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A New Class of Histone H2A Mutations in *Saccharomyces cerevisiae* Causes Specific Transcriptional Defects In Vivo

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Nucleosomes have been shown to repress transcription both in vitro and in vivo. However, the mechanisms by which this repression is overcome are only beginning to be understood. Recent evidence suggests that in the yeast *Saccharomyces cerevisiae*, many transcriptional activators require the SNF/SWI complex to overcome chromatin-mediated repression. We have identified a new class of mutations in the histone H2A-encoding gene *HTA1* that causes transcriptional defects at the SNF/SWI-dependent gene *SUC2*. Some of the mutations are semidominant, and most of the predicted amino acid changes are in or near the N- and C-terminal regions of histone H2A. A deletion that removes the N-terminal tail of histone H2A also caused a decrease in *SUC2* transcription. Strains carrying these histone mutations also exhibited defects in activation by LexA-GAL4, a SNF/SWI-dependent activator. However, these H2A mutants are phenotypically distinct from *snf/swi* mutants. First, not all SNF/SWI-dependent genes showed transcriptional defects in these histone mutants. Second, a suppressor of *snf/swi* mutations, *spt6*, did not suppress these histone mutations. Finally, unlike in *snf/swi* mutants, chromatin structure at the *SUC2* promoter in these H2A mutants was in an active conformation. Thus, these H2A mutations seem to interfere with a transcription activation function downstream or independent of the SNF/SWI activity. Therefore, they may identify an additional step that is required to overcome repression by chromatin.

In eukaryotic cells, DNA is complexed with histones and other proteins into chromatin (see reference 75 for a review). The primary component of chromatin is the nucleosome, which consists of approximately 146 bp of DNA wrapped around an octamer of histones (two histone H2A-H2B dimers and one $[H3\text{-}H4]_2$ tetramer; see reference 75 for a review). A growing body of evidence from both in vivo and in vitro studies has shown that the structure of chromatin influences gene expression (see references 25 and 53 for reviews). Biochemical experiments have shown that histones can repress transcription in vitro (see reference 53 for a review) and that transcriptional activators can overcome this repression (21, 41, 45, 79, 81, 82). In vivo experiments in *Saccharomyces cerevisiae* have shown that the loss of transcriptional activators or of activator binding sites can be suppressed by mutations in histone genes (27, 30, 37, 57). These results suggest that one function of transcriptional activators in vivo is to antagonize chromatin-mediated repression.

In vivo studies of histone mutants have also suggested that histones play a variety of roles in transcriptional regulation. Small deletions and point mutations that alter the flexible N-terminal tails of different yeast histones have been shown to cause specific changes in transcription (see reference 25 for a review). Analysis of deletions and single amino acid changes in the N-terminal region of histone H4 has demonstrated that certain changes in the H4 N terminus abolish repression of the yeast silent mating-type cassettes (35, 36; see reference 25 for a review). Some of these H4 mutants also prevent efficient repression by the α2 repressor (60) and repression of genes adjacent to telomeres (2). Similar analysis has shown that deletion of a different but overlapping N-terminal region of H4 prevents efficient activation of the *GAL1* and *PHO5* genes (22), while deletions in the N-terminal tail of histone H3 lead to hyperactivation of the *GAL1* gene (43). No transcriptional role has previously been demonstrated for the N-terminal tails of histones H2A and H2B, although the presence of at least one of the two tails is required for viability (63). Also, a reduction of histone H2A-H2B gene dosage can restore transcription to certain inactivated promoters (18, 30). These in vivo experiments, then, show that changes in histones can lead to many kinds of transcriptional defects. Therefore, histones may participate in distinct interactions with other proteins at different promoters.

Few single amino acid changes outside the N-terminal regions of histones H3 and H4 have been shown to affect transcription. However, the relative abundance of mutations that alter the N-terminal tails of histones H3 and H4 may reflect the experimental emphasis on these regions of H3 and H4 rather than a concentration of regulatory functions within these regions. Many other histone regions could conceivably affect transcription. For example, determination of the X-ray crystal structure of the histone octamer at high resolution has indicated that the C termini of histones are also flexible and may therefore be available for regulated interactions with DNA or other proteins (3, 4). Additionally, the more central regions of histones (especially those exposed to the surface) could easily be involved in interactions with DNA, histones, or other proteins in ways that affect transcription.

Analysis of histone mutants has provided evidence that a complex of yeast proteins that includes SNF2/SWI2, SNF5, SNF6, SWI1, and SWI3 may be required for transcriptional activators to overcome chromatin-mediated repression (11, 30, 55; see reference 77 for a review). The SNF/SWI complex is required for transcription of a large number of diversely regulated genes (see reference 77 for a review) and is also required for the function of several activators from *S. cerevisiae* and other organisms (40, 56, 84). Mutations in *SNF* and *SWI*

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TABLE 1. *S. cerevisiae* strains used

Strain	Relevant genotype[a]
FY393	*MAT***a** (*hta1-htb1*)Δ::*LEU2*
FY604	*MAT*α (*hta2-htb2*)Δ::*TRP1*
FY406	*MAT***a** (*hta1-htb1*)Δ::*LEU2* (*hta2-htb2*)Δ::*TRP1* <pSAB6>
FY986	*MAT***a** (*hta1-htb1*)Δ::*LEU2* (*hta2-htb2*)Δ::*TRP1* <pJH161> [Δ(5-21)]
FY987	*MAT*α (*hta2-htb2*)Δ::*TRP1 hta1-200* (S20F)
FY988	*MAT*α (*hta2-htb2*)Δ::*TRP1 hta1-300* (G30D)
FY989	*MAT*α (*hta2-htb2*)Δ::*TRP1 hta1-750,760* (N75S K76E)
FY990	*MAT*α (*hta2-htb2*)Δ::*TRP1 hta1-950* (L95S)
FY991	*MAT*α (*hta2-htb2*)Δ::*TRP1 hta1-1170* (L117S)
FY458	*MAT***a** *snf2*Δ*1*::*HIS3*
FY992	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pLW1*
FY993	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pLW1 hta1-200* (S20F)
FY994	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pLW1 hta1-300* (G30D)
FY995	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pLW1 hta1-950* (L95S)
FY996	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pRB1840*Δ*Spe*
FY997	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pRB1840*Δ*Spe hta1-200* (S20F)
FY998	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pRB1840*Δ*Spe hta1-300* (G30D)
FY999	*MAT*α (*hta2-htb2*)Δ::*TRP1 ura3-52*::*pRB1840*Δ*Spe hta1-950* (L95S)
FY1000	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140*
FY1001	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140 hta1-200* (S20F)
FY1002	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140 hta1-300* (G30D)
FY1003	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140 hta1-750,760* (N75S K76E)
FY1004	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140 hta1-950* (L95S)
FY1005	*MAT*α (*hta2-htb2*)Δ::*TRP1 spt6-140 hta1-1170* (L117S)

[a] All strains also carry the alleles *his3*Δ*200*, *trp1*Δ*63*, *lys2-128*δ, *ura3-52*, and *leu2*Δ*1*, except that FY604, FY992, and FY996 carry *LYS2* and FY458 carries *LEU2*, *TRP1*, and the insertion mutation *his4-912*δ. For strains carrying mutations in *HTA1*, the predicted amino acid changes are shown in parentheses and are designated as described in Table 2. Strains carrying the *ura3-52*::*pLW1* and *ura3-52*::*pRB1840*Δ*Spe* alleles contain *lacZ* reporter genes with zero or one *lexA* operator, respectively. All strains except FY458 were constructed for this study.

genes cause alterations in chromatin structure and defects in transcription at the *SUC2* gene (30, 44, 49, 56). These changes in chromatin structure and transcription are partially reversed by a deletion of *HTA1-HTB1*, one of two gene pairs encoding histones H2A and H2B (30); likewise, amino acid changes in histones H3 and H4 suppress transcriptional defects in *snf/swi* mutants (37, 57). These and other results suggest that the SNF/SWI complex activates transcription by overcoming chromatin-mediated repression (30; see reference 77 for a review). Consistent with this model, purified SNF/SWI complexes from yeast and mammalian cells have been shown to alter nucleosomes in vitro (20, 34, 39). The exact mechanism of SNF/SWI action remains unknown, although the suppression of *snf/swi* mutations by decreased H2A-H2B gene dosage suggests that the SNF/SWI complex may destabilize interactions in the nucleosome between H2A-H2B dimers and the $(H3\text{-}H4)_2$ tetramer (30).

