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Mutations in both the structured domain and N-terminus of histone H2B bypass the requirement for Swi–Snf in yeast

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The chromatin elements targeted by the ATP-dependent, Swi–Snf nucleosome-remodeling complex are unknown. To address this question, we generated mutations in yeast histone H2B that suppress phenotypes associated with the absence of Swi–Snf. Sin$^-$ (Swi–Snf-independent) mutations occur in residues involved in H2A–H2B dimer formation, dimer–tetramer association, and in the H2B N-terminus. The strongest and most pleiotropic Sin$^-$ mutation removed 20 amino acid residues from the H2B N-terminus. This mutation allowed active chromatin to be formed at the *SUC2* locus in a *snf5*Δ mutant and resulted in hyperactivated levels of *SUC2* mRNA under inducing conditions. Thus, the H2B N-terminus may be an important target of Swi–Snf *in vivo*. The *GCN5* gene product, the catalytic subunit of several nuclear histone acetytransferase complexes that modify histone N-termini, was also found to act in conjunction with Swi–Snf. The phenotypes of double *gcn5*Δ*snf5*Δ mutants suggest that histone acetylation may play both positive and negative roles in the activity of the Swi–Snf-remodeling factor.

Keywords: chromatin remodeling/histone H2B/transcription/yeast Swi–Snf complex

Introduction

The nucleosomal organization of eukaryotic chromosomes acts as an effective barrier to the interaction of DNA-binding proteins with their recognition sequences. The basic repeating unit of chromatin, the nucleosome core particle, is a tripartite protein structure composed of one H3–H4 tetramer and two H2A–H2B dimers, around which ~146 bp of DNA are wrapped (Arents *et al*., 1991; Luger *et al*., 1997). The core particle is held together through numerous histone–histone and histone–DNA interactions (Arents *et al*., 1991; Luger *et al*., 1997), and it is the strength of these interactions which inhibits DNA-binding factors from accessing their sites in chromatin templates (Kornberg and Lorch, 1992; Parenjape *et al*., 1994; Polach and Widom, 1995). The repressive effects of nucleosomes can be counteracted by multiprotein factors that interact with chromatin to remodel nucleosomes. Chromatin-remodeling factors can be grouped into several different functional classes. One class, which includes the Swi–Snf, NURF, Rsc, ACF and CHRAC complexes, uses energy from ATP hydrolysis to disrupt nucleosome structure (for review see Cairns, 1998). As a result of this activity, the Swi–Snf- and NURF-remodeling factors facilitate transcription-factor binding to nucleosomal templates both *in vitro* and *in vivo* (Côté *et al*., 1994, 1998; Imbalzano *et al*., 1994; Kwon *et al*., 1994; Kingston *et al*., 1996; Burns and Peterson, 1997; Mizuguchi *et al*., 1997), and *in vivo* their primary function is in transcription (Laurent *et al*., 1990, 1991; Hirschhorn *et al*., 1992; Winston and Carlson, 1992). The *in vivo* roles of the other ATP-dependent remodeling factors are still unknown.

A fundamental question, and one that relates to the mechanism by which transcription-coupled, ATP-dependent remodeling factors act, is which elements of chromatin these factors target *in vivo*. The prototype of such factors, the evolutionarily conserved Swi–Snf complex, is able to disrupt histone–DNA contacts on monosomes *in vitro* and to alter nucleosomal arrays, which more closely resemble the structure of chromatin *in vivo* (Côté *et al*., 1994, 1998; Imbalzano *et al*., 1994, 1996; Kwon *et al*., 1994; Logie and Peterson, 1997; Schnitzler and Kingston, 1998). Although yeast Swi–Snf can bind to special DNA structures (Quinn *et al*., 1996), it is not clear whether DNA is the chromatin component targeted by its disrupting activity; the core histones themselves are also potential targets. In either case, the net result of Swi–Snf activity is a weakening in histone–DNA interactions and the promotion of a chromatin state that could lead to the eventual removal of histones from DNA and the opening up of factor-binding sites (Chen and Workman, 1994; Côté *et al*., 1994; Owen-Hughes *et al*., 1996).

Nucleosome core particles deficient in H2A–H2B dimers have been shown to facilitate transcription-factor binding *in vitro* (Hayes and Wolffe, 1992) and to enhance transcription on nucleosomal arrays (Hansen and Wolffe, 1994). This is consistent with the idea that the removal of H2A–H2B dimers from chromatin templates might be a regulated step during activated transcription *in vivo*. Genetic studies in yeast support the view that Swi–Snf might assist transcription by altering the intranucleosomal interactions of H2A–H2B dimers with the H3–H4 tetramer. Mutations that suppress transcriptional defects resulting from alterations in the yeast Swi–Snf complex (Sin$^-$ or Swi–Snf-independent mutations) have been identified in the genes encoding histones H3 and H4, and several of the H4 mutations occur in amino acid residues predicted to be involved in the stable association of the dimer with the tetramer (Winston and Carlson, 1992; Kruger *et al*., 1995; Santisteban *et al*., 1997; Wechser *et al*., 1997). In addition, depletion of H2A–H2B dimers *in vivo* by mutation, or *in vitro* by histone-binding proteins, has been reported to bypass or enhance Swi–Snf function (Hirschhorn *et al*., 1992; Chen *et al*., 1994; Côté *et al*., 1994). However, there is no direct evidence that Swi–Snf

targets these particular chromatin constituents *in vivo*. No physical interaction has been reported between any of the four core histones and components of the Swi–Snf complex, and *in vitro* Swi–Snf on its own cannot remove histones from DNA (Owen-Hughes *et al*., 1996; Schnitzler *et al*., 1998). Finally, no Sin$^-$ mutations have been identified in histone H2A or H2B coding sequences. Indeed, a novel class of yeast H2A mutations has been found that results in Swi–Snf$^-$ phenotypes in strains that contain the wild-type chromatin-remodeling complex (Hirschhorn *et al*., 1995).

We have investigated whether histone H2B plays a role in Swi–Snf function *in vivo*. Using site-directed mutagenesis, we created Sin$^-$ mutations in residues that occur in two different domains of H2B. Sin$^-$ mutations in residues of the structured α-helical domain suppressed a subset of swi–snf phenotypes. The α-helical domain is responsible for both histone–histone and histone–DNA interactions in the nucleosome core particle (Arents *et al*., 1991; Luger *et al*., 1997), and the H2B Sin$^-$ mutations are predicted to alter H2A–H2B dimer assembly or H2A–H2B dimer–H3–H4 tetramer association. A second and novel Sin$^-$ mutation that resulted from a large deletion of the H2B N-terminus suppressed a wider range of swi–snf defects. The highly charged histone N-termini protrude from the nucleosome core particle and engage in interactions with internucleosomal DNA, adjacent nucleosomes, and non-histone proteins (Hecht *et al*., 1995; Edmondson *et al*., 1996; Luger *et al*., 1997). These interactions affect both core particle accessibility and the formation of higher order or compacted chromatin structure (Allen *et al*., 1982; Schwarz and Hansen, 1994; Fletcher and Hansen, 1995, 1996; Schwarz *et al*., 1996). In the absence of Swi–Snf, the H2B N-tail deletion allowed the formation of transcriptionally active chromatin at the Swi–Snf-regulated *SUC2* locus. This suggests that the H2B N-terminus might play an inhibitory role in chromatin structure that is antagonized by Swi–Snf. In support of this view, a portion of intracellular Snf5 protein was found to co-immunoprecipitate with histone H2B.

The histone N-termini are targeted by another group of chromatin remodeling activities, the histone acetyltransferases (HAT), which also enhance transcriptional activation on chromatin templates (reviewed in Grunstein, 1997; Struhl, 1998). As the result of HAT activity, acetyl groups are placed on the ε amino groups of specific lysine residues (Kuo *et al*., 1996; Zhang *et al*., 1998), resulting in positive-charge neutralization and a weakening of histone N-tail interactions with DNA or non-histone proteins (Cary *et al*., 1982; Garcia-Ramirez *et al*., 1995; Edmondson *et al*., 1996). We investigated the relationship between histone N-tail acetylation and ATP-dependent chromatin remodeling by combining a *GCN5* deletion with a *SNF5* deletion. *GCN5* encodes the catalytic subunit of several nuclear HAT complexes which show specificity for the N-termini of nucleosomal histones H2B and H3 and are required for activated transcription *in vivo* (Brownell *et al*., 1996; Kuo *et al*., 1996, 1998; Grant *et al*., 1997; Wang *et al*., 1998; Zhang *et al*., 1998). The phenotypes of double *gcn5*Δ*snf5*Δ mutants provide further genetic evidence that the Gcn5–HAT and Swi–Snf chromatin-remodeling pathways perform overlapping functions in activated transcription (Pollard and Peterson, 1997; Roberts and Winston, 1997). Moreover, at some Swi–Snf regulated genes, histone acetylation may play an inhibitory role.

