Evidence that Swi/Snf directly represses transcription in *S. cerevisiae*

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Many studies have established that the Swi/Snf family of chromatin-remodeling complexes activate transcription. Recent reports have suggested the possibility that these complexes can also repress transcription. We now present chromatin immunoprecipitation evidence that the Swi/Snf complex of *Saccharomyces cerevisiae* directly represses transcription of the *SER3* gene. Consistent with its role in nucleosome remodeling, Swi/Snf controls the chromatin structure of the *SER3* promoter. However, in striking contrast to activation by Swi/Snf, which requires most Swi/Snf subunits, repression by Swi/Snf at *SER3* is dependent primarily on one Swi/Snf component, Snf2. These results show distinct differences in the requirements for Swi/Snf components in transcriptional activation and repression.

Received May 24, 2002; revised version accepted July 2, 2002.

The *Saccharomyces cerevisiae* Swi/Snf complex is the founding member of a large family of ATP-dependent chromatin-remodeling complexes that have been well characterized as transcriptional activators (Kingston and Narlikar 1999; Sudarsanam and Winston 2000; Vignali et al. 2000; Narlikar et al. 2002). Genetic and biochemical studies from both yeast and humans have provided strong evidence that Swi/Snf complexes can be recruited to the promoters of specific genes (Peterson and Workman 2000). Once at a promoter, these complexes can remodel nucleosomes to facilitate the binding of transcription factors to their sites on nucleosomal DNA (Peterson and Workman 2000; Vignali et al. 2000).

In addition to their roles as transcriptional activators, several studies have suggested that Swi/Snf complexes serve as transcriptional repressors (Sudarsanam and Winston 2000; Urnov and Wolffe 2001). This idea arose both from studies of specific genes and from whole-genome expression analyses (for review, see Sudarsanam and Winston 2000; see also Angus-Hill et al. 2001). In addition, biochemical experiments have shown that Swi/Snf complexes can remodel nucleosomes in both directions between an inactive and a remodeled state (Lorch et al. 1998; Schnitzler et al. 1998). Although these reports support a role for Swi/Snf in repression of transcription, no experiments have tested whether Swi/Snf repression in vivo is direct or indirect, and if it involves the nucleosome-remodeling activity of Swi/Snf. Recent studies of two Swi/Snf-related complexes, Isw2 and RSC, have suggested that these complexes play direct roles in repression of transcription (Goldmark et al. 2000; Kent et al. 2001; Damelin et al. 2002; Ng et al. 2002).

The experiments presented in this paper investigate the repression of the *S. cerevisiae SER3* gene by Swi/Snf. Our results strongly suggest a direct role for Swi/Snf in transcriptional repression via controlling chromatin structure. Surprisingly, and in contrast to Swi/Snf activation, Swi/Snf repression has a strong requirement for only one Swi/Snf component, the Snf2 ATPase.

