Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure

Joel N. Hirschhorn, Steven A. Brown,¹ Chris D. Clark,² and Fred Winston³

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115 USA

Changes in chromatin structure have frequently been correlated with changes in transcription. However, the cause-and-effect relationship between chromatin structure and transcription has been hard to determine. In addition, identifying the proteins that regulate chromatin structure has been difficult. Recent evidence suggests that a functionally related set of yeast transcriptional activators (SNF2/SWI2, SNF5, SNF6, SWI1, and SWI3), required for transcription of a diverse set of genes, may affect chromatin structure. We now present genetic and molecular evidence that at least two of these transcriptional activators, SNF2/SWI2 and SNF5, function by antagonizing repression mediated by nucleosomes. First, the transcriptional defects in strains lacking these *SNF* genes are suppressed by a deletion of one of the two sets of genes encoding histones H2A and H2B, (*hta1-htb1*) Δ . Second, at one affected promoter (*SUC2*), chromatin structure is altered in *snf2/swi2* and *snf5* mutants, and this chromatin defect is suppressed by (*hta1-htb1*) Δ . Finally, analysis of chromatin structure at a mutant *SUC2* promoter, in which the TATA box has been destroyed, demonstrates that the differences in *SUC2* chromatin structure between *SNF5*⁺ and *snf5* mutant strains are not simply an effect of different levels of *SUC2* transcription. Thus, these results strongly suggest that SNF2/SWI2 and SNF5 cause changes in chromatin structure and that these changes allow transcriptional activation.

[Key Words: Yeast; chromatin; transcriptional activation; SNF and SWI proteins]

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Understanding the mechanisms by which genes are transcriptionally activated and repressed is central to understanding gene regulation. Numerous studies have shown a correlation between changes in transcription and alterations in chromatin structure (for review, see van Holde 1988; Grunstein 1990a). These studies have suggested that the positions of nucleosomes, the primary components of chromatin, may affect transcription. However, the cause-and-effect relationship between changes in transcription and alterations in chromatin structure is unclear.

Recent genetic and biochemical data strongly support the view that nucleosomes play an important role in transcriptional regulation. Studies in the yeast *Saccharomyces cerevisiae* have provided genetic evidence that histones, the protein components of nucleosomes, affect transcription. For example, alterations in histone stoichiometry restore transcription to promoters disrupted by certain transposon insertion mutations (Clark-Adams et al. 1988). In addition, depletion of histone H4 or mutations in genes encoding histone H4 can result in in-

creased or decreased levels of transcription (for review, see Grunstein 1990b). Genetic and biochemical data also suggest that some transcriptional activators may function to alleviate the repressive effects of chromatin structure (Fascher et al. 1990; Croston et al. 1991; Workman et al. 1991). However, aside from histones, the proteins involved in the regulation of chromatin structure are largely unknown.

A series of genetic experiments led us to believe that three yeast transcriptional activators, SNF2/SWI2 (hereafter referred to as SNF2 for simplicity), SNF5, and SNF6, might function by altering chromatin structure. The *SNF* genes were originally identified by mutations causing defects in transcription of *SUC2*, a glucose-repressible gene that encodes the enzyme invertase (Carlson et al. 1981; Neigeborn and Carlson 1984). *SNF2*, *SNF5*, and *SNF6* were also shown to be required for the expression of a number of diversely regulated genes (Abrams et al. 1986; Estruch and Carlson 1990; Happel et al. 1991) and thus appear to encode transcriptional activators.

Two genetic studies provided the link between SNF proteins and chromatin structure (for review, see Winston and Carlson 1992). The first study, in which suppressors of *snf2* and *snf5* mutations were isolated, identified the gene *SPT6* (Neigeborn et al. 1986). A separate study, in which suppressors of transposon insertion mu-

Present addresses: ¹Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114 USA; ²Department of Genetics, Stanford University, Stanford, California 94305 USA.

³Corresponding author.

tations were isolated, identified not only *SPT6* but also the gene pair *HTA1-HTB1*, one of two sets of genes encoding the histones H2A and H2B (Winston et al. 1984; Clark-Adams et al. 1988; Fassler and Winston 1988). Furthermore, mutations in *SPT6* and in *HTA1-HTB1* were shown to cause many similar phenotypes (Clark-Adams et al. 1988; Fassler and Winston 1988). Because *spt6* mutations suppress *snf2*, *snf5*, and *snf6* mutations and because *spt6* mutations cause many of the same phenotypes as do *hta1-htb1* mutations, it seemed possible that the histone mutations might also suppress *snf2*, *snf5*, and *snf6*. This suppression, presented in this paper, indicates that the function of SNF2, SNF5, and SNF6 transcriptional activators might involve chromatin.

More recent genetic studies have shown that SNF2, SNF5, and SNF6 are part of a larger group of functionally related proteins. This set of SNF proteins overlaps with a set of proteins named SWI1, SWI2, and SWI3 (Stern et al. 1984). Mutations in *SWI* genes, isolated on the basis of defects in transcription of the *HO* gene, also affect transcription of a diversely regulated set of genes (Stern et al. 1984; Peterson and Herskowitz 1992). Recent work showed that *SWI2* is identical to *SNF2* (Laurent et al. 1991; K. Nasmyth, pers. comm.). In every case tested, mutations in *snf2*, *snf5*, *snf6*, *swi1*, and *swi3* cause defects in transcription of a diverse set of genes, including *SUC2*, Ty elements, *INO1*, *GAL1*, *GAL10*, and *HO* (for review, see Winston and Carlson 1992). Interestingly, suppressors of *swi1* mutations have identified a gene (*SIN1/SPT2*) that shows similarity to HMG proteins (nonhistone components of chromatin), further strengthening the idea that the function of the SNF and SWI activators is related to chromatin (Kruger and Herskowitz 1991).

