A yeast H2A–H2B promoter can be regulated by changes in histone gene copy number

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The two divergently transcribed H2A–H2B gene pairs in yeast are differentially regulated as a function of the copy number of histone genes. Transcription of an *HTA2–lacZ* reporter gene is independent of histone gene copy number. Transcription of an *HTA1–lacZ* gene can be repressed or derepressed, depending on the number of *HTA* plus *HTB* genes in cells. Regulation by histone gene dosage is dependent on a negative site in the *HTA1–HTB1* promoter and the products of regulatory genes that act through this site. The level of H2A plus H2B protein in the cell may signal the response to histone gene copy number, suggesting that transcription of the *HTA1–HTB1* locus can be autogenously regulated. This phenomenon may be used, in part, to maintain the balanced synthesis of histones, a critical parameter in nucleosome assembly.

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The majority of histone genes in eukaryotic cells show replication-dependent expression—their transcription is activated near the G₁–S phase boundary (Hereford et al. 1982; Heintz et al. 1983; Sittman et al. 1983; Artishevsky et al. 1984), and histone mRNA is accumulated during the period of chromosome duplication (Hereford et al. 1981; Heintz et al. 1983). Because the assembly of histones into nucleosomes occurs almost exclusively at replication forks (Stillman 1986), temporal regulation of histone gene expression may ensure that new histones are available as soon as DNA is replicated. In mammals, sequences both upstream and downstream of histone genes participate in cell-cycle control. The upstream sequences are part of histone gene promoters and include histone subtype-specific regulatory elements (Hanly et al. 1985; Sive et al. 1986; Artishevsky et al. 1987; LaBella et al. 1987). These elements are the binding sites for factors that activate transcription as cells enter S phase (Dailey et al. 1986; Artishevsky et al. 1987; Fletcher et al. 1987). The downstream sequences occur within the histone RNAs and are part of a structure involved in histone mRNA processing (Birchmeier et al. 1983; Stauber et al. 1986; Capasso et al. 1987). These sequences are postulated to contribute to increased histone mRNA accumulation during chromosome replication (Capasso et al. 1987; Luscher and Schumperli 1987). In budding yeast, periodic transcription is regulated by both positive and negative promoter elements that have been identified upstream of several histone genes (Osley et al. 1986). Genes encoding regulatory proteins that act through the negative element have recently been identi-

fied (Osley and Lycan 1987). In yeast, the role played by downstream sequences in cell-cycle regulation has not been clearly defined; these sequences have been postulated to have a regulatory function, as periodic accumulation of H2B mRNA can be observed during S phase even when the H2B gene is constitutively transcribed (Lycan et al. 1987).

Besides temporal control, histone biosynthesis is subject to a second, poorly understood regulatory event that establishes the appropriate quantitative level of each of the four core histone proteins. Equimolar amounts of these proteins are found in eukaryotic chromatin (Albright et al. 1979), suggesting that equivalent numbers of the core histones are synthesized. The importance of keeping histone synthesis balanced has been shown by recent studies in *Saccharomyces cerevisiae*. Gene dosage alterations that create imbalances in core histone stoichiometry (e.g., as a consequence of increasing or decreasing the ratio of H2A–H2B dimers to H3–H4 tetramers) perturb cellular functions such as chromosome segregation (Meeks-Wagner and Hartwell 1986) and transcription (Norris and Osley 1987; Clark-Adams et al. 1988; Han and Grunstein 1988). It is therefore likely that cells have regulatory mechanisms to balance the synthesis of these essential chromosomal proteins.

We analyzed the phenomenon of balanced histone synthesis by examining the effects of changes in histone gene copy number on the expression of the two yeast loci that encode H2A and H2B (Hereford et al. 1979). We found that the two loci respond very differently to the number of histone genes in cells. The expression of one

H2A–H2B locus is independent of histone gene copy number. However, the second H2A–H2B locus is regulated as a function of histone gene copy number: When the number of H2A–H2B loci reduced, this locus produces more mRNA; when the number of H2A–H2B loci increased, it synthesizes less mRNA. This regulation occurs primarily at the level of transcription, apparently in response to the levels of H2A and H2B protein in the cell. The histone proteins affect (either directly or indirectly) the products of regulatory genes (Osley and Lycan 1987) that act through a negative site (Osley et al. 1986) in the promoter of the regulated locus. These data suggest that at least one yeast histone locus is autoregulated. Thus, besides their structural role as constituents of nucleosomes, H2A and H2B may also have a regulatory role in determining the frequency with which transcription of some histone genes is initiated. We propose that this phenomenon may be used to help balance the synthesis of histones, thereby maintaining an equimolar stoichiometry among the four core histone proteins.

Results

Decreasing histone gene copy number differentially affects the expression of the two HTA–HTB loci

In *S. cerevisiae*, H2A and H2B are encoded by two non-allelic loci, *HTA1–HTB1* and *HTA2–HTB2*, each of which contains a divergently transcribed pair of H2A and H2B genes (Hereford et al. 1979). Although both loci are expressed (Hereford et al. 1981), their expression is not equivalent, with the *HTA2–HTB2* locus accounting for the production of at least two-thirds of the H2A and H2B RNA in the cell (M.A. Osley, unpubl.). We reported previously that reducing the *HTA–HTB* copy number by one-half produces variable phenotypes in yeast, depending on which locus is absent (Norris and Osley 1987). A $\Delta hta2\text{--}htb2$ mutant appears indistinguishable from a wild-type strain, whereas a $\Delta hta1\text{--}htb1$ mutant shows alterations in a wide variety of intracellular processes. The differential effects of the deletions are the consequence of differences in the expression of the two *HTA–HTB* loci as a function of histone gene copy number. Northern blot analysis showed that wild-type and $\Delta hta1\text{--}htb1$ strains produce equivalent amounts of H2B2 mRNA, whereas a $\Delta hta2\text{--}htb2$ mutant produces significantly more H2B1 mRNA than a wild-type strain. These data could account for the biological effects of the *HTA–HTB* deletions if it is assumed that the major consequence of deleting the *HTA1–HTB1* locus is a decrease in the intracellular levels of H2A and H2B. This view is supported by the finding that the yeast genome appears to have nucleosome-free regions in a $\Delta hta1\text{--}htb1$ strain (Norris et al. 1988).