Results and Discussion

Repression of SER3 *is dependent primarily on the Snf2 ATPase*

To investigate the role of Swi/Snf in transcriptional repression, we chose to study the *S. cerevisiae SER3* gene, which encodes an enzyme required for serine biosynthesis (E. Albers, pers. comm.). Genome-wide expression analyses of *snf2*Δ and *swi1*Δ mutants identified *SER3* as a gene strongly repressed by Swi/Snf in rich medium (Holstege et al. 1998; Sudarsanam et al. 2000). Surprisingly, repression of *SER3* is three times more dependent on Snf2 than on Swi1 (Sudarsanam et al. 2000). This result contrasts with analyses of genes activated by Swi/Snf, including *HO*, *SUC2*, and Ty1 elements, which suggest an equal dependence on Snf2, Swi1, and most other Swi/Snf subunits (Winston and Carlson 1992). To investigate further the requirement for Swi/Snf components for both activation and repression, we tested eight mutants, each lacking a different Swi/Snf subunit, for the levels of Ty1 and *SER3* mRNAs by Northern analysis (Fig. 1). As expected, seven of these *swi/snf* mutants had at least an 80% decrease in Ty1 mRNA levels, showing that these subunits are strongly required for activation of Ty1 transcription. Snf11 was not required for Ty1 activation, which is expected because Swi/Snf mutant phenotypes were not detected previously for a *snf11*Δ mutant (Treich et al. 1995). In striking contrast to Swi/Snf activation, only Snf2, the catalytic ATPase subunit, was strongly required for repression of *SER3*. Two *snf2* mutations, *snf2*Δ and *snf2-798* (an allele encoding a K to A change of amino acid 798 that no longer has ATPase activity; Khavari et al. 1993), caused a 50-fold or greater increase in *SER3* mRNA levels. The derepression of *SER3* in the *snf2-798* mutant indicates that the ATPase activity of Snf2 is important for its role in repression. Among the remaining Swi/Snf subunits tested, *swp73*Δ, *swi1*Δ, and *swi3*Δ showed a moderate repression defect, whereas *snf5*Δ, *snf6*Δ, *swp29*Δ, and *snf11*Δ mutants had wild-type levels of repression. Therefore, repression of *SER3* was dependent predominantly on a single subunit, Snf2. These results suggest a fundamental difference in the mechanisms by which Swi/Snf confers repression and activation.

The lack of a role for Snf5 in repression of *SER3* emphasizes the difference between Swi/Snf activation and repression, as several previous studies have shown that Snf5 plays important roles in Swi/Snf complex formation and chromatin-remodeling activity. First, in vitro stud-

[*Key Words*: Swi/Snf; repression; chromatin remodeling; *Saccharomyces cerevisiae*]
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Article and publication are at http://www.genesdev.org/cgi/doi/10.1101/gad.1009902.

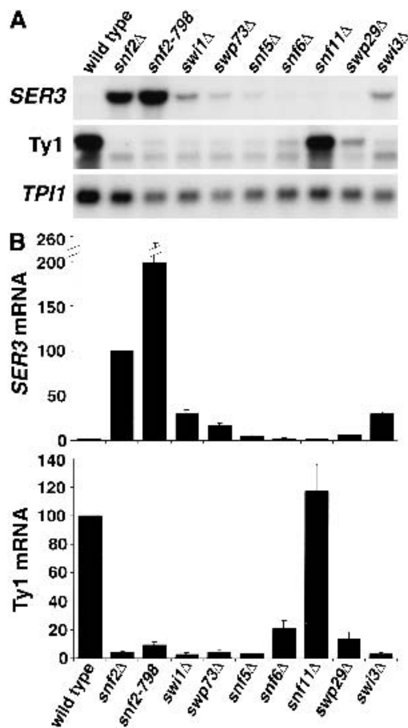

Figure 1. Repression of *SER3* is strongly dependent on Snf2. (*A*) Northern analysis of *SER3*, Ty1, and *TPI1* mRNAs. RNA was isolated from wild-type (FY2082), *snf2*Δ (FY2083), *snf2-798* (FY2084), *swi1*Δ (FY1852), *swp73*Δ (FY1702), *snf5*Δ (FY1658), *snf6*Δ (FY2085), *snf11*Δ (FY2086), *swp29*Δ (FY2087), and *swi3*Δ (FY2088) strains grown in YPD to 1–2 × 10^7 cells/mL. (*B*) Quantitation of Northern analysis. The relative levels of the *SER3* (*top* bar graph) and Ty1 (*bottom* bar graph) mRNAs were measured by PhosphorImager (Molecular Dynamics) and normalized to the level of *TPI1* mRNA. The level of *SER3* mRNA in the *snf2*Δ strain and Ty1 mRNA in the wild-type strain were set to 100. Each value represents the average and standard error of at least three independent experiments.

ies of human Swi/Snf defined the Snf5 homolog INI1 as one of four core members of the complex that are sufficient to reconstitute a level of remodeling activity equivalent to that of the complete complex (Phelan et al. 1999). Second, in *snf5*Δ mutants the size of the remaining Snf2-containing complex is ~700 kD, a significant decrease from the 2-MD Swi/Snf complex in wild-type cells (Peterson et al. 1994; Geng et al. 2001). Third, in studies of activation, a *snf5* mutation has been identified that blocks nucleosome remodeling by Swi/Snf in vivo, but does not block complex formation (Geng et al. 2001). Therefore, Snf5 plays critical roles in many contexts; however, it does not appear to play any significant role in *SER3* repression.