Results

Sin$^-$ mutations of histone H2B

To determine whether Sin$^-$ alleles could be generated in histone H2B, we targeted two regions for site-directed mutagenesis: the central α-helical domain, which mediates the interactions of H2B with other histones and with the DNA superhelix, and the protruding N-terminus, which interacts with internucleosomal DNA and adjacent nucleosomes (Figure 1). First, we altered residues that were predicted to be important for the interactions of H2B with histones H2A or H4. In the first α-helical domain (α1) of H2B-1 (Luger *et al*., 1997), we changed individually or in combination three conserved tyrosines (Y40, Y43, Y45) to glycines. These residues fall at the H2A–H2B dimer interface and can be crosslinked *in vitro* to a conserved proline residue (P27) in histone H2A (DeLange *et al*., 1979). Thus, mutations in these residues could affect H2A–H2B dimer assembly or stability and lead to nucleosomes deficient in dimers, a situation that can bypass the requirement for Swi–Snf *in vivo* (Hirschhorn *et al*., 1992) and potentiate Swi–Snf function *in vitro* (Chen and Workman, 1994; Côté *et al*., 1994). In α-helical domain 2 (α2), we changed a fourth conserved tyrosine residue (Y86) to glycine. This tyrosine forms a hydrophobic cluster with two conserved histone H4 tyrosines (Y72, Y88) at the dimer–tetramer interface (Kleinschmidt and Martinson, 1984; Zweidler, 1992; Santisteban *et al*., 1997), and when mutant, could destabilize the nucleosome core particle by perturbing the association of H2A–H2B dimers with the H3–H4 tetramer. Indeed, when the corresponding H4 tyrosine residues were mutated to glycine, a Sin$^-$ phenotype resulted (Santisteban *et al*., 1997). Next, we reconstructed a series of short, in-frame deletions in the H2B-1 N-terminus (Schuster *et al*., 1986; Lenfant *et al*., 1996; Recht *et al*., 1996). Three of these mutations removed residues predicted to be involved in internucleosomal DNA interactions (Δ3–22, Δ14–31, Δ3–32), while the fourth (Δ30–37) removed residues that interact with the DNA superhelix as the H2B N-terminus exits the core particle (Luger *et al*., 1997). The rationale for targeting the H2B N-terminus was twofold. First, this histone domain is involved in the formation of higher order or compacted chromatin structure (Allen *et al*., 1982; Schwarz and Hansen, 1994; Fletcher and Hansen, 1995, 1996; Schwarz *et al*., 1996; Luger *et al*., 1997), which might be targeted by Swi–Snf. Secondly, histone N-tails are required for *in vitro* nucleosome remodeling by the *Drosophila* NURF complex (Georgel *et al*., 1997).

Using the technique of plasmid shuffle (Boeke *et al*., 1984), we introduced each *htb1* mutation into both *SNF5* and *snf5*Δ strains (Abrams *et al*., 1986; Laurent *et al*., 1990, 1991) that contained non-functional *HTB1* and *HTB*2 genes (Materials and methods). The *snf5*Δ mutation prevents assembly of the Swi–Snf complex *in vivo* and is thus null with respect to Swi–Snf phenotypes (Peterson *et al*., 1994; Cao, 1998). Only the N-terminal deletion *htb1*Δ30–37 and the triple mutant *htb1*Y40G, Y43G, Y45G were unable to support cell viability (Table I). The residues defined by these two lethal mutations are thus

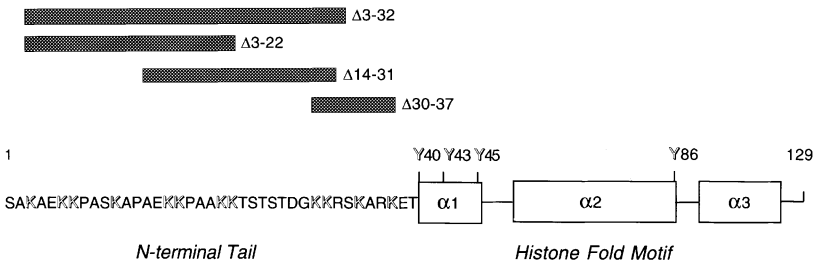

Fig. 1. Targeted mutagenesis in two structural domains of histone H2B. The amino acid residues of the N-terminal extension are indicated, with lysine residues (K) highlighted. Deletions in this region are shown as black boxes above the residues of the N-terminus. The three α-helical regions and the two β loops of the C-terminal histone fold domain are shown along with the positions of four conserved tyrosine (Y) residues targeted for change to glycine (G).

Table I. Effects of H2B mutations on viability and growth of *SNF5* and *snf5*Δ strains

H2B mutation	Viability[a]	Doubling time[b] (h)	
		SNF5	*snf5*Δ
wild type	+	1.8	3.9
Δ3–22	+	1.8	2.4
Δ3–32	+	1.9	4.2
Δ14–31	+	1.7	4.2
Δ30–37	–	n.d.	n.d.
Y40G	+	1.8	4.3
Y45G	+	1.7	4.1
Y86G	+	1.7	3.9
Y40G, Y43G, Y45G	–	n.d.	n.d.

[a]Viability was determined after loss of the resident YCp50-*HTB1* plasmid.
[b]Doubling time of *SNF5* or *snf5*Δ*htb1-1htb2-1* strains containing pRS314-*htb1* plasmids was determined in SD-medium–tryptophan. n.d., not determined.

Table II. Effects of H2B mutations on *HO–lacZ* expression in *snf5*Δ strains

Strain	H2B mutation	β-galactosidase units[a]
SNF5	wild type	103
	Δ3–22	165
*snf5*Δ	wild type	7.5
	Δ3–22	9.4
	Δ3–32	13.6
	Δ14–31	27.3
	Y40G	16.4
	Y45G	6.6
	Y86G	11.4

[a]Mean Miller units from three independent transformants assayed in duplicate (variation <20%).

implicated in some essential aspect of nucleosome assembly, stability or function. We note that the removal of amino acids 30–37 from the H2B2 subtype has been reported to confer viability in another strain background (Lenfant *et al*., 1996).

None of the viable *htb1* mutations conferred obvious phenotypes in wild-type cells, including temperature-sensitive or slow-growth, amino acid auxotrophies, or an inability to grow on carbon sources other than glucose (data not shown). This is in marked contrast to mutations in some of the same domains of histones H2A and H4, which result in a wide spectrum of mutant phenotypes in a wild-type background. For example, deletion of amino acid residues 4–20 from the H2A N-terminus produces Swi–Snf$^-$ phenotypes (Hirschhorn *et al*., 1995), while H4 Y→G mutations confer cell-growth or viability defects (Santisteban *et al*., 1997).

Viable *snf5*Δ*htb1* mutants were examined for phenotypes associated with defects in the Swi–Snf complex. Mutations in *SWI–SNF* genes cause pleiotropic phenotypes, including slow growth, clumpy colony morphology, and the failure to induce transcription of a subset of genes, most notably *SUC2*, *INO1* and *HO* (Neigeborn and Carlson, 1984; Stern *et al*., 1984; Abrams *et al*., 1986; Peterson *et al*., 1991; Hirschhorn *et al*., 1992; Winston and Carlson, 1992; Kruger *et al*., 1995). With one exception, none of the *htb1* mutations suppressed the slow-growth phenotype of *snf5*Δ (Table I). The exception was the H2BΔ3–22 N-tail deletion, which strongly complemented the *snf5*Δ growth defect, decreasing doubling time in supplemented minimal medium from ~4 h to 2.4 h, close to the 1.8 h doubling time of a wild-type strain. The growth suppression was also apparent on rich medium (YPD) plates, where the colony sizes of a *snf5*Δ*htb1*Δ*3–22* mutant were almost as large as those of a *SNF5HTB1* strain (Figure 3A).

The H2B mutations fell into two classes with respect to suppression of the transcriptional defects of *snf5*Δ mutants. The first class, which contained the point mutations, Y40G and Y86G, and the N-tail deletions, Δ14–31 and Δ3–32, partially suppressed a subset of transcriptional defects. The two point mutations and the Δ14–31 N-tail deletion weakly suppressed the inositol deficiency of a *snf5*Δ mutant (Figure 2A), a measure of the cell's ability to induce transcription of the *INO1* gene (Figure 2B), as well as the decrease in *HO–lacZ* expression (Table II). The H2B Δ3–32 mutation weakly suppressed only the *HO* transcriptional defect (Table II). Thus, mutations in two different H2B domains can partially bypass the requirement for Swi–Snf at the same set of genes. This suggests that these genes have a similar chromatin enviroment and are therefore affected in equivalent ways by the chromatin-remodeling complex.