The SNF and SWI proteins have been proposed to participate in a common function, possibly as a heteromeric complex (Laurent et al. 1991; Peterson and Herskowitz 1992). This function may require helicase activity because SNF2 has homology to known helicases (Davis et al. 1992; Laurent et al. 1992). In addition, although the SNF and SWI proteins have not been shown to bind to DNA, at least SNF2, SNF5, and SNF6 appear to play a direct role in transcriptional activation because *lexA*-SNF protein fusions can activate transcription by a *lexA* operator (Laurent et al. 1990, 1991; Laurent and Carlson 1992).

In this paper we provide evidence that at least two of these proteins, SNF2 and SNF5, function by altering chromatin structure. First, we show that *(hta1-htb1)Δ* suppresses multiple transcriptional defects of *snf2* and *snf5* mutants, including a defect in *SUC2* transcription. We then show that the chromatin from *snf2* and *snf5* mutants exhibits a different pattern of cleavage by micrococcal nuclease (MNase) over the *SUC2* promoter region than does chromatin from *SNF*⁺ strains. In addition, the suppression of *snf2* and *snf5* by *(hta1-htb1)Δ* is reflected in the MNase cleavage patterns over this region. Finally, by examining strains carrying mutations in the *SUC2* TATA box, we show that the differences in chromatin structure are not caused by different levels of

transcription. Taken together, these results indicate that SNF2, SNF5, and the other SNF and SWI proteins activate transcription by altering chromatin structure. Because SNF2 has sequence homologs in many other organisms (Tamkun et al. 1992; other references are cited in Laurent et al. 1992), this mechanism of activation may be functionally conserved throughout eukaryotes.

Results

Decreased histone gene dosage suppresses mutations in certain SNF genes

Yeast strains carrying mutations in *SNF2*, *SNF5*, or *SNF6* are defective for transcription of a diversely regulated set of genes, including *SUC2*, Ty elements, *INO1*, *GAL1*, and *GAL10* (Neigeborn and Carlson 1984; Happel et al. 1991; Peterson et al. 1991; Peterson and Herskowitz 1992). Preliminary evidence indicated that *(hta1-htb1)Δ*, a deletion of one of two gene pairs that encode histones H2A and H2B, might suppress *snf2*, *snf5*, and *snf6* mutations for the defects caused in *SUC2* expression (Clark-Adams 1988). This deletion is believed to reduce the levels of histones H2A and H2B in vivo (Clark-Adams et al. 1988).

To examine more rigorously the suppression of *snf* mutations by *(hta1-htb1)Δ*, we constructed a set of isogenic strains, differing only with respect to their *SNF* and *HTA1-HTB1* genotypes. We then measured the levels of invertase, the *SUC2* gene product, in these strains. Because *SUC2* is transcriptionally regulated, measurement of invertase levels reflects the level of *SUC2* mRNA (Carlson 1987). Our results (Table 1) show that the *SUC2* transcriptional defects of *snf2*, *snf5*, and *snf6* mutants

Table 1. The effects of *snf*, *hta1-htb1*, and *suc2* mutations on *SUC2* gene expression

Relevant genotype ^a	Invertase activity ^b	
	<i>HTA1-HTB1</i>	<i>(hta1-htb1)Δ</i>
<i>SNF</i> ⁺ <i>SUC2</i> ⁺	100	98
<i>snf2</i>	13	38
<i>snf5</i>	7	60
<i>snf6</i>	12	37
<i>snf1</i>	<1	<1
<i>snf4</i>	<1	<1
<i>suc2-104</i>	5	4
<i>snf5 suc2-104</i>	<1	1

^aRelevant genotype indicates the *SUC2* and *SNF* genotypes. The two columns of data show the secreted invertase activities for strains that are *HTA1-HTB1*⁺ or *(hta1-htb1)Δ*, respectively, in conjunction with the indicated *SUC2* and *SNF* genotypes. The strains used for these assays include all of the strains listed in Table 2 with the exception of FY637. In some cases, the invertase values represent the average from more than one strain.

^bUnits shown are micromoles of glucose released per minute per 100 mg of dry weight cells. Values shown are the average of at least two different derepressed cultures; two different amounts of cells were assayed for each culture. All repressed cultures had <1 unit of activity. All standard errors were <15%.

are suppressed by (*hta1-htb1*) Δ : Strains carrying (*hta1-htb1*) Δ in combination with a *snf2*, *snf5*, or *snf6* mutation express invertase at a level three- to ninefold above that observed in strains carrying only the *snf* mutations. Northern hybridization analysis confirms that *SUC2* mRNA levels in wild-type, (*hta1-htb1*) Δ , *snf5*, and *snf5* (*hta1-htb1*) Δ strains correlate with the levels of invertase activity measured for these strains (data not shown). This suppression suggests a functional interaction between SNF proteins and histones.

Suppression by the histone mutation (*hta1-htb1*) Δ is specific to *snf2*, *snf5*, and *snf6* mutations in all cases tested; that is, suppression does not occur by generally increasing *SUC2* transcription. First, (*hta1-htb1*) Δ does not detectably affect normal *SUC2* expression in a *SNF*⁺ background, under either repressing (high glucose) or derepressing (low glucose) conditions (Table 1). In addition, (*hta1-htb1*) Δ does not suppress a mutation in the *SUC2* TATA box (*suc2-104*; Table 1). Finally, (*hta1-htb1*) Δ has no detectable effect on *snf1* or *snf4* mutations (Table 1); *snf1* and *snf4* mutations are believed to impair *SUC2* transcription by a different mechanism than *snf2*, *snf5*, and *snf6* mutations (Neigeborn et al. 1986). Thus, suppression of *snf2*, *snf5*, and *snf6* by (*hta1-htb1*) Δ is unlikely to be the result of a nonspecific increase in transcription at *SUC2*. More probably, this histone deletion suppresses a specific transcriptional defect caused by *snf2*, *snf5*, and *snf6* mutations.

