Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription

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Transcription of many yeast genes requires the SWI/SNF regulatory complex. Prior studies show that reduced transcription of the *HO* gene in *swi* and *snf* mutants is partially relieved by mutations in the *SIN1* and *SIN2* genes. Here we show that *SIN2* is identical to *HHT1*, one of the two genes coding for histone H3, and that mutations in either can result in a Sin$^-$ phenotype. These mutations are partially dominant to wild type and cause amino acid substitutions in three conserved positions in the structured domain of histone H3. We have also identified partially dominant *sin* mutations that affect two conserved positions in the histone-fold domain of histone H4. Three *sin* mutations affect surface residues proposed to interact with DNA and may reduce affinity of DNA for the histone octamer. Two *sin* mutations affect residues at or near interfaces between (H2A–H2B) dimer and $(H3–H4)_2$ tetramer subunits of the histone octamer and may affect nucleosome stability or conformation. The ability of mutations affecting the structure of the histone octamer to relieve the need for SWI and SNF products supports the proposal that the SWI/SNF complex stimulates transcription by altering chromatin structure and can account for the apparent conservation of SWI and SNF proteins in eukaryotes other than yeast.

[*Key Words*: chromatin; *HO* gene; RNA polymerase carboxy-terminal domain]

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A major challenge in studies of gene regulation is to explain the roles played by chromatin structure in governing gene expression (for review, see Paranjape et al. 1994; Wolffe 1994). The fundamental repeating unit of chromatin, the nucleosome core particle, comprises 146 bp of DNA wrapped around a protein core, the core histone octamer (Kornberg 1974). The core histone octamer in solution, free of DNA, behaves as a self-assembling tripartite protein assembly, containing two (H2A–H2B) heterodimers and one $(H3–H4)_2$ tetramer (Eickbush and Moudrianakis 1978). The crystallographic structure of the histone octamer at 3.1 Å resolution reveals that the assembly is tripartite, with two (H2A–H2B) dimers flanking a centrally located $(H3–H4)_2$ tetramer (Arents et al. 1991). All four types of histone chain in the octamer share a common structural motif, the histone fold, a highly helical domain of ~65 amino acid residues; three of the chains have additional extrafold helical segments. The four chains also contain a number of residues at their amino termini that are unstructured and not imaged in the crystal structure. A pattern of positively charged residues on the surface of the histone octamer matches the expected path of the negative charges of the DNA duplex (Arents and Moudrianakis 1993). This pattern is particularly notable for the surface of the $(H3–H4)_2$ tetramer.

Genetic analysis is providing in vivo information on how chromatin affects gene expression (for review, see Grunstein et al. 1992; Winston and Carlson 1992; Peterson 1994; Wolffe 1994). Several studies have shown the importance of the unstructured amino-terminal domains of histones H3 and H4 in yeast for viability (Morgan et al. 1991) and (for histone H4) for silencing of the *HML* and *HMR* loci (Kayne et al. 1988; Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990; Hecht et al. 1995). The amino-terminal segment of H4 is also necessary for

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efficient induction of *GAL1* and *PHO5* genes (Durrin et al. 1991; Wan et al. 1995).

The *SWI1*, *SWI2* (*SNF2*), *SWI3*, *SNF5*, and *SNF6* genes of yeast code for components of what appears to be a general transcription regulator that works in conjunction with gene-specific regulators (Winston and Carlson 1992; Peterson 1994; Carlson and Laurent 1994). Inactivating any of these genes leads to decreased transcription of *HO*, *SUC2*, *INO1*, *ADH1*, and *GAL1* genes 3- to 50-fold. Recent studies demonstrate that these SWI and SNF proteins assosciate to form a macromolecular complex (Cairns et al. 1994; Peterson et al. 1994).

Clues to the function of the SWI/SNF complex have come from identifying mutations in genes that bypass or partially relieve the transcriptional defects of *swi* and *snf* mutants. These observations led to the hypothesis that chromatin structure is the target of the SWI/SNF complex (Kruger and Herskowitz 1991; Hirschhorn et al. 1992; Peterson and Herskowitz 1992; Winston and Carlson 1992). In particular, mutations in the *SIN1* and *SIN2* genes partially relieve the requirement for *SWI1*, *SWI2*, and *SWI3* to transcribe the *HO* gene (Sternberg et al. 1987). The *SIN1* gene codes for a highly charged nuclear protein that binds nonspecifically to DNA and has a distinctive composition, suggesting that it may be a nonhistone chromatin protein like HMG1 (Kruger and Herskowitz 1991). Deletion of one of the two sets of genes coding for histones H2A and H2B partially relieves the requirement of the SWI/SNF complex for transcription of the *SUC2* gene (Hirschhorn et al. 1992).

In the present work we have characterized mutations of the *SIN2* gene that partially bypass the requirement for the SWI/SNF complex. We have found that *SIN2* is one of the two genes coding for histone H3 and that the *sin2* mutations cause amino acid substitutions in the histone-fold domain of histone H3. We have obtained similar mutations in the gene coding for histone H4. These observations support the hypothesis that the SWI/SNF complex functions by antagonizing the repressive constraints of chromatin structure.

Results

SIN2-1 *is a mutation in histone H3 gene* HHT1 *and is complemented by both histone H3 genes*

SIN2 was cloned by screening for a low-copy plasmid able to complement a *sin2-1* mutation. Because *sin2-1* is partially dominant to $SIN2^+$ (data not shown; see Table 1A), we expected to see only partial complementation by a plasmid carrying wild-type *SIN2*. One plasmid (designated pSINc) was obtained; its complementing activity resided on a 2.7-kb *Hin*dIII fragment (pH-2) (Fig. 1) containing the *HHT2* gene, one of two genes coding for histone H3 in yeast (Smith and Andrésson 1983). Segregation analysis (see Materials and methods) demonstrated that *HHT2* and *SIN2* are different genes.

