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Analysis of a Mutant Histone H3 That Perturbs the Association of Swi/Snf with Chromatin

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We have isolated new histone H3 mutants in *Saccharomyces cerevisiae* that confer phenotypes indicative of transcriptional defects. Here we describe the characterization of one such mutant, encoded by the *hht2-11* allele, which contains the single amino acid change L61W in the globular domain of H3. Whole-genome expression analyses show that the *hht2-11* mutation confers pleiotropic transcriptional defects and that many of the genes it affects are normally controlled by the Swi/Snf chromatin remodeling complex. Furthermore, we show that Swi/Snf occupancy at two promoters, *PHO84* and *SER3*, is reduced in *hht2-11* mutants. Detailed studies of the *PHO84* promoter suggest that the *hht2-11* mutation impairs Swi/Snf association with chromatin in a direct fashion. Taken together, our results strongly suggest that the integrity of the globular domain of histone H3 is an important determinant in the ability of Swi/Snf to associate with chromatin.

In eukaryotic cells, nucleosomes pose a structural barrier to factors that use chromatin as the substrate for various cellular functions, including transcription, replication, recombination, and repair. The four core histones—H2A, H2B, H3, and H4—are the major protein components of chromatin, and it has become increasingly clear that they play crucial and active roles in the regulation of these essential functions. In particular, our understanding of the dynamic function of chromatin has significantly advanced as a result of studies of its role in the regulation of transcription (33).

One approach to defining the interplay between chromatin and transcription has been to study the protein complexes that act upon chromatin and alter its properties. One class of such complexes functions by covalently modifying specific residues within histones (6, 24). Most complexes identified thus far direct their activities toward residues located in the tails, particularly the N-terminal tails, of the core histones (65). Modifications, including acetylation, methylation, phosphorylation, and ubiquitylation, have been associated with specific chromatin effects that ultimately affect transcription. The combination of posttranslational modifications present on a particular histone is thought to constitute a "histone code" that dictates the property of that particular nucleosome (17, 52, 58).

Members of a second class of complexes, referred to as chromatin-remodeling complexes, utilize the energy from ATP hydrolysis to remodel nucleosomes in order to facilitate subsequent binding of proteins to a particular site on DNA (33, 54, 60). The yeast Swi/Snf complex is the founding member of this family, and both it and other members of this class function as activators as well as repressors of transcription (31). Genetic and biochemical experiments have shown that Swi/Snf is recruited to gene promoters by transcription factors and to subsequently alter chromatin structure (40). However, despite our basic understanding of the function of this complex, relatively little is known regarding the specific interactions that occur between Swi/Snf and chromatin.

An alternative strategy to defining the role of chromatin in transcriptional regulation has been to study the effects of histone mutations on gene expression (49). The N-terminal tails of histones H3 and H4 have been the focus of extensive mutational analyses. For example, genetic experiments in yeast have implicated the tails of H3 and H4 in maintaining silencing at the silent mating-type loci and at telomeres (20, 56). Recent whole-genome transcriptional studies have also shown that the N-terminal tail of histone H3 plays a general role in transcriptional repression (44). A number of experiments have also started to elucidate the function of the globular domains of the histone proteins in gene expression. Histone mutations have been isolated that bypass the requirement of Swi/Snf in transcriptional activation (22, 25, 41, 45, 61). Specific mutations within the globular domain of histone H2A have also been shown to affect transcription (21). Finally, recent genetic experiments have identified a nucleosomal surface that is required to maintain proper transcriptional silencing (38), as well as additional residues in the histone H3 and H4 globular domains that are important for normal levels of silencing (48, 57). In particular, K79 in histone H3, which is important for transcriptional silencing, has been shown to be the target of methylation by the Dot1 protein (9, 27, 36, 59).

The experiments presented in the present study provide new insights into the relationship between the Swi/Snf complex and chromatin. Specifically, we describe the isolation of a novel histone H3-globular domain mutant in *Saccharomyces cerevisiae* that appears to directly impair the association of Swi/Snf with chromatin. Combined with previous studies, our experiments strongly suggest that not only can Swi/Snf regulate chromatin structure but that the nature of the chromatin environment itself can influence how efficiently Swi/Snf is able to associate with specific promoters.

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MATERIALS AND METHODS

Yeast strains, genetic methods, and media. All *S. cerevisiae* strains used in the present study (listed in Table 1) are *GAL2*$^+$ derivatives of the S288C strain

TABLE 1. *S. cerevisiae* strains

Strain	Genotype
FY84	*MAT***a** *his3Δ200 leu2*Δ1 *ura3-52 lys2-128*δ
FY2160	*MAT***a** *his3Δ200 leu2*Δ1 *ura3-52 lys2-128*δ *hht2-11*
FY2161	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 lys2-128*δ*hht2*Δ::*URA3*
FY2162	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4* <pDM9>
FY2163	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4* <pDM18>
FY2164	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4* <pAAD11>
FY2165	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4* <pAAD180>
FY2166	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4 met15Δ0 SNF5-C18myc*::*KanMX4* <pDM18>
FY2167	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4 met15Δ0 SNF5-C18myc*::*KanMX4* <pAAD11>
FY2168	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4* <pDM18>
FY2169	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 snf2*Δ::*LEU2* <pDM18>
FY2170	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 snf2*Δ::*LEU2* <pDM18>
FY2171	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 snf2*Δ::*LEU2* <pAAD11>
FY2172	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 arg82*Δ::*KanMX4* <pDM18>
FY2201	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4 ser33*Δ::*KanMX4* <pDM18>
FY2202	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*LEU2* (*hht2-hhf2*)Δ::*HIS3 Ty912Δ35-lacZ*::*his4 ser33*Δ::*KanMX4* <pAAD11>
FY2203	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 snf2*Δ::*LEU2 ser33*Δ::*KanMX4* <pDM18>
FY2234	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-128*δ (*hht1-hhf1*)Δ::*HIS3* (*hht2-hhf2*)Δ::*HIS3 snf2*Δ::*LEU2* <pAAD11>
FY2235	*MAT*α *his3Δ200* or *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 SNF5-C18myc*::*kTRP1 pho4*Δ::*KanMX4*

background (64). Replacements of the *HHT1-HHF1* locus with *LEU2* and the *HHT2-HHF2* locus with *HIS3* were achieved, respectively, by transformations with pUK192 digested with *Hin*dIII and pUK431 digested with *Eco*RI and *Hin*cII (plasmids are gifts from M. Grunstein [29]). The (*hht1-hhf1*)Δ::*HIS3* alleles were generated by replacing the *LEU2* gene in (*hht1-hhf1*)Δ::*LEU2* cells with the *HIS3* gene amplified from pRS413 (8). The *hht2*Δ::*URA3* alleles were constructed by replacing the *HHT2* gene with the *URA3* gene from pRS426 (11). The *Ty912Δ35-lacZ*::*his4* reporter gene is a derivative of the *Ty912Δ44-lacZ*::*his4* allele previously described (14) containing an extra 225 bp of the ε region. The *SNF5*::*C18Myc*::*kanMX4* constructs were obtained by replacing *K. lactis TRP1* within the *SNF5*::*C18Myc*::*kTRP1* fusion (30) with PCR-amplified *kanMX4* from pRS400 (8). Construction of *snf2*Δ::*LEU2* alleles has been described previously (10). Generation of the *SER33*Δ::*kanMX4* alleles has been described previously (30). Strain FY2235 (*pho4*Δ::*KanMX4*) was obtained through crosses with a commercially available deletion strain (Research Genetics). Deletion of the *ARG82* gene was achieved by replacing the open reading frame (ORF) with the PCR-amplified *kanMX4* gene from plasmid pRS400. Integration of the *hht2-11* allele in the genome was performed by first replacing the *HHT2* gene with a *URA3-TRP1* cassette and then cotransforming the resulting strain with a *Bgl*II-*Sma*I fragment derived from pAAD11 and pRS413. His^+ transformants were then selected and then screened for Ura^- and Trp^- phenotypes. Correct integration was confirmed by PCR. Cells containing the integrated version of *hht2-11* as the sole source of histone H3 display phenotypes very similar to cells expressing the mutant histone protein from a plasmid as the only source of H3 (data not shown).