Given the success of the genetic studies of histone mutants and of *snf/swi* mutants, and the likelihood that new histone mutants would be useful and informative, we were interested in obtaining new mutations in histones H2A and H2B that had specific effects on transcription. We reasoned that such mutations might reveal different aspects of chromatin-mediated regulation than had been previously discovered by mutagenesis of histones H3 and H4. In addition, we hoped to use these new histone mutations to understand further the role of SNF/SWI proteins, since previous genetic data had suggested a functional link between the SNF/SWI complex and histones H2A and H2B.

In this report, we present the isolation and analysis of a previously undescribed class of mutations in the histone H2A-encoding gene *HTA1* that cause specific defects in transcription: they prevent high-level transcription of the SNF/SWI-dependent *SUC2* gene. These mutations occur at many sites in *HTA1* but show some bias toward codons corresponding to the N and C termini of histone H2A. In addition, we demonstrate that a deletion of the N-terminal tail of histone H2A causes a *SUC2* transcriptional defect, thereby providing evidence for a role in transcription for this histone domain. However, since these *hta1* mutants do not inhibit the formation of active chromatin, they are unlikely to block SNF/SWI function. These results lead us to propose a model in which relief from chromatin repression at *SUC2* (and perhaps at other SNF/SWI-dependent genes) may occur in at least two steps. In the first step, SNF/SWI proteins alter chromatin structure, but histones remain associated with the promoter. In the second step, repression by histones is finally relieved, perhaps by their removal from DNA. The histone mutations described in this report would interfere with this second step at *SUC2* and other genes. Thus, analysis of this new class of histone H2A mutations provides insight into another possible mechanism of regulation by chromatin structure.

MATERIALS AND METHODS

Strains and genetic methods. Yeast strains are listed in Table 1 and are isogenic to S288C except that all strains are *GAL2*$^+$ (78). Lowercase indicates a mutant allele, and uppercase indicates a wild-type allele. Standard methods were used for mating, sporulation, and tetrad analysis (59). Strains carrying the (*hta1-htb1*)Δ::*LEU2* allele were constructed as described previously (30). Strains carrying the (*hta2-htb2*)Δ::*TRP1* allele were constructed by transformation with the *Sty*I fragment of plasmid pJH21. Transformants were shown to be correct by Southern hybridization analysis. The *snf2*Δ*1*::*HIS3* (1) and *spt6-140* (69) alleles have been described previously. Strains carrying plasmid-borne *hta1* mutations were constructed by transformation of the strain FY406 with *HIS3*-based single-copy plasmids containing the *hta1* mutations (Table 2). Loss of the wild-type *HTA1*-containing plasmid (pSAB6) was achieved by selection on 5-fluoroorotic acid (5FOA). Strains carrying integrated *hta1* mutations were constructed by transformation of an (*hta1-htb1*)Δ::*LEU2* strain (FY393) or an (*hta2-htb2*)Δ::*TRP1* strain (FY604) with *URA3*-based integrating plasmids carrying the *hta1* mutations (Table 2). The plasmids were linearized with *Eag*I to direct integration to a site 3′ to the *HTB1* gene. The transformants were transferred to media containing 5FOA to select for cells that had lost the plasmid by recombination. Transformants derived from FY393 were then screened for loss of the *LEU2*

marker (indicating replacement of the null allele by the mutant *hta1* allele); transformants derived from FY604 were screened for a mutant phenotype (indicating replacement of the wild-type allele with a mutant allele). DNA prepared from Leu$^-$ or phenotypically mutant transformants was analyzed by PCR and subsequent restriction digestion to ensure that the mutant alleles had been correctly integrated. Some derivatives of FY393 carried an extra copy of the *HTA2-HTB2* locus, likely due to disomy of chromosome II; crosses were performed to eliminate this extra histone locus, and strains used in this study did not have this extra copy (31). Strains carrying the S20F, G30D, N75S K76E, and L117S alleles showed high rates of spontaneous diploidy; isolates used in these studies were diploids (31). Strains carrying *lexA* reporters integrated at *ura3-52* were constructed by transformation with plasmid pLW1 or pRB1840ΔSpe linearized with *Stu*I. The resulting strains carry the alleles *ura3-52*::*pLW1* or *ura3-52*::*pRB1840ΔSpe*, respectively.

Yeast strains were transformed by the lithium acetate method as described previously (59). *Escherichia coli* HB101 (5), DH5α (5), FB600 (a derivative of MC1065 [15] containing the *pyrF* and *hisB* alleles), and MH1 (26) were transformed as described previously (5).

Media. All media, including YPD, SC, and media containing 5FOA, were made as described previously (59) except that all rich media were supplemented with 80 mg of tryptophan per liter. YPraf and YPgal solid media contained 2% raffinose and 2% galactose, respectively, as the sole carbon sources and were supplemented with 1 μg of antimycin A per ml; YEP-plus-0.05% glucose medium contained 0.05% glucose as the sole carbon source. Media lacking inositol were made as described previously (64).

Enzymes and protease inhibitors. Restriction enzymes, *Taq* polymerase, T4 DNA ligase, DNA polymerase I (Klenow fragment), T4 polynucleotide kinase, avian myeloblastosis virus reverse transcriptase, and Vent polymerase were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and from New England BioLabs (Beverly, Mass.). Micrococcal nuclease (MNase) was purchased from Boehringer Mannheim Biochemicals. All enzymes were used and stored according to the instructions of the supplier or as described previously (30).

DNA preparation and analysis. Plasmids and genomic DNA from *S. cerevisiae* (32) and plasmids from *E. coli* (5) were prepared as described previously. Analysis and purification of DNA restriction fragments were performed as described previously (68). Southern blot hybridization analysis was performed as described previously (30). PCR analysis of yeast genomic DNA was performed as described previously (33) except that 1 μl of DNA was used instead of intact yeast cells.

Hybridization probes. Plasmids used as probes for RNA analysis were as follows: for Ty, B161 (29); for *TPI1*, pHB59 (6); and for *TUB2*, pYST138 (67). The probe for indirect end labeling of the *SUC2* promoter was a fragment from the 5′ *SUC2* coding region described previously (30). Plasmids were nick translated by using a kit from Boehringer Mannheim Biochemicals.

Plasmids. Plasmids used to integrate *lexA* reporters at *ura3-52* were pLW1 (containing the *GAL1* TATA box upstream of *lacZ*) and pRB1840ΔSpe (similar to pLW1 but containing one *lexA* operator upstream of the *GAL1* TATA box). These plasmids, provided by Lena Wu, were derived from similar 2μm-containing plasmids (pLR1Δ1 [76] and pRB1840, identical to 1145 in reference 8); 2μm sequences were deleted by digestion and religation with *Spe*I. Plasmid pSH17-4 contains LexA-GAL4 on a 2μm *HIS3* plasmid (28). To construct the plasmid used for disruption of *HTA2-HTB2*, the internal *Sty*I fragment of YIp5TRT2 (52) was cloned into the *Sty*I site of pBR322, creating plasmid pJH20. The internal *Bgl*II fragment of *HTA2-HTB2* in pJH20 (containing the promoters and 5′ coding regions of the histone genes) was replaced by the *TRP1* gene, which was excised on a *Bam*HI-*Bgl*II fragment, creating plasmid pJH21. For *HTA1*-containing plasmids, *Bam*HI-*Sac*II *HTA1-HTB1* fragments were cloned into the vectors pRS306 (*URA3* integrating), pRS313 (*HIS3 CEN*), and pRS316 (*URA3 CEN*) (65). Plasmids pSAB6 (10) and pJH23 contain the *Bam*HI-*Sac*II *HTA1-HTB1* fragment cloned into pRS316 and pRS313, respectively. pJH55 was constructed from pJH23 by several rounds of site-directed mutagenesis (38) using the oligonucleotides 1954Nco (CTTAATATAGTTACCATGGAGAAGCAATTTAATT CC), 2492Xba (GATGTAGTATCTAGAAGAGAAGTACAGATTGG), 3185Spe (CCTATATAGACTAGTCAAACCACAAATAAACC), and 3737Eag (GATA TTTACTCGGCCGTAAAATTGTGTTCTCG). These oligonucleotides create the following restriction sites: *Nco*I at 49 to 54 bp 3′ to the *HTA1* stop codon, *Xba*I at 85 to 90 bp 5′ to the *HTA1* ATG, *Spe*I at 31 to 36 bp 5′ to the *HTB1* ATG, and *Eag*I at 121 to 126 bp 3′ to the *HTB1* stop codon. The oligonucleotide 1954Nco (and therefore pJH55) also differs from the sequence of pJH23 at the nucleotide 36 bp 3′ to the *HTA1* stop codon. These base changes and restriction sites do not affect *HTA1* or *HTB1* function (31). pJH65 contains the *Bam*HI-*Sac*II fragment of pJH55 cloned into pRS306. Plasmids carrying mutant alleles of *HTA1* are listed in Table 2 and in the next section.

