strain and in two different *snf2* mutants, *snf2*Δ and *snf2-798*. The *snf2-798* mutation encodes a K798A amino acid change, which impairs the Snf2 ATPase activity (33), which is critical for Swi/Snf chromatin remodeling activity. This analysis revealed two aspects of *SUC2* regulation in the rDNA. First, in a wild-type genetic background, when cells are grown under conditions derepressing for *SUC2* transcription, *SUC2* mRNA levels are significantly reduced when *SUC2* is in the rDNA compared to when *SUC2* is at its natural location (Fig. 2; compare lanes 2 and 7). This result is consistent with previous studies demonstrating silencing of RNA polymerase II-dependent transcription in the rDNA (45, 57). Second, in both *snf2* mutants tested, the rDNA silencing of *SUC2* is abolished, as *SUC2* mRNA is present at a high level (Fig. 2; compare lane 7 to lanes 9 and 10). The finding that the *snf2-798* mutation causes a silencing defect strongly suggests that the Swi/Snf remodeling activity is required for rDNA silencing of *SUC2*. A similar derepression was observed in a *snf5*Δ mutant (data not shown). In contrast,

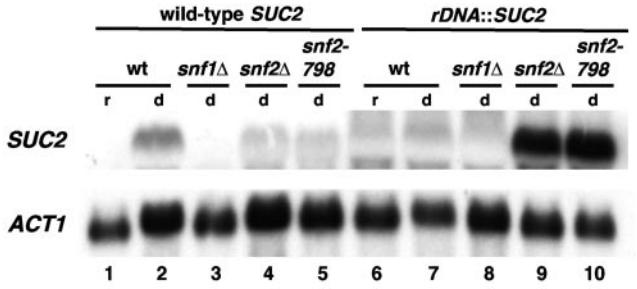

FIG. 2. Swi/Snf represses transcription of *SUC2* in the rDNA. Northern analysis of *SUC2* mRNA levels was performed on strains that contain *SUC2* in its normal genomic location (lanes 1 to 5) or in the rDNA (lanes 6 to 10). Strains were grown in YPD (repressing conditions; lanes r) and then shifted to YEP plus 0.05% glucose (derepressing conditions; lanes d) for 2 h 45 min. *ACT1* served as a loading control. The strains analyzed are as follows: lanes 1 and 2, FY78; lane 3, FY49; lane 4, FY328; lane 5, FY2084; lanes 6 and 7, FY2313; lane 8, FY2314; lane 9, FY2316; and lane 10, FY2321. wt, wild type.

Snf1, a protein kinase that activates *SUC2* transcription independently from Swi/Snf (68), does not play any role in rDNA silencing of *SUC2* (Fig. 2, lane 8). Taken together, these data suggest that Swi/Snf represses *SUC2* transcription in the rDNA, the opposite of its role in activation of *SUC2* at its natural location.

Analysis of the chromatin structure of the *SUC2* promoter in rDNA compared to its normal position. Previous studies showed that under derepressing conditions, a *snf2*Δ mutation causes changes in chromatin structure over the *SUC2* promoter. These studies demonstrated that in wild-type strains, the *SUC2* promoter region is generally sensitive to digestion by MNase. However, in *snf2*Δ mutants, MNase digestion is more inhibited in particular regions of the promoter, strongly suggesting the presence of nucleosomes over the TATA and the region between the TATA and upstream activation sequence (UAS) (19, 24, 71) (Fig. 3). To determine whether *snf2*Δ also causes changes in *SUC2* chromatin structure when *SUC2* is in the rDNA, we performed indirect end-labeling analysis of MNase-digested chromatin for cells grown under conditions derepressing for *SUC2* transcription (Materials and Methods). In contrast to what was found for *SUC2* at its natural location, our results revealed that a *snf2*Δ mutation causes little if any detectable effect on *SUC2* chromatin structure in the rDNA. In this location, the *SUC2* MNase cleavage pattern is the same in both wild-type and *snf2*Δ backgrounds, with an MNase cleavage pattern for both strains similar to the active, wild-type form at the normal *SUC2* location (Fig. 3). In particular, in both strains MNase cleavage occurs over the TATA and the region between the TATA and the UAS. These results suggest that Swi/Snf is not required to maintain *SUC2* in an active chromatin structure when it is in the rDNA; however, this active structure is not sufficient to allow expression in the presence of wild-type Swi/Snf (see Discussion).

