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The Effect of Histone Gene Deletions on Chromatin Structure in *Saccharomyces cerevisiae*

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As a way of studying nucleosome assembly and maintenance in *Saccharomyces cerevisiae*, mutants bearing deletions or duplications of the genes encoding histones H2A and H2B were analyzed. Previous genetic analysis had shown that only one of these mutants exhibited dramatic and pleiotropic phenotypes. This mutant was also the only one that contained disrupted chromatin, suggesting that the original phenotypes were attributable to alterations in chromosome structure. The chromatin disruption in the mutant, however, did not extend over the entire genome, but rather was localized to specific regions. Thus, while the arrangement of nucleosomes over the *HIS4* and *GAL1* genes, the telomeres, and the long terminal repeats (δ sequences) of Ty retrotransposons appeared essentially normal, nucleosomes over the *CYH2* and *UBI4* genes and the centromere of chromosome III were dramatically disrupted. The observation that the mutant exhibited localized chromatin disruptions implies that the assembly or maintenance of nucleosomes differs over different parts of the yeast genome.

THE YEAST *SACCHAROMYCES CEREVISIAE* contains two genes for histone H2A (designated *HTA1* and *HTA2*) and two genes for histone H2B (designated *HTB1* and *HTB2*) (*1*). These genes are located in two divergently transcribed gene pairs, an *HTA1-HTB1* gene pair on chromosome IV and an *HTA2-HTB2* gene pair on chromosome II (*1, 2*). The regulation of these two histone gene loci has been characterized in considerable detail. At one level, the timing of their transcription is precisely controlled so that histone genes are transcribed only during the late G_1 and early S phases of the cell cycle (*3, 4*). At a second level, the amounts of transcripts from the two loci are controlled. Surprisingly, the latter regulation, which probably ensures that H2A and H2B remain in stoichiometric balance with H3 and H4 (*2, 5, 6*), is mediated solely through the *HTA1-HTB1* gene pair (*2, 7*). Thus, in an *HTA1-HTB1* Δ*hta2-htb2* mutant, the intracellular level of transcripts from the *HTA1-HTB1* gene pair increases to compensate for the Δ*hta2-htb2* mutation; in a Δ*hta1-htb1*, *HTA2-HTB2* mutant, however, the intracellular level of transcripts from the *HTA2-HTB2* gene pair does not change (*2*).

As a result of this differential regulation, strains bearing deletions of the individual gene pairs exhibit different phenotypes. Thus, whereas an *HTA1-HTB1*, Δ*hta2-htb2* mutant is essentially wild type, a Δ*hta1-htb1*, *HTA2-HTB2* mutant is altered in the mitotic and meiotic cycles, the heat-shock response, germination, exit from stationary phase, and transcription (*2, 6*). The latter phenotypes were postulated to be caused by general changes in chromatin structure which, in turn, were caused by the inability of the remaining *HTA2-HTB2* locus to compensate for the Δ*hta1-htb1* mutation (*2, 6*). In this report, we directly tested this hypothesis by analyzing the arrangement of nucleosomes in isogenic mutants bearing various deletions and duplications of the two *HTA-HTB* gene pairs.

In the simplest model, decreased histone mRNA levels in the Δ*hta1-htb1*, *HTA2-HTB2* mutant would lead to a decreased intracellular concentration of H2A-H2B dimers. If this were to lead to a decreased number of nucleosomes and if nucleosomes were uniformly distributed along chromosomes, then the length of the linker DNA separating nucleosome cores should increase in the Δ*hta1-htb1*, *HTA2-HTB2* mutant. However, nucleosome ladders, which were produced by treating purified nuclei with micrococcal nuclease and separating the resulting DNA fragments on a 1% agarose gel, were essentially identical in all of the strains (Fig. 1). To rule out the possibility that the putative change in linker length was very small and hence indistinguishable on a 1% agarose gel, we also analyzed the digested DNA on a 5% acrylamide gel. Again, no difference in nucleosomal repeat length was detected (*8*). Therefore, the decreased level of histone gene transcripts in the Δ*hta1-htb1*, *HTA2-HTB2* mutant did not result in an increase in the length of the linker DNA between nucleosome cores, at least not in bulk chromatin.