Mating, transformation, sporulation, and tetrad analysis were performed by standard procedures previously described (43). Rich (yeast extract-peptone-dextrose [YPD]), synthetic dextrose (SD), synthetic complete (SC), omission (SC−), 5-fluoroorotic acid (5-FOA), and sporulation media were prepared as previously described (43). Where indicated, drugs were added to the following final concentrations: 150 mM for hydroxyurea, 15 mM for caffeine, and 3% (vol/vol) for formamide. Selection for cells harboring the *kanMX4* gene was conducted on YPD medium containing 200 μg of G418 sulfate (Gibco) per ml.

Plasmid DNA construction. pDM9 was constructed by amplifying the *HHT1-HHF1* locus from plasmid pCC64 (12) and ligating it into pRS416 (8) by using *Hin*dIII and *Xma*I. pDM18 is based on the pRS414 plasmid (8) and contains the wild-type *HHT2-HHF2* region except for the following mutations that were created by using a site-directed mutagenesis kit (Stratagene): *Afl*II and *Rsr*II sites were generated 3′ and 5′ of *HHT2*, respectively; *Hpa*I and *Bgl*II sites were created 3′ and 5′ of the *HHF2* gene, respectively. Plasmids harboring mutant versions of *HHT2* were obtained by using random PCR mutagenesis and gap-repair as described below. pAAD180 was derived by subcloning a *Pvu*II fragment containing the *hht2-11–HHF2* region (from pAAD11) into pRS424 (11). The plasmids carrying the different *hht2* alleles are named as follows: pAAD3 for *hht2-3*, pAAD5 for *hht2-5*, pAAD7 for *hht2-7*, pAAD11 for *hht2-11*, pAAD41 for *hht2-41*, pAAD44 for *hht2-44*, pAAD48 for *hht2-48*, pAAD49 for *hht2-49*, pAAD51 for *hht2-51*, pAAD56 for *hht2-56*, and pAAD57 for *hht2-57*.

Random PCR mutagenesis of the *HHT2* gene. Mutations within the *HHT2* ORF were obtained by random PCR mutagenesis and gap repair as described below. PCR mutagenesis was done by a procedure similar to a previously described method (21). Plasmid pDM18 was used as a template for PCRs with the primers FO4 (5′-GGATCCCCCGGGGGTAATATGTAGACAGTGATT-3′) and FO125 (5′-GGGCGTCCTACGGATGGGAGTTGG-3′). This reaction generates a product encompassing the entire *HHT2* gene, as well as 263 bp 5′ and 303 bp 3′ of the ORF. Several identical pools of product were generated by using 35 cycles of PCR (94°C for 30 s, 65°C for 20 s, and 72°C for 45 s) with standard concentrations of $MgCl_2$ (1.5 mM) and deoxynucleoside triphosphates (0.2 mM for each). After this round of PCR, 1 μl from a particular pool was used as a template for a second round of PCR under the conditions described above. The resulting products were then cotransformed into strain FY2162 with an *Afl*II-*Rsr*II fragment derived from pDM18 which retains the *TRP1* and *CEN* sequences, as well as >150 bp of homology with each end of the PCR products but which lacks the *HHT2* ORF. Trp^+ transformants were then selected for loss of the wild-type plasmid pDM9 by selection on 5-FOA medium and tested for phenotypes.

Approximately 25,000 transformants were screened for several phenotypes. These phenotypes included inability to grow on media containing galactose or raffinose as the sole carbon source (Gal^- and Raf^- phenotypes) and on media lacking inositol (Ino^- phenotype). We also screened for defects in growth at elevated (37°C, Ts^- phenotype) or reduced (14°, Cs^- phenotype) temperatures. From these analyses, we focused on 11 alleles that displayed one or more of the above phenotypes. Although some mutants initially appeared to have Gal^- or Raf^- phenotypes, further analysis revealed that these phenotypes were only observed when the cells were transferred from 5-FOA medium (to select for loss of the wild-type plasmid) directly onto media containing either galactose or raffinose a sole carbon source. This phenomenon was also seen to some degree in cells harboring a wild-type copy of *HHT2*. We do not know the cause or relevance of this effect.

Whole-genome expression analyses. Independent isolates of strains with genotypes identical to either FY2163 or FY2164 were grown in YPD medium and collected at a density of 1×10^7 to 3×10^7 cells/ml. For the 14°C microarray experiments, *HHT2* and *hht2-11* cells were grown at 30°C to early log phase and then shifted to 14°C for ca. 24 h. The viability of the mutant cells was not significantly affected by the 24-h shift to the nonpermissive temperature. At 14°C, the division time for *HHT2* cells is ~10 h. Under the same conditions, *hht2-11* mutant cells divide one time and then essentially stop growing. These cells do not

TABLE 2. Predicted amino acid substitutions and phenotypes conferred by the *hht2* alleles

Allele	Amino acid change(s)	Score[a]								
		YPD	YPD at 14°C	YPD at 37°C	YPD + HU	YPD + Form.	YPD + Caff.	SC−Lys[b]	SD	SD−ino
HHT2		5	5	5	5	5	5	1	5	5
hht2-3	F104S	4	4	1	3	4	1	1	5	5
hht2-5	E50G, G132S	3	1	1	0	0	0	3	4	3
hht2-7	F78Y, R129Stop	2	2	0	2	0	0	1	4	4
hht2-11	L61W	3	0	2	0	0	0	4	3	1
hht2-41	Q68R	4	4	3	1	2	2	4	4	4
hht2-44	Q93R	4	3	3	2	4	3	4	4	4
hht2-48	L60P, F84L	4	4	0	0	0	1	4	5	2
hht2-49	K37E	4	4	4	2	3	0	0	4	4
hht2-51	F54L	4	4	2	2	4	3	3	5	5
hht2-56	Y41C	3	2	3	2	2	0	0	3	3
hht2-57	I112T	3	2	2	2	3	1	1	4	4

[a] Mutant phenotypes were scored in strains in which the indicated *hht2* allele encoded the only source of histone H3 in the cell. Growth was scored on a scale of 5 to 0, where 5 represents wild-type growth, and 0 represents no growth. Media and conditions are as described in the legend to Fig. 2.

[b] Growth on SC-Lys scores for Spt$^-$ phenotypes. All strains contain the *lys2-128*δ mutation, an insertion of a Ty δ element in the ORF of the *LYS2* gene (15a). In a wild-type (*HHT2*) background, this mutation causes a Lys$^-$ phenotype (growth score of 1). Some *hht2* alleles suppress this defect (growth scores of 2 to 4), whereas some strengthen the defect (growth score of 0). These *hht2* alleles are likely to affect transcription at this locus (see the text).

appear to stop growing at any particular phase of the cell cycle. Total RNA was isolated by using the hot phenol method (5). The RNA was cleaned up by using the RNeasy Minikit (Qiagen). Slides were prepared by either the Harvard Medical School Biopolymers Facility or by the Whitehead Institute Center for Microarray Technology. Both types of slides are glass arrays spotted with 70-mer oligonucleotides corresponding to 6,388 ORFs (from Qiagen). Sample labeling (performed by reverse transcription-direct incorporation of cyanine dyes), analyses, hybridization, and slide washes were performed as described at the Whitehead Institute Center for Microarray Technology web site (http://www.whitehead.mit.edu/CMT/Microarrayhome.html) with the following modifications. Slides were incubated for 20 min in prehybridization solution (3.5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate, 10 mg of bovine serum albumin/ml) prewarmed to 50°C. A total of 20 pmol of each of the labeled samples was pooled and concentrated in a Speed-Vac (Savant). The samples were then resuspended in ~130 μl of hybridization solution [3× SSC, 0.1% SDS, 0.1 mg of salmon sperm DNA/ml, 0.2 mg of tRNA/ml, 0.4 mg of poly(A)/ml], heated at 90°C for 5 min, and applied to slides placed in CMT hybridization chambers (Corning). The chambers were then placed in a 50°C water bath for an overnight incubation. Slides were scanned by using a GenePix 4000B scanner (Axon Instruments), and the data were analyzed by using the GenePix Pro 4.0 program.