Because yeast has two genes coding for histone H3 (*HHT1* and *HHT2*), we anticipated that *sin2-1* might be an alteration of *HHT1*. To determine whether this is so, we recovered the *HHT1* locus from a *sin2-1* strain by gap repair (see Materials and methods). As shown in Table 1A, the *HHT1* locus recovered from a *sin2-1* strain exhibited this behavior: this plasmid increased *HO* expression in a *swi1* strain to 0.7 unit in comparison to an *HHT1* plasmid, which produced 0.1 unit. *sin2-1* is thus a mutation in the *HHT1* gene and is designated *hht1-1*. The Sin^- phenotype conferred by the *hht1-1* plasmid was not as strong as in the strain with a chromosomal *sin2-1* mutation (1.1 vs. 0.7 unit), perhaps because the latter strain contains fewer copies of the wild-type *HHT1* gene. We determined the nucleotide sequence of the *hht1-1* locus and found three differences from the previously published sequence (see Materials and methods; Smith and Andrésson 1983), only one of which resulted in an amino acid change, a substitution of histidine for arginine at position 116.

Table 1. *The* sin2-1 *mutation is an alteration of the* HHT1 *gene and can be complemented by both* HHT1 *and* HHT2

Strain (plasmid insert)		HO–LacZ Activity
A.	*swi1 SIN2* (none)	0.1
	swi1 SIN2 (*HHT1*)	0.1
	swi1 SIN2 (*hht1-1*)	0.7
	swi1 sin2-1 (none)	1.1
B.	*swi1 sin2-1* (none)	1.1
	swi1 sin2-1 (*HHT1*)	0.4
	swi1 sin2-1 (*HHT2*)	0.4
	swi1 SIN2 (none)	<0.1

β-Galactosidase activities from three independent transformants were assayed; averages are presented and varied <15%.
(A) Low-copy plasmids (pHHT1, pSinC, YCp50) containing the indicated inserts were introduced by transformation into *swi1*Δ *sin2-1 HO–lacZ* strain WK3-7c or into the *swi1*Δ SIN^+ *HO–lacZ* strain WK1-9a.
(B) Low-copy plasmids (pHHT1, pHHT1-1, YCp50) containing the indicated inserts were introduced into the *swi1*Δ *SIN2* strain WK1-9a or *swi1*Δ *sin2-1 Saccharomyces cerevisiae HO–lacZ* strain WK3-7c by transformation. The insert designated *hht1-1* contains the *HHT1* locus recovered from the *sin2-1* strain WK3-7c by repair of a gapped plasmid (see Materials and methods).

The chromosomal *sin2-1* mutation can be complemented equally well by both *HHT1* and *HHT2*: Low-copy plasmids carrying either gene reduced *HO* expression in the $swi1^-$ *sin2-1* strain from 1.1 to 0.4 unit (Table 1B). The level of *HO* expression in these strains is still greater than in the $swi1^-$ $SIN2^+$ strain (≤0.1 unit), indicating that *sin2-1* is partially dominant.

sin2-1 *and* sin1 *mutations result in the same set of transcriptional alterations*

sin1 mutations partially restore transcription of the *INO1* gene in strains with a truncation of the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II (Peterson et al. 1991). To determine whether *sin2-1* has similar ability, we measured *INO1*

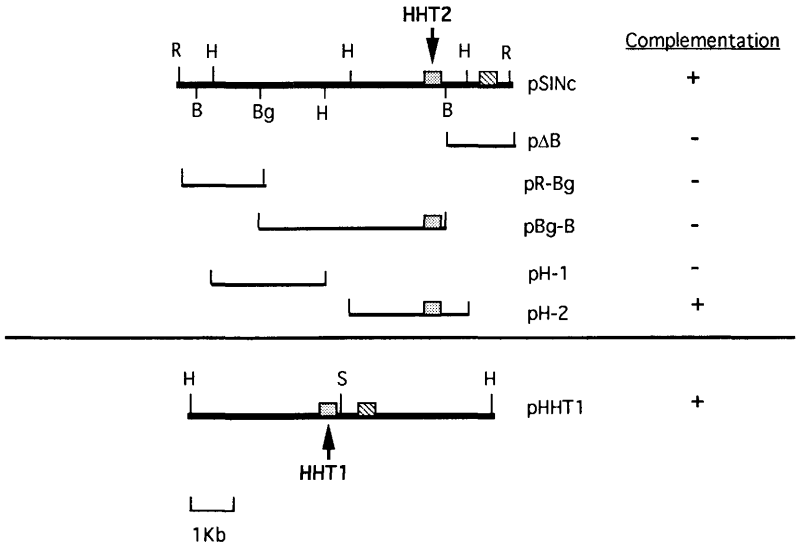

Figure 1. Structure of the plasmid inserts that partially complement *sin2-1* mutant strains. Restriction sites are: *Eco*RI (R), *Hin*dIII (H), *Bam*HI (B), *Bgl*II (Bg), and *Sma*I (S). Hatched rectangles indicate positions of histone H4 genes; stippled rectangles indicate positions of histone H3 genes (Smith and Andrésson 1983).

RNA levels in isogenic strains carrying wild-type or mutant alleles of *RPB1* (which codes for the largest subunit of RNA polymerase II) and plasmids with *sin2-1* or *sin1-2* mutations (a semidominant allele of *SIN1*), or no insert (Fig. 2). *INO1* transcripts, assayed by primer-extension analysis, were observed in the wild-type *RPB1* strain (lane 1), but not in the Sin$^+$ strain carrying the *RPB1* truncation (*rpb1-103*) (lane 2). *INO1* expression levels increased to ~15% of wild-type levels in the *rpb1-103* strain carrying *sin2-1* or *sin1-2* plasmids (lanes 3,4). The *sin2-1* mutation thus behaves like *sin1-2* in partially reversing the *INO1* transcriptional defect because of truncation of the CTD. The *sin1-2* and *sin2-1* mutations did not improve the growth of *rpb1-103* mutants on rich medium (C. Peterson, unpubl.).