The second class of suppressors included a single, semi-dominant mutation—the H2BΔ3–22 N-tail deletion. This was the only H2B mutation that allowed *snf5*Δ to grow on raffinose-containing medium (Figure 3A; and data not shown), which reflects the cell's ability to induce transcription of the *SUC2* gene (Hirschhorn *et al*., 1992, 1995; Roberts and Winston, 1997). It was also the strongest transcriptional suppressor of all of the H2B Sin$^-$ mutations. In the presence of the N-tail deletion, *INO1* and *SUC2*

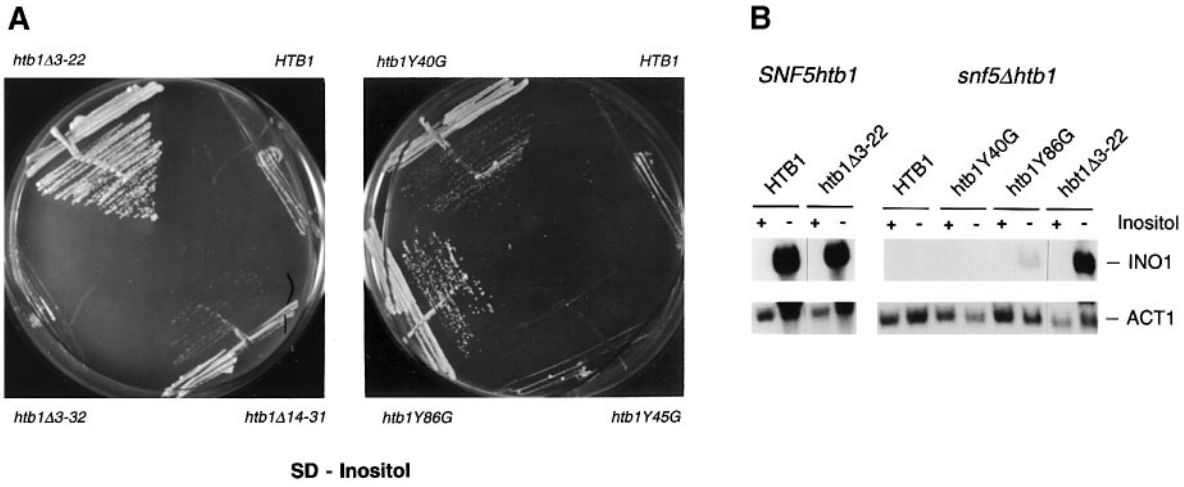

Fig. 2. Effects of H2B mutations on the Ino⁻ phenotype of *snf5*Δ strains. (**A**) Growth of *snf5*Δ*htb1* mutants on supplemented SD-inositol medium. (**B**) *INO1* transcription. *SNF5* and *snf5*Δ strains with wild-type *HTB1* or *htb1* mutations Y40G, Y86G and Δ3–22, were grown in supplemented SD-inositol medium in the absence (inducing) or presence (repressing) of 100 μM inositol. The levels of *INO1* transcript were measured by Northern blot analysis, with *ACT1* mRNA serving as an internal loading control.

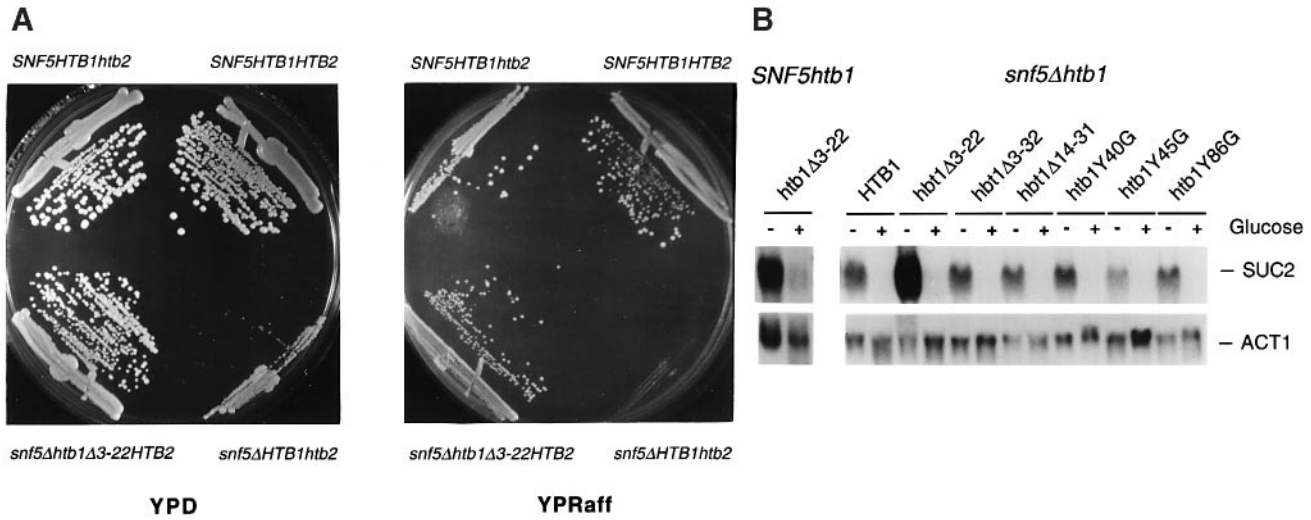

Fig. 3. Effects of H2B mutations on the Raff⁻ phenotype of *snf5*Δ strains. (**A**) Growth of *SNF5* and *snf5*Δ strains containing wild-type *HTB1* or *htb1*Δ3–22 on YPD and YP + raffinose plates. (**B**) *SUC2* transcription. *SNF5* and *snf5*Δ strains with wild-type *HTB1* or each of the six viable *htb1* alleles were grown under repressing (+glucose) or inducing (–glucose) conditions. The levels of *SUC2* mRNA were measured by Northern blot analysis, with *ACT1* mRNA serving as an internal loading control.

mRNA levels could be activated to high levels under inducing conditions, while repressed levels of the two mRNA species remained unaffected (Figures 2B and 3B). Only the defect in *HO–lacZ* expression could not be suppressed by the H2BΔ3–22 mutation (Table II). Thus, the region of the H2B N-terminus defined by the deleted residues might play a key role in the chromatin structure of a subset of Swi–Snf targeted genes.

Together, the results provide the first evidence that Sin⁻ mutations can be generated in histone H2B. These mutations occur in a region where Sin⁻ mutations have also been identified in histone H4 (the dimer–tetramer interface) (Kruger *et al*., 1995; Santisteban *et al*., 1997), and in two other regions where no other Sin⁻ histone mutations have previously been reported (the H2A–H2B dimer interface and the N-terminal-tail domain). The H2B N-tail deletion Δ3–22 is one of the strongest and most pleiotropic of the characterized histone Sin⁻ mutations, suggesting that the H2B N-terminus might be a general target of Swi–Snf *in vivo*.

Effects of H2B Sin⁻ mutations on nucleosome stability

To determine whether any of the H2B Sin⁻ mutations altered bulk nucleosome assembly or stability, we measured the superhelical density of the endogenous 2-micron plasmid in *SNF5* and *snf5*Δ strains that contained wild-type H2B or the H2B mutations Δ3–22, Y40G, Y45G, or Y86G (Figure 4). Each time a nucleosome is assembled onto a closed circular DNA molecule, a single superhelical turn is introduced (Worcel *et al*., 1981). This can be visualized as a distribution of topoisomers when the extracted plasmid DNA is electrophoresed through a chloroquine–agarose gel (Lenfant *et al*., 1996; Wechser *et al*., 1997). A decrease in plasmid superhelical density would be mainifest as a shift in the distribution of

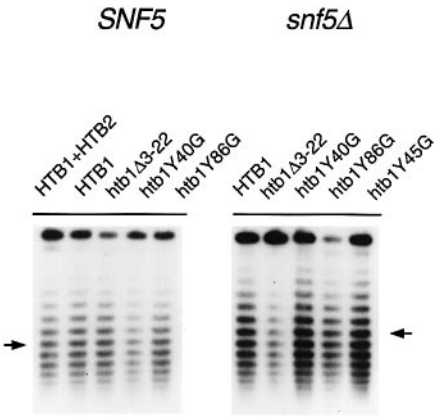

Fig. 4. Effects of H2B mutations on 2-micron plasmid topoisomer distribution. Total DNA was isolated from *SNF5* and *snf5*Δ strains containing wild-type *HTB1* or the indicated *htb1* mutations and electrophoresed through a 0.7% agarose gel containing 25 μg/ml chloroquine. The topoisomer distribution of endogenous 2-micron plasmid DNA was identified by Southern blot analysis. The arrows indicates the center of the topoisomer distribution.

topoisomers, indicating an impairment in the ability of nucleosomes to supercoil DNA *in vivo* (Lenfant *et al*., 1996; Wechser *et al*., 1997). No such shift was observed in the distribution of plasmid topoisomers isolated from either *SNF5* or *snf5*Δ strains that contained the H2B Sin$^-$ mutations. Thus, none of the H2B mutations apparently alters nucleosome assembly or stability in a way that leads to nucleosome loss *in vivo*, and their effects on nucleosome structure must therefore be more subtle.