Results from all microarray experiments, as well as details on the analysis of the data, can be found at http://genetics.med.harvard.edu/%7Ewinston/hht2-11.html. For experiments comparing expression profiles of cells grown at 30°C, three independent samples of cells expressing either *HHT2* or *hht2-11* as the only source of histone H3 were analyzed. Two independent samples of each wild-type and mutant cells were assayed for the experiments performed at 14°C. To avoid misleading data resulting from biased incorporation of one cyanine dye over the other, *HHT2* and *hht2-11* samples were reciprocally labeled with Cy3-dUTP and Cy5-dUTP in different experiments (see http://genetics.med.harvard.edu/%7Ewinston/hht2-11.html for details). MIPS functional classification of the affected genes in *hht2-11* cells and statistical significance calculations were carried out by using the FunSpec web-based tool described elsewhere (42). The statistical significance of the overlap between affected genes in *hht2-11* and *snf2*Δ cells, as well as between genes affected by *hht2-11* at 30 and 14°C, was determined by using a hypergeometric distribution calculation. *P* values were calculated by using the hypergeometric distribution calculator at http://www.alewand.de/stattab/tabdiske.htm.

Northern hybridization analysis. Cell growth and RNA isolation were performed as described above. Total RNA was separated on a 1% agarose gel and transferred to nylon membrane. The probe specific to the *PHO84* mRNA was synthesized by PCR amplification of the first 246 bases of the ORF. The *SER3* probe has been described previously (30). The *SNR190* probe was obtained through PCR amplification of the entire gene. All probes were radiolabeled with [α-^{32}P]dATP by random priming (5). Quantitation of relative levels of mRNA was performed by using a PhosphorImager (Molecular Dynamics). Based on the Northern blot, microarray, and chromatin immunoprecipitation experiments presented in here (see Results), as well as on previously reported findings (34, 50), growth in YPD medium is a condition that results in a significant level of activation of the *PHO84* gene. The expression level of *PHO84* in our experimental conditions in wild-type cells is ca. 63% of that seen in cells in which the *PHO* pathway is constitutively activated through a deletion for the gene encoding Pho80 (data not shown).

Chromatin immunoprecipitation experiments. Chromatin immunoprecipitation experiments were performed as previously described (30). Pho4 chromatin immunoprecipitation was performed with a rabbit polyclonal antibody specific to the Pho4 protein (a gift from Dennis Wykoff and Erin O'Shea). Quantitative radioactive PCR was performed as previously described (28). Primers used to detect the *SER3* promoter have been previously described (30). The *PHO84* promoter was amplified with primers spanning a region from positions −377 to −35 (+1 = ATG). Primers to amplify a region within the *POL1* gene have been described previously (26).

Indirect end-labeling analysis of *SER3* chromatin structure. Analysis of *SER3* chromatin structure was performed as previously described (30). Briefly, logarithmically growing cells were collected and spheroplated. Spheroplasts were then treated with increasing concentrations of micrococcal nuclease (MNase). Naked DNA was also analyzed in these assays to determine preferential cut sites for the MNase. After these treatments, the DNA was isolated, digested with *Bgl*II, and resolved on a 1.5% agarose gel. The DNA was then subjected to indirect end-labeling analysis with a *SER3*-specific probe.

RESULTS

Isolation of novel histone H3 mutants. To better understand the contribution of histone H3 in transcriptional regulation, we performed a screen for new histone H3 mutants. Transformants containing mutagenized copies of a plasmid-borne *HHT2* gene were screened for a variety of phenotypes indicative of transcriptional defects, such as inositol auxotrophy (Ino$^-$ phenotype) and for sensitivity to high and low temperatures (Ts$^-$ and Cs$^-$ phenotypes; see Materials and Methods). Table 2 shows the amino acid changes and phenotypes conferred by 11 alleles isolated in this screen. DNA sequence analysis showed that most encode single-amino-acid changes and that all of the predicted amino acid changes are within the globular domain of the protein (Table 2). The finding that some mutants confer an Ino$^-$ phenotype and that most suppress an insertion mutation in the *LYS2* gene (Spt$^-$ phenotype) suggests that these alleles affect transcription (for a review of these phenotypes, see references 18 and 63). The breadth and strength of the phenotypes conferred by the different *hht2* alleles isolated reveal that mutations within the