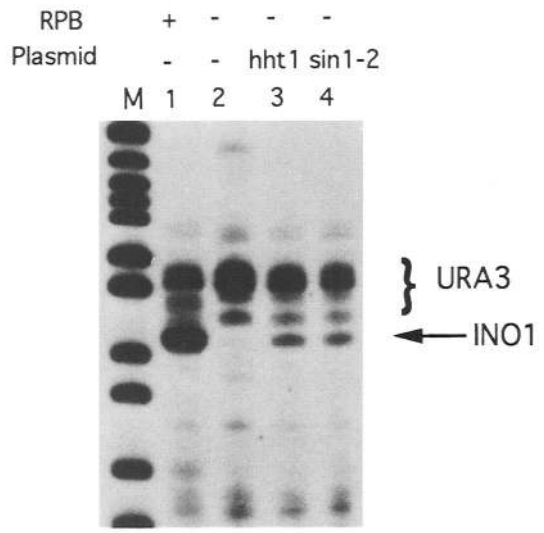

Figure 2. Partial suppression of *INO1* transcriptional defect of *rpb* mutants by *sin2-1*. Plasmids expressing either a wild-type *RPB1* gene (RP112) or *rpb1-103* (Ry2203), which has a truncated CTD, were introduced into JAY47. Plasmids that contained *sin2-1* (*hht1-1*; see Fig. 4), *sin1-2*, or no insert (YCp50 alone) were introduced into these strains, which were grown in glucose under inducing conditions for *INO1* transcription. RNA was isolated, and transcription of *INO1* was measured by primer-extension analysis as described in Materials and methods. Strains carried the plasmids indicated: (Lane *1*) RP112, YCp50; (lane *2*) Ry2203, YCp50; (lane 3) Ry2203, phht1-1; (lane *4*) Ry2203, psin1-2 (Kruger and Herskowitz 1991).

We also examined whether *sin2-1*, like *sin1-2*, could suppress the transcriptional defect caused by insertion of a yeast δ element in the 5′ region of *LYS2* (Winston et al. 1984). A strain containing a *lys2-128δ* allele (WK51-5d) exhibits a Lys$^-$ phenotype because insufficient *LYS2* transcript is produced (Simchen et al. 1984). Introduction of a *sin2-1* mutation into this strain on a low-copy plasmid conferred a Lys$^+$ phenotype (data not shown; see Fig. 3).

The *sin2-1* mutation thus behaves like the *sin1-2* mutation in several respects: Both partially relieve the requirement of *SWI1* for transcription of *HO*, the requirement of full-length CTD for transcription of *INO1*, and the effect of a δ element on transcription of *LYS2*.

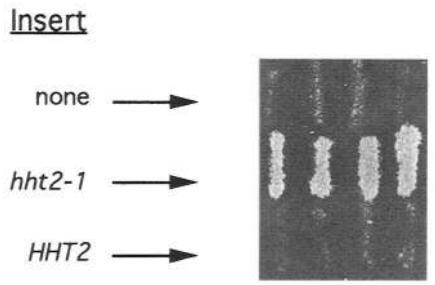

Figure 3. *hht2-1* causes an Spt$^-$ phenotype. The alleles described above were introduced into *swi1*$^-$ *hht2Δ lys2-128δ* strain WK51-5d on *URA3* plasmids. Four colonies from each transformation were tested for growth on minimal medium lacking lysine.

Introduction of the sin2-1 *change into* HHT2

As described above, both histone H3 genes partially complement the *sin2-1* mutation (Table 1B), an alteration in *HHT1*. The amino acid sequences of the H3 proteins coded by *HHT1* and *HHT2* are identical (Smith and Andrésson 1983), and either gene is sufficient for cell viability (Smith and Stirling 1988). Because of the apparent equivalence of *HHT1* and *HHT2*, we wished to determine whether a change from arginine to histidine at position 116 of H3 coded by *HHT2* would also cause a Sin$^-$ phenotype. A single base-pair change was introduced into *HHT2* to create the *hht2-1* allele, which should cause production of such an altered H3. A low-copy plasmid carrying this mutation was introduced into yeast strain WK51-5d, which is deleted for *HHT2* and contains appropriate markers to score both the Sin$^-$ and Spt$^-$ phenotypes (*swi1*$^-$ *lys2-128δ HHT1 hht2Δ*). Cells carrying the *hht2-1* plasmid exhibited a Lys$^+$ (Spt$^-$) phenotype (Fig. 3). These transformants also exhibited a Sin$^-$ phenotype (Table 2): The *hht2-1* plasmid increased *HO–lacZ* expression in the *swi1*$^-$ strain >10-fold. In contrast, the *swi1 hht2Δ* strain carrying a control plasmid with no insert expressed *HO* at low levels and was Lys$^-$ (Table 2; Fig. 3). We draw two conclusions from these observations: (1) Production of the mutant H3-Sin$^-$ protein from either *HHT1* or *HHT2* is sufficient to create both Sin$^-$ and Spt$^-$ phenotypes, and (2) simple deletion of *HHT2* is not sufficient to cause either the Sin$^-$ or Spt$^-$ phenotype.

Identification of additional mutations with a Sin$^-$ phenotype that affect histone H3

To identify additional changes in histone H3 which, like *sin2-1*, confer a dominant Sin$^-$ phenotype, we mutagenized plasmids containing the *HHT2* gene in vitro with hydroxylamine (see Materials and methods) and then introduced these plasmids into a *swi1Δ HHT1 HHT2 HO–lacZ* strain (CY240). Transformants were then screened for those that exhibited increased β-galactosidase activity in a plasmid-dependent manner. Plasmids from five transformants were obtained in this way.