Although both *HTA–HTB* loci are periodically transcribed at the G₁–S phase boundary in the cell cycle (Hereford et al. 1982), the results with deletion mutants indicated that the *HTA1–HTB1* locus can be additionally regulated in response to the number of *HTA* and

HTB genes in the cell. This locus can therefore be considered to exhibit dosage compensation. To study this phenomenon in more detail, we have used either an *HTA1*– or *HTA2*–*lacZ* reporter gene to monitor the expression of the two *HTA–HTB* loci. Each fusion gene contains DNA sequences encoding the first 13 amino acids of either the *HTA1* or *HTA2* gene preceded by the entire intergenic region from the *HTA1–HTB1* or *HTA2–HTB2* locus. We have reported previously that periodic transcription of an *HTA1*–*lacZ* fusion gene is regulated by sequences located in the *HTA1–HTB1* intergenic region (Osley et al. 1986). If these or other sequences in the same intergenic region also regulate the response of this locus to histone gene dosage, we would expect expression of the fusion gene to be dependent on *HTA–HTB* copy number.

The fusion genes were integrated in single copy into the genome of three isogenic strains: a wild-type strain that contained the normal complement of *HTA–HTB* loci, and either a $\Delta hta1\text{--}htb1$ or $\Delta hta2\text{--}htb2$ mutant. The levels of *HTA*–*lacZ* mRNA produced by each fusion gene and *HTB1* or *HTB2* mRNA produced by each endogenous *HTB* gene were then measured. Figure 1 shows that in these strains the fusion genes behave qualitatively like their counterpart full-length *HTB* genes. The levels of the two *HTA2–HTB2* transcripts are the same in all the strains (Fig. 1b). In contrast, the levels of the two *HTA1–HTB1* transcripts are higher in both deletion mutants than in the wild-type strain (Fig. 1a). For example, the *HTA1* fusion transcript is 4-fold more abundant in the $\Delta hta1\text{--}htb1$ strain and 14-fold more abundant in the $\Delta hta2\text{--}htb2$ strain. The levels of *HTB1* mRNA are also elevated in the $\Delta hta2\text{--}htb2$ strain (5-fold higher than in the wild-type strain). It is not immediately apparent why the abundance of *HTB1* mRNA is lower than *HTA1* fusion mRNA in the same mutant. One possibility may be that the full-length *HTB1* gene is regulated at more levels than the fusion gene; for example, we have reported previously that the *HTB1* gene is regulated both transcriptionally and post-transcriptionally, whereas an *HTA1*–*lacZ* gene is only regulated transcriptionally (Lycan et al. 1987). Even if this is the case, the results indicate that transcriptional control plays an important role in this regulatory phenomenon. We therefore used the fusion gene to study the transcriptional aspect of dosage regulation.

Expression of the HTA1–HTB1 locus is repressed by extra copies of histone genes

If transcription of the *HTA1–HTB1* locus is regulated by *HTA–HTB* copy number, we might expect this locus to be repressed when extra copies of histone genes are present. To examine the effects of elevated histone gene copy number, we first integrated an *HTA1*– or *HTA2*–*lacZ* fusion gene into the genome of a wild-type yeast strain. The two *lacZ* reporter strains were then individually transformed with high-copy-number plasmids carrying complete copies of each of the four histone loci. As shown in Figure 2 (lanes 1–5), the levels of the two

Figure 1. Effect of a decrease in *HTA-HTB* copy number on the expression of the two loci encoding H2A and H2B. A *HIS3⁺ LEU2⁺* plasmid carrying either an *HTA1-lacZ* (pfusA1-3His) or *HTA2-lacZ* (pfusA3-5His) fusion gene was integrated in single copy into the *LEU2* locus of three isogenic yeast strains: strain GRF167, which contains both the *HTA1-HTB1* and *HTA2-HTB2* loci (WT), strain DN106, which contains a deletion of the *HTA1-HTB1* locus (Δ hta1-htb1), and strain DN105, which contains a deletion of the *HTA2-HTB2* locus (Δ hta2-htb2). Each of these strains was grown into midexponential phase, and total RNA was prepared. The levels of *HTA1* or *HTA2* fusion mRNA, *HTB1* or *HTB2* mRNA, and *RP51A* mRNA were measured by a quantitative S1 nuclease digestion assay after annealing with specific end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. (a) Levels of mRNAs from genes controlled by the *HTA1-HTB1* promoter; (b) levels of mRNAs from genes controlled by the *HTA2-HTB2* promoter. Because the same probe was used to detect *HTA1-lacZ* and *HTA2-lacZ* mRNA, the differences in the autoradiographic signals represent real differences in the expression of these two genes. Any unlabeled bands represent reannealed probes.

HTA2-HTB2 transcripts are only nominally affected by the presence of any one of the extra histone loci. An exception is seen in a strain transformed with a high-copy-number *HTA2-HTB2* plasmid (Fig. 2, lane 5), where almost eightfold more *HTB2* RNA accumulates. This increase is proportional to the copy number of the plasmid that carries the *HTA2-HTB2* locus (data not shown). In contrast, the *HTA1-HTB1* locus again shows dosage compensation (Fig. 3, left). The level of *HTA1-lacZ* mRNA is reduced 10-fold in a strain transformed with a high-copy-number *HTA2-HTB2* plasmid (lane 2) and 20-fold in a strain transformed with a high-copy-number *HTA1-HTB1* plasmid (lane 3). The two H3-H4 gene pairs in high copy number also reduce the levels of

HTA1-lacZ mRNA but by only two- to threefold (lanes 4 and 5). The differential effects of H2A-H2B versus H3-H4 plasmids on *HTA1-lacZ* transcript levels is not the result of differences in the copy number of the histone containing plasmids; each plasmid is present at ~10 copies per cell (data not shown). Thus, transcription of the *HTA1-lacZ* gene is more sensitive to the copy number of *HTA-HTB* genes. We find that even one to two extra copies of an *HTA-HTB* locus can cause repression: *HTA1* fusion mRNA levels are reduced almost 10-fold when the *HTA1-HTB1* locus is introduced into yeast on a low-copy-number plasmid (lane 7).

The levels of endogenous *HTB1* mRNA also change in cells transformed with high-copy-number *HTA-HTB* plasmids (Fig. 3, left). However, there are again quantitative differences in the effects of histone gene dosage on the regulation of a full-length *HTB1* gene versus an *HTA1-lacZ* gene; the amount of full-length histone transcript is consistently less affected by copy number. For example, a high-copy-number *HTA2-HTB2* plasmid reduces the amount of chromosomal *HTB1* mRNA by three- to fivefold (lane 2). A similar effect is seen on the

Figure 2. Expression of the *HTA2-HTB2* locus is not affected by the presence of histone genes in high copy number. A *HIS3⁺ LEU2⁺* plasmid carrying an *HTA2-lacZ* fusion gene (pfusA3-5His) was integrated in single copy into the *LEU2* locus of wild-type strain GRF167. This strain was subsequently transformed with high-copy-number histone plasmids: (Lane 1) YE24 (vector); (lane 2) pDN1 (*HTA1-HTB1*); (lane 3) pCC65 (*HHT1-HHF1*); (lane 4) pCC66 (*HHT2-HHF2*); (lane 5) pCC223 (*HTA2-HTB2*). The levels of *HTA2-lacZ*, *HTB2*, and *RP51A* mRNAs were measured by a quantitative S1 nuclease digestion assay after hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. Any bands not identified represent reannealed probes.