Swi1 and Swp73 play minor roles in SER3 *repression*

The modest requirement for some Swi/Snf subunits in *SER3* repression could be due either to a direct effect on Swi/Snf's repression activity or to an indirect effect by a role in Snf2 stability. In support of the second possibility, we observed that Snf2 levels are decreased in *swi1*Δ and *swp73*Δ mutants (Fig. 2A, cf. lanes 1, 3, and 5), consistent with previous work that suggested that the stability of Swi/Snf components is mutually dependent (Peterson and Herskowitz 1992). Therefore, we tested whether the modest defect in *SER3* repression observed in *swi1*Δ and *swp73*Δ mutants was caused by reduced Snf2 levels. To do this, we overexpressed a functional *lexA–SNF2* fusion in *swi1*Δ, *swp73*Δ, and *snf2*Δ strains and assayed Snf2 protein levels (Fig. 2A) and *SER3* mRNA levels (Fig. 2B). In all strains, overexpression of *lexA–SNF2* resulted in a level of Snf2 greater than that of a wild-type strain (Fig. 2A). However, the elevated level of Snf2 failed to fully repress *SER3* in either the *swi1*Δ or *swp73*Δ mutants (Fig. 2B). As expected, overexpression of *lexA–SNF2* complemented the *snf2*Δ repression defect (Fig. 2B). We conclude from these results that Swi1 and Swp73 likely play direct, albeit minor roles in Swi/Snf repression of *SER3*.

Snf2 and Snf5 are physically present at the SER3 *promoter*

A major question regarding the function of Swi/Snf in transcriptional repression is whether its role is direct or indirect. To address this question, we used the method of chromatin immunoprecipitation to examine whether Snf2 is physically present at the *SER3* promoter. To allow specific immunoprecipitation of Snf2, we used a version of Snf2 fused to 18 copies of the Myc epitope. The *SNF2–Myc* allele encodes a functional Snf2 protein as it fully complemented all phenotypes caused by a *snf2*Δ mutation (data not shown). Our results show that Snf2 was physically present at the *SER3* promoter (Fig. 3, cf.

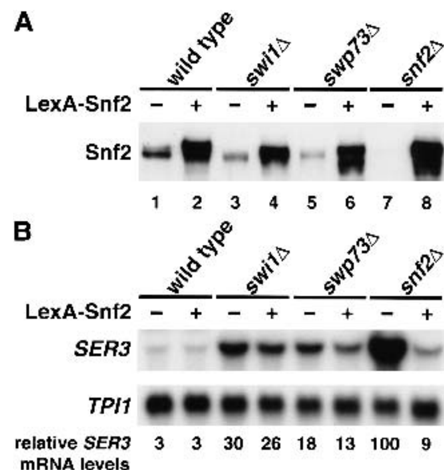

Figure 2. A subset of Swi/Snf subunits plays a minor role in repression. (*A*) Western analysis of wild-type (FY2089), *swi1*Δ (FY2091), *swp73*Δ (FY2093), and *snf2*Δ (FY2095) strains containing pJAM198 (no LexA–Snf2), or wild-type (FY2090), *swi1*Δ (FY2092), *swp73*Δ (FY2093), and *snf2*Δ (FY2096) strains containing pJAM200 (expressing LexA–Snf2). Strains were grown to 1–2 × 10^7 cells/mL in YPD medium supplemented with 200 µg/mL G418 to maintain selection for the plasmids. Equal amounts (50 µg) of whole-cell extracts were separated by SDS-PAGE and Western-blotted using an anti-Snf2 antibody. (*B*) Northern analysis of *SER3* and *TPI1* was performed on RNA isolated from the same cultures used in *A*. Each value represents the average level of *SER3* mRNA normalized to *TPI1* mRNA from two independent experiments.