Isolation of *hta1* mutants. DNA fragments containing mutant *hta1* alleles were generated by PCR. For isolation of plasmids whose designations begin with A, the primers were the oligonucleotides HTA15′ (CTCATGCTGTTATTTGTTA TTGCCC) and HTA13′ (GAACAAAGCACAGAATGTGTTTGC), and the template was plasmid pJH55. These primers generate PCR products extending from 295 bp upstream of the *HTA1* ATG to 267 bp downstream of the *HTA1* termination codon. Except as noted below, several identical pools of PCR products were generated by using 35 cycles of PCR (94°C for 15 s, 57°C for 15 s, 72°C for 60 s), using conditions described previously (85) except that the reaction volume was 100 μl. After this round of PCR, 1 μl of each reaction product was used as the template for a second identical round of 35 cycles. The molar ratio of product to starting material was approximately 10^5, which corresponds to approximately 17 exponential cycles of amplification. Since the *HTA1* coding region is about 400 bp long, and the fidelity of *Taq* polymerase is at best 10^{-5} errors per nucleotide (71), we expected approximately 7% of the PCR products to contain a mutation in the *HTA1* coding region. To introduce the PCR products into yeast cells, we used a single-step method based on gap repair as described previously (46). The PCR products were cotransformed into strain FY406 with an *Nco*I-*Xba*I fragment of pJH55 which retains *HIS3* and *CEN* sequences as well as approximately 200 bp of homology with each end of the PCR products but lacks the *HTA1* coding region. Strain FY406 carries deletions of the *HTA1-HTB1* and *HTA2-HTB2* chromosomal loci and a *URA3*-marked plasmid containing the *HTA1-HTB1* locus. His$^+$ transformants were selected; these arose from cells in which the gap in pJH55 had been repaired. We found that addition of the PCR products led to an approximately twofold stimulation of transformation efficiency (31). Thus, about one-half of the transformants probably repaired the gapped plasmid by using sequences from the wild-type plasmid already present in strain FY406 and were therefore phenotypically wild type. The remainder of the transformants presumably utilized the PCR products to repair the gapped plasmid and therefore contained potential *hta1* mutations. Since we expected at least 7% of the PCR products to contain a mutation in *HTA1*, we expected at least 3.5% of the transformants to contain *hta1* mutations.

His$^+$ transformants were selected and screened for phenotypes (see Results for details). Transformants were screened directly for dominant phenotypes of the repaired plasmids. After transfer to media containing 5FOA, which selects for cells that have lost the *URA3*-marked plasmid carrying the wild-type *HTA1-HTB1* locus, transformants were screened for recessive phenotypes. As an internal control for the level of mutagenesis, we also screened transformants for sensitivity to 5FOA, which indicates inability to lose the wild-type plasmid and therefore a complete loss-of-function mutation in *HTA1*, and suppression of the insertion mutation *lys2-128*δ, which probably indicates partial loss of function (18, 31). Of 2,200 transformants tested, 83 were 5FOA resistant and 70 showed an Spt$^-$ phenotype, suggesting that the proportion of transformants carrying *hta1* mutations was at least 6%. (One percent of transformants from control experiments with no PCR products were either 5FOA resistant or Spt$^-$.)

For isolation of plasmids whose designations begin with 23A, pJH23 was used as the template and the primers were the oligonucleotides JH13 (GTCAC GACGTTGTAAAACGACGG) and JH14 (GAAACGCCCGTAGAGATAT GTGG). These primers generate PCR products extending from near the polylinker in the plasmid backbone to 321 bp upstream of the *HTA1* ATG. PCR conditions were as follows: 20 mM Tris (pH 8.3), 25 mM KCl, 0.05% Tween 20, 0.01% gelatin, 1 mM each deoxynucleoside triphosphate, 5.5 mM $MgCl_2$, 0.6 mM $MnCl_2$, 20 pmol of each primer, and 1 U of *Taq* polymerase. PCR was performed for 40 cycles (93°C for 45 s, 55°C for 45 s, 72°C for 2 min). These conditions were more mutagenic than those described above (31). The PCR products were digested with *Bam*HI and *Pfl*MI and cloned into pJH23. The resulting library was transformed into FY406, and His$^+$ transformants were selected and screened for phenotypes as described above.

Plasmids bearing mutant *hta1* alleles were rescued from yeast cells as described previously (32). When yeast strains contained both *HIS3*- and *URA3*-based plasmids, the *E. coli* FB600 was used for rescue, and plasmids complementing the *hisB* but not the *pyrF* mutation were isolated. All plasmids used in this study were transformed into FY406 to confirm that the plasmid confers a mutant phenotype. Plasmids were sequenced with a Sequenase 2.0 kit (U.S. Biochemical, Cleveland, Ohio). The isolated plasmids, base changes in the *HTA1* coding region, and predicted amino acid changes were as follows (the A of the ATG is base 1, and the initial methionine is amino acid 1; the number of the base and the nucleotide change are given after the plasmid name, and the amino acid changes are in parentheses and designated as described in Table 2): A22-1D, C-59 to T and T-364 to C (S20F and S122P); A34-5R, T-58 to C (S20P); A37-26D, A-64 to G (K22E); A37-15D, T-74 to C (L25S); 23A3, T-74 to C, A-84 to G, T-183 to C, and A-190 to T (L25S and K97M); A29-8D, G-89 to A (G30D); A35-41R, T-95 to C and A-341 to G (V32A and H114R); A35-31R, C-149 to T (P50L); 23A13; C-161 to A and T-342 to A (T54N and H114Q); 23A5, A-224 to G and A-226 to G (N75S and K76E); 23A1, A-235 to G (R79G); A34-34-1R and A34-34-2R, A-271 to G and A-341 to T (N91D and H114L); A34-3R, T-284 to C (L95S); A35-2R, A-341 to G (H114R); A35-4R, T-350 to C (L117S); and A37-14D and A37-15R, A-361 to T (K121stop). Plasmids whose designations end with a D were identified on the basis of a dominant phenotype. All mutant alleles chosen for further study (except alleles encoding the K22E, L25S, and H114R changes) were subcloned into an unmutagenized *HTA1*-containing plasmid (pJH55 or pJH65); the subcloned portions were completely sequenced on both strands. The resulting plasmids are listed in Table 2 and are identical to pJH55 or pJH65 except that they carry mutant *HTA1* alleles. Plasmids pJH162, pJH163, and pJH164 are identical to plasmids A37-26D, A37-15D, and A35-2R, respectively. The plasmid encoding the H114Q mutation (pJH106) was constructed by site-directed mutagenesis (38) using the oligonucleotide HTA1H114Q (GCCAAACATCCAACAAAACTTGTTGCCAAAG) and subsequent subcloning into pJH55, pJH161, which encodes a deletion of residues 5 to 21, was constructed by cloning the *Mun*I-*Pfl*MI fragment of TS2 (63) into pJH55.