Figure 3. Expression of the *HTA1-HTB1* locus is repressed by the presence of histone genes in high copy number. (Left) A wild-type (*Hir*⁺) strain (M30-2B) with a copy of an *HTA1-lacZ* fusion gene integrated at the *HTA1-HTB1* locus was transformed with high-copy-number histone plasmids: (Lane 1) YEp24 (vector); (lane 2) pCC223 (*HTA2-HTB2*); (lane 3) pDN1 (*HTA1-HTB1*); (lane 4) pCC65 (*HHT1-HHF1*); (lane 5) pCC66 (*HHT2-HHF2*); (lane 6) pCC67 (*HTA1-HTB1* and *HHT1-HHF1*); (lane 7) this same strain was also transformed with a low-copy-number histone plasmid YCp50-*HTA1-HTB1* (*HTA1-HTB1*_{cen}). (Right) A *hir3* mutant strain (*Hir*⁻; M30-3A) with a copy of an *HTA1-lacZ* fusion gene integrated at the *HTA1-HTB1* locus was transformed with two high-copy-number plasmids: (Lane 1) YEp24 (vector); (lane 2) pCC223 (*HTA2-HTB2*). The levels of *HTA1-lacZ*, *HTB1*, and *RP51A* mRNAs were measured in a quantitative S1 nuclease digestion assay after hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. Unidentified bands represent reannealed probes.

level of *HTB1* transcripts produced from a high-copy-number *HTA1-HTB1* plasmid (lane 3): only two- to fourfold more *HTB1* mRNA accumulates, which correlates with a fivefold reduction in *HTB1* transcripts on the basis of a plasmid copy number of 10–20. The quantitative difference in the response of the full-length *HTB1* gene again suggests that it is regulated at more than the transcriptional level.

The HTA1-HTB1 locus may be regulated by the products of the locus

The response of the *HTA1-HTB1* locus to an increase in *HTA-HTB* copy number is explained most easily by a model of autogenous feedback regulation. We propose that the products of either *HTA-HTB* locus, that is, H2A and H2B, can specifically repress (either directly or indirectly) transcription of the divergently transcribed *HTA1-HTB1* gene pair (Osley et al. 1986). However, an

alternative explanation for the results is that there is normally a limiting amount of a histone- or *HTA-HTB*-specific positive transcription factor in cells. If the *HTA2-HTB2* locus competes more efficiently than the *HTA1-HTB1* locus for this factor, this could account for the disproportionate level of *HTA1-HTB1* and *HTA2-HTB2* transcripts in wild-type cells. Hence, derepression of the *HTA1-HTB1* locus in a Δ *hta2-htb2* strain might occur because of decreased competition for the factor; repression of the same locus on the addition of extra *HTA-HTB* loci might represent increased competition for the factor. To address this question, high-copy-number plasmids carrying the entire *HTA1-HTB1* or *HTA2-HTB2* intergenic region (where transcriptional regulatory sequences reside), but only truncated *HTA* and *HTB* genes, were transformed into a wild-type strain that contained an *HTA1-lacZ* reporter gene (Fig. 4A). In contrast to the strong repression caused by extra copies of complete *HTA-HTB* loci (lanes 2 and 4), multiple copies of *HTA-HTB* intergenic sequences do not decrease the levels of *HTA1-lacZ* mRNA (lanes 3 and 5). The amount of fusion mRNA actually increases slightly

Figure 4. Expression of the *HTA1-HTB1* locus is not affected by the presence of *HTA-HTB* regulatory sequences in high copy number. (A) A wild-type (*Hir*⁺) strain (M30-2B) containing a copy of an *HTA1-lacZ* fusion gene integrated at the *HTA1-HTB1* locus was transformed with high-copy-number histone plasmids: (Lane 1) YEp24 (vector); (lane 2) pDN1 (*HTA1-HTB1*); (lane 3) pHTA1-HTB1_p (*HTA1-HTB1* intergenic region); (lane 4) pCC223 (*HTA2-HTB2*); (lane 5) pHTA2-HTB2_p (*HTA2-HTB2* intergenic region). (B) A *hir3* mutant (*Hir*⁻) strain (M30-3A) containing a copy of an *HTA1-lacZ* fusion gene integrated at the *HTA1-HTB1* locus was transformed with high-copy-number histone plasmids: (Lane 1) YEp24 (vector); (lane 2) pHTA1-HTB1_p (*HTA1-HTB1* intergenic region); (lane 3) pHTA2-HTB2_p (*HTA2-HTB2* intergenic region). The levels of *HTA1-lacZ* and *HTB1* mRNAs were measured in a quantitative S1 nuclease assay after hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. Bands not labeled represent reannealed probes.

under these conditions, suggesting that some inhibitor may be titrated out by the promoter sequences on the plasmids. Thus, histone promoter sequences in high copy number are not sufficient, by themselves, to confer repression. These data suggested that an entire histone-coding region is required for the regulatory effect.

We then asked whether a high-copy-number plasmid carrying a single full-length *HTA* or *HTB* gene could repress transcription of the *HTA1* fusion gene. Two high-copy-number plasmids, one carrying a full-length *HTB1* gene and the other carrying a full-length *HTA1* gene plus a full-length but nonfunctional *HTB1* gene that harbors a frameshift mutation at amino acid 80 (Rykowski et al. 1981), were transformed into an *HTA1-lacZ* reporter strain (Fig. 5). In contrast to the effects of plasmids carrying full-length copies of both an *HTA* and an *HTB* gene (lanes 2 and 3), plasmids carrying a single functional *HTA* or *HTB* gene do not cause repression of the fusion gene (lanes 4 and 5). In addition, the amount of *HTB1* mRNA that is produced is now proportional to the ex-

pected copy number of these plasmids, suggesting that the *HTB1* gene carried on the plasmids is also no longer regulated. These data support the idea that both a full-length *HTA* gene plus a full-length *HTB* gene must be present in high copy number in the same cell to repress transcription. The results also suggest that the products of the *HTA* and *HTB* genes—the H2A and H2B proteins—may be the actual signals of feedback regulation, as a full-length but nonfunctional *HTB1* gene cannot substitute for a wild-type *HTB1* gene (lane 5).