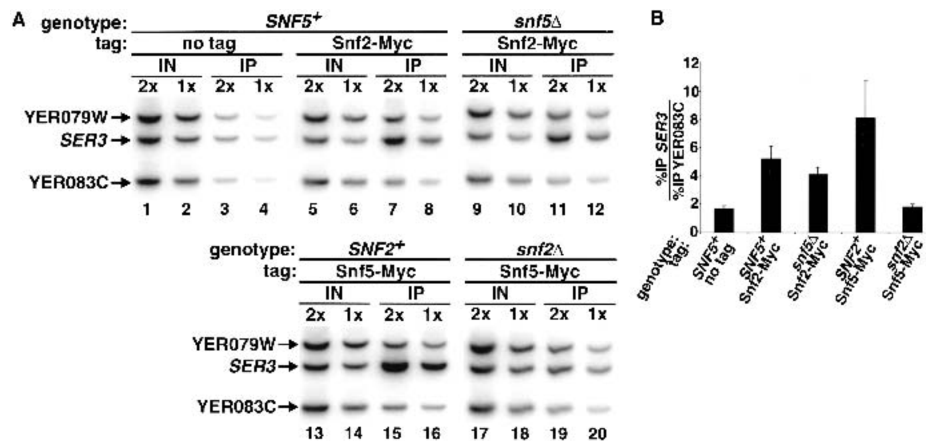

Figure 3. Snf2 and Snf5 are recruited to the *SER3* promoter. (*A*) Chromatin immunoprecipitations were performed on wild-type (FY2103) and *snf5*Δ (FY2102) strains expressing Snf2–Myc and on wild-type (FY2101) and *snf2*Δ (FY2104) strains expressing Snf5–Myc. An untagged strain (FY1338) was used as a negative control. Snf2–Myc and Snf5–Myc were immunoprecipitated with A14 anti-Myc antibody (Santa Cruz) from strains grown in YPD medium. The PCR products correspond to the promoter region of *SER3* and the promoter regions of two flanking genes, *YER079W* and *YER083C*, which serve as negative controls. One set of PCR reactions from twofold dilutions of each chromatin sample is shown. (*B*) Quantitation of chromatin immunoprecipitation. The %IPs of *SER3* and *YER083C* were calculated for each strain. Each value represents the average ratio of %IP *SER3* to %IP *YER083C* with the standard error from three independent experiments.

lanes 3,4 and 7,8). Consistent with the observation that Snf5 was not required for *SER3* repression (Fig. 1), Snf2 remained associated with the *SER3* promoter in a *snf5*Δ strain (Fig. 3, lanes 11,12). The physical association of Snf2 with the *SER3* promoter, taken together with the strong requirement for Snf2 in *SER3* repression, strongly suggests that Snf2 is a direct repressor of *SER3* transcription.

We also used chromatin immunoprecipitation to test for the presence of Snf5, a Swi/Snf subunit not required for *SER3* repression. As for Snf2, we used a functional SNF5–Myc fusion protein. These results show that Snf5–Myc was also present at the *SER3* promoter (Fig. 3, lanes 15,16). Consistent with a loss of repression of *SER3* observed in the *snf2*Δ mutant, the association of Snf5 with *SER3* was dependent on Snf2 (Fig. 3, lanes 19,20). The presence of Snf5 suggests that the entire Snf/Swi complex associates with the *SER3* promoter, although many of the subunits, including Snf5, are not required for *SER3* repression.