RNA preparation and analysis. RNA was prepared as described previously (14). For analysis of Ty mRNA, cells were grown in YPD at 20°C to a density of 1×10^7 to 2×10^7 cells per ml. Electrophoresis, transfer to GeneScreen (New England Nuclear), and hybridization analysis were performed as described previously (68). The amount of RNA in each lane was standardized by hybridization to pYST138 (67), which contains *TUB2*. For analysis of *SUC2* mRNA, cells were grown in low-glucose media for 2.75 h as described previously (30) except that the cells were grown at 20°C. Primer extension analysis was performed on 20 μg of RNA, using primers specific for *SUC2* (suc2-3) and *SNR6* (U6.48-72) as described previously (57). The relative levels of *SUC2* and *SNR6* RNAs were determined for each sample by using a PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.). The *SUC2*/*SNR6* ratio of each sample was normalized to the *SUC2*/*SNR6* ratio of a wild-type reference sample that was included in all experiments. Average ratios and standard errors were calculated for each strain and then normalized so that the average *SUC2*/*SNR6* ratio for the wild-type samples was 100%. The *SUC2* sequencing ladder was generated by using the suc2-3 primer and a CircumVent kit from New England BioLabs.

Preparation of yeast chromatin and indirect end-labeling analysis. Yeast chromatin from derepressed cultures was prepared as described previously (30) except that cells were grown at 20°C. Chromatin from repressed cultures was prepared identically except that after being washed with water, cells were transferred to YPD (2% glucose) rather than YEP plus 0.05% glucose. Indirect end-labeling analysis (48, 83) of chromatin structure in the *SUC2* promoter region was performed by using MNase and *Hin*fI exactly as described previously (30).

β-Galactosidase assays. Cells were grown to a concentration of 1×10^7 to 2×10^7 cells per ml at 20°C. Crude extracts were prepared and assayed for β-galactosidase activity as described previously (59). β-Galactosidase levels were normalized to the total protein concentration as determined with a Bradford protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). Levels of the LexA fusion proteins in the extracts were determined by immunoblotting analysis as described previously (69), using polyclonal antibodies that were directed against the LexA protein and provided by R. Brent, except that the ECL kit from Amersham (Amersham, Buckinghamshire, England) was used for detection of the primary antibody.

RESULTS

Isolation of new *hta1* mutations that affect gene expression. We set out to identify mutations in the histone H2A-encoding gene *HTA1* that cause specific transcriptional defects. To achieve this goal, we created a strain (FY406) that carries deletions of the loci that encode histones H2A and H2B (*HTA1-HTB1* and *HTA2-HTB2*). This strain is viable because it also carries a low-copy-number plasmid marked by *URA3* and containing the wild-type *HTA1-HTB1* locus. To identify *hta1* mutants, we first mutagenized the *HTA1* gene by using PCR and then transformed the PCR products along with a gapped plasmid marked by *HIS3* into strain FY406. In such a transformation, the PCR product and gapped plasmid can undergo homologous recombination to generate an intact plasmid containing a mutagenized *HTA1* gene (see reference 46 for a description of this technique). We then tested the transformants for both dominant and recessive mutant phenotypes caused by the plasmids (Fig. 1; see Materials and Methods).

In screening for new *hta1* mutants, we focused on phenotypes that would reflect changes in transcription of genes for which chromatin structure has been implicated as a regulatory factor. Specifically, since SNF/SWI proteins are thought to activate transcription in part by overcoming chromatin-mediated repression (30; see reference 77 for a review), we screened for H2A mutants that cause defects in transcription of the SNF/SWI-dependent genes *SUC2* and *INO1* and the *GAL1-GAL10* locus. In addition, since histone H4 mutations have been shown to cause derepression of the silent mating-type cassettes and consequently lead to sterility (see reference 25 for a review), we screened transformants for the inability to mate in the hope of identifying a similar class of mutations in *HTA1*. We also screened transformants for cold sensitivity (Cs^- phenotype) (15°C), heat sensitivity (37°C), and auxotrophy on minimal medium (SD) in an effort to identify other interesting *hta1* mutations. For each of these phenotypes, we screened for both dominant and recessive phenotypes.

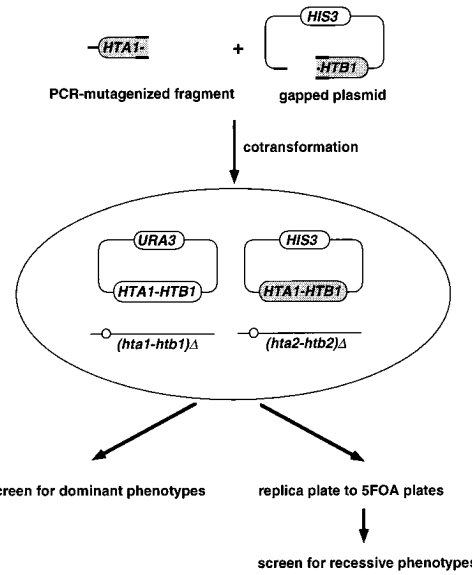

FIG. 1. A scheme for generating *hta1* mutants, based on that of Muhlrad et al. (46). Strain FY406, which already contains the *CEN URA3 HTA1-HTB1* plasmid pSAB6, was transformed with a PCR-mutagenized *HTA1* fragment and an appropriate gapped plasmid marked by *HIS3* that contains homology to the ends of the PCR product (approximately indicated by the thick lines; see Materials and Methods for details). Homologous in vivo recombination between the gapped plasmid and the PCR product gives rise to transformants that carry a mutagenized *HTA1* gene on an intact plasmid marked by *HIS3*. These transformants can be screened directly, by replica plating, for dominant phenotypes. In addition, the transformants can be replica plated to media containing 5FOA, which selects for transformants that have lost the *URA3*-marked plasmid carrying the wild-type *HTA1* gene; the 5FOA-resistant transformants can then be screened for recessive phenotypes caused by the plasmid containing the mutant *HTA1* gene.

From approximately 3,350 transformants, we identified 16 independent plasmids that confer a growth defect on media containing raffinose as the carbon source (Raf^-), a phenotype that reflects a defect in expression of *SUC2* (13). Several of the Raf^- mutants were also Gal^- or cold sensitive for growth. No plasmids that confer the other tested phenotypes (Ino^-, sterility, heat sensitivity, or auxotrophy) were identified.

Sequence analysis and initial characterization of *hta1* mutations. We sequenced the *HTA1* coding region from each of the 16 mutant candidates and found that every plasmid encodes at least one change in the 132-amino-acid H2A protein sequence (see Materials and Methods). Since many of these plasmids have more than one mutation in *HTA1*, we subcloned fragments containing individual mutations into unmutagenized plasmids and analyzed the phenotypes of the single *hta1* mutations. In 14 cases, the single *hta1* mutations (or in one plasmid a pair of adjacent mutations) caused a Raf^- phenotype and allowed viability in the absence of any other H2A-encoding gene (Table 2). We then integrated many of these mutations into the genome, replacing the wild-type *HTA1* locus (Table 2). These integrated mutations, when in strains carrying a deletion of *HTA2-HTB2*, caused the same Raf^-, Gal^-, and Cs^- phenotypes as were seen with plasmid-borne mutations (31) (Fig. 2). Therefore, these strains carrying the integrated *hta1* mutations and no other source of histone H2A were used for most of the subsequent analysis. Since the mutant phenotypes were stronger at 20°C than at 30°C (31), all subsequent experiments were performed at this temperature.