If feedback regulation is a response to the intracellular concentration of H2A plus H2B, it might possibly be triggered by the levels of H2A–H2B heterodimers. Several studies have indicated that the formation of nucleosomes in vivo is dependent on an equimolar ratio of H2A–H2B dimers to H3–H4 tetramers (Meeks-Wagner and Hartwell 1986; Clark-Adams et al. 1988). An increase or decrease in *HTA*–*HTB* copy number might be expected to produce an imbalance in the stoichiometric ratio. If this imbalance is the effector of *HTA1*–*HTB1* regulation, then any situation that restores the correct stoichiometric ratio should also abolish the regulatory signal. Thus, an *HTA1-lacZ* gene should not be repressed when all four core histone genes are present in high copy number in the same cell. However, a plasmid carrying both the *HTA1*–*HTB1* locus plus the *HHT1*–*HHF1* locus still results in significant repression of the fusion gene (Fig. 3, left, lane 6). These results suggest that the absolute levels of H2A and H2B in the cell (either as monomers or as heterodimers) might be more critical for the regulatory effect.

Figure 5. The expression of the *HTA1*–*HTB1* locus is repressed by the functional products of *HTA* plus *HTB* genes. A wild-type (*Hir*⁺) strain (M30-2B) with a copy of an *HTA1-lacZ* gene integrated at the *HTA1*–*HTB1* locus was transformed with high-copy-number histone plasmids: (Lane 1) YE24 (vector); (lane 2) pCC223 (*HTA2*–*HTB2*); (lane 3) pDN1 (*HTA1*–*HTB1*); (lane 4) pHTB1 (the intergenic region from the *HTA1*–*HTB1* locus plus a full-length *HTB1* gene); (lane 5) pHTA1-*HTB1*_{fs} (a complete *HTA1*–*HTB1* locus with a full-length *HTA1* gene and an *HTB1* gene that contains a frameshift mutation at amino acid 80). The levels of *HTA1-lacZ*, *HTB1*, and *RP51A* mRNAs were measured in a quantitative S1 nuclease assay following hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. Unlabeled bands are reannealed probes.

Feedback regulation requires a negative site in the HTA1–HTB1 promoter and HIR gene products that act through this site

Functional analysis of the *HTA1*–*HTB1* promoter has identified two classes of regulatory sequences: reiterated upstream activating sequences (UAS) elements that activate transcription periodically and a unique negative site (Osley et al. 1986). Deletion of the negative site leads to derepressed transcription of the *HTA1*–*HTB1* locus and the independence of this locus from cell-cycle controls. Because of its effects on the level of *HTA1*–*HTB1* transcription, the negative site was the likely element to regulate the response to *HTA*–*HTB* copy number. To test this possibility, an *HTA1-lacZ* reporter gene that contains a deletion of the negative site was integrated into the genome of a wild-type yeast strain. We showed previously that this mutant fusion gene was derepressed almost fivefold compared to a wild-type fusion gene (Osley et al. 1986). The strain containing the mutant gene was subsequently transformed with a high-copy-number *HTA2*–*HTB2* plasmid (Fig. 6). Whereas a wild-type fusion gene is repressed by the amplified *HTA2*–*HTB2* locus (Fig. 3, left, lane 2), the mutant gene remains derepressed under the same conditions (Fig. 6, lane 2). These results support the idea that transcriptional regulation plays a significant role in autogenous

Figure 6. A negative site in the *HTA1-HTB1* promoter is required for feedback repression. A plasmid (p $\text{fusA1}_{\Delta\text{neg}}$ -*lacZ*) carrying an *HTA1-lacZ* fusion gene that contains a 50-bp deletion of a negative site found in the *HTA1-HTB1* promoter was integrated at the *leu2* locus in a wild-type (Hir^+) strain (DB747) or *hir3* mutant (Hir^-) strain (M30-9C). These strains were subsequently transformed with high-copy-number plasmids YE24 (vector) and pCC223 (*HTA2-HTB2*). The levels of *HTA1-lacZ* and *HTB1* mRNAs were measured in a quantitative S1 nuclease assay, following hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography. Unidentified bands represent reannealed probes.

feedback control and identify the negative site as a necessary element in this response. Because we have been unable to identify a similar negative site in the *HTA2-HTB2* promoter (C. Higgins and M.A. Osley, unpubl.), these data also provide a possible explanation for the failure of the *HTA2-HTB2* locus to be feedback-controlled.

We also examined the importance of the negative site in feedback regulation by another approach. We previously constructed plasmids carrying hybrid *CYC1-lacZ* genes regulated by *CYC1* UAS elements, *CYC1* UAS elements plus the *HTA1-HTB1* negative site, or isolated *HTA1-HTB1* UAS elements (Osley et al. 1986). Each of these plasmids was transformed into three isogenic strains: a wild-type strain, and a $\Delta\text{hta1-htb1}$ or $\Delta\text{hta2-htb2}$ mutant. We have shown previously that the isolated negative site represses *CYC1-lacZ* transcription >10-fold in wild-type strains (Osley et al. 1986). If this site is solely responsible for regulating the response to *HTA-HTB* copy number, we would expect that it would not repress the *CYC1-lacZ* gene when the *HTA-HTB* copy number is reduced, because in these situations an *HTA1-lacZ* gene is derepressed (cf. Fig. 1). As shown in Table 1, the expression of a *CYC1* fusion gene regulated by the *HTA1-HTB1* UAS does not change significantly

among the three strains. In contrast, in the $\Delta\text{hta1-htb1}$ mutant, the repression of the *CYC1-lacZ* gene by the *HTA1-HTB1* negative site is partly eliminated. However, the negative site still efficiently represses the *CYC1-lacZ* gene in the $\Delta\text{hta2-htb2}$ mutant, a strain that shows 14-fold derepression of an *HTA1* fusion gene. We must therefore conclude that although the negative site is a necessary element for regulating the feedback response, it may not be sufficient. It may also be the case that the isolated negative site is a more sensitive monitor of the feedback signal and that the differences in its regulation in the two deletion mutants reflect both qualitative (H2A and H2B subtypes) and quantitative (H2A and H2B levels) differences in this signal between the strains.