Nonetheless, on closer examination, the nucleosome ladder in the Δ*hta1-htb1*, *HTA2-HTB2* mutant appeared to be superimposed on a slightly diffuse or smeared background (Fig. 1). This suggested that some chromosomal regions in the mutant were particularly sensitive to nuclease digestion. To test this possibility, we transferred DNA from the gel to nylon filters and performed DNA blot analysis (*9*), using radioactively labeled probes from a number of different regions of the yeast genome.

We first analyzed the structure of chromatin around various genes. Two of the genes, *HIS4* (*10*), which is involved in histidine biosynthesis (Fig. 1 and Table 1), and *GAL1* (*11*), which is involved in galactose catabolism (Table 1), exhibited essentially wild-type nucleosome ladders in all of the experimental strains, although a slight smearing of the two ladders was evident in the Δ*hta1-htb1*, *HTA2-HTB2* mutant. We therefore conclude that the structure of chromatin over *HIS4* and *GAL1* is essentially unaffected in any of the histone mutants. Two other genes, however, *UBI4* (*12*), which encodes ubiquitin (Fig. 1 and Table

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1), and *CYH2* (*13*), which encodes ribosomal protein L29 (Table 1), exhibit dramatically altered chromatin structures in the Δ*hta1-htb1* mutant. When the latter genes were used as probes, a uniform smear was detected with very little hint of nucleosomal structure.

We next characterized the chromatin structure of nongenic DNA. The first region analyzed was the centromere of chromosome III (*CEN3*), a region whose chromatin structure has been finely detailed (*14*). Once again, the Δ*hta1-htb1*, *HTA2-HTB2* mutant exhibited an altered nucleosome ladder (Fig. 1 and Table 1). The disruption of chromatin over *CEN3*, however, appeared to be less severe than that seen over either *UBI4* or *CYH2*, since a nucleosome ladder could still be distinguished. The second nongenic region analyzed was the telomere (*15*). Using as a probe either terminal C(1-3)A sequences or the internal Y sequence, we found that all of the mutants exhibited essentially identical telomere chromatin structures (Table 1) (*16*).

Finally, we analyzed the chromatin structure of the long terminal repeats, known as δ sequences, which flank Ty retrotransposons (*17*). We were prompted to do so by the observation that a solo δ sequence, when inserted into the promoter of *HIS4*, abolished the production of wild-type *HIS4* transcript in *HTA1-HTB1*, *HTA2-HTB2* cells but not in Δ*hta1-htb1*, *HTA2-HTB2* mutants (*6*, *18*). In order to explain this observation, it was hypothesized that the Δ*hta1-htb1* mutation altered nucleosome structure over δ sequences, which then led to suppression of the δ effects on transcription (*6*). However, the nucleosomal patterns over δ sequences were very similar in all of the strains, although a slight smearing was evident in the Δ*hta1-htb1*, *HTA2-HTB2* mutant (Fig. 1 and Table 1). Since the probe used in this study recognizes all δ sequences in the cell, however, we cannot rule out the possibility that nucleosomes over individual δ sequences might be dramatically disrupted.

This somewhat surprising result led us to analyze the transcriptional properties of the histone mutants in more detail. Specifically, we asked whether the presence of altered nucleosomes over a gene was accompanied by changes in the steady-state level of its transcript (Fig. 2). Since an increase in transcription rate appears to correlate with an increase in nuclease sensitivity (*19*), we expected that the open configurations over the *CYH2* and *UBI4* genes might lead to