Previous work showed that *snf2*, *snf5*, and *snf6* strains, but not *snf1* or *snf4* strains, were defective for transcription of the yeast transposable elements Ty1 and Ty2 (Ciriacy et al. 1991; Happel et al. 1991). Therefore, we also determined whether deletion of the *HTA1-HTB1* locus suppresses the defect of *snf2*, *snf5*, and *snf6* strains for transcription of these Ty elements. Northern hybridization analysis (Fig. 1) demonstrates that (*hta1-htb1*) Δ reproducibly suppresses the Ty transcription defect of *snf2* and *snf5* mutants. The effect of (*hta1-htb1*) Δ on the *snf6* defect is less clear (Fig. 1, lanes 7, 8). In the experiment shown, (*hta1-htb1*) Δ does not appear to suppress the Ty transcription defect of *snf6*. In other experiments weak suppression has been observed (data not shown).

Figure 1. A deletion of *HTA1-HTB1* suppresses the Ty transcription defect in *snf2* and *snf5* mutant strains. Total RNA was prepared from each strain and subjected to Northern analysis. Ten micrograms of RNA was run in each lane. The filter was hybridized with a Ty probe and rehybridized with *TUB2* and *TPI1* probes for standardization. The strains were (from left to right): FY120, FY710, FY458, FY724, FY712, FY714, L896, and L897.

Figure 2. Suppression of growth defects of *snf* mutants by (*hta1-htb1*) Δ . Strains were grown in patches on a YPD plate and then replica plated onto media containing galactose as a sole carbon source (YPgal), YPD, media lacking inositol (– inositol), and – inositol media supplemented with inositol (+ inositol). Plates were photographed after 2 days of growth at 30°C. The strains were as follows (from left to right): (Row 1) FY120 and FY710; (row 2) FY722 and FY723; (row 3) FY458 and FY724; (row 4) FY712 and FY714.

Consistent with these results, other studies have shown that *snf6* mutations display somewhat different genetic interactions than do *snf2* and *snf5* mutations (Laurent et al. 1991; Laurent and Carlson 1992).

Many *snf* mutants also show growth defects on media containing galactose as a carbon source and on media lacking inositol (Neigeborn and Carlson 1984; Peterson et al. 1991). In the most extensively studied case, *snf2* (*swi2*) mutants, these defects are probably the result of a reduced level of transcription of *GAL10*, *GAL1*, and *INO1* (Peterson et al. 1991; Peterson and Herskowitz 1992). Therefore, we determined whether the Gal[–] and Ino[–] phenotypes of *snf* mutants are also suppressed by (*hta1-htb1*) Δ . These results show that similar to the effects seen on *SUC2* expression, (*hta1-htb1*) Δ suppresses these phenotypes in *snf2*, *snf5*, and *snf6* mutants but not in *snf1* mutants (Fig. 2; data not shown). Thus, a deletion of one of the two gene pairs encoding histones H2A and H2B suppresses multiple defects of *snf2*, *snf5*, and *snf6* mutants.

Mutations in *SNF5* alter *SUC2* chromatin structure

The suppression of *snf* mutations by (*hta1-htb1*) Δ suggests that these SNF proteins activate transcription by altering chromatin structure. To test this possibility, we studied the chromatin structure of the *SUC2* promoter

region in *SNF5*⁺ and *snf5* mutant strains by examining sensitivity to cleavage by MNase. MNase cleavage of DNA is usually altered by nucleosomes (for review, see van Holde 1988); thus, differences in the pattern of MNase cleavage between different strains are likely to reflect differences in nucleosome position or structure.

To determine the sites of MNase sensitivity in the *SUC2* promoter region, we isolated chromatin from four strains that were grown under derepressing conditions for *SUC2* transcription (low glucose) and that differ only in their *SNF5* and *HTA1-HTB1* genotypes: wild type, *snf5*, (*hta1-htb1*) Δ , and *snf5* (*hta1-htb1*) Δ . We then performed indirect end-labeling experiments (Nedospasov and Georgiev 1980; Wu 1980) as described in Materials and methods. In this technique, chromatin is subjected to partial digestion with MNase. DNA is then purified, digested to completion with a restriction enzyme, and subjected to Southern hybridization analysis, using a short probe immediately adjacent to one of the restriction enzyme sites. By this method, the positions of MNase cleavage sites in the *SUC2* promoter region can be determined with respect to a defined restriction site.

The results of the indirect end-labeling experiments (Fig. 3) reveal that two regions of the *SUC2* promoter show significant and reproducible differences in MNase cleavage patterns between different strains. First, at two sites near the TATA box, MNase cleaves both naked DNA and chromatin from a wild-type (*SNF*⁺) strain (Fig. 3, sites E and F). However, chromatin from a *snf5* mutant is nearly completely protected from cleavage at these sites. This protection and the distance of ~145 bp between sites D and G are consistent with the presence of a nucleosome (diagramed in Fig. 6, below). The chromatin from a *snf5* (*hta1-htb1*) Δ double mutant shows partial cleavage at sites E and F (Fig. 3, lanes 13–17). The effects of (*hta1-htb1*) Δ suggest that in a *snf5* background, this histone mutation causes either a reduction or alteration of nucleosomes over the *SUC2* promoter region, leading to increased transcription of *SUC2*.

Differences in chromatin structure also occur in a region between the *SUC2* TATA box and upstream activation sequences (UAS). In this region, three adjacent MNase sites (Fig. 3, sites A, B, and C) are affected by *snf5* and (*hta1-htb1*) Δ mutations. In naked DNA and in chromatin from wild-type strains, only sites B and C are cleaved. In contrast, chromatin from a *snf5* strain exhibits protection from cleavage at site C and partial protection at site B and is sensitive to cleavage at site A. The altered cleavage pattern in the *snf5* mutant is also consistent with a nucleosome over this region: blockage of sites B and C and induced cutting adjacent to the putative nucleosome at site A (diagramed in Fig. 6). Chromatin from a *snf5* (*hta1-htb1*) Δ double mutant shows partial cleavage at all three sites, again consistent with the suppression of *snf5* by (*hta1-htb1*) Δ .

Alterations in chromatin structure do not require transcription or a functional TATA box

Previous genetic results, combined with our results on

MNase cleavage of *SUC2* chromatin, are consistent with the model that changes in chromatin structure at *SUC2* cause transcriptional changes. However, these results are also consistent with the possibility of the reverse relationship: Changes in the level of transcription cause changes in chromatin structure. This latter possibility is supported by experiments showing that RNA polymerase-induced supercoiling during transcription can affect histone–DNA interactions (Liu and Wang 1987; Clark and Felsenfeld 1991; Lee and Garrard 1991) and that the TATA box-binding protein TFIID can compete with histones for DNA binding in vitro (Workman and Roeder 1987).