Snf2 controls the chromatin structure at the SER3 *promoter*

Previous in vivo and in vitro studies have established that activation by Swi/Snf occurs via an alteration of chromatin structure (Kingston and Narlikar 1999; Peterson and Workman 2000; Vignali et al. 2000; Narlikar et al. 2002). To test whether repression by Swi/Snf also involves chromatin changes, we compared the sensitivity of the *SER3* promoter to cleavage by micrococcal nuclease (MNase) in *SNF2*+ (repressed) and *snf2*Δ (derepressed) strains. The results of indirect end-labeling experiments revealed two significant sets of differences in the *SER3* MNase digestion pattern between the two strains (Fig. 4A). First, in the *SER3* promoter region surrounding a consensus TATA site, there was little MNase cleavage in the *SNF2*+ strain; however, two sites (A and B) were hypersensitive to MNase digestion in the *snf2*Δ strain. Second, three sites further 5′ of the TATA were cleaved by MNase in *SNF2*+ chromatin (C,D, and E), but were protected in chromatin isolated from an *snf2*Δ strain. In addition, a new MNase cleavage site (F) appeared. To determine if the altered MNase cleavage pattern in the *snf2*Δ mutant is caused by the loss of Snf2 rather than the increased level of transcription, we assayed the MNase cleavage pattern of *SNF2*+ and *snf2*Δ strains in which transcription of *SER3* is greatly reduced by a mutation in the *SER3* TATA element (*ser3-100*). Although this TATA mutation caused a 10-fold reduction in *SER3* mRNA levels in an *snf2*Δ mutant (Fig. 4B), the changes in the MNase cleavage pattern were identical to those observed at the wild-type *SER3* promoter (Fig. 4A). These results strongly suggest that Snf2 is required to maintain a repressive chromatin structure over the *SER3* promoter.

Our findings for Swi/Snf, taken together with recent reports that show the physical presence of RSC and Isw2 at the promoters of repressed genes (Goldmark et al. 2000; Kent et al. 2001; Damelin et al. 2002; Ng et al. 2002), provide strong evidence for nucleosome-remodeling complexes acting directly to repress transcription. Although we have shown that Swi/Snf associates with the *SER3* promoter, the mechanism of Swi/Snf recruitment to this promoter remains unknown. Swi/Snf may be recruited through interaction with a DNA-binding protein, in a manner similar to Isw2 recruitment by the Ume6 repressor (Goldmark et al. 2000; Kent et al. 2001). Alternatively, Swi/Snf might have binding specificity for a particular chromatin structure at the *SER3* promoter.

There are several possible mechanisms by which Swi/Snf could repress transcription. First, based on in vitro experiments showing that Swi/Snf can catalyze remodeling of nucleosomes in either direction between the inactive and remodeled states (Lorch et al. 1998; Schnitzler et al. 1998), Swi/Snf might create an inactive nucleosome conformation at *SER3* that prevents transcription-factor access to the promoter. Second, Swi/Snf could fa-