The three plasmids that conferred a stronger Sin$^-$ phenotype contained the same mutation, designated *hht2-3*, which resulted in a threonine to isoleucine change at position 118. Both of the plasmids that gave the weaker Sin$^-$ phenotype contained the same mutation, designated *hht2-2*, which resulted in a glutamic acid to lysine change at residue 105. The *hht2-3* mutation yielded 2.0 unit of β-galactosidase activity; the *hht2-2* mutation exhibited 0.5 unit (Table 3). By comparison, the parent *HHT2* plasmid yielded <0.2 unit, and the *hht1-1* mutation yielded 0.8 unit. The apparent relative strengths of the different *sin* mutations should be viewed with some caution, as these mutations might affect plasmid copy number. A summary of the changes in histone H3 that cause a Sin$^-$ phenotype is shown in Figure 4. The three mutations (*hht2-1, hht2-2,* and *hht2-3*) affect residues conserved in all of the sequenced H3 genes (Wells and Brown 1991).

Table 2. HHT2 *containing the same mutation (*hht2-1*) as found in* sin2-1 *(*hht1-1*) exhibits a Sin$^-$ phenotype*

Strain (plasmid insert)	HO–LacZ activity
swi1 hht2Δ (none)	<0.1
swi1 hht2Δ (*hht2-1*)	1.0
swi1 hht2Δ (*HHT2*)	<0.1

The indicated inserts, carried on low-copy vector pRS314, were introduced into strain WK51-5d (*swi1Δ HO–lacZ lys2-128 hht2Δ*). β-Galactosidase assays were performed on three cultures of each strain; averages are presented and varied <15%.

Table 3. *Comparison of Sin$^-$ phenotypes of* hht1 *and* hht2 *mutations*

Strain (plasmid insert)	HO–LacZ activity
swi1 HHT1 HHT2 (*HHT2*)	<0.2
swi1 HHT1 HHT2 (*hht1-1*)	0.8
swi1 HHT1 HHT2 (*hht2-2*)	0.5
swi1 HHT1 HHT2 (*hht2-3*)	2.0
SWI1 HHT1 HHT2 (*HHT2*)	5.0

Low-copy plasmids (pSin2c, pHHT1-1, pHHT2-2, pHHT2-3, pHHT2) were introduced into *swi1Δ* strain WK1-9a or into *SWI1* strain WK50-4c by transformation; β-galactosidase activities from three separate transformants were measured. The *hht1-1* mutation is the original *sin2-1* mutation crossed onto a plasmid. Averages are presented and varied <15%.

Identification of mutations with a Sin$^-$ phenotype that affect histone H4

Given that the histone-fold domains of H3 and H4 are paired and thus clustered in a defined sector of the histone octamer, we wished to determine whether mutations affecting histone H4 could also yield a Sin$^-$ phenotype. Yeast has two histone H4 genes, *HHF1* and *HHF2*, which code for identical proteins (Smith and Andrésson 1983). Either is sufficient for viability (Smith and Stirling 1988).

We looked for mutations in *HHF2* that have a Sin$^-$ phenotype by a strategy similar to that used to identify Sin$^-$ mutations of *HHT2* (see Materials and methods) and isolated six independent plasmids. These plasmids increased HO–LacZ activity to 0.4 unit, clearly above the *HHF2*$^+$ background (≤0.1 unit) but only 5% of the level observed in the *SWI1*$^+$ strain (8.6 unit) (Table 4). Sequencing of the *HHF2* genes from these plasmids revealed a change from valine-43 to isoleucine in three mutants, from arginine-45 to histidine in two mutants, and from arginine-45 to cysteine in one mutant (Fig. 4). These mutations affect residues that are invariant among all sequenced histone H4 proteins (Wells and Brown 1991).

To determine whether the Sin$^-$ versions of histone H4 can support the essential functions of histone H4, we constructed strains in which the Sin$^-$ derivatives are the

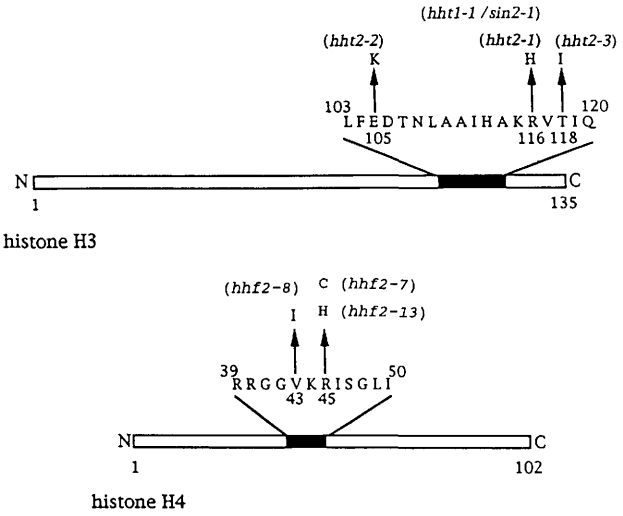

Figure 4. Amino acid changes in histones H3 and H4 resulting from *sin* mutations. The changes in histones H3 and H4 that give a dominant Sin$^-$ phenotype are indicated. Nucleotide changes in the H3 genes were as follows: *hht1-1* (*sin2-1*): CGT → CAT; *hht2-2*: GAA → AAA; *hht2-3*: ACT → ATT. Nucleotide changes in H4 genes were as follows: *hhf2-7*, CGT → TGT; *hhf2-8*, GTC → ATC; *hhf2-13*, CGT → CAT. Amino acid positions are numbered with alanine as the first residue of histone H3 and serine as the first residue of histone H4. Sequence information is from Smith and Andrésson (1983).

sole source of H4. This was accomplished using the procedure of Kim et al. (1988), in which the chromosomal *HHF1* and *HHF2* genes are deleted; viability of the strain is maintained by expression of *HHF2* from the *GAL1* regulatory region. We have introduced a second, low-copy plasmid into this strain, which carries a wild-type H4 gene, a histone H4 gene with a *sin* mutation, or no H4 gene. In the latter case, the yeast strain grew on galactose-containing medium but not on glucose medium (Fig. 5). The strain carrying a wild-type H4 gene grew equally well on both media. Two of the mutant H4 derivatives (*hhf2-13* [R → H] and *hhf2-8* [V → I]) allowed growth on glucose medium, indicating that they were able to function well as sole source of histone H4. The third mutant (*hhf2-7* [R →C]) grew considerably less well on glucose medium. The ability of these Sin$^-$ versions of H4, especially *hhf2-8* and *hhf2-13*, to support viability indicates that these proteins must be competent for assembly of histone octamers.