We reported previously on the isolation and partial characterization of *hir* (histone regulation) mutants that are defective in the regulation of the *HTA1-HTB1* locus (Osley and Lycan 1987). The mutations derepress transcription of this locus and relieve it from cell-cycle controls. Because the *HIR* gene products act (either directly or indirectly) through the negative site in the *HTA1-HTB1* promoter, we wished to determine whether *hir* mutants show altered feedback regulation. An *HTA1-lacZ* fusion gene was integrated into the genome of *hir1*, *hir2*, and *hir3* mutants, which were then subsequently transformed with high-copy-number plasmids carrying each of the four histone loci. No transformants arose in any of the *hir* strains upon the introduction of plasmids carrying either the *HTA1-HTB1*, *HHT1-HHF1*, or *HHT2-HHF2* locus, although *HIR*⁺ strains can be transformed with these same plasmids. Because the *hir* mutations cause derepressed expression of each of these three histone loci (Osley and Lycan 1987), we assume that the production of H2A and H2B or H3 and H4 by histone

Table 1. Effects of isolated regulatory elements from the *HTA1-HTB1* promoter on feedback regulation

Strain	pLGΔ312	pLG-CCR15	pLG-UAS
GRF167	1.0	0.08	1.9
DN105FOA	1.0	0.05	2.1
DN106FOA	1.0	0.24	2.6

High-copy-number *URA3*⁺ plasmids carrying a *CYC1-lacZ* fusion gene controlled by the *CYC1* UAS elements (pLGΔ312), the *CYC1* UAS elements plus the 67-bp *HTA1-HTB1* negative site (pLG-CCR15), or three 16-bp UAS elements from the *HTA1-HTB1* promoter (pLG-UAS) were transformed into three isogenic yeast strains: a wild-type strain (GRF167), a $\Delta\text{hta2-htb2}$ strain (DN105FOA), and a $\Delta\text{hta1-htb1}$ strain (DN106FOA). Each strain was grown to midexponential phase in selective medium, and the levels of β -galactosidase were measured in cell extracts. The numbers represent relative values (to the level of enzyme produced by a *CYC1-lacZ* gene controlled by the *CYC1* UAS elements) obtained from assays performed on duplicate cultures in at least three separate experiments. Miller units of β -galactosidase produced by a *CYC1-lacZ* gene controlled by the *CYC1* UAS elements are 26.2 (GRF167), 29.7 (DN105FOA), and 4.5 (DN106FOA).

genes carried on high-copy-number plasmids is grossly unbalanced in the mutant backgrounds. This imbalance may lead to deleterious effects on some essential cellular processes and, hence, result in lethality. This view is supported by the observation that only high-copy-number plasmids that carry complete *HTA1-HTB1* or *HHT-HHF* loci are unable to transform *hir* mutants; high-copy-number plasmids carrying either individual *HTA1* or *HTB1* genes or *HTA-HTB* regulatory sequences can transform wild-type and *hir* strains with almost equal efficiency. A low-copy-number plasmid carrying a complete *HTA1-HTB1* locus is also able to transform *hir* mutants.

Only the *HTA2-HTB2* locus can be transformed into *hir* mutants on a high-copy-number plasmid. We assume that this locus has no effect on cell viability because it is the only one of the four histone loci whose expression is not derepressed by the *hir* mutations (Osley and Lycan 1987). This fortuitous situation allowed us to examine the effects of a high-copy-number *HTA2-HTB2* plasmid on gene expression in a *hir3* mutant background (Fig. 3). We find that the *HTA1-lacZ* gene is no longer repressed by elevated *HTA2-HTB2* copy number in the *hir* strain (right); the fusion gene is actually derepressed in the mutant compared to a wild-type strain (cf. Fig. 3, left, lane 1 to right, lane 1). The effects of the *hir* mutation are not limited to high-copy-number repression because the same mutation also eliminates repression resulting from a low-copy-number *HTA1-HTB1* plasmid (data not shown). In addition, the *hir3*-associated derepression of the *HTA1-lacZ* gene is unaffected by high-copy-number plasmids that carry either *HTA-HTB* regulatory sequences (Fig. 4B) or individual *HTA1* or *HTB1* genes. Finally, the levels of *HTA2-lacZ* mRNA are not altered in a *hir3* mutant, either in the presence or in the absence of a high-copy-number *HTA2-HTB2* plasmid. It is therefore likely that the *HIR* gene products have a direct role in the feedback regulation of the *HTA1-HTB1* locus. Their function in this regulatory response may be coupled, in some way, to the intracellular levels of H2A and H2B.

An aspect of cell-cycle-regulated transcription is altered in a Δ hta1-htb1 strain

One of the characteristic phenotypes of *hir* mutant strains is a failure to turn off transcription of the *HTA1-HTB1* locus following a block in chromosome replication (Osley and Lycan 1987). Because we have shown that *hir* mutations also affect feedback regulation, it is possible that feedback regulation, itself, mediated through *HIR* proteins, is actually responsible for the cell-cycle response. We therefore asked whether a *Hir*⁻ phenocopy could be created in *HIR*⁺ cells merely by deleting an *HTA-HTB* locus. As indicated in Figure 7, a Δ hta1-htb1 strain (lanes 3 and 4) behaves as if it were a *hir* mutant because, unlike a wild-type strain (lanes 1 and 2), transcription of an *HTA1-lacZ* fusion gene is not repressed after inhibition of chromosome replication. This result is consistent with the idea that feedback reg-

Figure 7. A *Hir*⁻ phenocopy can be created by deleting the *HTA1-HTB1* locus. An *HTA1-lacZ* fusion gene was integrated at the *LEU2* locus in three isogenic yeast strains; strain GRF167, which contains both *HTA-HTB* loci (WT); strain DN106, which contains a deletion of the *HTA1-HTB1* locus (Δ hta1-htb1); and strain DN105, which contains a deletion of the *HTA2-HTB2* locus (Δ hta2-htb2). These strains were grown into midexponential phase, and one-half of each culture was treated for 30 min with 0.2 M HU to block DNA replication. The levels of *HTA1-lacZ* and *RP51A* RNAs in the untreated (-) and treated (+) cultures were measured in a quantitative S1 nuclease digestion assay, following hybridization to end-labeled probes, electrophoresis on denaturing polyacrylamide gels, and autoradiography.

ulation may play a role in this particular cell-cycle response; because the intracellular levels of H2A and H2B are presumably decreased in a Δ hta1-htb1 mutant, the *HIR* proteins may not be able to repress transcription.

Figure 7 also shows that a Δ hta2-htb2 mutant (lanes 5 and 6) is still phenotypically *Hir*⁺, as *HTA1-lacZ* transcription is turned off following the replication block. Although this strain is derepressed for *HTA1-HTB1* transcription, it can clearly respond normally to at least one aspect of cell-cycle control. Thus, in this strain, feedback regulation can be separated from cell-cycle regulation. This is not the case in a Δ hta1-htb1 mutant, where the two regulatory phenomena are apparently connected.