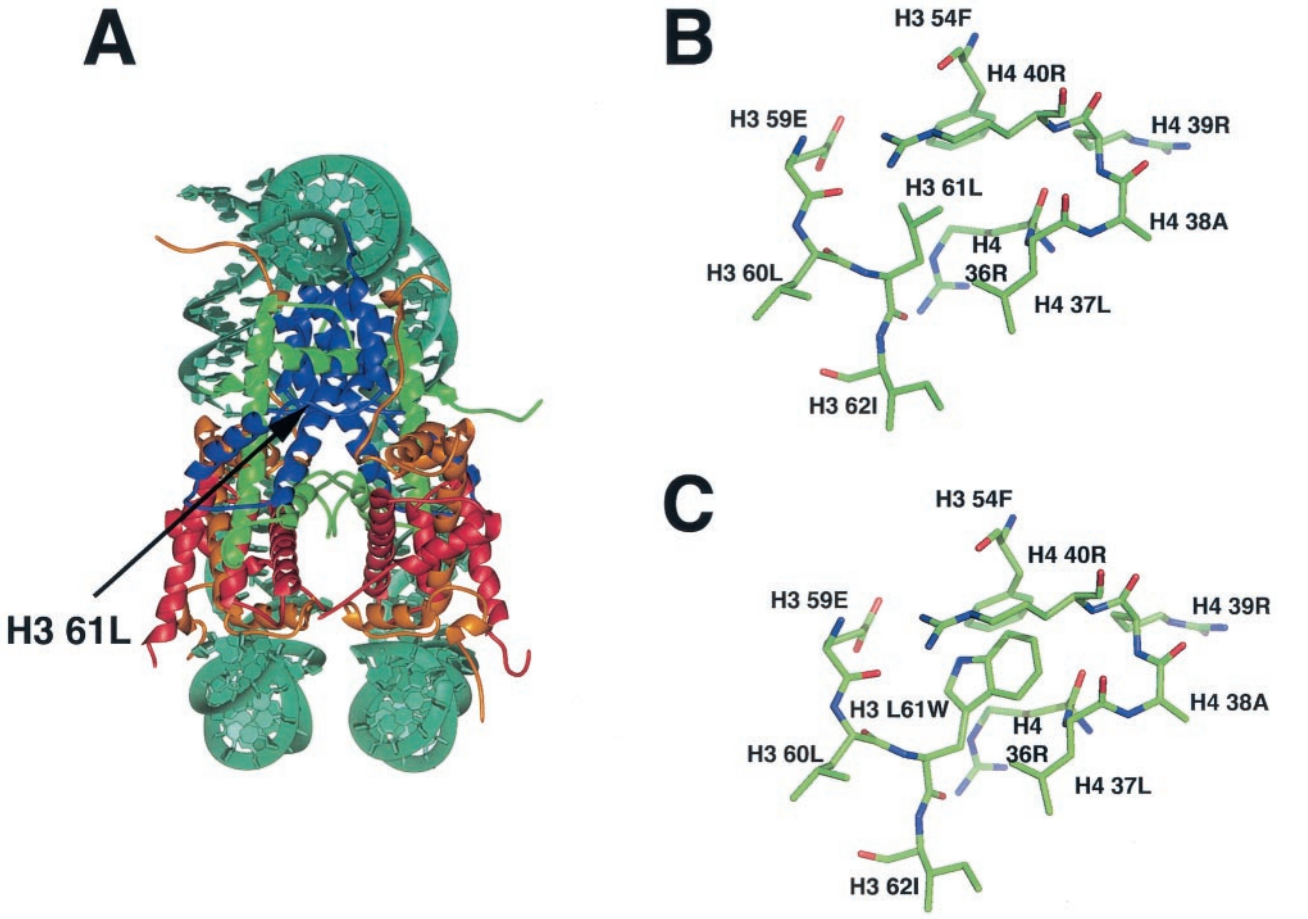

FIG. 1. Location and nature of the amino acid substitution encoded by the *hht2-11* allele. (A) Side view of the yeast nucleosome core particle as solved by White et al. (62). Part of the DNA is removed for clarity. The histone proteins are depicted as follows: H2A is in gold, H2B is in red, H3 is in blue, and H4 is in green. The arrow indicates the location of 61L within one of the two histone H3 proteins. (B and C) Close-up views of the region of interest showing the wild-type structure (B) and the predicted structure modeled with the L61W substitution (C). These figures were kindly provided by Cindy L. White and Karolin Luger.

globular domain of histone H3 can cause a wide range of phenotypic defects.

Analysis of the *hht2-11* mutant. To learn more about the relationship between histone H3 and transcription, we decided to focus our analysis on the *hht2-11* allele, since this mutant confers the phenotypes most strongly suggestive of transcriptional defects. Furthermore, the conditional Cs^- phenotype allows the analysis of this mutant under conditions that strengthen its effects and might therefore reveal additional characteristics of the mutant. The *hht2-11* allele encodes a single amino acid change at position 61 of H3, from a leucine to a tryptophan (H3 L61W). Leucine 61 is located within the globular domain of the protein in the loop region connecting the N-terminal helix and the first helix of the histone fold domain and lies on an interface between histones H3 and H4 (Fig. 1A). The substitution to a tryptophan is not predicted to confer any major structural change in the nucleosome, but the residue does appear to pack comfortably at the H3-H4 interface, possibly increasing hydrophobic interactions between H3 and H4 (Fig. 1B and C). These new interactions would result in increased H3-H4 tetramer stability that might ultimately lead to a stabilization of the H3 N-terminal helix (C. White and K. Luger, unpublished data). This stabilization might pose a barrier to the function of factors that operate by altering nucleosome-DNA interactions to mediate cellular processes, such as chromatin remodeling complexes in transcriptional regulation.

The nature and breadth of the phenotypes conferred by the *hht2-11* mutation are shown in Fig. 2A. Interestingly, we found that many of these defects, including the Ino^- phenotype, are shared with cells lacking the Swi/Snf chromatin remodeling complex (*snf2*Δ cells, compare the second and fourth rows in Fig. 2A), thus providing phenotypic evidence that the H3 L61W mutant and Swi/Snf might be functionally related. To examine the relationship between *hht2-11* and *snf2*Δ in greater detail, we also analyzed a *snf2*Δ *hht2-11* double mutant. This analysis (Fig. 2A, bottom row) showed that for most phenotypes examined (sensitivity to caffeine and formamide and the Spt^- phenotype) the double-mutant phenotype is the same as the stronger of the two single-mutant phenotypes. For two other phenotypes (inositol auxotrophy and growth on SD medium) the double mutant is less severe (see Discussion).

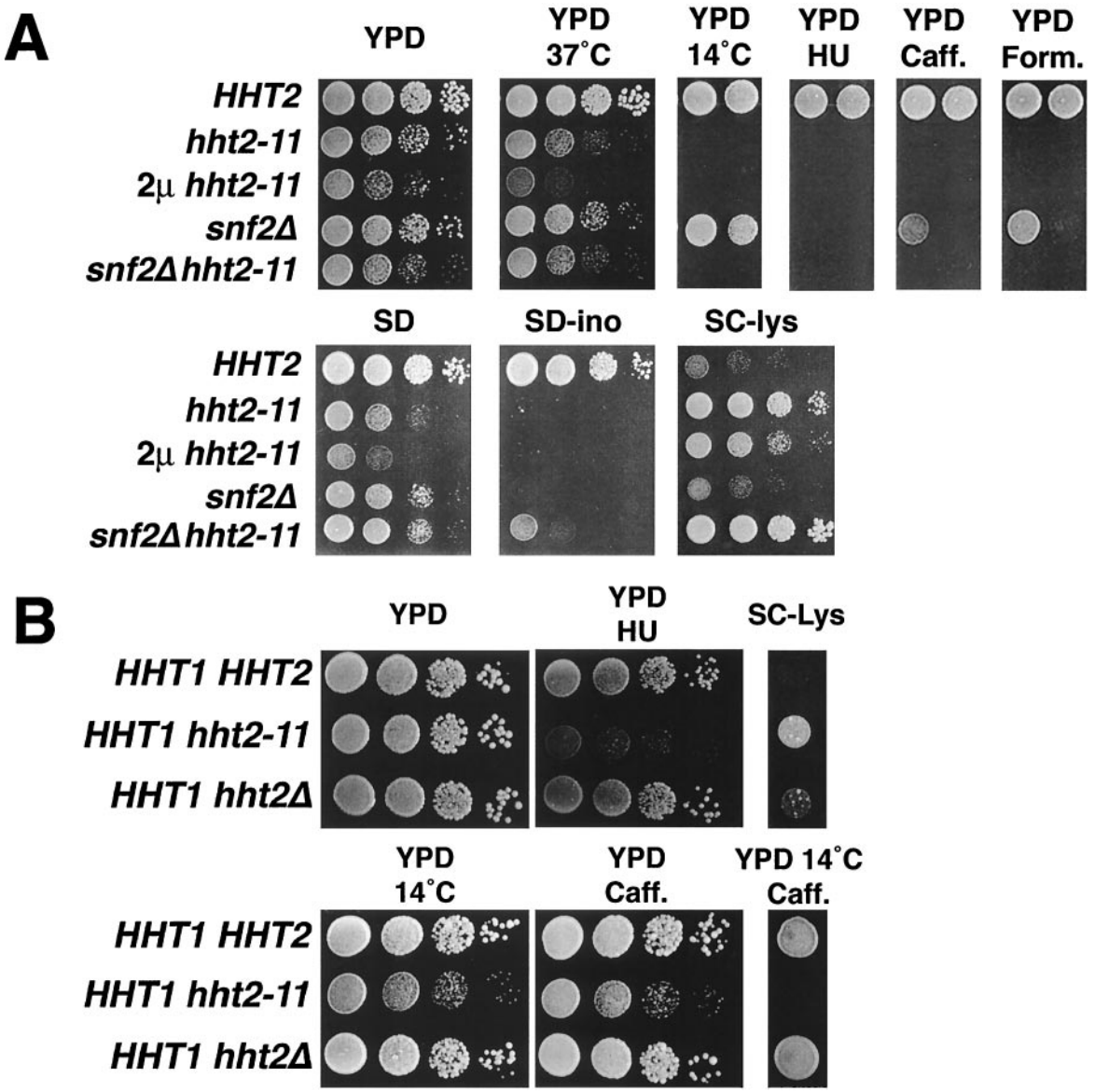

FIG. 2. Phenotypic analyses of strains harboring the *hht2-11* allele. (A) Phenotypes conferred by *hht2-11* when present as the sole source of histone H3. The mutant strains used were as follows: *HHT2* (strain FY2163), *hht2-11* (FY2164), 2μm *hht2-11* (FY2165), *snf2*Δ (FY2170), and *snf2*Δ *hht2-11* (FY2234). Cells were grown to saturation overnight in YPD medium, washed with H_2O, and then spotted in a dilution series from 7×10^6 to 7×10^3 cells/ml on the indicated medium (see Materials and Methods). For some phenotypes, only the two most concentrated spots are shown for simplicity. An Spt^- phenotype corresponds to growth on SC−Lys medium (see Table 2). The plates were then incubated at either 30°C or at the indicated temperature for the following times: YPD, 2 days; YPD at 37°C, 2 days; YPD at 14°C, 11 days; YPD + HU, 7 days; YPD + caffeine (Caff.), 5 days; YPD + formamide (Form.), 3 days; SD, 3 days; SD−inositol (ino), 4 days; and SC−Lys, 3 days. (B) Dominant phenotypes conferred by *hht2-11*. The strains used are as follows: *HHT1 HHT2* (strain FY84), *HHT1 hht2-11* (strain FY2160), and *HHT1 hht2*Δ (strain FY2161). Cells were grown and spotted as described above, except that for "SC-Lys" and "YPD 14°C Caff." the spots shown are at concentrations of 8×10^7 cells/ml. The plates were then incubated as described above, except for the "YPD 14°C Caff. plate," which was incubated for 30 days.