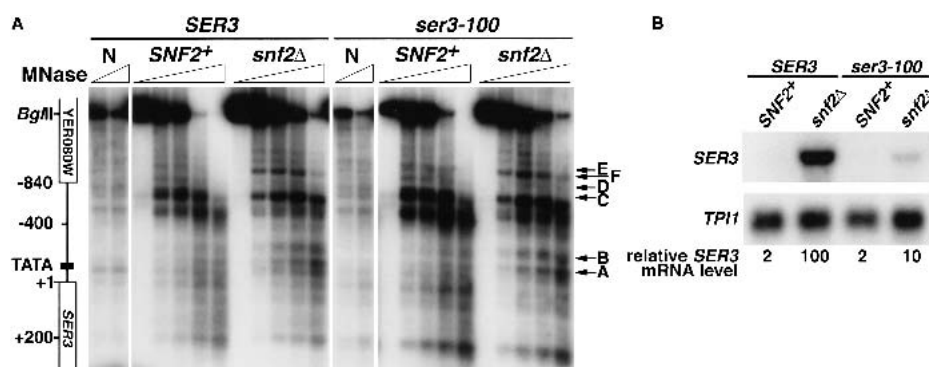

Figure 4. Swi/Snf regulates chromatin structure over the *SER3* promoter. (*A*) *SNF2*+ (FY2097), *snf2*Δ (FY2098), *SNF2*+ *ser3-100* (FY2099), and *snf2*Δ *ser3-100* (FY2100) strains were grown in YPD medium to 1–2 × 10^7 cells/mL. Spheroplasts were isolated and incubated with increasing amounts of MNase. DNA was isolated, digested with *Bgl*II, and subjected to indirect end-labeling analysis using a probe that anneals to +301 to +529 (+1 = ATG) in the coding sequence of *SER3*. All strains were deleted for the *SER33* ORF to prevent cross-hybridization of the *SER3* probe. The *SER3* genomic region is diagramed on the *left*, and the approximate positions of altered MNase cleavage sites are marked with arrows on the *right*. N denotes the naked DNA controls. (*B*) Northern analysis of *SER3* was performed on RNA isolated from the same strains listed in *A*. The level of *SER3* mRNA was normalized to *TPI1* and set to 100 for the *snf2*Δ strain. The average and standard error for *SER3* mRNA levels from four independent experiments are reported as follows: *SNF2*+, 2.2 ± 0.7; *snf2*Δ, 100; *SNF2*+ *ser3-100*, 1.5 ± 0.3; *snf2*Δ *ser3-100*, 10 ± 2.

cilitate the binding of a transcriptional repressor of *SER3*. Finally, Swi/Snf nucleosome remodeling could facilitate a subsequent step required for repression, such as histone modification. Recent studies have shown that some Swi/Snf-related complexes associate with histone deacetylase activity (Narlikar et al. 2002). Identification of the *cis*-acting elements and other *trans*-acting factors required for *SER3* regulation should provide additional insights into the mechanism by which Swi/Snf represses transcription.

Materials and methods

S. cerevisiae strains and methods

All *S. cerevisiae* strains used in this study (Table 1) are derivatives of a *GAL2*+ S288C strain (Winston et al. 1995). Standard strain construction methods and media recipes were as described previously (Rose et al. 1990). The *snf2*Δ*::LEU2* (Cairns et al. 1996), *snf2-798* (K to A change of amino acid 798; Khavari et al. 1993), *swp73*Δ*1::LEU2* (Cairns et al. 1996), *swi1*Δ*1::LEU2* (Peterson and Herskowitz 1992), and *snf5*Δ*2* (Sudarsanam et al. 1999) alleles have been described previously. Strains containing *snf6*Δ*::kanMX* and *snf11*Δ*::kanMX* were constructed through crosses with commercially available deletions (Research Genetics). The *ser33*Δ*::kanMX*, *swp29*Δ*::kanMX*, and *swi3*Δ*::kanMX* alleles were constructed by replacing the open reading frames with the *kanMX* marker (Baudin et al. 1993; Lorenz et al. 1995; Brachman et al. 1998). *SER33*, a gene with 82% identity to *SER3*, was deleted in strains FY2097, FY2098, FY2099, and FY2100 to prevent cross-hybridization to the *SER3* probe used in our chromatin analysis experiments. Deletion of *SER33* had no effect on *SER3* mRNA levels (data not shown). The *SNF2–C18Myc* and *SNF5–C18Myc* alleles marked by *K. lactis TRP1* were generated by introducing 18 copies of the Myc epitope at the C-terminal end of the *SNF2* and *SNF5* genes by PCR-mediated integration using plasmid pWZV88. Both strains were wild type for all phenotypes tested, including growth on glucose and raffinose and in the absence of inositol (data not shown). They also maintained complete repression of *SER3* (data not shown). The *ser3-100* mutants, which carry a TATAAA → CCTAGG mutation in a putative TATA box at –103 to –98 (+1 = ATG) within the *SER3* promoter, were constructed by two-step gene replacement using plasmid pJAM196.