To address the cause-and-effect relationship between changes in transcription and chromatin structure at *SUC2*, we examined *SUC2* chromatin structure under conditions where transcription was greatly reduced independently of SNF functions. To create these conditions, we mutated the *SUC2* TATA box, generating the *suc2-104* allele in which the TATA box at –133 (TATAAA) is replaced by a *KpnI* site (GGTACC). In an otherwise wild-type background, this mutation reduces *SUC2* transcription by ~20-fold to a level at or below that measured for a *snf5* mutant (Table 1).

We then examined MNase cleavage patterns in the *suc2-104* mutant background and discovered that the TATA box mutation has virtually no effect on *SUC2* chromatin structure, in spite of its strong effect on *SUC2* transcription (Fig. 4; cf. with Fig. 3). In addition, chromatin from a *snf5* (*hta1-htb1*) Δ strain carrying the *suc2-104* allele has a partly wild-type MNase cleavage pattern (Fig. 4, lanes 13–17), despite nearly undetectable levels of *SUC2* expression (Table 1). Therefore, the level of *SUC2* transcription does not determine *SUC2* chromatin structure. Rather, the chromatin structure of the *SUC2* promoter region is apparently determined by the presence or absence of SNF5 function.

Close comparison of Figures 3 and 4 reveals that MNase cleavage at a site near the TATA box (site F) is slightly diminished in chromatin from *suc2-104* strains in comparison to chromatin from *SUC2* strains. This difference is likely the result of the mutation of a favored MNase cleavage site (TATAAA; Dingwall et al. 1981; Hörz and Altenburger 1981) to a less preferred site (GGTACC), as naked DNA derived from a *suc2-104* strain is also cleaved less efficiently at site F than is naked DNA from a *SUC2* strain (cf. Fig. 3, lane 2, with Fig. 4, lane 2). This difference in cleavage also suggests that site F is located at the TATA box.

Mutations in SNF2 also alter chromatin structure at SUC2

We have shown that mutations in *SNF5* cause changes in chromatin structure in the *SUC2* promoter region. Because the SNF5 protein is thought to be functionally related to at least four other proteins, SNF2, SNF6, SWI1, and SWI3, we wanted to test whether a mutation in another member of this group would also cause changes in chromatin structure at *SUC2*. SNF2 is of particular in-

Figure 3. *snf5* mutants have altered chromatin structure over the *SUC2* promoter region. Chromatin from the indicated strains was digested with differing amounts of MNase and subjected to indirect end-labeling analysis as described in Materials and methods. The probe used was in the 5'-coding region of *SUC2* adjacent to a *HinfI* site. Approximate positions of promoter elements (Sarokin and Carlson 1984) and MNase cleavage sites in the *SUC2* promoter region are indicated at left; positions of size markers are indicated at right. Sources of DNA were as follows: Naked DNA from FY120 (lanes 1,2,23); chromatin from FY120 (lanes 3–7); chromatin from FY711 (lanes 8–12); chromatin from FY714 (lanes 13–17); and chromatin from FY710 (lanes 18–22). Chromatin from each strain was digested with 0, 1, 3, 10, or 30 units of MNase. Naked DNA was digested as follows: (Lanes 1,23) 0.3 units; (lane 2) 1 unit.

terest because it is evolutionarily conserved throughout eukaryotes and contains motifs found in helicases (Davis et al. 1992; Laurent et al. 1992; Tamkun et al. 1992). Therefore, we used indirect end-labeling to examine chromatin structure in *snf2* and *snf2 (hta1-htb1)*Δ strains (Fig. 5).

Mutations in *SNF2* cause essentially the identical changes in the chromatin structure of the *SUC2* promoter region as do mutations in *SNF5* (cf. Fig. 5 with Fig. 3). In particular, in *snf2* mutants, sites B, C, E, and F are protected from cleavage, whereas cleavage at site A is induced. *snf2 (hta1-htb1)*Δ chromatin is nearly identical to *snf5 (hta1-htb1)*Δ chromatin; cleavage at sites B, C, E, and F is partially restored, whereas cleavage at site A is reduced. These data, combined with the other phenotypic similarities of *snf2* and *snf5* mutants, argue that

SNF2 and *SNF5* play a similar role in activating transcription by altering chromatin structure.

Discussion

Our results indicate that *SNF2* and *SNF5* serve as transcriptional activators by overcoming the repressive effects of chromatin structure. This conclusion is based on three lines of evidence. First, mutations in these *SNF* genes are suppressed by a deletion of one of the two loci encoding histones H2A and H2B, *(hta1-htb1)*Δ. Second, *snf2* and *snf5* mutants have altered chromatin structure at the *SUC2* promoter; these alterations are partly reversed by *(hta1-htb1)*Δ. Finally, a mutation of the *SUC2* TATA box has no effect on the chromatin structure of the *SUC2* promoter region, even though it reduces tran-

Figure 4. Mutation of the *SUC2* TATA box does not affect chromatin structure at *SUC2*. Indirect end-labeling analysis was performed as described in the legend to Fig. 3. Sources of DNA were as follows: Naked DNA from FY716 (lanes 1,2,23); chromatin from FY716 (lanes 3–7); chromatin from FY719 (lanes 8–12); chromatin from FY721 (lanes 13–17); and chromatin from FY718 (lanes 18–22). All strains contained the TATA mutation *suc2-104*. Amounts of MNase were as described in the legend to Fig. 3, except lane 23 was digested with 1 unit of MNase.