If H3 L61W alters the property of the nucleosome, for example, by increasing the structural stability as described above, the mutation would be predicted to behave phenotypically as a gain-of-function mutation. Alternatively, if H3 L61W impaired H3 function, for example, by causing reduced levels of H3, it would behave as a loss-of-function mutation. To distinguish between these possibilities, we performed two genetic tests. First, we tested the effect of overexpression of the *hht2-11* allele from a multicopy plasmid. These strains displayed all of the *hht2-11* mutant phenotypes (Fig. 2A), making it unlikely that the effects seen are a result of reduced levels of H3. Second, we tested whether any of the phenotypes conferred by the *hht2-11* mutant are dominant. This was done by constructing strains that contained the *hht2-11* allele, as well as a wild-type copy of *HHT1*, the second histone H3-encoding gene in yeast. As shown in Fig. 2B, the *hht2-11* mutant displayed several dominant phenotypes compared to either wild-type or *hht2*Δ cells. These results strongly suggest that the effects

TABLE 3. Genes whose expression is most highly affected by *hht2-11*

Temp (°C)[b]	ORF	Gene[a]	Fold increase (+) or decrease (−)
30	YOL052C-A	*DDR2*	10 (+)
	YER081W	*SER3**	9 (+)
	YOL155C		7 (+)
	YNL160W	*YGP1*	7 (+)
	YIL169C		5 (+)
	YHR136C	*SPL2**	5 (−)
	YBR296C	*PHO89*	4 (−)
	YML123C	*PHO84**	3 (−)
	YDR367W		3 (−)
	YGR234W	*YHB1**	2 (−)
14	YBR040W	*FIG1*	14 (+)
	YIL082W-A	*TyB*	10 (+)
	YKL221W	*MCH2**	8 (+)
	YFR026C		8 (+)
	YBR116C		7 (+)
	YHR215W	*PHO12**	21 (−)
	YBR296C	*PHO89*	20 (−)
	YBR093C	*PHO5**	18 (−)
	YAR071W	*PHO11**	17 (−)
	YHR136C	*SPL2**	14 (−)

[a] Genes affected by the *hht2-11* and *snf2*Δ mutations in a similar fashion are indicated by an asterisk.

[b] There was a highly significant overlap in the number of genes affected by *hht2-11* at either temperature ($P \ll 10^{-9}$).

caused by H3 L61W are the result of a mutation that alters the function of histone H3 and are not simply due to reduced H3 function.

Whole-genome expression analysis of *hht2-11* cell. To obtain a comprehensive view of the transcriptional defects conferred by the *hht2-11* mutation, we performed whole-genome expression analyses by using microarrays. These experiments were performed on cells grown at both the permissive temperature (30°C) or after a shift to the nonpermissive temperature (14°C). At the permissive temperature, the *hht2-11* mutation resulted in increased expression of ~3% of the genes and decreased expression of ~0.4% of the genes. Interestingly, whereas the percentages of the genes whose expression is increased were similar whether the cells were grown at 30°C or grown at 14°C, we observed a 10-fold increase in the numbers of genes whose expression was reduced when the cells were shifted to 14°C. Based on the structural information discussed above, the fact that the *hht2-11* mutation is less permissive to transcription at 14°C might reflect either an increased stabilization of the H3-H4 tetramer or an increased dependence on normal H3-H4 interactions for transcriptional activation at this temperature. The inability of *hht2-11* cells to grow at the nonpermissive temperature might be due in part to the marked increase in abnormally downregulated genes in the mutant. Among the affected genes, we see enrichment of genes involved in several functional categories, including phosphate metabolism, lysosomal and vacuolar degradation, and cell rescue, defense, and virulence (see Tables A1 and A2 at http://genetics.med.harvard.edu/%7Ewinston/hht2-11.html).

Table 3 lists the genes that are most highly affected by the *hht2-11* mutation. Strikingly, among these genes, nearly 50% are affected in a similar fashion by a *snf2*Δ mutation (53). To further explore a potential correlation between H3 L61W and Swi/Snf functions, we performed a genomewide comparison of the transcriptional defects displayed by the histone mutant with those reported for cells deleted for *SNF2* (53). This analysis (Fig. 3) shows that, for all pairwise comparisons (with the possible exception of the comparison shown in the top panel of Fig. 3B), there is a statistically significant overlap between those genes whose expression is similarly affected by both the *hht2-11* and the *snf2*Δ mutations. A comparison of our *hht2-11* data with microarray data published for mutations in three other factors that affect chromatin dynamics—Rsc30, Hda1, and Isw2 (3, 7, 16)—showed reduced statistical significance in the overlap in the case of *isw2*Δ versus *hht2-11*, and no significant overlap in the cases of *rsc30*Δ versus *hht2-11* and *hda1*Δ versus *hht2-11* (see Fig. A1 to A3 at http://genetics.med.harvard.edu/%7Ewinston/hht2-11.html), possibly suggesting some degree of specificity in the effects of *hht2-11* on Swi/Snf. We note, however, that the correlation we observe between *hht2-11* and *snf2*Δ by itself, although suggestive, does not demonstrate that the two mutations affect common functions. For example, unrelated mutations that activate the stress response by different mechanisms might be expected to also show statistically significant correlations in the number of genes they affect. Nevertheless, our microarray data, together with the phenotypic and structural data discussed above, suggested the possibility that nucleosomes containing the H3 L61W mutant protein might in some cases be resistant to the function of the Swi/Snf complex.

H3 L61W reduces the association of Swi/Snf with the *SER3* and *PHO84* promoters. To test the possibility that nucleo-

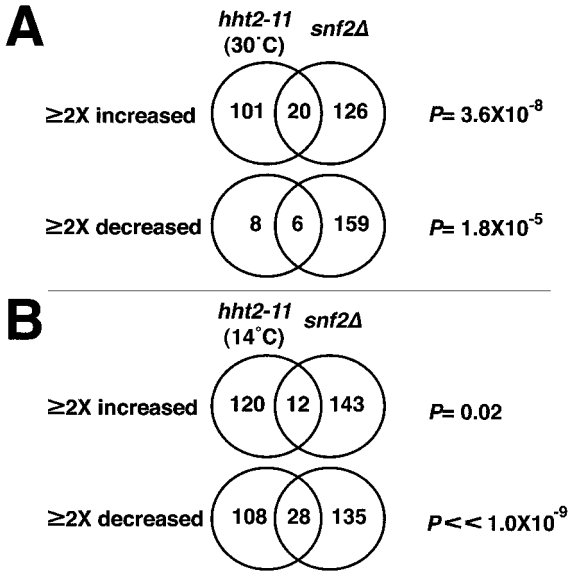

FIG. 3. Overlap in the number of genes whose expression is affected by the *hht2-11* and *snf2*Δ mutations. (A) A Venn diagram showing the number of genes whose expression is either increased or decreased in *hht2-11* and *snf2*Δ cells grown at 30°C and the overlap in the two data sets with the corresponding *P* value. The data from 3,639 genes were used for this comparison. (B) Same as for panel A, except that the data for the *hht2-11* mutant was obtained from experiments in which the cells were shifted to 14°C. The data from 2,955 genes were used for this comparison.