Swi/Snf is a general repressor in rDNA. Since Swi/Snf silences the transcription of *SUC2* in the rDNA, we asked whether Swi/Snf has a general role in rDNA silencing. To do

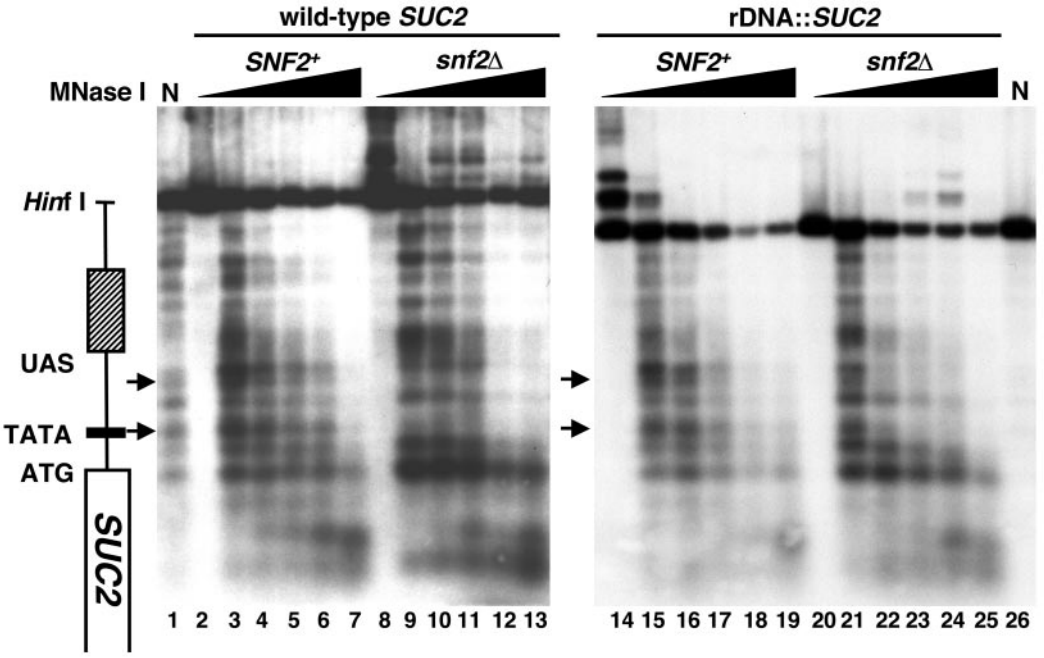

FIG. 3. MNase analysis of *SUC2* chromatin structure. Strains were grown in YPD medium to 10^7 cells/ml and then shifted to derepressing conditions as described in Materials and Methods. Spheroplasts were isolated and then incubated with increasing amounts of MNase as described in Materials and Methods. DNA was purified, digested with HinfI, and subjected to indirect end-labeling analysis using a probe that anneals to +140 to +296 (+1 = ATG) in the coding sequence of *SUC2*. The *SUC2* genomic region is diagramed on the left. The positions of two prominent sites that differ in levels of MNase sensitivity for *SUC2* in its wild-type location are marked with arrows. N denotes the naked DNA controls (lanes 1 and 26). The strains used were FY78 (*SNF2*; lanes 2 to 7), FY328 (*snf2*Δ; lanes 8 to 13), FY2313 (*SNF2* rDNA::*SUC2*; lanes 14 to 19), and FY2316 (*snf2*Δ rDNA::*SUC2*; lanes 20 to 25).