Plasmid DNA construction and analysis

Plasmids were constructed and isolated from *Escherichia coli* by standard methods (Ausubel et al. 1988). pJAM196 was constructed by ligating an *Eco*RI DNA fragment containing *SER3* sequence from –340 to +252 (+1 = ATG) into pRS406 (Christianson et al. 1992) and subsequently changing the sequence of a TATA box (TATAAA) to an *Avr*II site (CCTAGG) using the Stratagene QuikChange Site-Directed Mutagenesis Kit. pJAM198 and pJAM200 are derivatives of pRS423 (Christianson et al. 1992) and pLEXA-SNF2 (Laurent et al. 1991), in which the *HIS3* marker has been replaced with the *kanMX* marker (Brachmann et al. 1998) by plasmid gap repair (Muhlrad et al. 1992).

Northern hybridization analysis

Cells were grown in YPD media to a concentration of 1–2 × 10^7 cells/mL. To maintain plasmids pJAM198 and pJAM200, YPD media was supplemented with 200 µg/mL of G418 (Invitrogen). Total yeast RNA was prepared and separated on a 1% agarose gel as described previously (Ausubel et al. 1988). A probe specific to *SER3* was synthesized by PCR-amplification of DNA from +1378 to +1626 (+1 = ATG) and radiolabeled with [α-^{32}P]dATP by random priming (Ausubel et al. 1988). The *TPI1* and Ty1 probes have been described previously (Winston et al. 1987; Hirschhorn et al. 1992).

Western analysis

Yeast cultures were grown to 1–2 × 10^7 cells/mL, and whole-cell extracts were prepared by bead lysis. Protein concentrations were measured by Bradford assay (Bio-Rad). Equal amounts of extract were separated by SDS-PAGE and transferred to immobilon membrane (Millipore). Snf2 and LexA–Snf2 proteins were detected using an anti-Snf2 antibody (1:2000; Geng et al. 2001), followed by HRP-conjugated secondary antibody (1:5000) and chemiluminescence.

Chromatin immunoprecipitation

The procedure for chromatin immunoprecipitation was adapted from previously described methods (Dudley et al. 1999; Kuras and Struhl 1999). Briefly, cells from 200-mL YPD cultures were cross-linked by adding formaldehyde to a final concentration of 1%. Chromatin was prepared in FA lysis buffer containing 140 mM NaCl and no SDS. Cross-linked chromatin was sonicated to an average length of 500 bp with a size range from 200 to 1200 bp. Snf2–Myc and Snf5–Myc were immunoprecipitated from 1/10 of the cross-linked chromatin by a two-step method (Harlow and Lane 1999) using rabbit polyclonal anti-Myc A14 antibody (Santa Cruz) followed by IgG-sepharose beads (Pharmacia). Dilutions of input DNA (1/1000 and 1/2000) and immunoprecipitated DNA (1/2.5 and 1/5) were subjected to quantitative radioactive PCR as described (Larschan and Winston 2001), and the products were separated on a 7.5% nondenaturing polyacrylamide gel. The *SER3* promoter region was detected using a primer set that amplifies a 302-bp product from –424 to –123 (+1 = ATG). Primer sets amplifying a 358-bp product of YER079W