Figure 5. *snf2* mutants have altered chromatin structure over the *SUC2* promoter region. Indirect end-labeling analysis was performed as described in the legend to Fig. 3. Sources of DNA were as follows: Naked DNA from FY120 (lanes 1,2,18); chromatin from FY120 (lanes 3–7); chromatin from FY458 (lanes 9–12); chromatin from FY724 (lanes 13–17). Chromatin from each strain was digested with 0, 1, 3, 10, or 30 units of MNase. Naked DNA was digested as follows: (Lane 1) 0.3 units; (lanes 2,18) 1 unit.

scription of *SUC2* to a level at or below that measured for a *snf2* or *snf5* mutant. Thus, the differences in the level of *SUC2* transcription between wild-type and *snf* mutant strains are not responsible for the differences in chromatin structure between these strains. Rather, these results strongly suggest that SNF2 and SNF5 act directly on chromatin structure and that the resulting changes in chromatin structure cause changes in transcription.

MNase cleavage patterns provide the direct evidence that these SNF proteins activate transcription by altering chromatin structure. MNase has been shown to cleave preferentially the regions between nucleosomes; partial MNase digestion of bulk chromatin reveals a ladder of DNA fragments that are integral multiples of a nucleosome-sized fragment (for review, see van Holde 1988). Comparison of chromatin from different strains shows that in *snf2* and *snf5* mutants there is reproducibly much less cleavage by MNase at sites near the TATA box and between the TATA box and UAS than there is in *SNF*⁺ strains. In addition, cleavage is induced in these mutants (possibly by the adjacent binding of a nucleosome) just 3' to the UAS at a site that is not cut in naked DNA. Because these changes in *snf* mutants are mostly reversed by decreasing histone gene dosage, they are consistent with nucleosomes being present in *snf2* and *snf5* chromatin but absent, positioned differently, or otherwise modified in wild-type chromatin (Fig. 6). In addition, the distances between sites that are not protected from MNase cleavage in *snf* mutants are consistent with the size of a nucleosome.

Our data suggest that chromatin must be in an active

configuration over the *SUC2* promoter region for *SUC2* transcription to occur. In this model, the SNF and SWI proteins establish or maintain an active chromatin structure so as to allow increased accessibility of the *SUC2* promoter for binding of transcription factors. These could include general transcription factors (such as the TATA box-binding protein TFIID) and/or other as-yet-unidentified proteins required for *SUC2* transcription. One simple model that is consistent with our data is that the SNF and SWI activators function by removing or otherwise modifying nucleosomes to increase accessibility of the TATA box to TFIID (Fig. 6). This model is appealing because our results using strains without a functional *SUC2* TATA box demonstrate that SNF-dependent alterations in chromatin structure do not require a functional TFIID-binding site, suggesting that these chromatin structure changes precede TFIID binding.

Recently, Matallana et al. (1992) also examined the chromatin structure at *SUC2* in particular mutants, including *snf2* and *snf5* strains. Their general conclusions are consistent with ours (this paper and our unpublished data): Chromatin is altered in *snf* strains and appears similar in structure to the chromatin of wild-type cells grown under repressing conditions. However, we cannot make specific comparisons between the two sets of results, because many of the sites at which we observed alterations in MNase cleavage were not clearly detected in their experiments.

Previous studies suggest that the SNF and SWI proteins may have another role in activating transcription in addition to their effects on chromatin structure. These

Figure 6. A model for SNF2 and SNF5-dependent activation of *SUC2* transcription. Possible chromatin structures of the *SUC2* promoter region in different strains are depicted schematically. Approximate locations of MNase cleavage sites in chromatin from a *snf2* or *snf5* mutant (a) and from a *SNF*⁺ strain (b) are indicated by the arrows below each line; a small arrow represents reduced cleavage by MNase. The solid ovals represent nucleosomes; the broken ovals represent nucleosomes that have been removed or modified so as to increase accessibility of the *SUC2* promoter region. SNF and SWI proteins could help in either establishment or maintenance of the chromatin structure shown in b.

studies showed that LexA–SNF hybrid proteins can activate transcription of a reporter gene whose promoter contains a *lexA* operator (Laurent et al. 1990, 1991; Laurent and Carlson 1992), indicating that SNF proteins may also activate transcription by directly stimulating the transcription machinery. However, these results are also consistent with the possibility that SNF proteins activate transcription solely by altering chromatin structure, because the *lexA* reporter plasmid is almost certainly assembled into chromatin in the cell. By this model, LexA–SNF hybrid proteins could activate transcription by changing the chromatin structure of the reporter plasmid to an active configuration that is accessible to the transcriptional machinery. More detailed biochemical studies of SNF and SWI proteins will be required to distinguish between these and other possibilities.

SNF2 and SNF5 both contain regions that could directly modulate chromatin structure. *SNF2* encodes an evolutionarily conserved putative helicase; helicase activity could affect nucleosome stability, perhaps through local changes in supercoiling. This possibility is consistent with models discussed by Travers (1992). *SNF5* encodes a protein that is glutamine- and proline-rich (Laurent et al. 1990), a characteristic of some transcriptional activators (Mitchell and Tjian 1989). Sp1, which has been shown to overcome repression by chromatin in vitro (Croston et al. 1991), requires a glutamine-rich domain to activate transcription (Courey and Tjian 1988). Therefore, glutamine- and proline-rich regions may function as activation domains by altering chromatin structure.

The precise sites and mechanisms of action of the SNF and SWI proteins at the *SUC2* promoter are unknown. SNF and SWI proteins may activate transcription by directly altering chromatin structure between the UAS and the TATA box and over the TATA box. However, the changes observed in either of these regions could be indirect effects of other proteins binding to the *SUC2* promoter region. For example, the SNF and SWI proteins might enable the binding of an as-yet-unidentified activator to the chromatin in the *SUC2* UAS, either by altering chromatin structure in the UAS or by increasing the binding affinity of this activator for nucleosomes. The binding of this activator might then alter chromatin structure in adjacent promoter regions, in a manner similar to the effect of GRF2 binding to the *GAL1–GAL10* intergenic region (Fedor et al. 1988).