Discussion

We initiated a study of the mechanisms that regulate the balanced synthesis of histones in vivo. Very little is known about this aspect of histone biosynthesis, although it must be of critical importance to the cell because the unbalanced synthesis of these proteins produces pleiotropic phenotypes (Meeks-Wagner and Hart-

well 1986; Norris and Osley 1987; Clark-Adams et al. 1988). To study the balanced synthesis of H2A and H2B, we either increased or decreased the number of histone genes in yeast cells. We measured the effects of these changes in histone gene copy number on the regulation of the two *HTA-HTB* loci by using *HTA-lacZ* reporter genes to monitor their expression. We found that the expression of the *HTA2* gene is insensitive to the number of histone genes in the cell, whereas the *HTA1* gene is regulated by histone gene copy number. The *HTA1* gene produces 10- to 20-fold less RNA when between 1 and 10 additional copies of *HTA* and *HTB* genes are present and 4- to 14-fold more RNA when the *HTA-HTB* copy number is reduced by one-half. We have shown that transcription of the *HTA1-HTB1* locus can be regulated by *HTA-HTB* copy number; this regulation is dependent on a negative site in the *HTA1-HTB1* promoter and at least three gene products that act through the negative site. Our studies additionally demonstrate that the functional products of the *HTA* and *HTB* genes must be made to see the effects of gene dosage. Although we cannot eliminate the possibility that the histone proteins affect transcription only indirectly, the simplest interpretation of this result is that it is the amount of H2A plus H2B protein in the cell that is the actual feedback signal. We therefore propose that the transcription of the gene dosage-sensitive *HTA1-HTB1* locus can be autogenously regulated. This form of regulation is in addition to the temporal control of transcription that occurs at the G₁-S phase boundary.

We reported previously that the stability of mRNA produced by the *HTA1-HTB1* locus can also be affected by the copy number of this locus (Osley and Hereford 1981). The half-life of full-length *HTB1* mRNA is decreased approximately twofold in response to a chromosomal duplication of the *HTA1-HTB1* locus. However, our studies with an *HTA1* fusion gene indicate that changes in *HTA-HTB* copy number also affect the regulation of transcription. It is therefore likely that the effects of gene dosage on full-length *HTA1* or *HTB1* genes represent the contributions of transcriptional as well as post-transcriptional controls. Because the *HTA1* fusion gene does not appear to contain sequences that regulate mRNA stability (Lycan et al. 1987), this could also account for the quantitatively different responses of the full-length gene and fusion gene to altered histone gene dosage.

The observation that transcription of the *HTA1-HTB1* locus, but not the *HTA2-HTB2* locus, is under autogenous control may be accounted for by differences in both the *cis*- and *trans*-acting regulation of these two histone promoters. A search of the *HTA2-HTB2* promoter has identified several DNA sequences with similarity to the *HTA1-HTB1* UAS consensus element (Osley et al. 1986) but no sequences with similarity to the *HTA1-HTB1* negative site, which is required for feedback control (C. Higgins and M.A. Osley, unpubl.). In addition, *hir* mutations, which both derepress *HTA1-HTB1* transcription and eliminate autogenous control, have no effect on either the basal level of *HTA2-HTB2*

transcription (Osley and Lycan 1987) or on the failure of this locus to respond to histone gene copy number.

Although the *HTA2-HTB2* locus is not feedback-regulated, several observations suggest that transcription of both *HHT-HHF* loci might be autogenously controlled. First, a 15-bp sequence found in the 67-bp *HTA1-HTB1* negative site has been noted in the two *HHT-HHF* promoters (Breedon 1988; M. Smith, pers. comm.). Second, *hir* mutations have been shown to derepress transcription of both the *HTA1-HTB1* and the two *HHT-HHF* loci (Osley and Lycan 1987). However, it has been reported that when either *HHT-HHF* locus is deleted, the remaining locus does not produce more RNA (Cross and Smith 1988). Although these data suggest that the *HHT-HHF* loci are not dosage compensated, it is not known whether either of these two loci can be regulated by changes in *HTA-HTB* copy number.

Two models, not necessarily mutually exclusive, can be proposed to account for autogenous regulation of the *HTA1-HTB1* locus. In the first model, regulation occurs by direct protein-protein interactions among the factors regulating *HTA1-HTB1* transcription. One *HIR* gene could encode a repressor that binds to the *HTA1-HTB1* negative site; this repressor may interact with one or more additional transcription factors to inhibit the initiation of transcription. H2A and H2B would be corepressors, interacting (directly or indirectly) with the repressor to affect its DNA binding or activity. In this model of autogenous control, the histone proteins may function as classic regulatory molecules. This may account for the observation that transcription is repressed following a twofold change in *HTA-HTB* copy number, as if regulation is triggered by only small differences in histone levels. In the second model, autogenous regulation occurs through the well-known structural roles of H2A and H2B in nucleosomes, by the creation of altered chromatin structure. The formation or maintenance of nucleosomes in the *HTA1-HTB1* promoter could be altered by imbalances in the levels of H2A-H2B dimers. These chromatin alterations could affect the binding or activity of a transcription factor at the negative site (e.g., a *HIR* gene product) or other sites in the promoter. As a result, the initiation of transcription may either be stimulated or repressed. This model is supported by data demonstrating that both constituents of an H2A-H2B heterodimer (i.e., H2A plus H2B) are required for feedback regulation, and by the observation that yeast chromatin structure is altered when the levels of H2A-H2B dimers are reduced (Norris et al. 1988).

This is not the only example in yeast where changes in histone gene copy number have been found to affect transcription. When the number of histone genes are either reduced or elevated, the *his4-9128* mutant allele is suppressed and the cell becomes phenotypically His⁺. In this system, altered histone levels suppress the effects of the δ insertion in *HIS4* by changing the site where transcription is initiated: instead of using the initiation site in the δ element, the cell now utilizes the natural HIS4⁺ initiation site (Clark-Adams et al. 1988). This is in contrast to the effects of histone levels on *HTA1-HTB1*

transcription, where similar alterations appear to affect the frequency with which transcription is initiated. Histone levels differentially affect the transcription of these two loci in other ways as well. The most notable difference is the observation that the *his4-9128* allele is suppressed only in a Δ *hta1-htb1* strain; *HTA1-HTB1* transcription, on the other hand, is derepressed by a deletion of either *HTA-HTB* locus. A second difference is the finding that any histone locus in high copy number can suppress the *his4-9128* mutation. In contrast, *HTA1-HTB1* transcription is significantly repressed only when loci encoding H2A and H2B are present in high copy number. Finally, the effects of high-copy-number histone genes on *his4-9128* transcription can be abrogated by restoring the stoichiometric balance among the four core histones. *HTA1-HTB1* transcription is still repressed under the same conditions. Whether these differences reflect fundamentally different regulatory mechanisms remains to be determined.