TABLE 2. *hta1* mutations: plasmids, amino acid changes, and phenotypes

HIS3 CEN plasmid[a]	Amino acid change[b]	Phenotype[c]			*URA3* integrating plasmid[a]
		Raf	Gal	Cs	
pJH55	Wild type	+	+	+	pJH65
pJH161	Δ(5-21)	−	−/+	+	ND
pJH70	S20F	−	−	−	pJH79
pJH85	S20P	−	−/+	+	pJH91
pJH162	K22E	−/+	+	+	ND
pJH163	L25S	−/+	+	+	ND
pJH124	G30D	−	−	−	pJH89
pJH68	P50L	−/+	−/+	+	pJH87
pJH135	T54N	−/+	−/+	+	pJH83
pJH134	N75S, K76E	−	−/+	+	pJH82
pJH123	R79G	−/+	+	+	pJH81
pJH77	L95S	−	−/+	+	pJH90
pJH106	H114Q	−/+	−/+	+	ND
pJH164	H114R	−/+	−/+	+	ND
pJH67	L117S	−	−	−/+	pJH78
pJH69	K121stop	−/+	−/+	+	pJH88

[a] Plasmids shown encode the H2A amino acid changes shown in the second column and are otherwise identical to pJH55 or pJH65 (see Materials and Methods). ND, plasmid was not constructed.

[b] Predicted amino acid changes encoded by the plasmids in the first column are indicated by standard one-letter abbreviations; stop indicates a translation stop codon. The first and last letters indicate the wild-type and predicted mutant amino acid at the indicated H2A residue (we have counted the initial methionine as amino acid 1). Δ(5-21) indicates a deletion of amino acids 5 through 21 inclusively. Histone H2A is 132 amino acids in length.

[c] Strain FY406 was transformed with the plasmid shown in the first column; transformants were transferred to media containing 5FOA to select for loss of the wild-type histone H2A plasmid. Symbols indicate growth of the resulting strains. −, little or no growth after 3 days; +, growth similar to wild-type growth; −/+, growth after 3 days but significantly slower than wild-type growth. Growth was assayed on YPRaf at 20°C (Raf), YPGal at 20°C (Gal), or YPD at 15°C (Cs).

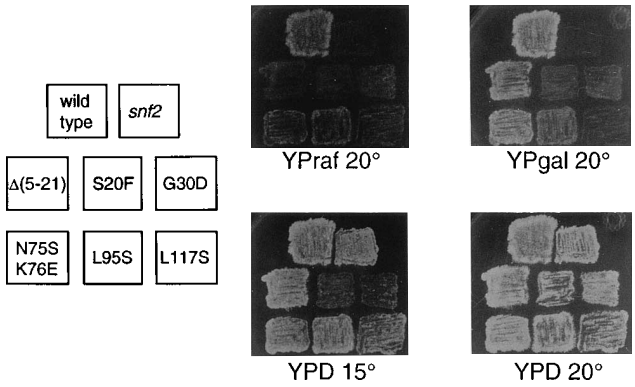

FIG. 2. Growth defects of *hta1* mutants. Strains were grown in patches on a YPD plate and then replica plated onto media containing raffinose as a sole carbon source (YPraf), media containing galactose as a sole carbon source (YPgal), and YPD. Plates were photographed after 3 days of growth at the indicated temperature. Strains (from left to right): top row, FY604 and FY458; middle row, FY986, FY987, and FY988; bottom row 3, FY989, FY990, and FY991. For the *hta1* mutants, the predicted amino acid changes in histone H2A are shown in the rectangles and are designated as described in Table 2.

The locations of the *hta1* mutations that cause a Raf$^-$ phenotype demonstrate that these mutations are found at several of the 132 codons of *HTA1* (Table 2). However, of the 14 different point mutations that cause this phenotype, five change amino acids between residues 20 and 30, and four change amino acids between residues 114 and 121 (Table 2). Thus, there is a mild clustering of changes near the N and C termini of the histone. Interestingly, one of the mutations (*hta1-1210*) changes the codon for lysine 121 to a stop codon, indicating that a loss of this C-terminal region can cause a Raf$^-$ phenotype. Furthermore, the viability of strains carrying this mutation suggests that this region of H2A is not essential for viability; previous analysis had shown that a region from amino acids 125 to 132 could be deleted without loss of viability, whereas deletion of amino acids 115 to 132 causes lethality (63).

Deletion of the N-terminal tail of H2A causes a Raf$^-$ phenotype. Since many of the *hta1* mutations cause amino acid changes at or near the N and C termini of H2A, and since a presumed truncation of the C terminus causes a Raf$^-$ phenotype, we tested whether a deletion of the N terminus would also cause a Raf$^-$ phenotype. Previous work showed that a strain carrying a deletion of amino acids 5 to 21 is viable (63); since this deletion removes amino acid 20 (at which we had identified two different Raf$^-$ mutations), we suspected that this deletion might cause a Raf$^-$ phenotype as well. Indeed, a strain carrying this Δ(5-21) deletion was Raf$^-$ (Fig. 2). The Raf$^-$ phenotype of this mutant was recessive (31). Thus, a deletion (or truncation) of either terminus of histone H2A, as well as single amino acid changes in histone H2A, can cause a Raf$^-$ phenotype.

Some *hta1* mutations are semidominant. Several of the mutant *HTA1* plasmids were isolated because they confer partially dominant Raf$^-$ phenotypes (the plasmids whose designations end in D are described in Materials and Methods). We tested each *hta1* mutation and found that those encoding the S20F, K22E, L25S, G30D, and K121stop changes were partially dominant to the wild-type allele (31); interestingly, these mutations are clustered at or near the termini of the histone. The dominance of these alleles suggests that the phenotypes they cause are due to a gain of function rather than a loss of H2A function. Also, a complete loss-of-function allele caused recessive lethality and not a Raf$^-$ phenotype (31). In addition, double-mutant combinations between semidominant alleles (such as those encoding S20F and G30D) were more strongly dominant (31), further suggesting that some of these *hta1* mutations are gain-of-function alleles. All of the double-mutant combinations tested were viable only in the presence of a wild-type *HTA1* gene (31).

Histone H2A mutants are defective for transcription of some, but not all, SNF/SWI-dependent promoters. To show that these *hta1* mutants are defective for *SUC2* transcription, we measured *SUC2* mRNA levels in a set of mutants that display strong Raf$^-$ phenotypes. Primer extension analysis demonstrated that the *hta1* mutants have reduced levels of *SUC2* mRNA (Fig. 3). Quantitation showed that they decrease the level of *SUC2* mRNA by up to 10-fold (Table 3). No effect was observed on transcription of *SNR6*, a gene transcribed by RNA polymerase III (9).

Since transcription of *SUC2* is dependent on SNF/SWI proteins and since the SNF/SWI proteins appear to activate transcription at least in part by altering chromatin structure, we tested whether the *hta1* mutations cause other transcriptional defects similar to those seen in *snf* and *swi* mutants. Many of the *hta1* mutants exhibited a Gal$^-$ phenotype (Table 2; Fig. 2). Since activation by GAL4 is dependent on SNF/SWI proteins (40, 56), we tested the ability of a LexA-GAL4 fusion protein to activate transcription in a set of *hta1* mutants that displayed a Gal$^-$ phenotype. β-Galactosidase assays using strains carrying a chromosomal *lexA* reporter demonstrated that activation mediated by LexA-GAL4 at this promoter is reduced three- to fivefold in the histone mutants (Table 4). Western blot (immunoblot) analysis showed that the level of the LexA-GAL4 protein is unaffected by the *hta1* mutations (31). Thus, the

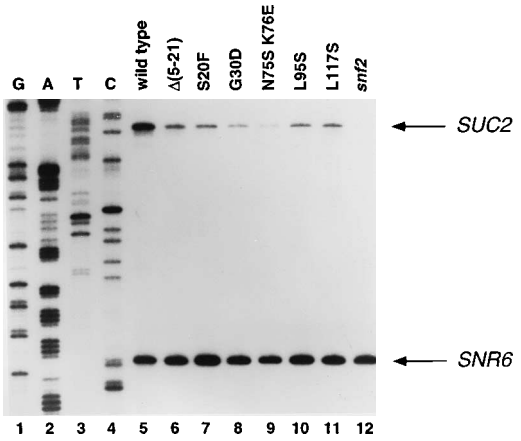

FIG. 3. Mutations in *HTA1* cause defects in *SUC2* transcription. Total RNA was prepared from strains grown under derepressing conditions for *SUC2* transcription; 20 μg of RNA from each strain was subjected to analysis by primer extension. Primers specific for *SUC2* and *SNR6* (U6), suc2-3 and U6.48-72, were used. The suc2-3 primer was used to generate a *SUC2* sequencing ladder (lanes 1 to 4) to confirm that the *SUC2* mRNA initiated at the correct site. Strains in lanes 5 to 12: FY604, FY986, FY987, FY988, FY989, FY990, FY991, and FY458, respectively.

function of the LexA-GAL4 activator is impaired in strains carrying histone H2A mutations. This result is consistent with the Gal$^-$ phenotype of the *hta1* mutants.