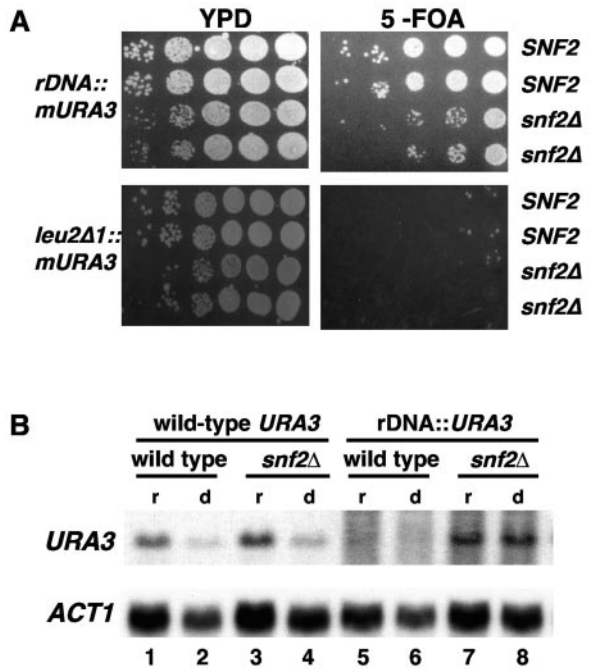

FIG. 4. Swi/Snf silences *mURA3* and *URA3* in the rDNA. (A) Swi/Snf silences expression of *mURA3* in the rDNA. Tenfold serial dilutions of stationary-phase cultures of *SNF2* (L1081 and L1084) or *snf2*Δ (L1079) strains containing the *mURA3-LEU2* cassette at the rDNA and *SNF2* (L1082 and L1083) or *snf2*Δ (L1085 and L1086) containing the *mURA3-LEU2* cassette at *leu2Δ1* were spotted onto 5-FOA and YPD media to monitor expression of *mURA3*. Loss of silencing is indicated by less growth on 5-FOA. (B) Swi/Snf represses the transcription of *URA3* in the rDNA. *URA3* mRNA levels were measured in four strains by Northern hybridization analysis. For *URA3* at its natural location, FY78 (wild type; lanes 1 and 2) and FY328 (*snf2*Δ; lanes 3 and 4) were used. For *URA3* in the rDNA, FY2313 (wild type; lanes 5 and 6) and FY2316 (*snf2*Δ; lanes 7 and 8) were used. Strains were grown under conditions both repressing (YPD) and depressing (shifted to 0.05% glucose) for *SUC2* transcription (see Materials and Methods). In low glucose, URA3 at its natural location is transcribed at a low level whereas URA3 in the rDNA is not significantly affected. *ACT1* served as a loading control.

this, we examined the expression of two forms of *URA3*, a gene not normally regulated by Swi/Snf. First, we used a previously established rDNA-silencing assay that measures expression of a modified *URA3* gene, *mURA3*, by spot tests on solid media (60). In this case, *URA3* expression is under control of a minimal *TRP1* promoter (60). We found that in *SNF2* strains, as expected, expression of the *mURA3* gene in the rDNA is reduced relative to its expression when integrated at *leu2Δ1*, reflecting transcriptional silencing in rDNA (Fig. 4A). In contrast, in *snf2*Δ strains, expression of *mURA3* in the rDNA is significantly greater than in the *SNF2* strains. In addition, we measured mRNA levels for the wild-type *URA3* gene present on the cassette that contains *SUC2* (Fig. 4B). In this construct, the *URA3* promoter is 1.9 kb from the *SUC2* promoter (Fig. 1) and therefore is unlikely to be regulated the same as *SUC2*. When *URA3* is in its natural location, there is no significant difference in the *URA3* mRNA levels between *SNF2* and *snf2*Δ strains. In contrast, when *URA3* is located in the rDNA, it is strongly silenced in *SNF2* strains and has a significantly increased mRNA level in *snf2*Δ mutants. These experiments provide strong evidence that Swi/Snf silences *URA3* specifically when it is located in the rDNA, suggesting that Swi/Snf is generally required for rDNA silencing of RNA polymerase II-transcribed genes.