These mutations did not appear to cause chromosome loss, as judged by inability of α cells carrying these mutations to mate as **a** or to produce α-factor (see Materials and methods).

Discussion

We describe a number of single amino acid substitutions in histones H3 and H4 that affect gene expression in specific ways. In particular, these altered histones partially relieve the requirement of a general transcription factor (SWI/SNF) for transcription of the *HO* gene. Analysis of one of the mutations affecting histone H3 shows that it also partially relieves two other transcriptional defects (one resulting from a truncated CTD, the other from the presence of a δ element). The *sin* mutations described here cause amino acid replacements in the histone-fold domains of histones H3 and H4 affecting three positions in H3 and two positions in H4. These findings have several implications. First, they identify specific sites within the structured domains of H3 and H4 that are necessary for nucleosome function. Second, they support the hypothesis that the SWI/SNF complex functions by altering chromatin structure. Finally, because the *sin* mutations are partially dominant and affect highly conserved proteins, it may be possible to exploit our findings to isolate Sin$^-$ derivatives of H3 and H4 proteins from other organisms.

SWI/SNF may activate transcription by altering chromatin structure

The finding that mutations in genes encoding histones or an HMG-like protein can relax the requirement for the SWI/SNF complex led to the hypothesis that SWI/SNF may function by antagonizing the repressive effects of chromatin (Kruger and Herskowitz 1991; Hirschhorn et al. 1992; Peterson and Herskowitz 1992; Winston and Carlson 1992). This hypothesis was strongly supported by results demonstrating that *swi2/snf2* and *snf5* mutations cause alterations in chromatin structure and that these alterations are suppressed by deletion of one of the two H2A/H2B gene pairs (Hirschhorn et al. 1992). Recent biochemical studies support this hypothesis: SWI/SNF complexes from yeast and from mammalian cells can stimulate binding of activator and basal transcription proteins to nucleosomal DNA in vitro (Côté et al. 1994; Imbalzano et al. 1994; Kwon et al. 1994).

How might chromatin structure control transcription of the *HO* gene? It is thought that the SIN products govern utilization of binding sites for the SWI4/SWI6 transcription factor in the *HO* upstream region (Andrews and Herskowitz 1989; Kruger 1991). These SWI4/SWI6-dependent upstream activating sequence (UAS) sites are nonfunctional in *swi1*, *swi2*, or *swi3* mutants but are

Table 4. *Sin$^-$ phenotype of* HHF2 *mutations*

Strain (plasmid insert)	HO–LacZ activity
swi1 HHF1 HHF2 (*HHF2*)	<0.1
swi1 HHF1 HHF2 (*hhf2-7*)	0.4
swi1 HHF1 HHF2 (*hhf2-8*)	0.4
swi1 HHF1 HHF2 (*hhf2-13*)	0.4
SWI1 HHF1 HHF2 (*HHF2*)	8.6

Low-copy plasmids (CP326, CP327, CP328) were introduced into *swi1*Δ strain CY240 or *SWI1* strain CY236 by transformation; β-galactosidase activities from two independent transformants were measured. In a separate experiment, two additional transformants of each strain showed similar activities. Averages are presented and varied <10%.

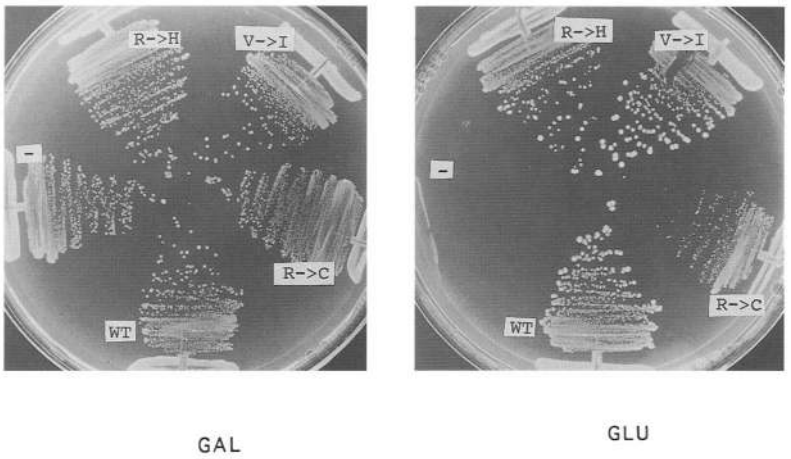

Figure 5. Growth ability of strains using histone H4-Sin$^-$ as sole histone H4. Plasmids carrying a wild-type histone H4 gene (WT; pUK499), no histone H4 gene (−), or various histone H4–Sin$^-$ mutations (*hhf2-7*, R → C; *hhf2-8*, V → I; *hhf2-13*, R → H) were introduced into a strain (UKY412) lacking chromosomal histone H4 genes but carrying a wild-type histone H4 gene under galactose control (pUK421). (*A*) Growth ability on minimal medium with galactose as carbon source. (*B*) Growth ability on minimal medium with glucose as carbon source. Plates were incubated at 30°C.

functional if the *swi* mutant also carries a *sin1* or *sin2* mutation. We thus propose that in the wild-type situation, the SWI/SNF complex relieves repression exerted on the SWI4/SWI6-binding sites by the structure of chromatin.