Other proteins have been proposed to activate transcription by increasing the accessibility of promoter regions for transcription factors. For example, Fascher et al. (1990) have shown that activation of *PHO5* transcription may involve removal of nucleosomes by the PHO4 transcriptional activator. Because SNF and SWI proteins are also required for *PHO5* expression (Abrams et al. 1986), PHO4 may function by recruiting SNF and SWI proteins to the *PHO5* promoter region, where they could cause changes in chromatin structure. Alternatively, SNF and SWI proteins may interact with the PHO4 protein or alter the *PHO5* chromatin structure to allow the

binding of PHO4 to nucleosomes in the *PHO5* promoter region. Similar roles for SNF and SWI proteins in aiding many different gene-specific transcriptional activators to function have been proposed previously (Laurent and Carlson 1992; Peterson and Herskowitz 1992).

The mechanisms by which SNF and SWI proteins activate transcription do not appear to be unique to yeast. SNF2 has extensive similarity to proteins in other eukaryotes, including the *Drosophila* transcriptional activator *brahma* (Tamkun et al. 1992). The *brahma* protein may activate transcription of homeotic genes by overcoming a repressive chromatin structure maintained by a large group of proteins, including Polycomb (for review, see Paro 1990). The Polycomb group of proteins may be analogous to a group of yeast SPT proteins that includes histones H2A and H2B (Swanson and Winston 1992). Thus, like the SNF and SWI proteins, *brahma* may activate transcription by overcoming repression mediated by chromatin. Finally, the *Drosophila* transcriptional activators *fushi tarazu* and *Bicoid* both require SNF and SWI proteins to function in yeast (Laurent and Carlson 1992; Peterson et al. 1992), further suggesting the presence of SNF and SWI functional homologs in other organisms.

One appealing possible role for SNF and SWI proteins would be to remove one or both H2A–H2B dimers from nucleosomes, perhaps leaving an H3–H4 tetramer bound to the DNA. This possibility is particularly intriguing because *snf/swi* mutations can be suppressed by a deletion of one of the two gene pairs encoding histones H2A and H2B, which probably results in lower levels of these histones in vivo. Dong and van Holde (1991) have shown that H3–H4 tetramers can be positioned on DNA in a manner similar to intact nucleosomes and that MNase cleavage of tetramer-bound DNA is enhanced relative to cleavage of nucleosomes. In addition, Baer and Rhodes (1983) have shown that RNA polymerase binds preferentially to nucleosomes that are deficient in H2A and H2B. Finally, histone octamers, but not H3–H4 tetramers, efficiently repress 5S RNA transcription in vitro (Tremethick et al. 1990; Almouzni et al. 1991). This model also raises the possibility that proteins functionally related to histones, such as the acidic SPT5 and SPT6 proteins (Swanson et al. 1990, 1991), could repress transcription by stabilizing the interaction of H2A–H2B dimers with DNA-bound H3–H4 tetramers, in a manner analogous to that of the acidic chromatin assembly factor nucleoplasmin (Dingwall et al. 1987). Other proteins implicated in affecting chromatin structure, including SIN1/SPT2, SPT4, and SPT16 (Kruger and Herskowitz 1991; Malone et al. 1991; Swanson and Winston 1992), might also affect histone–histone or histone–DNA interactions.

In conclusion, we have presented genetic and molecular data strongly suggesting that the yeast SNF2 and SNF5 transcriptional activators function by causing changes in chromatin structure. Because these SNF and SWI proteins control transcription of many different genes in yeast, this mechanism of activation appears to be widespread. Further genetic, molecular, and biochemical analyses will be necessary to elucidate the precise functions of these proteins and of other proteins that

may regulate chromatin structure. Finally, the strong evolutionary conservation of SNF2 and of histones indicates that an understanding of the mechanism by which these proteins control gene expression in yeast will contribute to the understanding of gene expression throughout eukaryotes.

Materials and methods

Strains and genetic methods

Yeast strains are listed in Table 2 and are isogenic to the strain S288C, except that they are *GAL2*⁺ [C. Dollard and F. Winston, unpubl.]. Uppercase letters denote the dominant (wild-type) allele, and lowercase letters denote the recessive (mutant) allele. Standard methods for mating, sporulation, and tetrad analysis were used [Rose et al. 1990]. A strain containing the (*hta1*–*htb1*)Δ::*LEU2* allele was constructed by transformation with the 3.8-kb *Bam*HI–*Nhe*I fragment of pJH8. *Leu*⁺ transformants were screened for suppression of the insertion mutations *his4*–*9128* and *lys2*–*1288*. A strain containing the *suc2*–*101*::*URA3* allele was constructed by transformation with the 2.2-kb *Hin*dIII fragment of pJH38. A strain containing the *suc2*–*104* allele was constructed by transforming strain FY637 with the 0.9-kb *Eco*RI–*Hin*dIII fragment of pJH42 and selecting for resistance to 5-fluoro-orotic acid. Strains carrying the *snf4*Δ1 [Celenza et al. 1989] and *snf6*Δ2 [Estruch and Carlson 1990] null alleles were kindly provided by M. Carlson (Columbia University, New York). Strains carrying the *snf1*Δ10, *snf2*Δ1::*HIS3*, and *snf5*–*5*::*URA3* null alleles were constructed as described [Abrams et al. 1986; Celenza and Carlson 1989]. During the course of these studies, we noticed that *snf5* mutants occasionally acquired weak second-site suppressors [J. Hirschhorn and F. Winston,

unpubl.]. These suppressors were detected by weak suppression of the insertion mutations *his4*–*9128* and *lys2*–*1288* and had no detectable effect on MNase cleavage patterns [J. Hirschhorn and F. Winston, unpubl.]. Nevertheless, the *snf5* strains used in this study were put through crosses to confirm the absence of additional mutations and were carefully monitored to ensure that phenotypes of strains grown in cultures were unchanged. For all alleles constructed in this study, transformants were shown to be correct by Southern hybridization analysis.

Yeast strains were transformed by the lithium acetate method as described [Rose et al. 1990]. *Escherichia coli* HB101 [Ausubel et al. 1988], TB1 [Bethesda Research Laboratories, Gaithersburg, MD], and GM237 [Arraj and Marinus 1983] were transformed as described [Ausubel et al. 1988].