*Active SUC2 chromatin is formed in a snf5*Δ *mutant in the presence of H2B*Δ*3–22*

Activation of *SUC2* transcription in wild-type strains is accompanied by a well-defined chromatin transition in which nucleosomes present at the TATA element and UAS region are selectively disrupted (Hirschhorn *et al*., 1992; Wu and Winston, 1997; Gavin and Simpson, 1997). To determine whether this transition occurred in a *snf5*Δ mutant when the H2BΔ3–22 Sin$^-$ mutation was present, we performed indirect end-labeling on micrococcal nuclease (MNase) treated *SUC2* chromatin isolated from *SNF5htb1*Δ3–22, *snf5*Δ*HTB1*, and *snf5*Δ*htb1*Δ3–22 cells grown under low glucose-inducing conditions (Figure 5). A diagnostic feature of the *SNF5* chromatin transition is the appearance of strong MNase cut sites flanking the *SUC2* TATA element, which is protected from digestion in the repressed chromatin state (Hirschhorn *et al*., 1992). In chromatin isolated from all three strains, this transition did not occur when cells were grown under conditions of glucose repression (data not shown). However, in both *SNF5htb1*Δ3–22 and *snf5*Δ*htb1*Δ3–22 chromatin, enhanced MNase cleavages occurred in the vicinity of the TATA box upon induction (lanes 2–4 and 10–12). In contrast, none of the enhanced cleavages occurred in *snf5*Δ*HTB1* chromatin (lanes 6–8), which retained the structure of the repressed state. Thus, the effect of the H2BΔ3–22 Sin$^-$ mutation at *SUC2* is direct, and active *SUC2* chromatin can be formed in the absence of Swi–Snf when the H2B N-terminus is partially deleted. This suggests that the H2B N-tail domain plays an inhibitory role in the chromatin structure of the *SUC2* gene, and that this inhibition is normally antagonized by Swi–Snf.

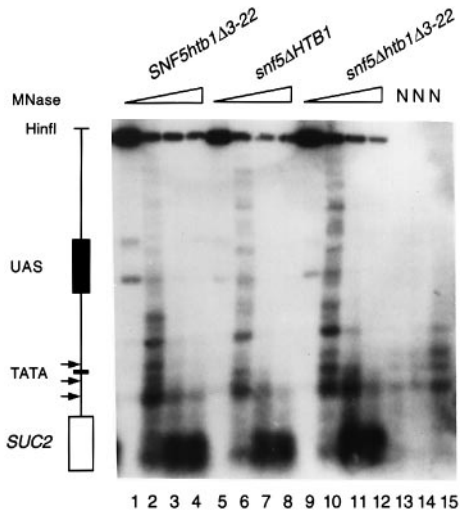

Fig. 5. Effect of H2BΔ3–22 mutation on *SUC2* chromatin structure. Nuclei were prepared from strains grown under conditions of *SUC2* induction and treated with 0, 1, 3 or 10 units of micrococcal nuclease (MNase). DNA was isolated, digested with the restriction enzyme *Hin*fI, and electrophoresed through a 2% agarose gel. Positions of MNase cleavage sites relative to a *Hin*fI site in the *SUC2* ORF were mapped by hybridization to a 166 bp *SUC2* fragment that abuts the *Hin*fI site. The arrows mark the positions of hypersensitive sites that appear upon destabilization of a nucleosome at the TATA box. Lanes: 1–4, *SNF5htb1*Δ3–22; lanes 5–8, *snf5*Δ*HTB1*; lanes 9–12, *snf5*Δ*htb1*Δ3–22; lanes 13–15, naked DNA treated with 0.3, 0.3 or 1.0 units MNase.

Snf5p interacts with histone H2B in vivo

Although the purified Swi–Snf complex can remodel nucleosomes *in vitro* (Logie and Peterson, 1997; Schnitzler *et al*., 1998), no direct interactions with the histone components of nucleosomes have been reported. One model to account for the bypass of Swi–Snf by the H2BΔ3–22 mutation is that in wild-type cells, Swi–Snf interacts with the H2B N-terminus and promotes a chromatin transition that is permissive for the action of another remodeling factor or for the binding of transcriptional activators. The N-tail deletion, then, might promote this same transition in the absence of Swi–Snf. As a first test of this model, we asked whether Snf5p was physically associated with histone H2B *in vivo*. A Flag-tagged *HTB1* gene was introduced into a *SNF5* strain to provide the only source of H2B in the cell. Strains containing Flag-H2B were indistinguishable from those containing wild-type H2B in growth rate. Next, we precipitated Flag-H2B from cell extracts using a Flag antibody resin, and asked whether Snf5p was present in the immunoprecipitates (Figure 6). Western blot analysis performed with polyclonal antibody against Snf5p showed that Snf5p specifically coprecipitated with Flag-H2B (Figure 6, lane 2): this association could be competed by addition of Flag peptide (lane 4), and although Snf5p showed some non-specific association with the antibody coated resin, it was present only in very low levels in control immunoprecipitations performed with extracts from a strain that contained untagged H2B (lane 6). Moreover, the interaction between Snf5p and Flag-H2B persisted when DNase I was present during immunoprecipitation. This implies that the association occurs through protein–protein interactions, either directly through Snf5p, another Swi–Snf component, or another protein.

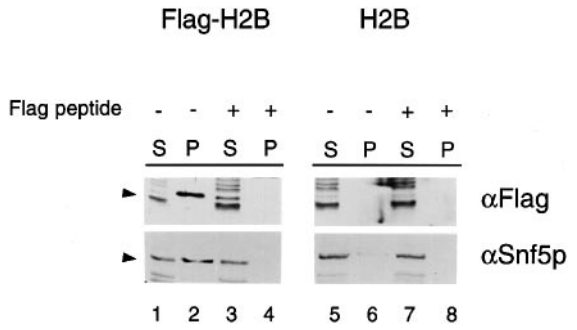

Fig. 6. Interaction of Snf5p with Flag-H2B *in vivo*. Whole-cell lysates were prepared from a *SNF5htb1-1htb2-1* strain containing either a Flag-*HTB1* gene or an untagged *HTB1* gene, and 1.2 mg of protein was incubated with Flag M2 monoclonal antibody affinity resin in the presence of DNase I. Following SDS–PAGE, Western blot analysis was performed using anti-Flag monoclonal antibody (αFlag) or polyclonal antibody against Snf5p (α-Snf5p). Lanes 1, 3, 5 and 7: IP supernatants (S) from 1/250 of input lysate for α-Flag Western or 1/33 of lysate for α-Snf5p Western; lanes 2, 4, 6 and 8: 1/80 of IP pellet (P) for α-Flag Western or entire IP pellet for α-Snf5 Western. Immunoprecipitations were performed in the absence (–) or presence (+) of 50 ng/ml of Flag peptide. The arrowheads indicate the bands corresponding to Flag-H2B and Snf5p. The second band from the bottom in lanes 1 and 3 represents Flag-H2B.

It has been shown that Swi–Snf can bind with nanomolar affinity to DNA *in vitro* (Quinn *et al*., 1996), implicating Swi–Snf–DNA interactions in the mechanism by which the remodeling complex functions. Our data provide the first demonstration that a Swi–Snf subunit physically interacts with a histone component of chromatin *in vivo*, presumably without the mediation of DNA. Although we assume that it is Snf5p which is present in the Swi–Snf complex that interacts with H2B, it is formally possible that the observed association also represents an interaction of free Snf5p with H2B. We do not know whether Snf5p contacts H2B directly, or indirectly though another histone constituent of nucleosomes, but the results are consistent with our genetic data that Swi–Snf might target the H2B N-terminus. However, we have been unable to test the prediction that Swi–Snf will no longer associate with H2B when the N-terminal residues 3–22 are missing because Flag-H2BΔ3–22, unlike untagged H2BΔ3–22, is unable to suppress the transcriptional defects of a *snf5*Δ mutant (unpublished observation).