Promoters enfeebled by insertion of a δ element appear to be very sensitive to alterations of chromatin structure. The transcriptional defect can be relieved by mutations in the H2A and H2B genes (Clark-Adams et al. 1988), in the histone H3 gene (this paper; Prelich and Wilson 1993), by altering the balance between (H2A–H2B) dimer and $(H3–H4)_2$ tetramer subunits (Clark-Adams et al. 1988), and by mutations in *SIN1* (Kruger and Herskowitz 1991). The molecular basis by which these mutations relieve the transcriptional block imposed by δ is not known.

Mutations affecting *SIN1* and histone H3 can also partially suppress the transcriptional defects resulting from truncation of the CTD of RNA polymerase II (Peterson et al. 1991; this paper). These observations led to the proposal that one function of the CTD may be to antagonize the effect of chromatin structure at the promoter (Peterson et al. 1991; Peterson 1994).

How do *sin* mutations exert these effects on transcription? One possibility is that the altered histones result in nucleosome depletion, which can activate transcription of some genes (Han and Grunstein 1988; Han et al. 1988). At least some of the *sin* mutations do not, however, appear to cause extensive depletion of nucleosomes, as cells are viable even when their sole source of histone H4 is a *sin* variant. We cannot, however, exclude the possibility that the *sin* mutations cause depletion of nucleosomes in a minority of genes. We favor the possibility that *sin* mutations lead to formation of an altered nucleosome in which binding of the $(H3–H4)_2$ tetramer to DNA, to the (H2A–H2B) dimer (as suggested by Hirschhorn et al. 1992; see also Hansen and Ausio 1992), or to other proteins (e.g., SIN1) is altered. Interestingly, calf thymus histone H3 can be cross-linked to HMG1 in vivo (Stros 1987). The *sin* mutations might allow more efficient binding of proteins essential for transcription initiation without the need for SWI/SNF to provide assistance.

Relation of the Sin$^-$ phenotype to the structure of the histone octamer

The *sin* mutations described here affect single amino acid residues in histones H3 or H4. When these five sites are examined relative to the nucleosomal model of Arents and Moudrianakis (1993), they appear to cluster within a small area of the $(H3–H4)_2$ tetramer (Fig. 6). Four of the changes are on the tetramer surface facing either the DNA or the (H2A–H2B) dimer subunit of the nucleosome. The fifth change involves not a surface residue but a residue that appears to buttress or lie just beneath a β bridge residue with potential to interact with DNA. All of these mutations thus appear, directly or indirectly, to be in positions that could alter the overall state of assembly of the nucleosome by modifying either histone–DNA contacts or histone dimer–tetramer contacts.

Histone H3

Position 105 (a change from glutamine to lysine in the *hht2-2* mutant) is on the side of helix II of the histone-fold facing the (H2A–H2B) interface but is not normally involved in dimer–tetramer contact. Such a change would shorten the separation across that interface from 4Å to ~2.0 Å. The introduction of a longer, charged side chain might contribute to the destabilization of that interface and shift the octamer equilibrium towards disassembly.

Position 116 (a change from arginine to histidine in the *hht1-1/sin2-1* and *hht2-1* mutants) affects a surface residue found directly in the DNA path of the nucleosome model (Arents and Moudrianakis 1993) and is normally expected to interact with the negative charges of the DNA backbone.

Position 118 (a change from threonine to isoleucine in the *hht2-3* mutant) is on the (H3–H4) tetramer surface

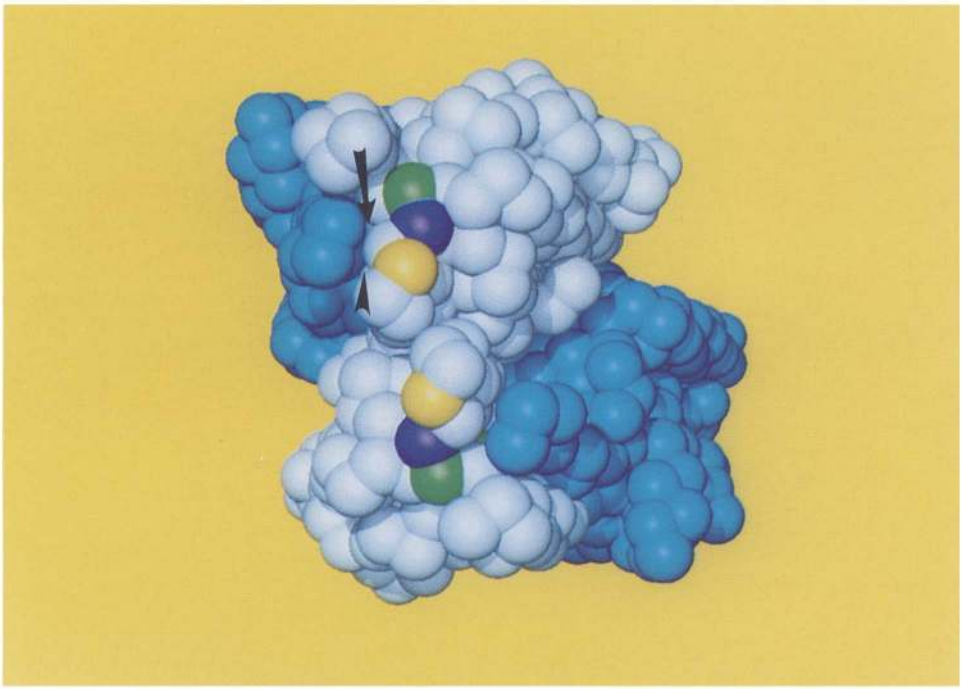

Figure 6. Positions of Sin$^-$ substitutions on the three-dimensional structure of the histone octamer. Histone H2A–H2B dimers are shown in blue; the $(H3–H4)_2$ tetramer is shown in white. The mutated sites are marked as follows: green, arginine-45 of H4; violet, threonine-118 of H3; yellow, arginine-116 of H3. Two other sites are barely visible in this assembly, as they are found deep in the cleft of the dimer–tetramer interfaces. These are residue 43 of H4, at the tip of the long arrow, and residue 105 of H3, at the tip of the arrowhead. Note that only half of the symmetric structure is marked by these arrows. Spheres indicate the C^α positions of the amino acid residues at twice the standard van der Waal radius for carbon atoms. [Image generated by MIDAS (Ferrin et al. 1988).]

flanked by two basic residues (arginine-116 of H3 and arginine-45 of H4), both within the proposed path of the DNA. Threonine may form hydrogen bonds directly with DNA atoms. Isoleucine might disrupt such a putative interaction either because of its bulk or its lack of H-bonding potential. Prelich and Winston (1993) identified an identical mutation that increases transcription of the *SUC2* gene in the absence of its upstream activation site and in the absence of *SNF5*.