Analysis of the effect of *snf2*Δ, *sir2*Δ, and *set1*Δ mutations on rDNA silencing of *SUC2*. Several other factors have been previously shown to be required for rDNA silencing (45, 57). The factor most extensively characterized and that is known to function directly in rDNA silencing is Sir2, a histone deacetylase (45, 57). To determine whether the Swi/Snf complex affects rDNA silencing indirectly by affecting other genes known to be required for rDNA silencing, we compared rDNA silencing of *SUC2* between *snf2*Δ and two other previously characterized silencing mutants, *sir2*Δ and *set1*Δ (7, 8, 60). We found that while each mutation caused increased *SUC2* mRNA levels, the *snf2*Δ mutation caused a significantly greater increase (Fig. 5; compare lane 4 to lanes 6 and 10). In *snf2*Δ *sir2*Δ and *snf2*Δ *set1*Δ double mutants, the *SUC2* mRNA levels are similar to those of the *snf2*Δ single mutant (Fig. 5; compare lane 4 to lanes 8 and 12). The greater defect in the *snf2*Δ mutant strongly suggests that at least a component of the control of silencing by Swi/Snf is independent of Sir2 and Set1.

We also used ChIP to test whether loss of Swi/Snf affects Sir2- or Set1-mediated silencing. First, we found that Sir2 is still associated with the rDNA repeat in *snf2*Δ mutants, although the distribution of Sir2 along the rDNA repeat in *snf2*Δ mutants is modestly different from that seen with wild-type strains (Fig. 6A) (28). This small change seems unlikely to

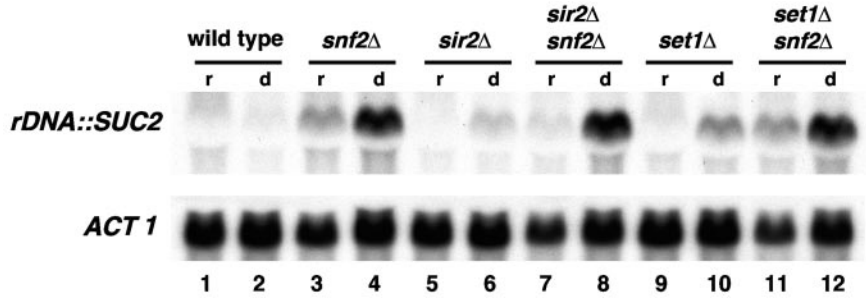

FIG. 5. Analysis of the effects of *sir2*Δ and *set1*Δ mutations on rDNA silencing of *SUC2*. Northern analysis of *SUC2* when integrated in the rDNA. Strains were grown in YPD (repressing conditions; lanes r) and then shifted to YEP plus 0.05% glucose (derepressing conditions; lanes d) for 2 h and 45 min. *ACT1* serves as a loading control. The strains analyzed are as follows: *SNF2* (FY2312), *snf2*Δ (FY2315), *sir2*Δ (L1075), *sir2*Δ *snf2*Δ (L1076), *set1*Δ (FY2318), and *set1*Δ *snf2*Δ (FY2319).