Media

All media were made as described [Rose et al. 1990]. YEP plus 0.05% glucose was identical to YPD except that the media contained 0.05% glucose instead of 2% glucose as the carbon source. YPsuc, YPrff, and YPgal media contained 2% sucrose, raffinose, or galactose, respectively, as the sole carbon source and contained 1 mg/liter of antimycin A. Solid media lacking inositol were made as described [Sherman et al. 1978].

Enzymes and protease inhibitors

Restriction enzymes, T4 DNA ligase, DNA polymerase I (Klenow fragment) and *Taq* polymerase were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN) and from New England BioLabs (Beverly, MA). MNase was purchased from Boehringer Mannheim Biochemicals and stored at 6000 U/ml at –20°C in 50% glycerol, 2 mM CaCl₂, and 50 mM Tris (pH 9.0). All enzymes were used according to the instructions of the supplier. All protease inhibitors were purchased from Sigma (St. Louis, MO), and stocks were prepared and stored according to the instructions of the supplier.

DNA preparation and analysis

Yeast genomic DNA [Hoffman and Winston 1987] and plasmids from *E. coli* [Ausubel et al. 1988] were prepared as described previously. Analysis and purification of DNA restriction fragments were performed as described previously [Swanson et al. 1991]. Southern blot hybridization analysis was performed as described in the instruction manual for GeneScreen [New England Nuclear, Boston, MA] using the dextran sulfate method.

Plasmids

The construction of a plasmid for generation of the (*hta1*–*htb1*)Δ::*LEU2* allele involved several steps. First, the *Hin*dIII site in pUC18 was changed to an *Nhe*I site by digestion with *Hin*dIII, filling in with Klenow, and religation, creating the plasmid pJH1. pJH1 was digested with *Bam*HI and *Hinc*II, and a 1.6-kb *Bam*HI–*Hpa*I *HTA1*–*HTB1* fragment from pCC72 [C. Clark and F. Winston, unpubl.] was inserted, creating plasmid pJH3. This *HTA1*–*HTB1* fragment extends from the *Hpa*I site 3' to *HTA1* to the *Bam*HI site 3' to *HTB1* but lacks the 1.1-kb *Hin*dIII promoter-containing fragment and 329 bp immediately adjacent to the *Bam*HI site [M. Osley, pers. comm.]. After passage through the *E. coli* strain GM237 (*dam*[–]), pJH3 was digested with *Xba*I and *Hin*dIII, and the *LEU2* gene was inserted into these sites, creating plasmid pJH8. For this insertion of *LEU2*, a 2.2-kb *Sal*I–*Xho*I fragment from YEp13 containing the *LEU2* gene was first cloned into the *Sal*I site of a derivative of

Table 2. *S. cerevisiae* strains

Strain	Relevant genotype ^a
FY56	<i>MAT</i> α
FY120	<i>MAT</i> α
FY710	<i>MAT</i> α (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY738	<i>MAT</i> α (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY722	<i>MAT</i> α <i>snf1</i> Δ10
FY723	<i>MAT</i> α <i>snf1</i> Δ10 (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY737	<i>MAT</i> α <i>snf2</i> Δ1:: <i>HIS3</i> <i>his3</i> Δ200 <i>his3</i> Δ200
FY458	<i>MAT</i> α <i>snf2</i> Δ1:: <i>HIS3</i>
FY724	<i>MAT</i> α <i>snf2</i> Δ1:: <i>HIS3</i> (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
L898	<i>MAT</i> α <i>snf4</i> Δ1
L899	<i>MAT</i> α <i>snf4</i> Δ1 (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY711	<i>MAT</i> α <i>snf5</i> – <i>5</i> :: <i>URA3</i>
FY712	<i>MAT</i> α <i>snf5</i> – <i>5</i> :: <i>URA3</i>
FY713	<i>MAT</i> α <i>snf5</i> – <i>5</i> :: <i>URA3</i> (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY714	<i>MAT</i> α <i>snf5</i> – <i>5</i> :: <i>URA3</i> (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
L896	<i>MAT</i> α <i>snf6</i> Δ2
L897	<i>MAT</i> α <i>snf6</i> Δ2 (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY637	<i>MAT</i> α <i>suc2</i> – <i>101</i> :: <i>URA3</i>
FY716	<i>MAT</i> α <i>suc2</i> – <i>104</i>
FY718	<i>MAT</i> α <i>suc2</i> – <i>104</i> (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>
FY719	<i>MAT</i> α <i>suc2</i> – <i>104</i> <i>snf5</i> – <i>5</i> :: <i>URA3</i>
FY721	<i>MAT</i> α <i>suc2</i> – <i>104</i> <i>snf5</i> – <i>5</i> :: <i>URA3</i> (<i>hta1</i> – <i>htb1</i>)Δ:: <i>LEU2</i>

^aAll strains also contain the alleles *his4*–*9128*, *lys2*–*1288*, *ura3*–*52*, and *leu2*Δ1, except for FY56 and FY458, which are *LEU2*, and FY737, which is *HIS4*. All strains were constructed for this study.

pUC18 (provided by D. Eisenmann, Stanford University, CA) and then excised by digestion with *Xba*I and *Hind*III. For construction of the *suc2-101::URA3* allele, the 1.1-kb *Hind*III fragment containing the *SUC2* promoter from pRB58 (Carlson and Botstein 1982) was cloned into pUC18, creating plasmid pJH35. pJH35 was then digested with *Mlu*I and *Nco*I, and a 1.1-kb *Hind*III fragment containing *URA3* was inserted between these sites after all sites were filled in with Klenow, creating plasmid pJH38. For construction of the *suc2-104* allele, the 0.9-kb *Eco*RI–*Hind*III *SUC2* promoter fragment of pRB58 was cloned into mp19, and the TATA box at –133 (TATAAA) was changed to a *Kpn*I site (GGTACC) by site-directed mutagenesis (Kunkel 1985) using the oligonucleotide 5'-CCTTCCTCCTGAAA-GAAACAGGTACCTAGATATGTATTATTCTTC. The resulting *Eco*RI–*Hind*III fragment was cloned into pUC19, creating plasmid pJH42.