Table 1. Saccharomyces cerevisiae *strains*

Strain	Genotype
FY1338	MATαura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ0 leu2Δ0
FY1658	MAT**a** ura3-52 his3Δ200 lys2-128δ snf5Δ2
FY1702	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 swp73Δ1::LEU2
FY1852	MAT**a** ura3Δ0 his3Δ200 ade8 met15Δ0 leu2Δ0 swi1Δ1::LEU2
FY2082	MAT**a** ura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ0
FY2083	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 met15Δ0 leu2Δ0 snf2Δ::LEU2
FY2084	MAT**a** ura3Δ0 snf2-798
FY2085	MAT**a** ura3Δ0 his3Δ200 snf6Δ::kanMX
FY2086	MAT**a** ura3Δ0 his3Δ200 snf11Δ::kanMX
FY2087	MAT**a** ura3Δ0 his3Δ200 swp29Δ::kanMX
FY2088	MAT**a** ura3Δ0 swi3Δ::kanMX
FY2089	MAT**a** ura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ0 <pJAM198>
FY2090	MAT**a** ura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ0 <pJAM200>
FY2091	MAT**a** ura3Δ0 his3Δ200 ade8 met15Δ0 leu2Δ0 swi1Δ1::LEU2 <pJAM198>
FY2092	MAT**a** ura3Δ0 his3Δ200 ade8 met15Δ0 leu2Δ0 swi1Δ1::LEU2 <pJAM200>
FY2093	MAT**a** ura3Δ0 his3Δ200 leu2Δ0 swp73Δ1::LEU2 <pJAM198>
FY2094	MATα ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 swp73Δ1::LEU2 <pJAM200>
FY2095	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 met15Δ0 leu2Δ0 snf2Δ::LEU2 <pJAM198>
FY2096	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 met15Δ0 leu2Δ0 snf2Δ::LEU2 <pJAM200>
FY2097	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 ser33Δ::kanMX
FY2098	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 ser33Δ::kanMX snf2Δ::LEU2
FY2099	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 ser33Δ::kanMX ser3-100
FY2100	MAT**a** ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 ser33Δ::kanMX snf2Δ::LEU2 ser3-100
FY2101	MATα ura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ0 leu2Δ0 SNF5-C18MYC::TRP1
FY2102	MAT**a** ura3Δ0 his3Δ200 lys2-128δ snf5Δ2 SNF2-C18MYC::TRP1
FY2103	MATα ura3Δ0 trp1Δ63 his3Δ200 lys2Δ0 met15Δ2 leu2Δ0 SNF2-C18MYC::TRP1
FY2104	MATα ura3Δ0 or ura3-52 trp1Δ63 his3Δ200 lys2Δ0 met15Δ2 leu2Δ0 or leu2Δ1 SNF5-C18MYC::TRP1 snf2Δ::LEU2

from –475 to –117 (+1 = ATG) that is 3.9 kb 5′ of *SER3* and a 273-bp product of YER083C from –301 to –29 (+1 = ATG) that is 4.4 kb 3′ of *SER3* were added to each PCR reaction as negative controls. The relative amount of each PCR product immunoprecipitated (%IP) was calculated as described previously (Larschan and Winston 2001). Specific binding of Snf2–Myc and Snf5–Myc to *SER3* was evaluated by calculating the ratio of the %IP of *SER3* to the %IP of YER083C for each strain.

Analysis of chromatin structure by MNase

Yeast strains were grown in YPD media to 1–2 × 10^7 cells/mL. Spheroplasts were isolated and subjected to micrococcal nuclease (MNase) digestion as adapted from previously described methods (Kent et al. 1993; Kent and Mellor 1995). Approximately 1.2 × 10^9 cells were incubated with 2 mg/mL zymolyase (ICN 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. MNase-treated DNA samples were digested completely with *Bgl*II, separated on a 1% agarose gel, and analyzed by indirect end-labeling (Hirschhorn et al. 1992). A 228-bp PCR product corresponding to base pairs +301 to +529 (+1 = ATG) of the *SER3* open reading frame was synthesized by PCR, radiolabeled by random priming (Ausubel et al. 1988), and used as the probe to detect *SER3* DNA. DNA fragments of lengths 1684, 1177, 868, and 228 bp, synthesized by PCR from the *SER3* region, were used as size standards to calculate positions of MNase cleavage.

Acknowledgments

We thank Andrea Duina, Jenny Wu, and Robert Kingston for critical comments on the manuscript; Nicholas Kent for helpful advice on the MNase experiments; Brehon Laurent for providing the anti-Snf2 antibody and the pLexA–SNF2 plasmid; Craig Peterson for providing the *snf2-798* allele; and Wolfgang Zachariae and Kim Nasmyth for providing the pWZV88 plasmid. This work was supported by grant GM32967 from the National Institutes of Health (to F.W.) and by Postdoctoral Fellowships from the Human Frontiers Science Program and the Canadian Institute for Health Research (to J.A.M).

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