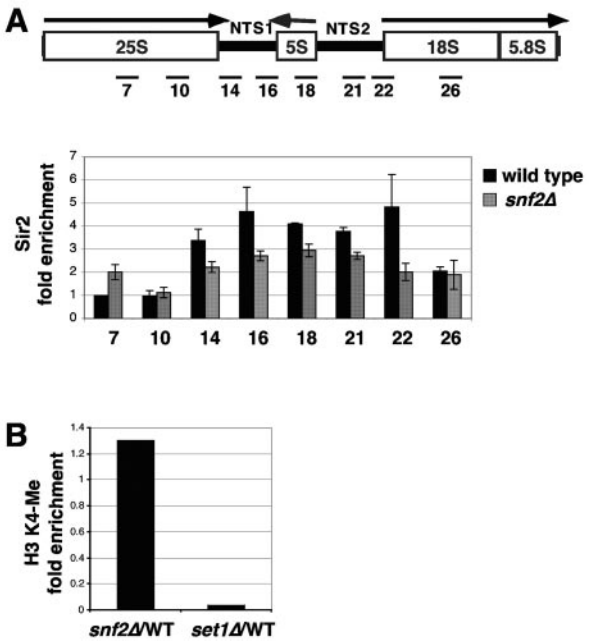

FIG. 6. Analysis of Sir2 and histone H3 methylation in *snf2*Δ mutants. (A) ChIP analysis of Sir2 in the rDNA. ChIP was performed across the rDNA repeat in both wild-type (FY2313) and *snf2*Δ (FY2316) strains. The positions of the regions analyzed by PCR in the ChIP experiments are shown at the top. The comparison between the wild-type and *snf2*Δ strains, normalized to an untranscribed region of the genome, is shown below (36). The PCR products correspond to those previously used (28). (B) ChIP analysis of histone H3 K4 methylation in wild-type and *snf2*Δ strains. ChIP was performed in three strains: the wild type (FY2313), *snf2*Δ (FY2316), and *set1*Δ (FY2318). Comparisons of *snf2*Δ and *set1*Δ to the wild type are shown.

account for the loss of silencing of RNA polymerase II-transcribed genes in the rDNA in *snf2*Δ mutants. This is particularly true for *SUC2*, as it is integrated in a position in the rDNA repeat that normally has very low levels of Sir2 (Fig. 6A) (28). We also examined the levels of histone H3 K4 methylation in wild-type and *snf2*Δ strains and found that they are the same (Fig. 6B). These results suggest that Swi/Snf controls rDNA silencing independently of Sir2 and Set1.

rDNA recombination is reduced in *snf2*Δ strains. Previous studies have shown that most mutations that impair rDNA silencing elevate the rate of mitotic recombination at the rDNA. This relationship has been demonstrated for mutations in *SIR2*, *TOP1*, *UBC2*, and *ZDS2* (7, 21, 56). The correlation is not perfect, however, as mutations in *SET1* impair rDNA silencing and yet have no effect on rDNA recombination (8), and mutations in *FOB1* also impair rDNA silencing and decrease rDNA recombination (15, 28, 35). To determine whether *snf2*Δ causes an effect on rDNA recombination, we compared rDNA mitotic recombination levels in wild-type, *snf2*Δ, and *sir2*Δ strains (see Materials and Methods). Surprisingly, in a *snf2*Δ mutant there is dramatic reduction in the rate of rDNA mitotic recombination, approximately 50-fold below that of the wild type (Table 2). In a *sir2*Δ mutant, as expected, the rate was elevated compared to that of the wild type. To test the epistatic relationship between *snf2*Δ and *sir2*Δ with respect to rDNA recombination, we also tested *snf2*Δ *sir2*Δ double mutants. Our results (Table 2) show that the *snf2*Δ *sir2*Δ double mutant still has a recombination rate below that of the wild type although greater than that of the *snf2*Δ single mutant. These results are consistent with the conclusion that the role of the Swi/Snf complex in rDNA silencing is distinct from that of Sir2 and other rDNA silencing factors previously identified.

Snf2 is also required for silencing at telomeres but not at *HM* loci. We also tested whether Swi/Snf is required for silencing at the two other known silenced regions in *S. cerevisiae*, telomeres and *HM* loci. To detect whether Swi/Snf has a role in telomeric silencing, we first performed spot tests using strains that have *URA3* near the right telomere of chromosome V. The results (Fig. 7A) show that a *snf2*Δ mutation causes increased expression of the *URA3* reporter compared to a wild-type background. To determine whether the increased *URA3* expression is caused at the transcriptional level, we performed Northern hybridization analysis. Our results show that the level of *URA3* mRNA is modestly but significantly increased in the *snf2*Δ mutants compared to the wild-type strain results (Fig. 7B). Thus, Swi/Snf is required for telomeric silencing. To determine whether Swi/Snf has a role in the silencing of the *HM* loci, we performed Northern hybridization analysis to assay for α1 mRNA expressed from *HML*α in a *MAT***a** strain. Our results (Fig. 7C) show that there is no detectable α1 mRNA in the *swi*/*snf* mutants tested, suggesting that the Swi/Snf complex is not required for silencing of the *HM* loci.