What is the biological significance of autogenous regulation? One role may be to keep the synthesis of H2A–H2B and H3–H4 in balance, thereby ensuring that the correct number of nucleosomes is formed or that the correct structure of nucleosomes is maintained. We have shown that a change in the nuclease sensitivity of chromatin results from an imbalance in core histone stoichiometry (Norris et al. 1988). Other studies have also shown that these imbalances result in pleiotropic cellular phenotypes (Meeks-Wagner and Hartwell 1986; Norris and Osley 1987; Clark-Adams et al. 1988). Therefore, feedback regulation may function in cellular homeostasis to control the assembly of histones into chromatin. A second function may be to alter the intracellular levels of particular H2A and H2B subtypes. The two *HTA-HTB* loci encode different protein subtypes (Wallis et al. 1980; Choe et al. 1982), but only one locus is feedback-regulated. Derepression or repression of the *HTA1-HTB1* locus could be used to either increase or decrease the proportion of subtypes that it encodes. If nucleosomes that are formed from these particular subtypes specifically affect some cellular processes, this mechanism may function to alter their distribution in chromatin. Finally, autogenous regulation may have a role in one or more aspects of cell-cycle-regulated transcription. A Δ *hta1-htb1* mutant fails to turn off transcription of an *HTA1-lacZ* reporter gene when chromosome replication is blocked. One interpretation of this result is that the intracellular levels of H2A and H2B affect the execution of this particular regulatory response. For example, when DNA synthesis is inhibited, the failure of histones to be assembled into nucleosomes at stalled replication forks may provide a sufficient regulatory signal to repress transcription. However, this cannot be the only mechanism to turn off transcription in S phase. Transcription of the *HTA2-HTB2* locus is also repressed following a replication block, although this locus is not regulated by histone levels or the products of identified *HIR* genes. It is therefore likely that cell-cycle regulation involves a combination of both temporal and feedback controls.

Materials and methods

Media and growth conditions

The following yeast media were used: YPD medium is 10 g of yeast extract, 20 g of peptone, and 20 g of glucose per liter. SD medium is 6.7 g of yeast nitrogen base without amino acids per liter, 2% glucose, and appropriate amino acid supplements (Sherman et al. 1982). YM-1 medium (Hartwell 1967) was used to grow cells to be treated with hydroxyurea (HU). Log phase cells were treated with 0.2 M HU for 30 min (Lycan et al. 1987). Yeast cells were transformed by the lithium chloride method (Ito et al. 1983), with selection on SD/2% agar plates lacking the appropriate amino acid.

Integrating plasmids

pfusA1-3His The construction of plasmid *pfusA1-3* has been described (Osley and Hereford 1982). This plasmid contains the 13 amino-terminal amino acids of *HTA1* fused to the *lacZ* structural gene, the entire intergenic region from the *HTA1-HTB1* locus, a truncated *HTB1* gene, and *ars* sequences present 3' to the *HTB1* gene. A 4.3-kb *Pst*I fragment containing the *LEU2* gene is present as a selectable marker. Plasmid *pfusA1-3His* was constructed by inserting a 1.7-kb *Bam*HI fragment containing the *HIS3* gene into a unique *Bam*HI site on *pfusA1-3*.

pfusA3-5His This plasmid is similar to *pfus1-3His*, except that it contains a fusion of the 13 amino-terminal amino acids of *HTA2* to the *lacZ* gene, the *HTA2-HTB2* intergenic region, a truncated *HTB2* gene, and sequences 3' to the *HTB2* gene.

pfus1 Δ neg-lacZ This plasmid is derived from *p* Δ 16', which has been described previously (Osley et al. 1986). It contains a fusion of the 13 amino-terminal amino acids of *HTA1* to the *lacZ* gene, the *HTA1-HTB1* intergenic region with a 50-bp deletion that removes the negative site, and a truncated *HTB1* gene. Plasmid *pfus1 Δ neg-lacZ* is an integrating form of *p* Δ 16' made by deletion of the CEN IV sequences on the latter plasmid.

High-copy-number replicating plasmids

High-copy-number plasmids were constructed using the origin of replication from the 2- μ circle plasmid YEp24 (Botstein et al. 1979).

pDN1 A 6.4-kb *Bam*HI fragment that contains the *HTA1-HTB1* locus (Hereford et al. 1979), including the full-length *HTA1* and *HTB1* genes, the entire intergenic region between *HTA1* and *HTB1*, and the *PRT1* (protein 1) gene 3' to *HTA1*, was inserted into the unique *Bam*HI site on plasmid YIp5 (Parent et al. 1985). A 2.3-kb *Eco*RI fragment containing the origin sequences from the high-copy-number 2- μ plasmid was then inserted into the unique *Eco*RI site of YIp5.

pCC223 A 3.7-kb *Eco*RI–*Bam*HI fragment containing the *HTA2-HTB2* locus (Hereford et al. 1979), which includes the full-length *HTA1* and *HTB1* genes, the entire intergenic region between these two genes, and the *PRT2* (protein 2) gene, was cloned into the *Eco*RI and *Bam*HI sites of YIp5. The 2.3-kb *Eco*RI fragment containing the 2- μ plasmid origin sequences was then cloned into the *Eco*RI site of YIp5.

pCC65 A 6.7-kb *Hind*III fragment containing the *HHT1-HHF1* locus (Smith and Anderson 1983; Smith and Murray

1983], which includes the full-length *HHT1* and *HHF1* genes and intergenic sequences between the two genes, as well as a gene of unknown function (*SMT1*), was inserted into the *HindIII* site on plasmid YEp24 [Clark-Adams et al. 1988].

pCC66 Two *HindIII* fragments of 2.6 and 1.5 kb containing the *HHT2–HHF2* locus [Smith and Anderson 1983; Smith and Murray 1983], which includes the full-length *HHT2* and *HHF2* genes and the intergenic sequences between these genes, were inserted into the *HindIII* site on plasmid YEp24 [Clark-Adams et al. 1988].

pCC67 A 6.4-kb *BamHI* fragment containing the *HTA1–HTB1* locus and a 5.4-kb *HindIII–BamHI* fragment containing the *HHT1–HHF1* locus were inserted into the *HindIII* and *BamHI* sites on plasmid pCGS42 [Clark-Adams et al. 1988].

pHTA1–HTB1_p A 1.1-kb *HindIII* fragment containing the entire *HTA1–HTB1* intergenic region and coding sequences for 13 amino acids of H2A-1 and 80 amino acids of H2B-1 was inserted into the *HindIII* site of Yip5. The 2.3-kb *EcoRI* fragment containing the 2- μ plasmid origin was then inserted into the unique *EcoRI* site on this plasmid.

pHTA2–HTB2_p A 0.97-kb *HindIII* fragment from the *HTA2–HTB2* locus, which contains the entire intergenic region and 13 amino acids of H2A-2 and 80 amino acids of H2B-2, was inserted into the *HindIII* site of Yip5. The 2.3-kb *EcoRI* fragment containing the 2- μ plasmid replication origin was then inserted into the *EcoRI* site on this plasmid.