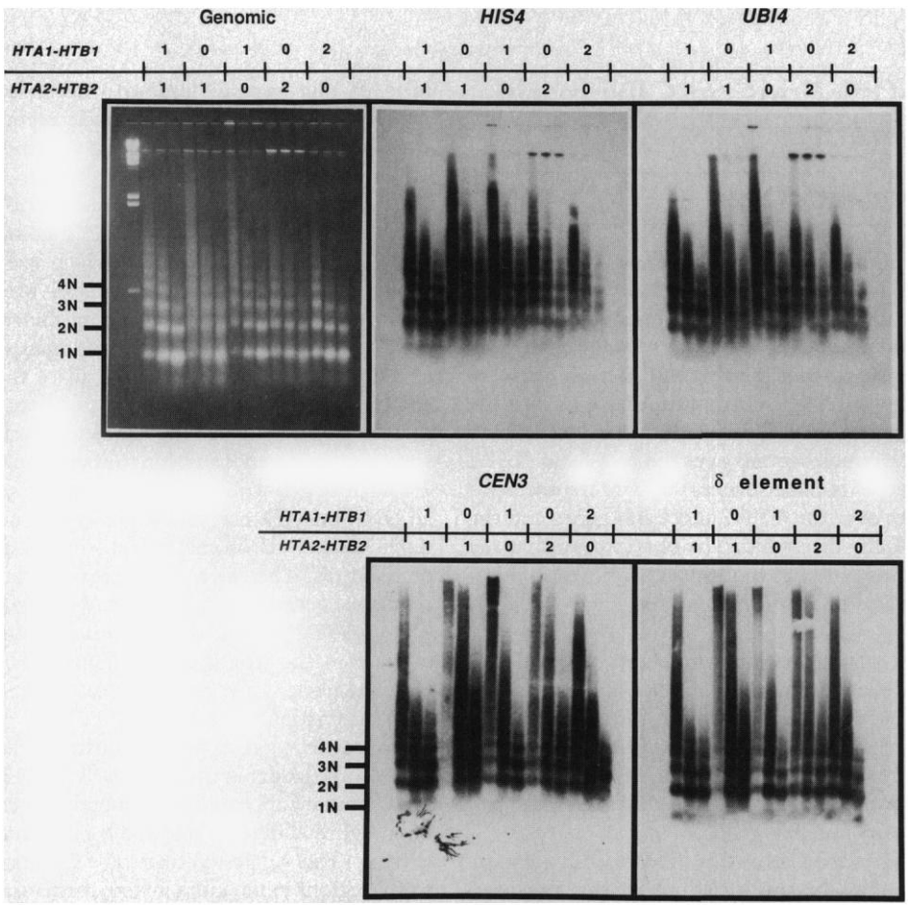

Fig. 1. Structure of chromatin in *Saccharomyces cerevisiae* strains bearing deletions or duplications of the *HTA-HTB* gene pairs. The following five isogenic yeast strains were used in this analysis (only the genetic complements of *HTA-HTB* gene pairs are listed): GRF167 (*HTA1-HTB1*, *HTA2-HTB2*); DN106 (Δ*hta1-htb1*, *HTA2-HTB2*); DN105 (*HTA1-HTB1*, Δ*hta2-htb2*); DN116 (Δ*hta1-htb1*, *HTA2-HTB2*, *HTA2-HTB2*); and DN115 (*HTA1-HTB1*, *HTA1-HTB1*, Δ*hta2-htb2*) (*2*). These strains were grown in YPD (*24*) at 30°C until mid logarithmic phase, and nuclei were purified as described by Nelson and Fangman (*25*), with the following two modifications: first, spheroplasts were made by digestion with Zymolyase (2.5 mg of Zymolyase 20T per gram of cells) rather than glusulase; second, all steps after spheroplast lysis contained, in addition to phenylmethylsulfonyl fluoride, chymostatin (0.1 μg/ml), aprotinin (1.7 μg/ml), pepstatin (1.0 μg/ml), phosphoramidon (1.1 μg/ml), and E-64 (7.2 μg/ml). Approximately 1×10^8 nuclei (75 μl of purified nuclei) were digested at 30°C with 10 μl of MNase buffer [6000 U of micrococcal nuclease (Sigma) per milliliter in 40 m*M* 1,4-piperazinediethanesulfonic acid (Pipes) (*p*H 6.3), 2 m*M* $CaCl_2$, 50% (v/v) glycerol] for 10, 30, or 60 min. To stop the digestions, SDS, NaCl, and EDTA were added to the reaction mixtures to final concentrations of 1%, 1*M*, and 20 m*M*, respectively. The nuclei were then phenol-extracted, and the released nucleic acids were ethanol-precipitated for 30 min on ice. The precipitate was centrifuged and then resuspended in ribonuclease (RNase) at 50 μg/ml for 1 hour at 37°C. The reaction was phenol-extracted, the DNA was ethanol-precipitated, and the pellet was resuspended in TE buffer [10 m*M* tris (*p*H 7.4) and 1 m*M* EDTA (*p*H 8.0)]. The first panel (genomic) shows the pattern generated after electrophoresis of this DNA on a 1% agarose gel. The numbers above each group of three indicate the number of *HTA1-HTB1* and *HTA2-HTB2* gene pairs in the strain from which the nuclei were purified. Within each group of three, the time of micrococcal nuclease digestion increases from left to right. Also shown in panel 1 is λ DNA digested with Hind III restriction endonuclease. The remaining panels show DNA blots with probes from the gene fragments described in Table 1. All the panels in the top row represent the same gel, while those in the bottom row represent a second gel.