Functional relationship between Gcn5–HAT and Swi–Snf chromatin-remodeling activities

HATs represent a second major class of chromatin-remodeling activities with roles in activated transcription (for reviews, see Grunstein, 1997; Struhl, 1998). These factors catalyze the reversible acetylation of specific lysine residues in the N-termini of all four core histones, neutralizing positive charge and loosening histone N-tail interactions with DNA or non-histone proteins (Garcia-Ramirez *et al*., 1995; Puerta *et al*., 1995; Edmondson *et al*., 1996; Fletcher and Hansen, 1996; Schwarz *et al*., 1996). One of the major transcription-coupled HAT activities in yeast is encoded by the *GCN5* gene (Brownell *et al*., 1996; Kuo *et al*., 1998; Wang *et al*., 1998). The evolutionarily conserved Gcn5–HAT is present in several multiprotein nuclear complexes, two of which (Ada and SAGA) target nucleosomal H3 and H2B histones for acetylation (Grant *et al*., 1997; Pollard and Peterson, 1997; Saleh *et al*., 1997). Deletion of the *GCN5* gene compromises expression of several genes that are also subject to Swi–Snf regulation, supporting the view that the Swi–Snf and Gcn5–HAT pathways might contribute overlapping functions to the activation of a common set of genes (Pollard and Peterson, 1997; Roberts and Winston, 1997). For example, wild-type Swi–Snf might promote a chromatin transition that allows a Gcn5-dependent HAT to acetylate lysine residues in the H2B N-tail, a modification that could be required for full transcriptional activation. Twelve lysine residues occur within the entire H2B N-terminal domain, eight of which are removed in the *htb1*Δ3–22 allele (Figure 1), and the H2B N-tail deletion might therefore be equivalent to the charge neutralization that accompanies acetylation of the N-terminus.

To test whether acetylation of the histone H2B N-terminus by a Gcn5-dependent HAT plays a role in conjunction with Swi–Snf, we first examined the effects of deletion of the *GCN5* gene on the phenotypes of a *snf5*Δ strain that contained wild-type H2B. As observed previously (Roberts and Winston, 1997), *gcn5*Δ*HTB1* mutants on their own showed a subset of the phenotypes of *snf5*Δ*HTB1* mutants. Common phenotypes included small colony size (Figure 7A), slow growth in supplemented minimal medium, and reduced expression of an *HO–lacZ* reporter gene (data not shown). In contrast, both *INO1* and *SUC2* transcription could be induced in a *gcn5*Δ*HTB1* mutant (Figure 7B and C), indicating that Gcn5p is dispensable for the activation of these genes or performs a redundant function with another HAT (Roberts and Winston, 1997). However, deletion of *GCN5* has also been reported to reduce both *INO1* and *SUC2* expression (Pollard and Peterson, 1997), suggesting that strain background may contribute to the severity of the *gcn*Δ transcriptional defects.

The double *gcn*Δ*snf5*Δ*HTB1* mutant was viable and exhibited a range of phenotypes, some of which were more severe than those of individual *snf5*Δ or *gcn5*Δ mutants. For example, the double mutant grew more slowly than each single mutant (Figure 7A; and data not shown) and had a novel Ts^- phenotype (data not shown). A synthetic slow-growth phenotype is also associated with the deletion of *GCN5* in a *swi1*Δ or *snf2*Δ mutant (Roberts and Winston, 1997), while another report found that *gcn5*Δ*swi1*Δ mutants are inviable (Pollard and Peterson, 1997). Other phenotypes of the double mutant were closer to those of single *snf5*Δ*HTB1* or *gcn5*Δ*HTB1* mutants. *INO1* transcription could not be activated in the double mutant (Figure 7B), the phenotype of a *snf5*Δ*HTB1* mutant, while *SUC2* transcription could be induced by low glucose, the phenotype of a *gcn5*Δ*HTB1* mutant (Figure 7C). Together, these results are consistent with the view that the Gcn5–HAT and Swi–Snf pathways have complex functional relationships *in vivo*, which are only revealed when double *gcn5*Δ*snf5*Δ mutants are analyzed. Moreover, the observation that deletion of *GCN5* suppressed the *SUC2* transcriptional defect of a *snf5*Δ mutant suggests that the Gcn5–HAT pathway could play an inhibitory role at *SUC2*.

Next, we examined the phenotypes of *gcn5*Δ*snf5*Δ double mutants that contained the H2BΔ3–22 N-tail deletion. The presence of this H2B mutation had no effect in a single *gcn5*Δ mutant, and neither suppressed nor

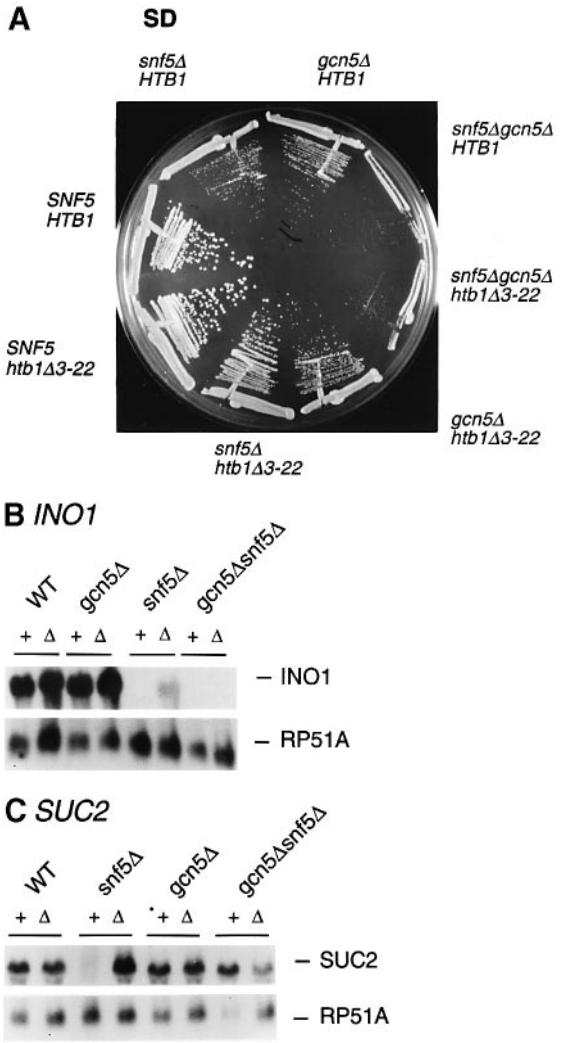

Fig. 7. Effects of a *gcn5*Δ mutation on the phenotypes of *snf5*Δ strains. Plasmids pRS315-HTB1, pRS314-HTB1, pRS314-htb1Δ3–22 or pRS315-htb1Δ3–22 were transformed into strains JR5-2A (*SNF5GCN5*), JR6-16A (*snf5*Δ*GCN5*), JR7-2B (*SNF5gcn5*Δ) and JR9-13C (*gcn5*Δ*snf5*Δ), and transformants were analyzed for their growth phenotypes and for the ability of the *INO1* and *SUC2* genes to be activated. (**A**) Growth on supplemented SD plates. (**B**) Induction of *INO1* transcription by growth in supplemented SD-inositol medium. (**C**) Induction of *SUC2* transcription by growth for 2.5 h in YP + 0.05% glucose. +, WT H2B; △, H2B△3-22.

enhanced any of its phenotypes (Figure 7 and data not shown). However, we predicted that if acetylation of the H2B N-terminus by a Gcn5p-dependent HAT occurred as a consequence of Swi–Snf activity and was responsible for the ensuing transcriptional effects, then the H2BΔ3–22 mutation might also bypass the requirement for Swi–Snf in a *gcn5*Δ*snf5*Δ double mutant. We found that the phenotypes of the triple mutant were no different from those of a *gcn5*Δ*snf5*Δ strain that contained wild-type H2B. In particular, *INO1* transcription remained uninducible (Figure 7B) and slow growth was not suppressed (Figure 7A). This indicates that the failure to acetylate the lysine residues in the first 22 amino acids of the H2B N-terminus does not account for all of the transcriptional defects of *gcn5*Δ*snf5*Δ mutants.