Further analysis demonstrated, however, that not all SNF/SWI-dependent genes are affected by these *hta1* mutations. Transcription of the yeast transposons Ty1 and Ty2 is strongly dependent on SNF/SWI proteins (17, 29) (Fig. 4; compare lanes 1 and 5). However, the *hta1* mutants did not have a decreased level of Ty mRNA; in fact, levels of Ty mRNA were increased in these mutants (Fig. 4). Similarly, strains carrying the S20P, P50L, T54N, N75S K76E, R79G, L95S, H114Q, and K121stop mutations did not have decreased levels of Ty mRNA (31). In addition, none of the mutant strains showed a growth defect on media lacking inositol, suggesting that SNF/SWI-dependent transcription of the *INO1* gene is not greatly decreased (31). Thus, not all SNF/SWI-dependent promoters are impaired by these *hta1* mutations. In addition, Northern (RNA) analysis showed that transcription of the non-SNF/SWI-dependent genes *TUB2* and *TPI1* is unaffected, also indicating that the transcriptional defects in the *hta1* mutants are specific to a subset of genes (31) (Fig. 4).

Histone H2A mutants may affect a chromatin change downstream or independent of SNF/SWI function. Since the *hta1* mutations affect transcription of some but not all SNF/SWI-dependent promoters, we considered the possibility that these histone mutations affect a step in transcription distinct from that regulated by the SNF/SWI complex. To analyze this possibility in greater detail, we compared the phenotypes caused by these *hta1* mutations and the phenotypes caused by *snf/swi* mutations with respect to genetic interactions with other mutations and with respect to their effects on chromatin structure.

First, to attempt to place the histone mutations in a pathway with respect to SNF/SWI function, we constructed double mutants that contain different *hta1* mutations in combination with an *spt6* mutation. Mutations in *spt6* suppress the transcriptional defects of *snf/swi* mutations and suppress the defects in *SUC2* chromatin structure seen in *snf/swi* mutations (7, 19, 50, 51). Thus, SPT6 appears to function downstream of the step at which SNF/SWI proteins function (77). If the histone mutations interfered with the step at which SNF/SWI proteins function, one might expect that these histone mutations would also be suppressed by *spt6* mutations. However, an *spt6* mutation did not suppress the Raf$^-$ and Gal$^-$ phenotypes of the *hta1* mutations encoding the S20F, G30D, N75S K76E, L95S, or L117S amino acid change—all *spt6 hta1* double mutants were Raf$^-$ and Gal$^-$. This result is consistent with the idea that the histone mutations interfere with a step that is downstream or independent of SPT6 function and, therefore, also downstream or independent of SNF/SWI function.

Second, we examined the chromatin structure of the *SUC2* promoter in the H2A mutants. Normally, when *SUC2* transcription is derepressed by a shift from high glucose to low glucose, characteristic changes in chromatin structure occur, as assayed by indirect end labeling (44, 54) (Fig. 5; compare lanes 3 to 7 with lanes 8 to 12). Previous work showed that these chromatin changes do not occur in *snf/swi* mutants; thus, chro-

TABLE 4. Activation by LexA-GAL4 in histone H2A mutants

Strain	H2A mutant[a]	Mean β-Galactosidase activity (U) ± SE[b]	% of wild-type activity
FY996	Wild type	246.5 ± 4.3	100.0
FY997	S20F	63.9 ± 3.6	25.9
FY998	G30D	85.4 ± 2.8	34.7
FY999	L95S	45.4 ± 2.9	18.4

[a] Amino acid changes are designated as described in Table 2.
[b] β-Galactosidase assays were performed as described in Materials and Methods on extracts from the strains listed in the first column and containing the LexA-GAL4 plasmid pSH17-4. Two or three transformants were assayed for each strain, and each transformant was assayed twice. Extracts from strains with reporters lacking the *lexA* operator contained less than 1 U of β-galactosidase activity.

TABLE 3. *SUC2* transcription in histone H2A mutants

H2A mutant[a]	*SUC2*/*SNR6* ratio[b] (%)
Wild type	100.0 ± 16.0
Δ(5-21)	16.0 ± 2.1
S20F	16.4 ± 3.8
G30D	10.8 ± 1.1
N75S K76E	12.8 ± 5.8
L95S	12.8 ± 5.2
L117S	46.1 ± 18.0
snf2	6.9 ± 5.3

[a] Amino acid changes are designated as in Table 2. Strains used are those listed in the legend to Fig. 3.
[b] Mean and standard error for the ratio of *SUC2*/*SNR6* mRNA levels in each strain. The mean *SUC2*/*SNR6* ratio has been arbitrarily set at 100% for the wild-type strain.

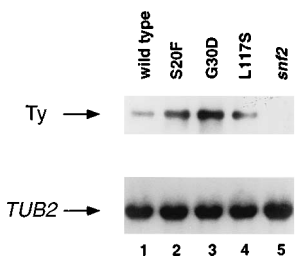

FIG. 4. Mutations in *HTA1* do not decrease transcription of Ty elements. Total RNA was prepared from each strain and subjected to Northern analysis; 10 μg of RNA was loaded in each lane. The filter was hybridized with a probe for Ty elements and then stripped and rehybridized with a probe for *TUB2* to demonstrate that equal amounts of RNA were loaded in all lanes. The strains were (from left to right) FY604, FY987, FY988, FY991, and FY458.

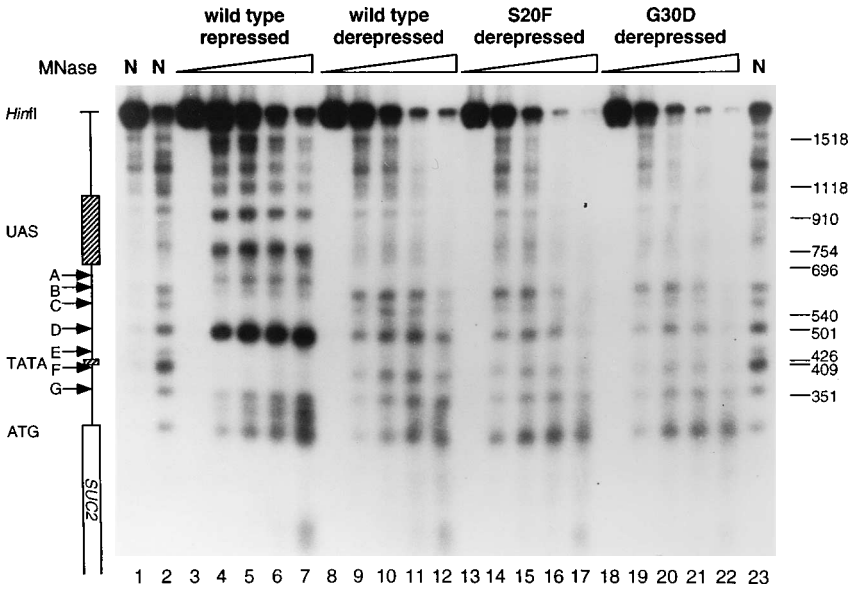

FIG. 5. Chromatin structure of the *SUC2* promoter region in *hta1* mutants grown under derepressing conditions is characteristic of chromatin from derepressed wild-type cells. Chromatin or naked DNA was digested with different amounts of MNase and subjected to analysis by indirect end labeling. The probe used was adjacent to a *Hin*fI site in the *SUC2* coding region. Approximate positions of *SUC2* promoter elements (61) and MNase cleavage sites within the *SUC2* promoter region are indicated at the left; positions of size markers are indicated at the right. DNAs subjected to analysis were as follows: naked DNA from FY604 (lanes 1, 2, and 23); chromatin from FY604 grown under repressing conditions (lanes 3 to 7) or derepressing conditions (lanes 8 to 12); chromatin from FY987 grown under derepressing conditions (lanes 13 to 17); and chromatin from FY988 grown under derepressing conditions (lanes 18 to 22). Chromatin was digested with 0, 1, 3, 10, or 30 U of MNase (from left to right within each group of five lanes). Naked DNA was digested with 0.3 U (lane 1) or 1 U (lanes 2 and 23) of MNase.