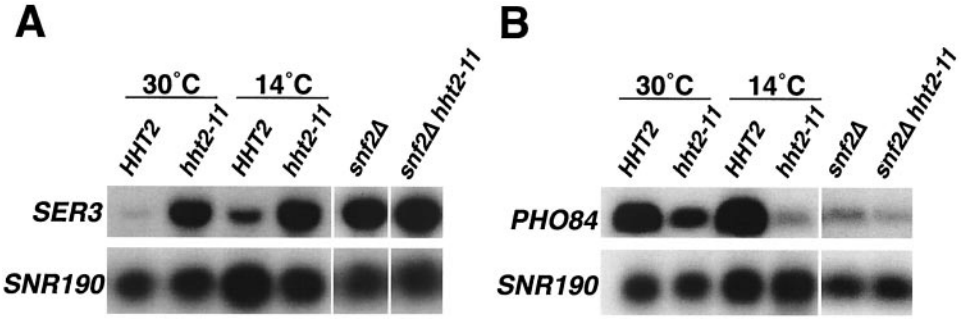

FIG. 4. The *hht2-11* and *snf2*Δ mutations affect *SER3* and *PHO84* expression in a similar manner. (A) Northern analysis of *SER3* mRNA levels in *HHT2* (strain FY2163), *hht2-11* (strain FY2164), *snf2*Δ (strain FY2169), and *snf2*Δ *hht2-11* (strain FY2171) strains. *snf2*Δ and *snf2*Δ *hht2-11* cells were grown at 30°C, whereas the *HHT2* and *hht2-11* cells were either grown at 30°C or shifted to 14°C for 24 h, as indicated. The levels of the *SNR190* transcript, used as a loading control, are also shown for each strain. The data shown is representative of at least three independent experiments. (B) Northern analysis of *PHO84* mRNA levels was performed with the same strains and conditions as described for panel A. The data shown are representative of at least three independent experiments.

somes containing H3 L61W are refractory to the chromatin remodeling activity of the Swi/Snf complex, we focused our analysis on two genes, *SER3* and *PHO84*, whose expression is strongly affected by both *hht2-11* and *snf2*Δ. Previous studies have strongly suggested that Swi/Snf directly represses *SER3* (30) and directly activates *PHO84* (51). All of the analyses presented in the present study have been carried out on material obtained from cells grown in rich (YPD) media, a condition that results in significant induction of the *PHO84* gene (see Materials and Methods and references 34 and 50). Consistent with our microarray results and with previously reported data (30, 51), Northern hybridization experiments showed that both *hht2-11* and *snf2*Δ caused increased levels of *SER3* mRNA and decreased levels of *PHO84* mRNA (Fig. 4). The finding that at the permissive temperature *hht2-11* affects transcription of these genes less than does *snf2*Δ suggests that H3 L61W mutant decreases but does not eliminate the ability of Swi/Snf to function at these genes. Analysis of *snf2*Δ *hht2-11* double mutants showed that the increase in *SER3* expression seen in *snf2*Δ strains is not further increased by the presence of the *hht2-11* mutation (Fig. 4A), a finding consistent with the notion that the two mutations affect *SER3* expression through effects on a common pathway. Similarly, we see a lack of additivity of the *hht2-11* and *snf2*Δ mutations in their effects on expression of *PHO84* (Fig. 4B), although in this case the results are harder to interpret due to the already very low levels of *PHO84* expression in *snf2*Δ cells.

To determine whether the histone mutant affects the association of the Swi/Snf complex with the *SER3* and *PHO84* promoters, we performed chromatin immunoprecipitation experiments directed against Snf5, a component of Swi/Snf. Our results show that *hht2-11* cells have a significantly decreased level of Swi/Snf physically associated with both the *SER3* and *PHO84* promoters compared to wild-type strains (Fig. 5). The level of Swi/Snf detected at *SER3* indicates that Swi/Snf still binds to this promoter to some degree, since previous work has shown that no detectable chromatin immunoprecipitation of Snf5 is observed in cells lacking Snf2, a situation in which the integrity of the Swi/Snf complex is compromised (30). These results are consistent with a model in which the H3 L61W mutant protein alters chromatin structure to reduce binding of Swi/Snf at these promoters.

Analysis of the chromatin structure at *SER3* in *hht2-11* cells. To determine the nature of any chromatin changes conferred by H3 L61W, we analyzed the chromatin structure at the *SER3* gene by indirect end labeling. The *SER3* promoter has been previously shown to have different sensitivities to MNase in *snf2*Δ mutants that correlate with increased transcription (30). Figure 6 shows results from indirect end-labeling experiments on chromatin that had been treated with MNase from *HHT2*, *hht2-11*, and *snf2*Δ cells. In *snf2*Δ cells, we observed changes in two regions of the *SER3* promoter compared to wild-type cells as previously described (30). In *hht2-11* cells, the MNase pattern is similar to that seen in *snf2*Δ cells, although not quite as extreme, suggesting that the H3 L61W mutant blocks most remodeling of the *SER3* promoter by Swi/Snf.

Evidence that H3 L61W directly affects the ability of Swi/Snf to associate with the promoter of the *PHO84* gene. The decreased level of Swi/Snf at *SER3* and *PHO84* might reflect a direct effect of H3 L61W on the ability of Swi/Snf to properly interact with the promoter chromatin of these genes. Alternatively, the histone mutant could act indirectly by controlling earlier events in pathways that would ultimately result in less Swi/Snf association at these promoters. To differentiate between a direct or indirect effect, we analyzed the events that occur at the promoter of the *PHO84* gene in greater detail.

The expression of *PHO84* under the conditions used in our assays is dependent upon both Swi/Snf and the Pho4 transcriptional activator (Fig. 4 and data not shown). To determine the relationship between these factors at *PHO84*, we performed additional chromatin immunoprecipitation experiments. In wild-type cells, we detected strong association of Pho4 at the *PHO84* promoter (Fig. 7). The Pho4 activator is required for the recruitment of Swi/Snf to the *PHO84* promoter since we observed a loss of Swi/Snf association at *PHO84* in a *pho4*Δ mutant (data not shown). Next, we tested whether Swi/Snf is required for efficient association of the Pho4 protein to the *PHO84* promoter. Deletion of *SNF2* results in a decrease, but not complete loss, of Pho4 binding, indicating that Swi/Snf is required for full Pho4 promoter occupancy at the *PHO84* gene (Fig. 7). This mutual dependence between Pho4 and Swi/Snf for chromatin association is also seen at the promoter of the *PHO5* gene (51). At *PHO5*, Pho4 is able to bind to the accessible UASp1 site in a Swi/Snf-independent manner but re-