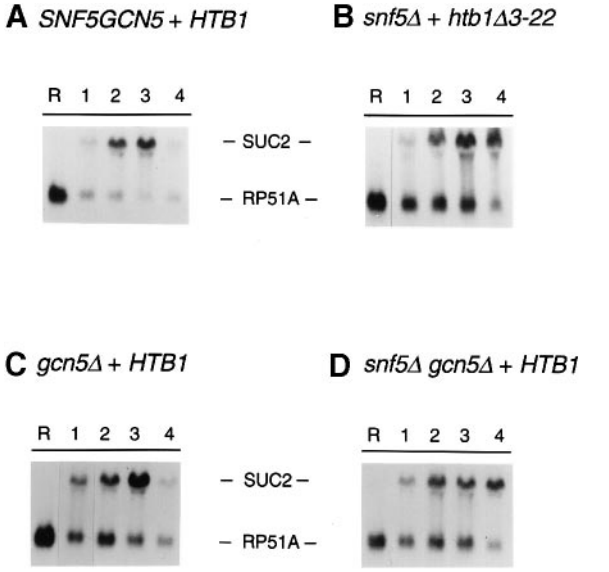

Fig. 8. Effects of deletion of the H2B N-terminus or deletion of *GCN5* on the accumulation of *SUC2* mRNA in a *snf5*Δ mutant. *SNF5HTB1* (**A**), *snf5*Δ*htb1*Δ3–22 (**B**), *gcn5*Δ*HTB1* (**C**) and *gcn5*Δ*snf5*Δ*HTB1* (**D**) strains were grown in high glucose repressing conditions (R) or shifted to low glucose inducing conditions (YP + 0.05% glucose). At 1, 2, 3 and 4 h after induction, the levels of *SUC2* mRNA were measured by Northern blot analysis, with *RP51A* mRNA serving as an internal loading control.

Deletion of the H2B N-terminus or of GCN5 alters the pattern of SUC2 transcription in the absence of Swi–Snf

When *SUC2* transcription is induced by low glucose in wild-type cells, mRNA levels peak between 2–3 h after induction and then decline rapidly (Cao, 1998). Although the molecular basis for this response is not known, one possibility is that Swi–Snf establishes, but is unable to maintain a transcriptionally active state. Because either the deletion of the H2B N-terminus or the deletion of *GCN5* relieved the barrier to *SUC2* activation in a *snf5*Δ strain, we asked whether these two conditions also affected the pattern of *SUC2* transcription once it had been activated. At hourly intervals after induction, *SUC2* mRNA levels were measured by Northern blot analysis on RNA isolated from *SNF5HTB1*, *snf5*Δ*htb1*Δ*3–22*, *gcn5*Δ*HTB1*, and *snf5*Δ*gcn5*Δ*HTB1* strains (Figure 8). In both *SNF5* and *gcn5*Δ strains, the pattern of *SUC2* mRNA accumulation was similar: by 3 h after induction, *SUC2* transcript levels peaked, and then precipitously declined over the next hour (Figure 8A and C). The presence of H2BΔ3–22 in these two strains did not in any way alter the pattern of *SUC2* transcription or affect the final levels of *SUC2* mRNA (data not shown). In contrast, in both *snf5*Δ*htb1*Δ*3–22* and *gcn5*Δ*snf5*Δ*HTB1* mutants, *SUC2* transcripts continued to accumulate with time (Figure 8B and D), and by 6 h after induction *SUC2* mRNA levels were 2.5–4 times higher than those measured in wild-type or *gcn5*Δ strains at 3 h (data not shown). These results indicate that in the absence of Swi–Snf, the deletion of either the H2B N-terminus or the deletion of *GCN5* creates a hyperactivated state of *SUC2* transcription. This additionally suggests that in wild-type cells, Swi–Snf acts during both the establishment of the induced state and the reversal to a transcriptionally inactive state. Although the H2B

N-terminus is postulated to play an inhibitory role in the establishment phase, it has no effect on the reverse transition as long as Swi–Snf is present.

Discussion

In this study, we have identified the first Sin⁻ mutations in histone H2B. Two weak Sin⁻ mutations occur in residues in the structured α-helical domain and a single strong and pleiotropic Sin⁻ mutation results from a partial deletion of the flexible N-terminus. The phenotypes associated with deletion of the H2B N-terminus suggest that this structural domain plays an inhibitory role in chromatin structure that is antagonized by Swi–Snf. Consistent with a role for the H2B N-terminus in Swi–Snf function, a fraction of intracellular Snf5 protein could be co-immunoprecipitated with H2B. A second chromatin-remodeling activity, a Gcn5-dependent HAT that targets histone N-termini for modification, was also found to act in conjunction with Swi–Snf. The Gcn5–HAT and Swi–Snf pathways have complex functional relationships *in vivo*, and at the *SUC2* locus, histone acetylation may play an inhibitory role.

Sin⁻ mutations of the H2B α-helical domain

The two weak Sin⁻ mutations that occur in the α-helical domain of H2B change residues involved in H2B association with H2A (Y40G) or H4 (Y86G) (DeLange *et al*., 1979; Kleinschmidt and Martinson, 1984; Zweidler, 1992). Both mutations have the potential to perturb nucleosome integrity, either by interfering with H2A–H2B dimer formation or by destabilizing dimer–tetramer interactions. Because other histone mutations with effects on dimer–tetramer stoichiometry or stability also suppress *Swi–Snf* mutations (Hirschhorn *et al*., 1992; Santisteban *et al*., 1997), this could be interpreted as a role for Swi–Snf in removing H2A–H2B dimers from the nucleosome core particle. However, *in vitro* data argue against such a mechanism, as Swi–Snf activity alone does not dissociate histones from DNA (Côté *et al*., 1994; Schnitzler *et al*., 1998). It is therefore more likely that the two H2B mutations alter nucleosome structure sufficiently so that some transcription factors are now able to bind to chromatin templates without the assistance of Swi–Snf. The nature of the nucleosome structural change induced by the H2B Sin⁻ mutations is not known. Sin⁻ mutations in H3 and H4 that occur at points of tetramer–DNA interaction have been shown to have variable effects on nucleosome structure. The H4 R45H, H3 R116H and H3 T118I Sin⁻ mutations, for example, appear to destabilize nucleosome structure (Kruger *et al*., 1995; Kurumizaka and Wolffe, 1997; Wechser *et al*., 1997), while the H3 E106K Sin⁻ mutation produces no apparent structural alteration (Kurumizaka and Wolffe, 1997). The two H2B Sin⁻ mutations do not lead to detectable nucleosome loss *in vivo*, and their effects on nucleosome structure must therefore be subtle.

The H2B Y86 residue and the H4 Y72 and Y88 residues form a hydrophobic cluster at the dimer–tetramer interface to create a molecular interaction that contributes to core particle integrity (Kleinschmidt and Martinson, 1984; Arents *et al*., 1991). An identical Sin⁻ phenotype (suppression of inositol auxotrophy) occurs when either H4 tyrosine residue (Santisteban *et al*., 1997) or H2B Y86 is changed to glycine, supporting the view that perturbation of dimer–tetramer contacts *per se* is able to bypass Swi–Snf during *INO1* activation. However, in contrast to the H2B Y86G mutation, the two H4 Sin⁻ mutations cause additional phenotypes. Both H4 mutations are partially dominant and cause a Ts⁻ phenotype in a *SWI–SNF* background (Santisteban *et al*., 1997), whereas the H2B mutation is recessive (J.Recht, unpublished observation) and does not confer a growth defect at any temperature. Thus, the H4 Y→G Sin⁻ mutations might cause other structural defects besides destabilizing dimer–tetramer contacts.

Sin⁻ mutation of the H2B N-terminus

All of the Sin⁻ mutations identified in histones H3 and H4 occur in residues that fall in the structured α-helical domain (Kruger *et al*., 1995; Wechser *et al*., 1997). The H2BΔ3–22 N-tail deletion represents the first Sin⁻ mutation to occur in the N-terminus of a histone, and it is additionally one of the most pleiotropic of the histone Sin⁻ mutations. Among three well-characterized Swi–Snf-regulated genes (*SUC2*, *INO1* and *HO*), only the *HO* gene did not show increased transcription in a *snf5*Δ*htb1*Δ3–22 mutant. The fact that deletion of the entire H2B N-terminus (*htb1*Δ3–32) does not produce the same effects in a *snf5*Δ mutant implies that it is not the absence of the N-tail domain *per se* that bypasses the requirement for Swi–Snf. Instead, the results suggest that the residues deleted from the H2B N-terminus could play a distinct role in the intracellular function of this histone.