Hybridization probes

Plasmids used as probes for RNA analysis were as follows: for Ty, B161 (Happel et al. 1991); for *TUB2*, pYST138 (Som et al. 1988); for *TP11*, pHB59 (H. Baker, pers. comm.). Probes used for RNA analysis and indirect end-labeling of *SUC2* were DNA fragments generated using the polymerase chain reaction (PCR) essentially as described (Ausubel et al. 1988) with pRB58 as a template. For mRNA analysis, the probe extended from +787 to +966, and for indirect end-labeling, the probe extended from +140 to +296, relative to the start of translation. ³²P-Labeled plasmids were prepared by nick translation using a kit from Boehringer Mannheim Biochemicals. ³²P-Labeled DNA fragments were prepared by primer extension with Klenow essentially as described for radiolabeling DNA fragments using random hexamers (Ausubel et al. 1988), except that 50 pmoles each of the two oligonucleotides that had been used for PCR were used as primers.

RNA preparation and analysis

For analysis of Ty mRNA, yeast RNA was prepared as described previously (Carlson and Botstein 1982) from cells grown in YPD to a density of 1×10^7 to 2×10^7 cells/ml. For analysis of *SUC2* mRNA, cells were grown in 10 ml of YPD to a density of 6×10^6 to 1×10^7 cells/ml, washed twice with 10 ml of water, and grown in 10 ml of YEP plus 0.05% glucose for 2.75 hr. Electrophoresis, transfer to GeneScreen (New England Nuclear), and hybridization analysis were performed as described previously (Swanson et al. 1991), except that for *SUC2* mRNA analysis, the wash conditions were two washes of 250 ml $2 \times$ SSC at 23°C for 15 min, followed by two washes of 250 ml $2 \times$ SSC, 0.5% SDS, at 57°C for 15 min. The amount of RNA in each lane was standardized by hybridization to pYST138 containing *TUB2* or to pHB59 containing *TP11*.

Invertase assays

Secreted invertase activity was determined by assaying whole cells (Goldstein and Lampen 1975), as described previously by Celenza and Carlson (1984). Glucose-repressed cultures were grown in YPD at 30°C to a density of 6×10^6 to 1×10^7 cells/ml. Derepressed cultures were prepared by washing these cultures twice with water, shifting to an equal volume of YEP plus 0.05% glucose, and growing at 30°C for 2.75 hr. For calculation of units of activity, cell number was converted to dry weight of cells; 1×10^7 cells are equivalent to 0.38 mg of cells, dry weight (Swanson and Winston 1992).

Preparation of yeast chromatin

Chromatin was prepared using a modified version of a protocol provided by B. Dunn (pers. comm.) One liter of cells was grown in YPD at 30°C to a concentration of 6×10^6 to 1×10^7 cells/ml. Cultures were washed twice with 330 ml of water and resuspended in 1 liter of YEP plus 0.05% glucose. After 2.75 hr of further growth at 30°C to relieve glucose repression, cells were washed with 330 ml of water and the cell pellet was weighed. Cells were washed with 30 ml of cold DTT solution (10 mM DTT, 20 mM potassium phosphate at pH 7.0, 1 M Sorbitol) and resuspended in 4 ml/gm cell weight of S buffer (1.1 M Sorbitol, 20 mM potassium phosphate at pH 7.0, 0.5 mM CaCl_2) containing 1 mg/ml of Zymolyase T100 (50,000 U/mg) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF; Sigma). Cells were shaken at 95 rpm at 30°C for 35 min; >95% of cells were converted to spheroplasts as monitored by lysis in a 50-fold excess of water. All subsequent steps were performed at 4°C, and all buffers contained protease inhibitors as described by Norris et al. (1988). SPC and Ficoll buffers are as described by Nelson and Fangman (1979). Spheroplasts were harvested by centrifugation in a Sorvall SA600 rotor (DuPont, Wilmington, DE) at 5000 rpm for 5 min and washed with 15 ml of SPC buffer. Spheroplasts were gently resuspended in 0.5 ml of SPC buffer and lysed by addition to 25 ml of Ficoll buffer with gentle stirring over 15 sec. The solution was poured back into the original tube and allowed to settle for 10 min. "Supernatant" (23.5 ml) was transferred to a new tube and centrifuged in an SA600 rotor at 13,200 rpm for 20 min. The pellet was resuspended in 17 ml of SPC buffer and recentrifuged in an SA600 rotor at 10,100 rpm for 10 min. The pellet, which contains nuclei, was resuspended in 2 ml/gm cell weight of SPC buffer, frozen on dry ice, and stored at –70°C.

Indirect end-labeling analysis of yeast chromatin

MNase (0, 1, 3, 10, or 30 units) was added to 200 μ l of nuclei and incubated at 37°C for 10 min. For naked DNA controls, yeast genomic DNA from ~8 ml of a saturated culture was brought to 1 M Sorbitol, 20 mM PIPES (pH 6.3), and 10 mM CaCl_2 (SPC plus 10 mM CaCl_2) in a final volume of 200 μ l before incubation with 0.3 or 1 unit of MNase at 37°C for 10 min. The excess CaCl_2 was required to overcome the chelation of divalent cations by DNA that had been treated with EDTA. Reactions were stopped by the addition of 40 μ l of 5 M NaCl, 8 μ l of 0.5 M EDTA (pH 8.0), and 20 μ l of 10% SDS. DNA was purified as described by Bloom and Carbon (1982), except that an additional extraction with chloroform/isoamyl alcohol was performed before the first ethanol precipitation and DNA was resuspended in 20 μ l of TE at the end. For subsequent restriction digestion and Southern analysis, 9 μ l was used; electrophoresis was through 2% Seakem ME agarose (FMC BioProducts, Rockland, ME), and subsequent steps were performed as according to the dextran sulfate method of the GeneScreen manual, except that filters were washed three to four times for 15 min with 500 ml of $2 \times$ SSC, 1% SDS, at 57°C. For the experiments shown, the restriction enzyme *Hinf*I was used; essentially identical results were obtained when different restriction enzymes and probes were used to study MNase cleavage patterns (data not shown).

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