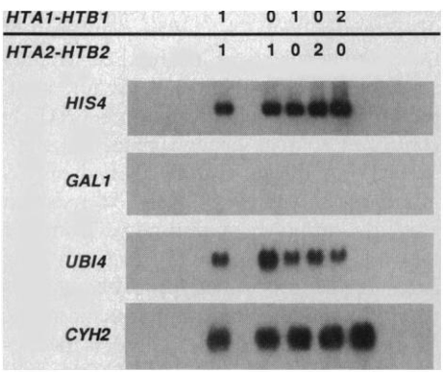

Fig. 2. Effect of histone mutations on the steady-state level of specific transcripts. Yeast strains GRF167, DN106, DN105, DN116, and DN115 were grown in YPD medium to early logarithmic phase. RNA was extracted and the purified RNA was subjected to RNA blot analysis (*4*). The numbers above each lane indicate the number of *HTA1-HTB1* and *HTA2-HTB2* gene pairs in the strain from which the RNA was purified. The top three panels represent the same gel, while the bottom panel represents another gel.

Table 1. Effect of histone mutations on chromatin structure. The *HIS4* probe consisted of a 2.9-kbp Eco RI fragment containing the 3′ end of *HIS4* (*10*). The *GAL1* probe consisted of a 275-bp Bam HI–Sal I fragment from the middle of the coding region of *GAL1* (*11*). The *UBI4* probe consisted of a 2.4-kbp Eco RI fragment containing all of *UBI4* as well as 5′ and 3′ flanking DNA (*12*). The *CYH2* probe consisted of a 590-bp Eco RI–Xho I fragment containing the 3′ end of the intron and the 5′ end of the second exon of *CYH2* (*13*). The *CEN3* probe consisted of a 60-nucleotide synthetic oligonucleotide constructed to have a sequence identical with the sequence immediately flanking *CEN3* (*14*). The C(1-3)A probe consisted of a 40-bp synthetic C(1-3)A oligonucleotide. The Y sequence probe consisted of an 800-bp Pvu I–Sac I fragment from pSZ220 (*15*). The δ element probe consisted of a 62-bp synthetic oligonucleotide constructed to have a sequence identical with nucleotides 2 to 63 of a yeast δ element (*18*). Plus (+) indicates normal nucleosome pattern.