matin from *snf/swi* mutants grown under derepressing conditions appears similar to that from wild-type cells grown under repressing conditions (30, 44). Therefore, if the *SUC2* transcriptional defect in the *hta1* mutants is due to a defect in these SNF/SWI-dependent alterations in chromatin structure, the chromatin structure of the *SUC2* promoter should be the same in *hta1* mutants as it is in *snf/swi* mutants or in wild-type cells grown under repressing conditions. However, examination of the chromatin structure of the *SUC2* promoter by using indirect end labeling and MNase demonstrated that the chromatin structure in the *hta1* mutants looks the same as that in wild-type strains grown under derepressing conditions (Fig. 5; lanes 13 to 17 and 18 to 22 resemble lanes 8 to 12 but are different from lanes 3 to 7). Thus, the defect in transcription observed in the *hta1* mutants is unlikely to be explained by a defect in SNF/SWI function.

To determine if any changes in *SUC2* chromatin could be detected in these *hta1* mutants, we also examined the chromatin structure of wild-type and *hta1* mutant strains grown under repressing conditions. Interestingly, *SUC2* chromatin from a strain carrying either the S20F or G30D amino acid change shows a partially derepressed structure compared with wild-type chromatin (Fig. 6). At some sites digested by MNase, the chromatin appears to be derepressed (A, B, and the site below G), and at other sites it appears to be intermediate (site C) or repressed (site F). These differences are best seen by comparing the relative intensities of the bands within lanes in Fig. 5 and 6. This result shows that the *hta1* mutations alter *SUC2* chromatin structure and that this altered structure prevents high levels of transcription when the *hta1* mutant strains are shifted to derepressing growth conditions. (*SUC2* transcription is undetectable in wild-type or *hta1* mutant strains grown under repressing conditions [31].) Furthermore, despite this altered chromatin structure, some of the SNF/SWI-dependent chromatin changes (at sites C and F) are observed for the *hta1* mutants. Thus, these results are consistent with the unimpaired action of the SNF/SWI complex in the H2A mutants. Taken together, these results suggest that the H2A mutants are defective in a step in transcriptional activation that most likely occurs downstream or independently of the SNF/SWI-mediated changes in chromatin structure.

DISCUSSION

In this study, we have isolated and characterized histone H2A mutations that prevent normal levels of transcription from some SNF/SWI-dependent promoters in *S. cerevisiae*. Specifically, we have found that *SUC2* mRNA levels are reduced up to 10-fold and that transcriptional activation by a

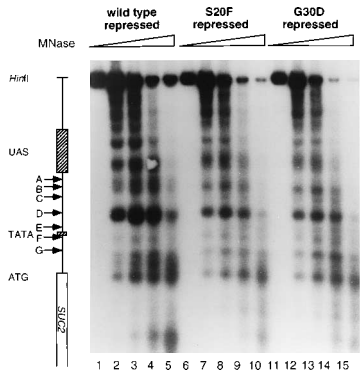

FIG. 6. Chromatin structure of the *SUC2* promoter region in wild-type and *hta1* mutant strains grown under repressing conditions. Indirect end-labeling analysis was performed as described in the legend to Fig. 5. Chromatin from strains FY604 (lanes 1 to 5), FY987 (lanes 6 to 10), and FY988 (lanes 11 to 15) was analyzed. The amounts of MNase were as described in the legend to Fig. 5.

LexA-GAL4 fusion protein is decreased. In addition, many of the mutants were Gal$^-$ and slowly growing, indicating that expression of several genes may be altered. However, not all SNF/SWI-dependent promoters are defective in these mutants, since Ty mRNA levels were not decreased. Therefore, these H2A mutants probably do not form nucleosomes that are resistant to SNF/SWI activity. Two other findings support this view. First, a suppressor of *snf/swi* mutations, *spt6*, did not suppress these *hta1* mutations, suggesting that the H2A mutations impair a step downstream or independent of the SNF/SWI-dependent step in transcriptional activation. Second, indirect end-labeling experiments showed that the chromatin structure of the *SUC2* promoter region in these H2A mutants is different from that seen in *snf/swi* mutants. These results suggest that the H2A mutations described in this report affect transcription at a previously unidentified step, distinct from that affected in *snf/swi* mutants.

We cannot rule out the possibility that the H2A mutations cause a decrease in *SUC2* mRNA levels by an indirect effect, such as changing the level of a protein that regulates *SUC2* transcription. However, these H2A mutations also decreased transcriptional activation by LexA-GAL4. This defect in activation by LexA-GAL4 is quite unlikely to be due to an indirect effect on the levels of a regulator of *SUC2* transcription. This possibility is also unlikely since mutations that affect the known activators of *SUC2* alter *SUC2* chromatin structure differently from the *hta1* mutations (30, 31, 44) (Fig. 5 and 6). We also do not believe that our H2A mutations interfere with a step subsequent to transcription initiation (such as transcriptional elongation) because *lacZ* reporters dependent on different activators were affected differently by the H2A mutations (31). In addition, at least some of these *hta1* mutations (encoding N75S K76E, L95S, and H114Q) were suppressed by *ssn6* mutations (31), which are known to affect transcription initiation at *SUC2* (62). We therefore favor a model in which these H2A mutations directly affect transcription initiation at *SUC2*. Since histone H2A is a component of chromatin, and since we observe changes in *SUC2* chromatin structure in the H2A mutants (Fig. 6), this effect is likely mediated by chromatin structure.

We have shown that the *SUC2* chromatin structure seen in the H2A mutants resembles the active chromatin that is seen in wild-type strains. Previous work has shown that *SUC2* chromatin from *snf/swi* mutants is in a different, inactive, state (30, 44). Theoretically, it is possible that the chromatin structure in the histone mutants did not show an inactive pattern because the level of *SUC2* transcription is somewhat higher in the *hta1* mutant strains than in the *snf/swi* mutant strains. This possibility is unlikely since strains carrying *snf/swi* mutations and a deletion of *HTA1-HTB1* have higher levels of *SUC2* transcription than are seen in the H2A mutants, but they have a less active chromatin structure (30). Furthermore, it is unlikely that the *hta1* mutations prevent or bypass the SNF/SWI-dependent chromatin changes at *SUC2* since some of these changes are still seen in the *hta1* mutants (Fig. 5 and 6). Thus, the wild-type pattern of chromatin in the H2A mutants likely reflects the unimpaired action of the SNF/SWI complex on chromatin in these strains.

Our results indicate that the step in transcription affected by the H2A mutants could be either in the same pathway and downstream from SNF/SWI function or in a different, independent pathway. Previous results showed that decreased dosages of genes encoding histones H2A and H2B can partially suppress *snf/swi* mutations (30), suggesting that H2A and H2B are downstream of SNF/SWI activity. Therefore, we favor a model in which the new H2A mutations block a step in overcoming chromatin-mediated repression that is downstream of the step mediated by the SNF/SWI proteins. This two-step model is diagrammed schematically in Fig. 7.