DISCUSSION

Our results have demonstrated that the Swi/Snf complex, previously shown to be required for the normal activation and repression of many genes in *S. cerevisiae*, also regulates transcriptional silencing in the rDNA and at telomeres. Our results provide strong evidence that the Swi/Snf-dependent mechanism acts independently of the histone-modifying enzymes Sir2 and Set1. First, *snf2*Δ causes a significantly greater defect in the rDNA silencing of *SUC2* than either *sir2*Δ or *set1*Δ. Second, in

TABLE 2. Deletion of *SNF2* reduces mitotic recombination in the rDNA

Relevant genotype[a]	No. of Ura auxotrophs/total no. of cells analyzed	No. of *URA3* markers lost/generation[b]	Mutant loss rate/ wild-type loss rate
Wild type	144/4132 (0.035%)	2.9×10^{-4}	
*sir2*Δ	2288/5261 (0.43%)	3.6×10^{-3}	12.4
*snf2*Δ	4/6412 (6.2×10^{-4})	5.1×10^{-6}	0.02
*snf2*Δ *sir2*Δ	21/3099 (6.8×10^{-3})	5.6×10^{-5}	0.19

[a] The strains used for these experiments were FY2313, wild type; L1075, *sir2*Δ; FY2316, *snf2*Δ; and L1076 and L1077, *snf2*Δ *sir2*Δ.
[b] The rate of mitotic recombination was determined by measuring the rate of loss of the *URA3* marker (number of Ura$^-$ auxotrophs/total number of cells analyzed) after 120 generations of growth in nonselective medium (as described in Materials and Methods).