Histone H4

Position 43 (a change from valine to isoleucine in the *hhf2-8* mutant) is not located on the tetramer surface but in the loop between helix I and strand A of the histone-fold of H4, just underneath the β bridge, where it appears to buttress bridge residues. Introduction of greater bulk in this crowded site might alter the position or orientation of the bridge and thus its DNA-binding potential.

Position 45 (a change from arginine to histidine in the *hhf2-13* mutant and to cysteine in the *hhf2-7* mutant) involves a surface, β bridge residue implicated in DNA binding (Arents and Moudrianakis 1993). A change to cysteine (loss of charge) is expected to have more pronounced consequences than a change to histidine, and this is what is observed when the cysteine variant serves as the sole source of H4 in the cell (Fig. 5).

It is striking that these five sites in two different histone chains are found clustered in a small region of the $(H3–H4)_2$ tetramer. Although the mutagenesis was random, our mutant hunt was not saturating and demanded that the mutations be at least partially dominant. Different mutant hunts might identify other positions for *sin* mutations.

It would be most appealing if the changes in chromatin structure caused by *sin* mutations mimic the effects of the SWI/SNF complex on wild-type nucleosomes. We do not know whether this is the case or whether the *sin* mutations bypass the need for SWI/SNF in an artificial manner. Biochemical studies to examine whether *sin* mutations affect the affinity of nucleosomal components for each other or the requirement of the SWI/SNF complex for binding of activator proteins in vitro (Côté et al. 1994; Kwon et al. 1994) should be revealing.

The Sin$^-$ phenotype and histone gene redundancy

The *sin* mutations affecting histones H3 and H4 were isolated in strains with two functional copies of each gene (see Table 5); thus, it is not surprising that these mutations are partially dominant. The Sin$^-$ phenotype requires production of a mutant histone H3 protein and is not simply attributable to the reduction in the amount of histone H3, indicating that mutant histone interferes

with the wild-type protein. This interference might occur within an individual octamer, or a mutant octamer might interfere with neighboring wild-type octamers.

It may be possible to exploit yeast for analysis of histone function in organisms such as *Drosophila*. It would be interesting, for example, to determine whether mutations in the SWI2/SNF2 homolog of *Drosophila*, *brahma* (Tamkun et al. 1992), are suppressed by Sin$^-$ versions of *Drosophila* histones H3 or H4. Such mutant histone genes could be generated by introducing the changes found in the yeast *sin* mutations into the *Drosophila* genes or by isolating *sin* derivatives of the *Drosophila* genes in yeast as we have done for the yeast H3 and H4 genes. The partial dominance of *sin* mutations might allow a mutant phenotype to be exhibited even though *Drosophila* has ~100 copies of the histone genes (Lifton et al. 1978).

The possibility that highly conserved components of chromatin are the targets for the SWI/SNF regulatory complex accounts nicely for the finding of homologs of the components of the SWI/SNF complex not only in *Drosophila* (Tamkun et al. 1992; Elfring et al. 1994) but also in mammalian systems (for review, see Carlson and Laurent 1994).

Materials and methods

Strains and general methods

Yeast strains are described in Table 5. pUK499 is a low-copy *URA3* plasmid that contains the wild-type histone H4 gene *HHF2* (Han et al. 1988); pUK421 is a low-copy *TRP1* plasmid that carries *HHF2* (Kim et al. 1988). General yeast genetic methods are described in Hicks and Herskowitz (1976) and references therein. A sensitive assay for loss of chromosome III is the ability of α cells to mate as **a** or their inability to produce α-factor: Loss of chromosome III even in a small fraction of cells in a colony allows cells to mate as **a** (Strathern et al. 1981) and eliminates α-factor production because of expression of the **a**-specific protease BAR1 (F. Chang and I. Herskowitz, unpubl.). Yeast strains were transformed as described by Ito et al. (1983). β-Galactosidase activity was quantitated in cells grown in culture as described by Miller (1972).

Isolation and analysis of a plasmid carrying HHT2

A genomic low-copy library (see Kruger and Herskowitz 1991) was used to transform yeast strain WK3-7c (relevant genotype: α *swi1Δ sin2-1 HO–lacZ URA3-52*), and ~5000 colonies were screened for reduced β-galactosidase activity as described by Kruger and Herskowitz (1991). Forty-three candidates were tested for plasmid dependence of the phenotype after selecting for loss of the plasmid on plates containing 5-fluoro-orotic acid (Boeke et al. 1984). Only one of the candidates (carrying plasmid pSINc) was plasmid dependent. The *Hin*dIII insert in pH-2 was further subcloned as *Bam*HI–*Hin*dIII fragments in M13mp18 and M13mp19 and was used for DNA sequencing using Sequenase. pHHT1 was constructed by subcloning a 7-kb *Hin*dIII fragment containing *HHT1* derived from MS191 (Smith and Andrésson 1983) into the *Hin*dIII site of YCp50.

To determine whether pSINc contained *SIN2*, a *Hin*dIII fragment from pSINc was inserted into YIp5, and the subsequent plasmid pYIPH3 was linearized with *Bgl*II and used to transform WK3-7c. A resultant transformant was mated with WK10-8d, sporulated, and 17 tetrads dissected. Of the 12 *swi1Δ sin2-1* spores, only 7 were Ura$^+$, indicating that *HHT2* and *SIN2* were not tightly linked and thus are different genes.