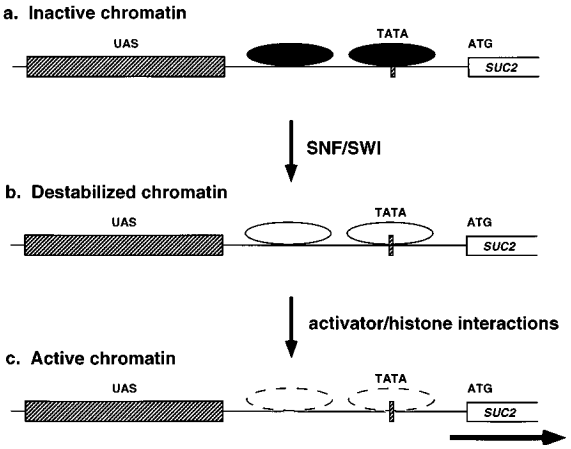

FIG. 7. A multistep model for overcoming chromatin-mediated repression at *SUC2*. Possible chromatin structures of the *SUC2* promoter region are depicted schematically as in reference 30. The solid ovals in panel a represent nucleosomes, the empty ovals in panel b represent destabilized or otherwise modified nucleosomes that do not affect micrococcal nuclease cleavage patterns but nonetheless repress transcription, and the broken ovals in panel c represent nucleosomes that have been modified or removed and that do not repress transcription. The histone mutants described in this article are proposed to impair the transition from the destabilized state (b) to the active state (c), while the SNF/SWI complex is proposed to facilitate the transition from the inactive state (a) to the destabilized state (b).

Since we attribute the decreased *SUC2* expression in these *hta1* mutants to repression of *SUC2* transcription by histones, this model implies that the SNF/SWI complex does not remove these histones from DNA. Rather, the SNF/SWI complex may reconfigure chromatin into an intermediate state in which histones, but not intact nucleosomes, are present (Fig. 7, first step). Other proteins could then facilitate the removal of these nonnucleosomal and perhaps destabilized histones (Fig. 7, second step); this step would be specifically impaired at some promoters by the histone mutations described in this report. Our model fits well with recent biochemical experiments showing that the purified SNF/SWI complex can bind to nucleosomes and alter the DNase I protection pattern without the removal of histones (20, 34). This model also is consistent with several studies that suggested that histones can be associated with promoters in nonnucleosomal conformations characterized by altered histone-DNA contacts and/or altered susceptibility to cleavage by nucleases (24, 42, 47, 66; see reference 53 for a review). In addition, destabilized chromatin states in which histones are more easily removed have been described (16, 80). It is important to note that our data are consistent with a model in which the two steps diagrammed in Fig. 7 occur simultaneously but are mediated by different proteins. In this model, the H2A mutants act independently rather than downstream of the SNF/SWI complex and trap chromatin in the destabilized configuration (Fig. 7b). It is also formally possible that the SNF/SWI complex participates in both steps diagrammed in Fig. 7 and that the H2A mutations specifically impair the second of these steps at some, but not all, SNF/SWI-dependent promoters. However, we do not favor this model, as it is not consistent with either the *spt6* suppression data or the in vitro studies of SNF/SWI (20).

Several results indicate that the mutations in histone H2A impair a step that normally occurs in transcriptional activation. We isolated multiple mutations at amino acids 20 and 114,

suggesting that the amino acid normally found at each of these locations is required for proper transcriptional activation. In addition, a deletion of either the C terminus or the N terminus of histone H2A caused the same transcriptional defects, suggesting that a normal function of these domains is to assist transcriptional activation. Since deletions can cause these defects, these results are consistent with an interaction between these regions of H2A and proteins that activate transcription by overcoming repression by chromatin, perhaps by removing histones from the promoter.

The semidominance of the histone mutations is also consistent with a model whereby a histone domain required for transcriptional activation has been partially destroyed. Histones that are competent for nucleosome assembly but are no longer suitable substrates for transcriptional activation would be expected to act in a semidominant fashion. Not all of the *hta1* mutations that we isolated are semidominant. The recessive *hta1* mutations may also cause defects in nucleosome assembly or stability in addition to defects in transcriptional activation; thus, the wild-type histone would be more frequently assembled into nucleosomes and mask the effects of the mutant histones.

The crystal structures of the histone octamer and of the histone octamer with DNA (3, 58) indicate that the positions of the amino acid changes that we identified are near the surface of the histone octamer. While these amino acids have been predicted to interact with DNA (4), they are also potential sites for interactions with other proteins, such as transcriptional activators and/or proteins that facilitate removal of histones from DNA. A defect in such interactions might result in a persistent association of histones with a promoter, thereby causing a transcriptional defect.

Biochemical experiments have suggested that at least two types of functions facilitate nucleosome removal. First, transcriptional activators have been shown to bind to nucleosomes and to displace histones from DNA in vitro (70, 72, 80). Second, nucleoplasmin, an acidic protein that binds histones and can assemble nucleosomes (23), can assist the in vitro removal of histones H2A and H2B from a nucleosome, with subsequent destabilization of the nucleosome (16). Interestingly, both the binding of an activator to nucleosomes and the nucleoplasmin-mediated removal of histones H2A and H2B are enhanced when the nucleosomes are destabilized by SNF/SWI proteins in vitro (20, 39). In addition, nucleoplasmin and activators appear to act synergistically in nucleosome disruption (16). Thus, as suggested by Chen et al. (16), transcriptional activators, a nucleoplasmin-like protein, or both may function at some promoters in vivo to remove histones and overcome chromatin-mediated repression (Fig. 7, second step). This step could theoretically be facilitated by destabilization of nucleosomes by the SNF/SWI complex (Fig. 7, first step). By this hypothesis, the H2A mutants would reduce transcription at *SUC2* and other genes by preventing the removal of destabilized histones. However, at different promoters, such as the δ promoter in Ty elements, other proteins and therefore other histone regions may be important for allowing removal of histones; at these promoters, the H2A mutants would not cause a transcriptional defect.

Currently, the only good candidate proteins in *S. cerevisiae* that might interact with destabilized nucleosomes and remove histones from a promoter are transcriptional activators. At *SUC2*, no promoter-binding transcriptional activators have yet been identified. However, other proteins that may contribute to this function are now being identified. Two recently identified genes, *SNF7* and *SNF8*, have been shown to be required for transcription of *SUC2* (73, 74). The products of these genes are not thought to be a part of the SNF/SWI complex, and mutations in these genes show genetic interactions with *spt6* and *ssn6* mutations similar to those of the histone *hta1* mutations (12, 31, 73). Thus, the *SNF7* and *SNF8* gene products could conceivably interact with histones and participate in overcoming repression by histones at the *SUC2* promoter; by this speculative model, the H2A mutations would impair interactions with SNF7 and SNF8.

Mutagenesis of the N terminus of histone H4 has identified amino acid changes that impair transcriptional activation of *GAL1* and other genes, providing compelling evidence for a role of the histone H4 N terminus in transcriptional activation in vivo (22). Since we have shown that removal of the H2A N terminus also impairs activation, it is possible that the H4 mutants and the H2A mutants described here are defective in similar steps in transcriptional activation. In this case, mutagenesis of the entire H4 coding region might identify amino acid changes outside of the N terminus of H4 that prevent activation, analogous to the non-N-terminal amino acid changes that we have identified in H2A. Alternatively, the H2A and H4 mutations might affect different aspects of transcriptional activation. Further studies, such as examination of *SUC2* transcription and chromatin structure in the H4 mutants, should help to distinguish between these possibilities.

In conclusion, this mutant hunt represents a screen for certain classes of defects after random mutagenesis of a histone gene. Our results show that single amino acid changes in this highly conserved protein can cause specific transcriptional defects while still allowing viability. We believe that by analyzing these mutants, we have identified a region of histone H2A that likely interacts with other proteins and participates in overcoming repression by nucleosomes. Further studies should shed more light on the mechanism by which this regulation of transcription occurs. In addition, the cold-sensitive alleles may provide a useful reagent for further biochemical and genetic studies. Thus, random mutagenesis of a gene encoding a conserved chromatin component, combined with subsequent genetic and molecular analysis, has both led to insights into the mechanisms by which chromatin affects transcription and provided useful reagents for further analysis. Had we used a different type of mutagenesis, screened for different phenotypes, or mutagenized a different histone gene, we likely would have found different classes of mutations. Thus, this general type of approach promises to help us to understand the role of each histone in vivo in many types of cellular functions.

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