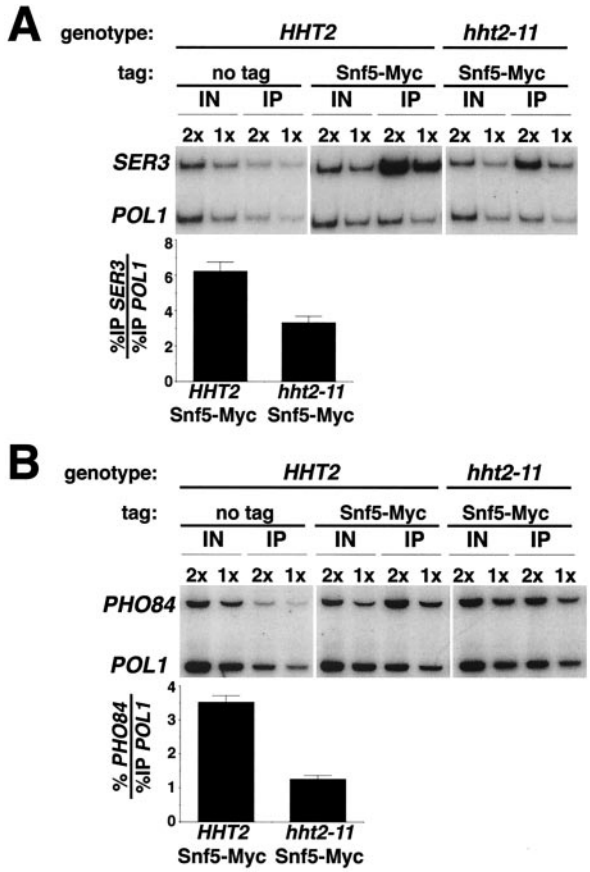

FIG. 5. Association of the Swi/Snf complex with the *SER3* and *PHO84* promoters is perturbed by the *hht2-11* mutation. (A) Chromatin immunoprecipitations were performed on *HHT2* (strain FY2166) and *hht2-11* (strain FY2167) strains expressing the Snf5-Myc protein. A strain lacking the Snf5-Myc fusion (no tag, strain FY2168) was used as negative control. Amplifications of the *SER3* promoter and the *POL1* region (used as an internal control) were performed on chromatin samples prior to immunoprecipitation (IN, input) and after immunoprecipitation with the A14 anti-Myc antibody (IP, immunoprecipitate). For each sample, two dilutions (2× and 1×) of the chromatin templates were used for the PCR amplification to ensure linearity. The percent immunoprecipitation values (%IPs) of *SER3* and *POL1* were measured, and the ratios are shown in the bar graph. Each value represents the average ratio of the *SER3* %IP to the *POL1* %IP with the standard error from three independent experiments. (B) Chromatin immunoprecipitations were performed as described in panel A, except that the PCR amplifications were directed toward the promoter of the *PHO84* gene instead of *SER3*. The bar graph shows the average ratios of the *PHO84* %IP to the *POL1* %IP with the standard error for each strain measured in three independent experiments.

quires chromatin remodeling in order to bind to the UAS2p site, which is assembled into a nucleosome (51, 55). Based on our data and the fact that the *PHO84* promoter has several Pho4 binding sites (37), it is plausible that a similar situation exists at *PHO84* and that Pho4 binds to some sites in a Swi/Snf-independent fashion and to others in a Swi/Snf-dependent fashion.

Two predictions can be made if H3 L61W directly impairs Swi/Snf association at the *PHO84* promoter. First, the histone mutant should cause a reduced level of Pho4 association at the *PHO84* promoter. This effect is predicted to be less than or equal to the defect measured in Swi/Snf-defective cells. As shown in Fig. 7, the *hht2-11* mutation does indeed cause reduced association of Pho4 at *PHO84* and this defect is less severe than that seen in *snf2*Δ mutants. A second prediction of the direct model is that the decreased level of association of Pho4 at the *PHO84* promoter in *snf2*Δ cells should not be further reduced by an *hht2-11* mutation, since both *snf2*Δ and *hht2-11* are affecting the same process (Swi/Snf function). Mutations that affect Pho4 binding in a Swi/Snf-independent fashion, such as those that affect the *PHO* pathway or that directly perturb Pho4 binding, would be expected to cause a greater reduction in Pho4 binding in combination with a *snf2*Δ mutation. Consistent with our prediction, we found that Pho4 binding at the *PHO84* promoter in *snf2*Δ cells is essentially unaffected when combined with the *hht2-11* mutation (Fig. 7), indicating that the two mutations affect a common pathway. These results strongly suggest that H3 L61W affects Swi/Snf binding to chromatin at a step subsequent to the initial binding of the Pho4 activator to the *PHO84* promoter. Because in vitro experiments have shown that Pho4 can directly interact with the Swi/Snf complex (35), the reduced binding of Swi/Snf in the context of H3 L61W is likely to be a direct effect on Swi/Snf association with chromatin rather than through an intermediate complex connecting Pho4 and Swi/Snf.

H3 L61W perturbs Swi/Snf chromatin association through a mechanism distinct from that involving the production of phosphoinositols. Recent studies have demonstrated a role for inositol polyphosphates in the regulation of chromatin remodeling (47, 51). Specifically, formation of the small molecules IP_4 and IP_5 appears to be required for proper transcription and chromatin remodeling at the *PHO5* promoter. In these experiments it was shown that the absence of the Arg82 IP_3 kinase resulted in decreased association of Swi/Snf at both the *PHO5* and *PHO84* promoters and that, at least in the case of *PHO5*, this occurred at a step after Pho4 binding (51). To test whether *hht2-11* affects Swi/Snf association at *PHO84* through an effect on the production of inositol phosphatases, we compared the extent of the defects in *SER3* and *PHO84* expression in *hht2-11* and *arg82*Δ cells. As shown in Fig. 8, the *hht2-11* mutation conferred stronger transcriptional defects at both *SER3* and *PHO84* than did an *arg82*Δ mutation, indicating that at least a component of the mechanism by which the *hht2-11* mutation affects the regulation of these genes is distinct from that involving the production of IP_4 and IP_5. These results support the hypothesis that H3 L61W directly affects Swi/Snf association with chromatin.

DISCUSSION

The results presented here provide strong evidence that the histone H3 globular domain plays an important role in ensuring proper association of the Swi/Snf complex with chromatin. Because this effect appears to be direct, these findings suggest that the nature of the chromatin environment at particular chromosomal sites can dictate how efficiently the Swi/Snf complex can be recruited and/or maintained. The broader implication of this idea is that gene-specific regulation by Swi/Snf occurs through two activities: direct recruitment of Swi/Snf by transcription factors and Swi/Snf-nucleosome interactions. This combination of activities could ensure proper transcrip-

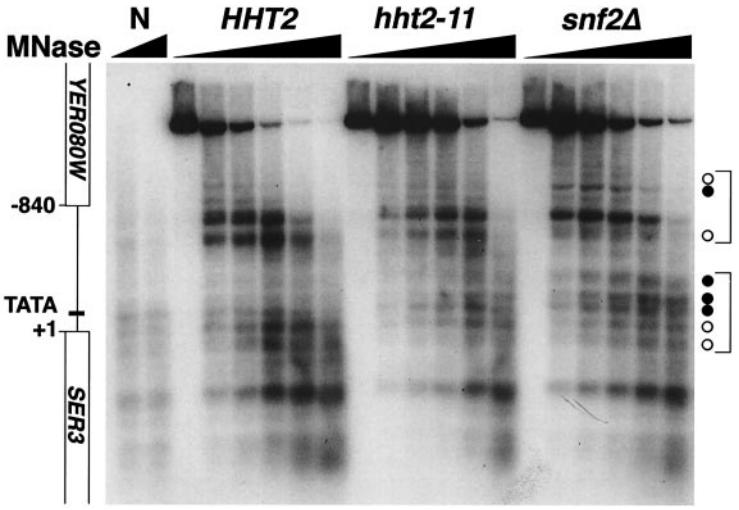

FIG. 6. H3 L61W causes chromatin changes at the *SER3* promoter. Chromatin was isolated from *HHT2* (strain FY2201), *hht2-11* (strain FY2202), and *snf2*Δ (strain FY2203) strains and treated with increasing amounts of MNase, as indicated. The resulting material was subjected to indirect end-labeling analysis. All strains were deleted for the *SER33* gene to prevent cross-hybridization with the *SER3* probe. Part of the *SER3* gene and upstream region is depicted on the left. The brackets indicate the chromatin regions most clearly affected by both the *hht2-11* and the *snf2*Δ mutations. Sites that become more sensitive to MNase digestion in either mutant are indicated with closed circles, whereas sites that become more resistant are indicated with open circles. N, naked DNA controls.