The N-termini of all four core histones are required *in vitro* to stimulate the ATPase activity of the related *Drosophila* remodeling factor, NURF (Georgel *et al*., 1997). These results indicate that histone N-tails are essential elements in the interaction of the nucleosome core particle with NURF and contribute to the mechanism by which this complex remodels chromatin. The observation that a *snf5*Δ*htb1*Δ3–22 mutant undergoes a wild-type chromatin transition at the *SUC2* locus also supports a direct role for the H2B N-terminus in the mechanism by which yeast Swi–Snf functions *in vivo*. Our results are most consistent with the view that residues 3–22 of the H2B N-terminus play an inhibitory role in the chromatin structure of the *SUC2* locus, and that wild-type Swi–Snf normally antagonizes this inhibition. Once the N-tail inhibition is relieved, this could promote a chromatin transition which is acted on by a second remodeling factor, producing the characteristic pattern of nucleosome destabilization (Hirschhorn *et al*., 1992; Gavin and Simpson, 1997; Wu and Winston, 1997), or which is permissive for the binding of transcriptional activators. Our finding that a fraction of intracellular Snf5p can be co-immunoprecipitated with epitope-tagged histone H2B supports a role for a direct interaction between Swi–Snf and H2B in the function of the yeast-remodeling complex. Alternatively, this association could reflect the interaction of Swi–Snf with nucleosome cores or with a non-histone protein that associates with H2B.

The *INO1* gene, but not the *HO* gene, can also be induced in a *snf5*Δ*htb1*Δ3–22 mutant, implying that the H2B N-tail residues play an inhibitory role only at a subset of the loci where Swi–Snf acts. Why would the H2B N-terminus be inhibitory to transcription at some

Table III. Yeast strains

Strain	Genotype	Source
W303-1A	*MAT***a**, *ura3-1, leu2-3,-112, ade2-1 trp1-1, his3-11,-15, can1-100, ssd1*	R.Rothstein
JR5-2A	*MAT***a**, *htb1-1, htb2-1, ura3-1, leu2-3,-112 ade2-1, trp1-1, his3-11,-15, can1-100, ssd1* <YCp50-*HTB1* or pRS314-*htb1**>	this study
JR2-19A	*MAT*α, *snf5-*Δ*2, ura3-1, leu2-3,-112 ade2-1, trp1-1, his3-11,-15, can1-100, ssd1*	this study
JR6-16A	*MAT*α, *snf5-*Δ*2, htb1-1, htb2-1, ura3-1, leu2-3,-112 ade2-1, trp1-1, his3-11,-15, can1-100, ssd1* <YCp50-*HTB1* or pRS314-*htb1**	this study
JR7-2B	*MAT***a**, *gcn5*Δ*::TRP1, htb1-1, htb2-1, ura3-1 leu2-3,-112, ade2-1, trp1-1, his3-11,-15, can1-100, ssd1* <YCp50-*HTB1* or pRS315-*htb1**>	this study
JR9-13C	*MAT***a**, *gcn5*Δ*::TRP1, snf5-*Δ*2, htb1-1, htb2-1, ura3-1 leu2-3,-112, ade2-1, trp1-1, his3-11,-15, can1-100, ssd1* <YCp50-*HTB1* or pRS315-*htb1**>	this study

pRS314-*htb1** and pRS315-*htb1** contain either *HTB1* or one of the viable *htb1* mutations (Δ3–22, Δ3–32, Δ14–31, Y40G, Y45G or Y86G).

genes but not at others? One possibility is that distinct N-tail residues interact with gene-specific, non-histone regulatory proteins that help package chromatin into an inaccessible state, much like the interactions of the histone H3 and H4 N-termini with Sir3p and Sir4p are proposed to establish silent chromatin at the *HM* loci and at telomeres (Hecht *et al*., 1995). Swi–Snf might in fact be targeted to loci where these interactions occur. A second possibility is that the acetylation state of particular lysine residues in the H2B N-terminus marks the chromatin at which Swi–Snf will act. Although hyperacetylated histone N-tails are associated with unfolded chromatin and gene activation (Lee *et al*., 1993; Fletcher and Hansen, 1995, 1996; Garcia-Ramirez *et al*., 1995; Puerta *et al*., 1995; Edmondson *et al*., 1996; Vattese-Dady *et al*., 1996; Ura *et al*., 1997; Kuo *et al*., 1998; Wang *et al*., 1998; Zhang *et al*., 1998), our study suggests that acetylation of H2B N-tail residues might in fact be inhibitory to the transcription of some genes, thereby creating a requirement for Swi–Snf (see below).

It is not known whether the N-termini of the three other core histones also play a role in the function of yeast Swi–Snf. Numerous studies indicate that the histone H3 and H4 N-termini have both unique and redundant functions in a variety of *in vivo* transcriptional processes (Fisher-Adams and Grunstein, 1995; Hecht *et al*., 1995; Edmondson *et al*., 1996; Lenfant *et al*., 1996; Ling *et al*., 1996), so it would not be surprising if individual histone N-tails played different roles in the function of Swi–Snf. Indeed, it is very likely that the H2A and H2B N-termini act at different points in the Swi–Snf pathway during *SUC2* induction. Deletion of a large portion of the H2A N-terminus in wild-type cells allows the chromatin transition associated with Swi–Snf to occur, but prevents *SUC2* from being activated (Hirschhorn *et al*., 1995). In contrast, in either the presence or absence of Swi–Snf, the H2B N-tail deletion allows both *SUC2* chromatin remodeling and transcription to occur. This suggests that the H2B N-tail may play a role during chromatin disruption itself, while the H2A N-tail is important for a step subsequent to nucleosome-remodeling to activate transcription (Hirschhorn *et al*., 1995).

Relationship between Gcn5–HAT and Swi–Snf chromatin-remodeling pathways

Our study has revealed a novel functional relationship between the pathways of histone acetylation and ATP-dependent chromatin remodeling during *SUC2* activation: deletion of the *GCN5* gene can suppress the inability of a *snf5*Δ*HTB1* mutant to induce *SUC2* transcription. *GCN5*, when present in nuclear complexes, acetylates N-terminal lysine residues of nucleosomal histones H3 and H2B (Grant *et al*., 1997), and like Swi–Snf, has been defined genetically as a transcriptional coactivator (Georgakopoulos and Thireos, 1992). However, the transcriptional phenotype of a *gcn5*Δ*snf5*Δ*HTB1* double mutant suggests that the Gcn5–HAT pathway plays an inhibitory role at *SUC2*. The similar phenotypes that occur upon deletion of either the H2B N-terminal residues or *GCN5* (induction and hyperactivation of *SUC2* transcription in the absence of Swi–Snf) support the idea that the Gcn5–HAT pathway exerts its inhibitory effects through the acetylation of lysine residues in H2B N-terminus. Thus, the acetylated form of the H2B N-terminus could be inhibitory when it is present in *SUC2* chromatin, thereby creating a requirement for Swi–Snf. Once Swi–Snf relieves this inhibition, this would permit another chromatin-remodeling factor to destabilize nucleosomes at *SUC2*, ultimately allowing transcriptional activators to bind to their recognition sequences. In support of this view, an identical chromatin transition occurs in nuclei isolated from *SNF5HTB1*, *snf5*Δ*htb1*Δ3–22, or *gcn5*Δ*snf5*Δ*HTB1* cells upon low glucose induction (J.Recht and M.A.Osley, unpublished data).

The Gcn5–HAT and Swi–Snf chromatin-remodeling pathways appear to have different functional relationships at other loci where Swi–Snf acts. For example, previous genetic studies indicated that the two pathways contribute overlapping or redundant functions during cell growth (Pollard and Peterson, 1997; Roberts and Winston, 1997), a phenotype that was also observed in the present study. In addition, at *INO1*, unlike at *SUC2*, the deletion of *GCN5* in a *snf5*Δ mutant is unable to bypass the requirement for Swi–Snf, even though the H2B N-terminus is postulated

to play an inhibitory role at both genes. This suggests that while the acetylation state of the H2B N-terminus might contribute to transcriptional inhibition at *INO1*, other, locus-specific factors make this gene dependent on the Gcn5–HAT pathway when Swi–Snf is absent.