	HTA1-HTB1 *HTA2-HTB2*	Δ*hta1-htb1* *HTA2-HTB2*	*HTA1-HTB1* Δ*hta2-htb2*	Δ*hta1-htb1* *HTA2-HTB2* *HTA2-HTB2*	*HTA1-HTB1* *HTA1-HTB1* Δ*hta2-htb2*
Genomic	+	Slightly disrupted	+	+	+
HIS4	+	+	+	+	+
GAL1	+	+	+	+	+
UBI4	+	Drastically disrupted	+	+	+
CYH2	+	Drastically disrupted	+	+	+
CEN3	+	Moderately disrupted	+	+	+
C(1-3)A	+	+	+	+	+
Y sequence	+	+	+	+	+
δ sequence	+	+	+	+	+

significant increases in the intracellular concentrations of the transcripts. However, the steady-state level of *CYH2* transcripts was essentially identical in all of the strains (Fig. 2), even though nucleosomes were dramatically disrupted over *CYH2* in the Δ*hta1-htb1*, *HTA2-HTB2* mutant. One interpretation of this result is that open chromatin configurations are not sufficient for inducing high transcription rates, a conclusion that agrees with earlier work in metazoan cells (*20*). An alternative explanation, however, is that *CYH2* transcription does increase in the Δ*hta1-htb1*, *HTA2-HTB2* mutant, but the excess transcript is posttranscriptionally degraded to maintain the proper stoichiometric balance among ribosomal proteins.

In contrast to *CYH2*, the steady-state level of transcripts from *UBI4* did increase about twofold in the Δ*hta1-htb1*, *HTA2-HTB2* mutant (Fig. 2). The elevated level of *UBI4* transcript in the Δ*hta1-htb1*, *HTA2-HTB2* mutant was expected since the heat-shock response is constitutively induced in this mutant (*2*), and *UBI4* transcription is induced by heat shock (*2, 21*). We also analyzed the transcription pattern of the *GAL1* gene, a gene that showed normal nucleosome patterns in all strains. Since the mutants had been grown in media containing glucose, conditions known to repress galactose catabolic genes (*22*), we expected that the gene would be transcriptionally quiescent. This prediction was verified (Fig. 2), which indicated that *GAL1* repression was unaffected by any of the histone mutations.

In conclusion, several points can be made regarding the results of this study. First, the chromatin disruptions in the Δ*hta1-htb1*, *HTA2-HTB2* mutant did not extend over entire chromosomes but were localized to individual regions, suggesting that either the assembly or maintenance of chromatin differs over different parts of the genome. Second, it should not be concluded that nucleosome-free regions of DNA necessarily exist in vivo over *UBI4* and *CYH2* in the Δ*hta1-htb1* mutant. Although the results presented in Fig. 1 and Table 1 support this interpretation, another possibility is that nucleosomes over these genes were unusually unstable in the Δ*hta1-htb1*, *HTA2-HTB2* mutant and hence dissociated from DNA during the preparation of nuclei. Alternatively, nucleosomes over these genes may have been in an extended "lexisome" configuration (*23*). Other less direct mechanisms could also account for the observed disruptions. Third, previous genetic analysis had suggested that the phenotypes of the Δ*hta1-htb1*, *HTA2-HTB2* mutant were caused by an underexpression of H2A and H2B (*2*). If this were the case, then the results in the present study suggest that a direct consequence of histone underexpression is location-specific disruptions in chromatin. This model, however, remains hypothetical as no definitive biochemical analysis has yet proven that H2A and H2B are in fact underrepresented in the mutant. Finally, although nucleosomes over *GAL1*, *HIS4*, telomeres, and δ sequences were concluded to be normal in the Δ*hta1-htb1*, *HTA2-HTB2* mutant, they do appear on closer examination to be slightly disrupted in this strain, since the nuclease-generated ladders are somewhat diffuse when compared to wild type. This result may be attributed to subtle changes in chromatin structure over these regions caused by the Δ*hta1-htb1* mutation; alternatively, it could be attributed to the inclusion in the total cell population of a small subpopulation with drastically disrupted chromatin structure. Notwithstanding this caveat, it is still true that different regions of the genome are differentially sensitive to the Δ*hta1-htb1* mutation.

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