tion levels of Swi/Snf-regulated genes and might allow for differential regulation of genes that use the same factor to recruit Swi/Snf to their promoters. This notion is supported by the finding that the presence of a linker histone, which affects chromatin structure by constraining nucleosomal DNA and by promoting formation of higher order chromatin structure, also results in the decreased ability of chromatin remodeling enzymes to bind and remodel in vitro-reconstituted nucleosomal arrays (23, 39). Similarly, histone variants incorporated into nucleosomes can influence chromatin structure and transcription and in some cases have been shown to functionally interact with remodeling complexes (1, 2, 4, 13, 15, 32). It will be of

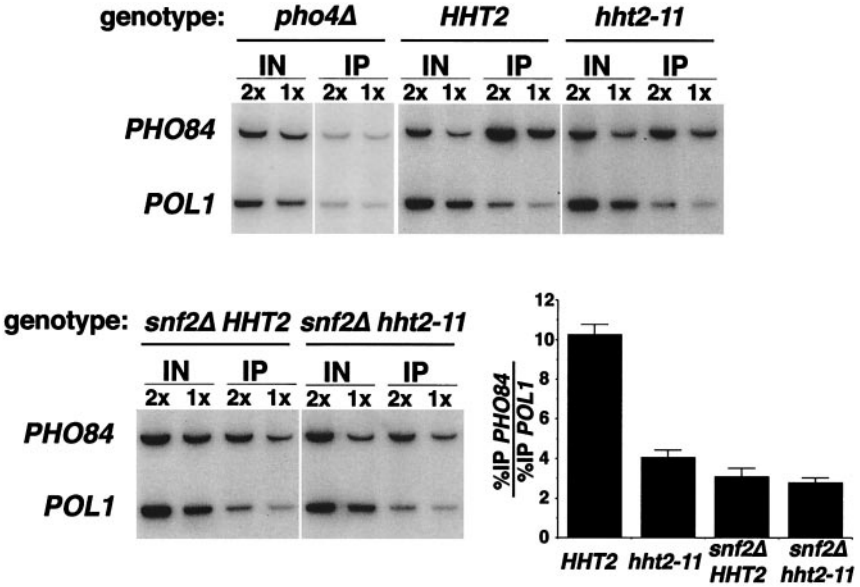

FIG. 7. Chromatin association of Pho4 at *PHO84* is decreased in *hht2-11* and *snf2*Δ cells, but the effects of the two mutations are not additive. Chromatin immunoprecipitations were performed and are presented as described in Fig. 5B, except that the chromatin samples were immunoprecipitated by using a polyclonal antibody directed against the Pho4 protein. The strains used were as follows: *HHT2* (strain FY2166), *hht2-11* (strain FY2167), *snf2*Δ *HHT2* (FY2169), *snf2*Δ *hht2-11* (strain FY2171), and *pho4*Δ (strain FY2235). To ensure that the enrichment of the *PHO84* signal detected is specific for the presence of Pho4 at the promoter, we performed control immunoprecipitations with either Pho4 antibody added to *pho4*Δ cells (first set of experiments shown here) or no Pho4 antibody added to *PHO4* cells (data not shown). In both cases, no enrichment for the *PHO84* promoter was observed compared to the *POL1* internal control. The %IP values for the *PHO84* and *POL1* regions were measured, and the average values and corresponding standard errors from at least three independent samples for each genotype are shown in the bar graph.

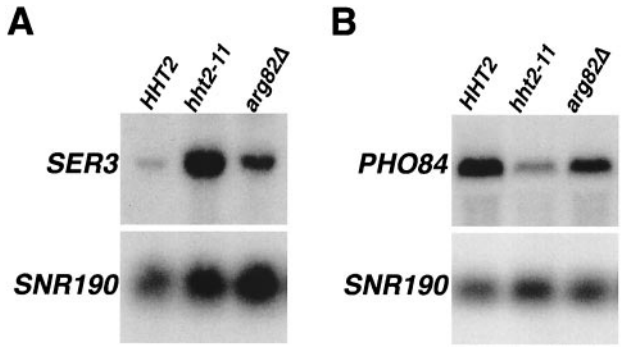

FIG. 8. The *hht2-11* mutation affects expression of *SER3* and *PHO84* to a greater degree than does an *arg82*Δ mutation. (A) Northern analysis of *SER3* levels in *HHT2* (strain FY2163), *hht2-11* (FY2164), and *HHT2 arg82*Δ (FY2172) cells. The levels of *SNR190* mRNA were measured and used as a normalization control. Shown here is one representative result from three independent experiments. (B) Northern analysis of *PHO84* levels was performed as described in panel A.

particular interest to determine which components of chromatin remodeling complexes regulate how efficiently and productively the complex will associate with any given chromatin environment. Recent advances in this regard include the finding that the bromodomain of Snf2 plays a crucial role in retention of Swi/Snf to acetylated nucleosomal templates (19).

What is the mechanism by which H3 L61W reduces the ability of Swi/Snf to associate with chromatin? One possibility is that this amino acid change alters a recognition site on the nucleosome that normally interacts with Swi/Snf. Alternatively, the mutant nucleosome might be more refractory to remodeling, resulting in the disassociation of Swi/Snf from chromatin. The latter model is supported by the prediction that the L61W mutation in histone H3 might result in increased hydrophobic interactions between histones H3 and H4 (White and Luger, unpublished). The effects of substituting other amino acids with different chemical properties at position 61 should lead to a better understanding of the mechanistic nature of H3 L61W. It is also formally possible that the H3 L61W mutant creates a chromatin environment that leads to reduced binding of either a ubiquitous or an activator-recruited factor that in turn affects Swi/Snf association with the promoter. Although in this scenario H3 L61W would not be exerting its effects through direct contact with Swi/Snf, it still implies that the globular domain of histone H3 is responsible for optimal Swi/Snf chromatin association at a step downstream from activator binding in vivo. Whereas the results presented in the present study suggest that the *hht2-11* and *snf2*Δ mutations can affect some functions through effects on common pathways, our studies do not rule out more indirect effects of H3L61W on either expression of factors required for recruitment of Swi/Snf or on components of Swi/Snf itself needed for chromatin association. Biochemical experiments with purified components will need to be conducted to more precisely ascertain the role of the globular domain of H3 in Swi/Snf chromatin association.

One prediction of the proposed model for the effect of the *hht2-11* mutation on the Swi/Snf complex might be that all of the genes regulated by Swi/Snf should be affected by the histone mutation. However, whereas we see a significant overlap of the genes affected in the two mutants, there are clearly many Swi/Snf-dependent genes not affected by the *hht2-11* mutation and vice versa. This observation can be reconciled with the proposed hypothesis in one of several ways. First, Swi/Snf might function in different ways at different genes and the H3 L61W mutant might only impair some of these activities. In addition, the *hht2-11* mutation might confer additional effects that would mask this specific relationship with the Swi/Snf complex. This possibility is supported by our observation that some of the *snf2*Δ phenotypes are suppressed by the *hht2-11* mutation (Gal^-, Raf^-, and Ino^- phenotypes; Fig. 2A and data not shown). The *hht2-11* mutation, therefore, might confer two opposing activities: first, in line with the proposed hypothesis, it confers resistance to Swi/Snf activity by decreasing the ability of Swi/Snf to associate with chromatin and second, it can also partially alleviate the requirement for Swi/Snf activity, perhaps by disrupting higher-order chromatin structure or through some other mechanism. As a result, only in cases where the former activity prevails over the latter will a transcriptional correlation with a *snf2*Δ mutation be observed.

The results presented here set the stage for further analysis of the relationship between nucleosomes and factors that function through interactions with chromatin. Specifically, detailed analyses of the effects of *hht2-11* on the functions of other chromatin remodeling complexes, such as the Ino80, RSC, and Isw complexes (46, 60), will be of interest. Furthermore, the isolation of extragenic suppressors of *hht2-11* should prove fruitful. For instance, suppressor mutations that alter the Swi/Snf complex or other complexes that interact with chromatin might be expected to be isolated by using such an approach, possibly leading to further insights into the physical and functional interactions that occur within the context of chromatin.

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