Recovery and analysis of the sin2-1 *mutation*

To recover the *HHT1* locus from a *sin2-1* strain, we employed the following procedure (Rothstein 1991). First, the *Eco*RI site of YCp50 was inactivated by digesting with *Eco*RI, filling in the cohesive ends with Klenow polymerase, and recircularizing to yield plasmid YCp50δR1. Into the *Hin*dIII site of this plasmid was inserted a 6-kb *Hin*dIII fragment from pMS361 (a gift from M. Smith, University of Virginia, Charlottesville) that contains an *HHT1* gene in which the *HHT1* coding sequences were replaced with an *Eco*RI site (see Smith and Stirling 1988). The resultant plasmid (pHHT1Δ) was digested with *Eco*RI and used to transform *sin2-1* strain WK3-7c. Four transformants were isolated and their plasmids recovered. Two of the plasmid restriction maps indicated that they had been repaired by the resident *HHT1* locus. These two plasmids conferred a Sin$^-$ phenotype when introduced into WK1-9a (see Table 1B). The recovered plasmid was designated phht1-1.

The *Hin*dIII–*Sma*I fragment containing *hht1-1* (*sin2-1*) was subcloned into M13mp18 and M13mp19, and the entire histone H3-coding region was sequenced using the primers 5-'CTAAAACTGATGACAATCAA-3' and 5-'GAAAAACATCTAA-

Table 5. *Strain list*

Strain	Genotype	Source/Reference
CY236	**a** *HO–lacZ ura3-52 leu2*$^=$	this study
CY240	**a** *swi1Δ:LEU2 HO–lacZ ura3-52 leu2*$^=$ *trp*$^-$	this study
JAY47	**a** *leu2*$^=$ *trp1-1 ura3-1 ade2-1 his3-11,15 can1-100 ho*	Archambault et al. (1991)
UKY412	**a** *ade2-101 his3Δ200 leu2*$^-$ *lys2-801 trp1Δ900 ura3-52 hhf1::HIS3 hhf2::LEU2* [pUK499]	Han et al. (1988)
WK1-9a	α *swi1Δ:LEU2 HO–lacZ ura3-52 leu2*$^=$	Kruger and Herskowitz (1991)
WK3-7c	α *swi1Δ:LEU2 HO–lacZ ura3-52 leu2*$^=$	this study
WK10-8d	α *HO–lacZ trp1-289 ura3-52 leu2*$^=$	this study
WK48-6c	**a** *swi1Δ:LEU2 sin2-1 HO–lacZ leu2*$^=$ *ura3-52*	this study
WK48-20a	α *hht2Δ:URA3 ura3-52 leu2*$^=$ *HO–lacZ*	this study
WK50-2a	**a** *hht2Δ:URA3 lys2-128δ ura3-52 leu2*$^=$ *HO–lacZ*	this study
WK51-5d	α *swi1Δ hht2Δ* lys2-128δ *HO–lacZ*	this study
WK50-4c	**a** *ura3-52 leu2 HO–lacZ*	this study

CATAAT-3′. Three polymorphisms were observed between *sin2-1* and *HHT1*: C → T at position 267; G → A at position 479; C → T at position 536 (numbering according to Smith and Andrésson, 1983).

Construction of hht2-1 *and* hht2Δ

In vitro mutagenesis of *HHT2* was performed essentially as described by Climie et al. (1990) using oligonucleotide 5-′GATAGTAACATGCTTAGCGTG-3′. The *hht2Δ* allele was constructed by first subcloning the *Hin*dIII fragment of pH-2 into pUC18, followed by digestion of the resultant plasmid with *Acc*I and *Bam*HI. A *Bam*HI–*Sma*I fragment containing *URA3* derived from CY243 (S. Michaelis, unpubl.) was subsequently ligated into the above vector. The *Hin*dIII fragment from the resultant plasmid, pUC:*hht2Δ*, was used for gene replacement as described by Orr-Weaver et al. (1983) and was confirmed by Southern hybridization.

In vitro mutagenesis of HHT2 *and* HHF2 *and screening for additional Sin⁻ alleles*

pH-2 was mutagenized with hydroxlamine (Mendenhall et al. 1988) with the following modifications: 10 μg of plasmid DNA in 100 μl 1 M potassium phosphate (pH 6.0) was treated with 100 μl fresh 1 M hydroxylamine at 70°C for 1.5 hr. Reactions were desalted over G-25 spin columns, ethanol precipitated, and resuspended in 10 μl of TE. Half of the resultant pool of mutagenized plasmid DNA was used to transform *swi1⁻* strain CY240. Approximately 1500 Ura⁺ transformants were patched and tested for β-galctosidase activity by filter assay. Six suppressors were obtained, five of which were plasmid dependent. Plasmid inserts were subcloned into M13mp18, and the entire *HHT2* gene of each insert was sequenced using the primers 5-′GGATGTTTGTATGATGTCCC-3′ and 5-′TTCCCGCTTTATATTCATGA-3′. Mutagenesis of the histone H4 gene *HHF2* was carried out in a similar manner by hydroxylamine mutagenesis of the *HHF2* plasmid pUK499 (Han et al. 1988) and transformation of strain CY240.

INO1 *primer-extension analysis*

For analysis of *INO1* transcripts, RNA was isolated as described by Peterson et al. (1991). Primer-extension analysis was performed on 20 μg of RNA as described by Kruger and Herskowitz (1991), using the primer 5-′GCTGTCTTCGTAACTACAGAC-3′ to measure *INO1* transcripts. Primer-extension products were analyzed on an 8% denaturing polyacrylamide gel followed by exposure to X-ray film (Peterson and Herskowitz 1992).

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W Kruger, C L Peterson, A Sil, et al.

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