Role of Swi–Snf and H2B N-terminus in turning off SUC2 transcription

The failure of wild-type cells to accumulate *SUC2* mRNA after 2–3 h of induction suggests that either Swi–Snf is unable to maintain transcription or it is involved in turning off transcription. However, when both Swi–Snf and the H2B N-tail residues 3–22 are absent, or when both Swi–Snf and *GCN5* are absent, *SUC2* transcription persists for an extended period of time. These results suggest a model in which Swi–Snf plays two roles at *SUC2*: (i) it establishes a transcriptionally active state; and (ii) it promotes the reverse transition to an inactive state. This dual role for Swi–Snf could result from its ability to reversibly modify chromatin structure. Yeast and human Swi–Snf have been shown to act catalytically on nucleosomes *in vitro*, and purified human Swi–Snf and a related yeast complex, Rsc, have been reported to promote an interchange between remodeled and unremodeled nucleosomes (Imbalzano *et al*., 1996; Owens-Hughes *et al*., 1996; Logie and Peterson, 1997; Lorch *et al*., 1998; Schnitzler *et al*., 1998). Thus, when the inhibitory effects of the H2B N-tail are relieved by deletion of residues 3–22, transcription might persist in the absence of Swi–Snf because nucleosomes remain in a persistently remodeled state. However, the H2B N-terminus is apparently dispensable for the reverse transition to unremodeled nucleosomes by the wild-type Swi–Snf complex.

An alternative model that is also consistent with both the genetic and biochemical data is that Swi–Snf normally functions only during the establishment and/or maintenance of the remodeled state, and that in its absence, remodeled nucleosomes spontaneously revert back to the unremodeled state (Owen-Hughes *et al*., 1996; Logie and Peterson, 1997). In this model, the H2B N-terminal domain and Gcn5p-dependent acetylation would be required to enhance the kinetics of the Swi–Snf-independent reverse transition.

Materials and methods

Yeast strains, media and growth conditions

The *Saccharomyces cerevisiae* strains used in this study are listed in Table III; they are all congenic or isogenic to W303. The *htb1-1* and *htb2-1* alleles were introduced by mating, and both contain a frame-shift mutation at amino acid 80 that leads to a stop codon (Schuster *et al*., 1986; Lenfant *et al*., 1996). pRS314 or pRS315 plasmids (Sikorski and Hieter, 1989) carrying *htb1* mutations were substituted for plasmid YCp50-*HTB1* (*CEN4URA3*) in all strains by 5-fluoro-orotic acid counter-selection (Boeke *et al*., 1984; Lenfant *et al*., 1996). The *snf5Δ2* and the *gcn5Δ::TRP1* alleles were introduced into all strains by mating.

Yeast strains were grown in rich or synthetic media and transformed with plasmids using standard procedures (Rose *et al*., 1990). YPD medium contains 1% yeast extract and 2% peptone (YP) supplemented with 2% dextrose and YPRaff medium contains YP supplemented with 2% raffinose and 1 μg/ml antimycin A. SD medium contains YNB supplemented with 2% dextrose and a drop-out mixture of amino acids and bases and SD-inositol medium contains inositol-free YNB (Difco).

To induce *SUC2* transcription, cells were grown in YPD medium (repressing conditions) to mid-log phase, washed two or three times with 50 ml distilled water, and transferred to YP medium containing 0.05% glucose (inducing conditions) for 2.75 h. *INO1* transcription was induced by transfer of cells grown to early-log phase in SD-inositol medium supplemented with 100 μM inositol (repressing conditions) to SD-inositol medium (inducing conditions), and growth was continued until mid-log phase.

Construction of htb1 mutations

All *htb1* mutations were created by oligonucleotide-directed mutagenesis, using an *HTB1 Bst*EII–*Not*I open reading frame (ORF) cassette inserted in M13 as template (Ausubel *et al*., 1989). Oligonucleotide sequences used for mutagenesis will be supplied upon request. Mutations were confirmed by DNA sequence analysis using the dideoxynucleotide chain termination method (Ausubel *et al*., 1989).

Plasmids

Plasmid YCp50-*HTB1* contains the *HTB1* ORF as a *Bst*EII–*Not*I cassette under control of the wild-type *HTA1-HTB1* promoter. pRS314-*htb1* (*CEN6 TRP1*) and pRS315-*htb1* (*CEN6 LEU2*) plasmids carry the *HTA1-HTB1* promoter and the *htb1* ORF mutations generated in M13 or the wild-type *HTB1* ORF. A Flag epitope-tagged *HTB1* gene with an in-frame fusion of the Flag epitope to the N-terminus of *HTB1*was constructed in a Flag-pET11d vector (a gift of Drs Robert Roeder and Alexander Hoffman). The Flag-*HTB1* ORF was isolated from this vector and substituted for the wild-type *HTB1* ORF in plasmid pRS314-*HTB1*. Plasmid p12 carries an *HO–lacZ* fusion gene that contains >2 kB of the *HO* 5′ regulatory region.

RNA analysis

Total RNA was extracted from 25 ml of cells grown under appropriate conditions of repression or induction, and 20 μg was analyzed by Northern blot analysis after electrophoresis through a 1.2% agarose-formaldehyde gel (Ausubel *et al*., 1989). The *SUC2* DNA probe contains *SUC2* ORF sequences between +131 and +770. The *INO1* DNA probe is a 0.6 kb *Pvu*II–*Bgl*II DNA fragment isolated from plasmid pJH318 (a gift from Dr S.Henry). *ACT1* and *RP51*A transcripts were identified with a 0.25 kB *Bgl*II–*Hin*dIII fragment and a 0.52 kb *Ava*II–*Sal*I fragment, respectively. All DNA probes were labelled by the method of random priming (Ausubel *et al*., 1989).

β-galactosidase assay

htb1 mutants were transformed with the *CEN3-URA3 HO–lacZ* reporter gene plasmid, p12 (a gift of Dr Kenneth Robzyk). Ten millilitre cultures were grown to mid-log phase in supplemented SD-uracil medium. β-galactosidase assays were performed in duplicate in permeabilized cells prepared from at least three independent transformants, and the results are expressed as Miller units (Perez-Martin and Johnson, 1998).

Measurement of 2μ plasmid DNA superhelical density

DNA was isolated from cells grown to mid-log phase in supplemented SD medium, using glass beads to lyse cells in the presence of protein denaturants (Kim *et al*., 1993). Twenty micrograms of total DNA was electrophoresed through a 0.7% agarose gel in Tris-phosphate buffer containing 25 μg/ml of chloroquine at 50 V for 27.5 h at 4°C. Topoisomers were transferred to a GeneScreen membrane (Dupont-NEN) and detected by hybridization to a 2.2 kb *Eco*RI fragment isolated from 2μ plasmid DNA and labelled by the method of random priming. Topoisomer distributions were quantitated by PhosphorImager analysis, using a Fuji PhosphorImager and MacBas software.

Immunological analysis

JR5-2A cells that contained pRS314-Flag-*HTB1* or pRS314-*HTB1* plasmids were grown in 150 ml YPD medium to a density of 1×10^7 cells/ml. Cell pellets were lysed with glass beads, and 30 μl of anti-Flag M2 affinity resin (Kodak) were added to 1.2 mg of protein in IP buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.5% NP-40 and 0.5 mg/ml BSA) in the presence of 250 units of DNase I (Boehringer Mannheim) for 2 h at 4°C. Protein bound affinity resin was washed three times with IP buffer in the presence of 5 mg/ml BSA and three times with IP buffer in the absence of BSA. Proteins were released rom the resin by boiling and analyzed by 7.5% (α-Snf5p) or 15% (α-Flag) PAGE (Ausubel *et al*., 1989). Proteins were transferred to Immobilon membranes (Millipore) for Western blot analysis as previously described (Recht *et al*., 1996), using a 1:2000 dilution of polyclonal antibody against Snf5p (a gift of Dr Brehon Laurent) or a 1:300 dilution of anti-Flag M2 monoclonal antibody. Detection was performed by enhanced chemiluminescence (Dupont-NEN).

Indirect end-labeling of SUC2 chromatin

Nuclei were prepared from 500 ml YP + 0.05% dextrose cultures that had been induced for 2.75 h as described by Hirschhorn *et al*. (1992), with the exception that nuclei were resuspended in S buffer containing 0.5 mM PMSF (Norris *et al*., 1988) before storage at –80°C. Indirect end-labeling of *SUC2* chromatin was performed on 200 µl of nuclei digested with 0, 1 or 3 units of micrococcal nuclease (MNase) for 5 min at 37°C. Chromosomal DNA prepared by the method of Wu and Winston (1997) was digested with 0.3 or 1.0 units of MNase in 200 µl SPC buffer containing 10mM $CaCl_2$ (Hirschhorn *et al*., 1992). DNA was digested with *Hin*fI and separated by electrophoresis through a 2% agarose gel. Southern blot analysis was performed after transfer to a GeneScreen membrane (Dupont-NEN), using a 166 bp *SUC2* probe fragment (+140 to +196) labelled by the random priming method. Hybridization and washes were performed according to the procedures described by Hirschhorn *et al*. (1992).

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