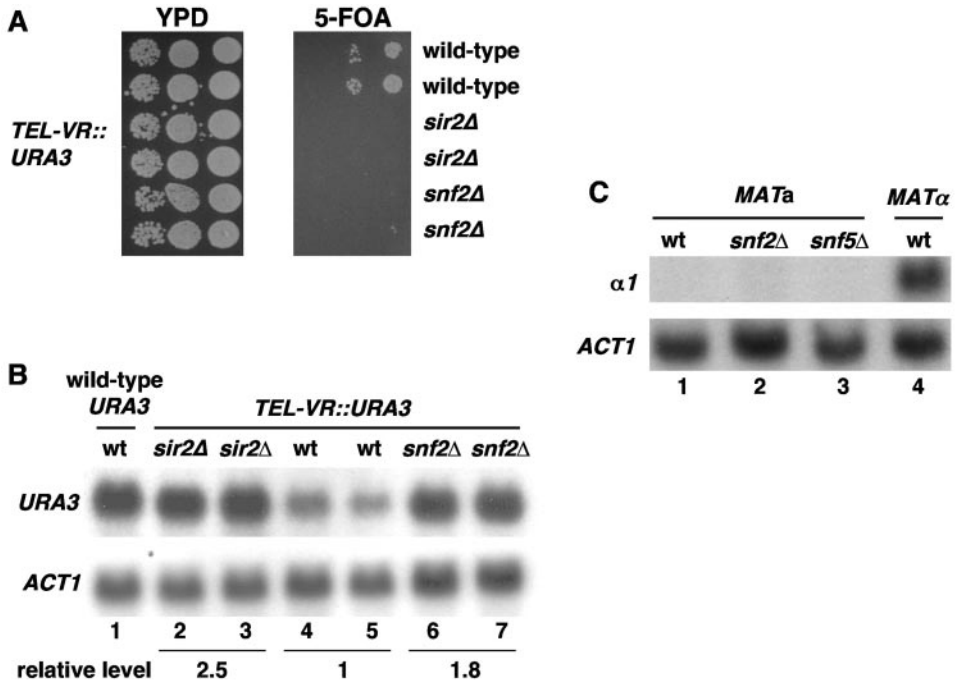

FIG. 7. *SNF2* is required for telomeric silencing. (A) Telomeric silencing phenotypes determined using a telomeric *URA3* reporter gene. Tenfold serial dilutions of stationary-phase cultures of wild-type (L1087 and L1088), *sir2*Δ (L1091 and L1092), or *snf2*Δ (L1089 and L1090) strains containing the *URA3* at the right telomere of chromosome V were spotted onto 5-FOA and YPD medium to monitor expression of the *URA3*. Loss of silencing is indicated by reduced growth on 5-FOA. (B) Swi/Snf represses *URA3* transcription at the telomere. Strains with *URA3* at its normal genomic location (lane 1) or integrated at the right telomere of chromosome V (lanes 2 to 7) were grown in SC medium supplemented with 100 mg of uracil/liter. mRNA levels of *URA3* and the loading control, *ACT1*, were measured by Northern analysis. The strains used in the experiment were as follows: lane 1, FY78; lane 2, L1091; lane 3, L1092; lane 4, L1087; lane 5, L1088; lane 6, L1089; and lane 7, L1090. The quantitation represents the relative level of *URA3* mRNA normalized to the level of *ACT1*. The numbers represent the averages for the pairs of strains shown. wt, wild type. (C) Swi/Snf has no detectable effect on *HML*α silencing. Three *MAT***a** strains in lanes 1 to 3 (wild type [wt], FY78; *snf2*Δ, FY328; *snf5*Δ, FY1658) and a *MAT*α strain, FY1856, were grown in YPD to 2×10^7 cells/ml. α1 and *ACT1* mRNA levels were measured by Northern analysis.

*snf2*Δ mutants, Sir2 is still associated with the rDNA and Set1-dependent histone methylation levels are normal. Third, in contrast to *sir2*Δ, *snf2*Δ does not alter nucleolar structure nor does it affect the association of Net1 with the nucleolus (data not shown). Finally, a *snf2*Δ mutation dramatically reduces rDNA recombination, a phenotype distinct from the increased levels in *sir2*Δ mutants and the unaffected levels in *set1*Δ mutants. These findings support the existence of a Swi/Snf-dependent mechanism for rDNA transcriptional silencing that acts independently of Sir2 or Set1.

The role of Swi/Snf in *SUC2* chromatin structure when *SUC2* is in its normal genomic location differs from that seen when *SUC2* is in the rDNA. At the normal *SUC2* genomic location, an active MNase cleavage pattern is dependent upon Swi/Snf, while in the rDNA, an active pattern is independent of Swi/Snf. Therefore, *SUC2* chromatin structure and its Swi/Snf dependence can be determined by genomic location. Furthermore, since *SUC2* chromatin structure is in the active conformation in either the presence or absence of Swi/Snf, the role of Swi/Snf in rDNA silencing must occur at a level other than that assayed by MNase sensitivity. Finally, the Swi/Snf independence of *SUC2* chromatin structure when *SUC2* is in the rDNA suggests that this active conformation may be dependent upon a different chromatin remodeling complex.

Our results have demonstrated that the absence of the Swi/Snf complex causes a drastic reduction in rDNA mitotic recombination. While mutations in *FOB1* and *HRM2-HRM4* also reduce rDNA mitotic recombination (42), those effects are not as severe as those caused by a mutation in *SNF2*. However, in similarity to *snf2*Δ, *fob1*Δ also impairs rDNA silencing (28). Our finding that *snf2*Δ is largely epistatic to *sir2*Δ with respect to recombination suggests that *snf2*Δ causes a change in rDNA chromatin that makes it inaccessible to recombination enzymes, even in the absence of Sir2 activity. This finding, combined with our MNase results, hints that the control of rDNA chromatin structure by Swi/Snf might occur at a higher-order level, a role that has been previously suggested for Swi/Snf (27).

An important question regarding the function of Swi/Snf in rDNA silencing is whether its role is direct or indirect. One obvious direct role is for Swi/Snf to directly control chromatin structure of nucleolar DNA. However, by ChIP experiments, neither Snf2 nor Snf5 were detectably associated with the rDNA (data not shown). In the most extensive experiments, Snf2 association was assayed across the entire rDNA repeat, at the *SUC2* promoter, and at the *URA3* promoter. In addition, by immunolocalization experiments, Snf2 was nuclear, in consistency with earlier findings (29, 38); however, Snf2 also appeared nucleolar in only a low percentage of cells (data not shown). While these negative results do not rule out the pos-

sibility that Swi/Snf functions directly in rDNA silencing, they leave open the possibility of a less direct role. For example, Swi/Snf might regulate a gene required for rDNA silencing. Regardless of the specific mechanism by which Swi/Snf controls rDNA silencing, our results have shown that it plays a prominent role in rDNA silencing that is independent of previously identified factors.

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