pHTB1 The *HTB1* gene was reconstituted in pUC13 first by insertion of the 0.8-kb *HindIII–BamHI* fragment, which contains the 3' end of the *HTB1* gene, into the *HindIII* and *BamHI* sites of pUC13 and then insertion of the 1.1-kb *HindIII* fragment, which contains the *HTA1–HTB1* intergenic region and the 5' end of the *HTB1* gene, into the unique *HindIII* site on this plasmid. The *URA3* gene was added as a 1.1-kb *BamHI* fragment into the unique *BamHI* site, and the 2.3-kb *EcoRI* origin fragment from the 2- μ plasmid was inserted into the *EcoRI* site.

pHTA1–HTB1_{fs} A 2.3-kb *EcoRI* fragment encompassing the 2- μ plasmid origin of replication was inserted into the *EcoRI* site of plasmid pMR2 [Rykowski et al. 1981]. This plasmid contains a complete *HTA1–HTB1* locus with a wild-type, full-length *HTA1* gene, the entire intergenic region, and a full-length *HTB1* gene with a frameshift mutation at amino acid 80.

pLG Δ 312 This *URA3* plasmid carries a *CYC1–lacZ* fusion gene controlled by the two *CYC1* UAS elements and *CYC1* TATA sequences [Guarente and Ptashne 1981]. This plasmid replicates at high copy number because it contains the 2- μ plasmid origin of replication.

pLG-UAS This plasmid contains a substitution of three consensus 16-bp UAS elements from the *HTA1–HTB1* locus for the *CYC1* UAS elements that are located in a 140-bp *SmaI–XhoI* fragment in plasmid pLG Δ 312 [Osley et al. 1986].

pLG-CCR15 A 67-bp *HindIII–SacI* fragment, which contains the negative site from the *HTA1–HTB1* locus, was inserted into the *SmaI* site located between the *CYC1* UAS elements and TATA region in plasmid pLG Δ 312 [Osley et al. 1986].

Low-copy-number replicating plasmids

The *URA3* CEN vector YCp50 [Johnston and Davis 1984] was used to provide histone genes in low copy number.

YCp50-*HTA1–HTB1* A 6.4-kb *BamHI* fragment containing the entire *HTA1–HTB1* locus was inserted into the *BamHI* site of YCp50.

Yeast strains

The strains DN105 (*MAT α his3 ura3 Δ hta2-htb2 :: URA3*) and DN106 (*MAT α his3 ura3 Δ hta1-htb1 :: URA3*), which were isolated by gene transplacement of wild-type strain GRF167 (*MAT α his3 ura3*), have been described previously [Norris and Osley 1987]. To measure the effects of a reduction in histone *HTA–HTB* copy number on the expression of the two *HTA–HTB* loci, *HTA1–lacZ* or *HTA2–lacZ* reporter genes were integrated into strain GRF167 and each deletion strain by plasmid integration. Plasmids pfusA1-3His and pfusA3-5His were partially digested with *SalI* to cut within the *LEU2* locus, and the resulting linearized plasmids were transformed into each of the three strains. Because the strains are Leu⁺, stable integrants were isolated by selection for histidine prototrophs.

Ura[–] derivatives of strains DN105 and DN106 were made by selection on SD plates containing 5-fluoro-orotic acid [Boeke et al. 1984]. These derivatives are DN105FOA and DN106FOA, and they were used to study the effects of a reduction in histone gene copy number on the expression of *CYC1–lacZ* genes carrying isolated *HTA1–HTB1* promoter elements.

Strain M30-2B (*MAT α ura3-52 trp1 his3 his7 leu2-3 leu2-112 ade2 HTA1–lacZ :: HTA1–HTB1 :: LEU2*) was used to measure the effects of high-copy-number histone genes on expression of the *HTA1–HTB1* locus in a wild-type strain. Strain M30-3A (*MAT α ura3-52 trp1 his3 leu2-3 leu2-112 HTA1–lacZ :: HTA1–HTB1 :: LEU2 hir3*) was used to measure the effects of the same high-copy-number histone genes in a *hir3* mutant (formerly *hir30*; Osley and Lycan 1987) background. Each of these strains contains a single copy of an *HTA1–lacZ* fusion gene integrated at the *HTA1–HTB1* locus. Strain GRF167 containing a copy of an *HTA2–lacZ* fusion gene integrated at the *leu2* locus was used to measure the effects of high-copy-number histone genes on expression of the *HTA2–HTB2* locus.

To study the role of the *HTA1–HTB1* negative site in dosage compensation, plasmid pfusA1 _{Δ neg}-*lacZ* was partially linearized with *SalI* to direct its integration to the *leu2* locus of wild-type strain DBY747 (*MAT α ura3-52 leu2-3 leu2-112 his3 trp1*) or *hir3* mutant strain M30-9C (*MAT α ura3-52 leu2-3 leu2-112 trp1 hir3*).

RNA analysis

Strains were grown in supplemented SD medium to midexponential phase of growth. Total RNA was extracted from 30–40 ml of cells, as described previously [Hereford et al. 1981]. Quantitative S1 nuclease digestion assays were performed with 20 μ g of RNA, as outlined in Lycan et al. (1987). Double-stranded probes were end-labeled with polynucleotide kinase. A 1.2-kb *XhoI–ClaI* fragment labeled at a *ClaI* site in *lacZ* was isolated from plasmid pLG Δ 312 and used to detect all fusion RNA species. A 700-bp *SacI–HindIII* fragment and a 600-bp *AccI–HindIII* fragment labeled at the *HindIII* site at amino acid 80 in *HTB1* and *HTB2*, respectively, were used to measure the levels of the two *HTB* RNAs. As an internal control, a *SalI–AvaII* fragment labeled at the *AvaII* site in the second exon of the *RP51A* gene [Teem and Rosbash 1983] was used to measure the level of RNA produced by this ribosomal protein gene. Fol-

lowing digestion with 1200 units of S1 nuclease enzyme (BRL) per milliliter, protected fragments were separated on 4% polyacrylamide/8 M urea gels and detected by autoradiography. Quantitation was performed with an LKB ultrascan laser densitometer. HTA-*lacZ* and HTB mRNA levels were calculated as the ratio of these mRNA species to the level of *RP51A* mRNA.

β-Galactosidase assay

Cells were grown in supplemented SD medium lacking uracil to mid-exponential phase of growth. Duplicate samples were centrifuged to pellet cells, and the resulting cell pellets were frozen at -80°C . Cells were lysed by vortexing with glass beads, and β -galactosidase was measured as described previously [Osley and Hereford 1982].

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