Histone Regulatory (*hir*) Mutations Suppress δ Insertion Alleles in *Saccharomyces cerevisiae*

Peter W. Sherwood*,† and Mary Ann Osley*

Program in Molecular Biology, Sloan Kettering Cancer Center, and †Cornell University Graduate School of Medical Sciences, New York, New York 10021*

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ABSTRACT

Changes in histone gene dosage as well as mutations within some histone genes suppress δ insertion mutations in the *HIS4* and *LYS2* loci of *Saccharomyces cerevisiae* by altering the site of transcription initiation. We have found that three *hi*stone *r*egulatory (*hir*) mutations, identified by their effects on the regulation of histone gene expression, suppress the same insertion mutations. In addition, we have examined whether any previously identified *spt* (*s*up*p*ressor of *T*y) mutations might suppress the δ insertion alleles because of effects on histone gene regulation. Our results demonstrate that mutations in the histone genes *SPT11/HTA1* and *SPT12/HTB1* and in three other *SPT* genes, *SPT1*, *SPT10* and *SPT21*, confer Hir⁻ phenotypes. The *spt1* mutation was found to be an allele of *HIR2* while the *spt10* and *spt21* mutations are not in any of the known *HIR* genes.

IN the yeast *Saccharomyces cerevisiae*, the expression of all four histone gene loci is linked to the cell cycle, with maximal accumulation of histone mRNAs occuring during the period of DNA replication (HEREFORD *et al.* 1981; HEREFORD, BROMLEY and OSLEY 1982; CROSS and SMITH 1988). Activation of histone gene transcription in late G_1 requires a functional *CDC4* gene product (WHITE *et al.* 1987), while subsequent repression of transcription in early S phase is dependent on the product of the *CDC7* gene (HEREFORD, BROMLEY and OSLEY 1982). Transcription of the histone genes is also prematurely repressed in S phase when DNA replication is inhibited with a drug such as hydroxyurea (LYCAN, OSLEY and HEREFORD 1987).

In addition to temporal regulation, some of the yeast histone genes are autogenously regulated. Transcription of the *HTA1-HTB1* locus is regulated in response to changes in the copy number of either of the two loci encoding H2A and H2B, while transcription of the *HTA2-HTB2* locus is only subject to temporal regulation (MORAN, NORRIS and OSLEY 1990). Since autogenous regulation of the *HTA1-HTB1* locus is mediated by the levels of H2A and H2B protein in the cell (MORAN, NORRIS and OSLEY 1990), it has been suggested that this form of regulation represents a mechanism by which the stoichiometric balance of the core histones is maintained. This may be important to the cell's physiology since imbalances in core histone stoichiometry produced by alterations in histone gene dosage result in pleiotropic effects on cellular processes as diverse as mitotic chromosome segregation (MEEKS-WAGNER and HARTWELL 1986), induction of the stress response (NORRIS and OSLEY 1987), transcriptional regulation (NORRIS and OSLEY 1987; CLARK-ADAMS *et al.* 1988; HAN and GRUNSTEIN 1988), and assembly or maintenance of chromatin structure (HAN *et al.* 1987, 1988; NORRIS, DUNN and OSLEY 1988).

We previously used a yeast strain containing an *HTA1-lacZ* fusion gene to screen for *trans*-acting mutations that affected the regulation of histone gene transcription (*hir* mutations, OSLEY and LYCAN 1987). We isolated five recessive mutants (*hir1-1*, *hir2-1*, *hir2-2*, *hir3-1*, *hir3-2*) representing three complementation groups and one dominant mutant (*HIR9-1*) representing a fourth complementation group (M. SPECTOR, unpublished results) based on their elevated transcription of the fusion gene. Three distinct phenotypes, representing effects on both temporal and autogenous regulation, have been associated with mutations in the *HIR1*, *HIR2* and *HIR3* genes. Each results from a loss of transcriptional repression, which in the case of the *HTA1-HTB1* locus, is conferred by a unique negative site in the promoter (OSLEY *et al.* 1986; OSLEY and LYCAN 1987; MORAN, NORRIS and OSLEY 1990). First, transcription of the *HTA1-HTB1*, *HHT1-HHF1* and *HHT2-HHF2* loci occurs at an elevated level throughout the cell cycle. In contrast, neither the periodicity nor the level of expression of the *HTA2-HTB2* locus is affected in the mutant strains. Second, transcription of all four of the histone gene loci is no longer repressed when DNA replication is inhibited. We have made use of this second phenotype in the present study to distinguish Hir⁺ from Hir⁻ strains. Finally, autogenous regulation of the *HTA1-HTB1* locus is abolished. This is manifest as the failure of this locus to be repressed in response to elevated

TABLE 1

Yeast Strains

Strain	Genotype	Source
PS24-2A	*MATαura3-52*	This study
PS21-2A	*MATαhir1-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS21-9A	*MATαhir1-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS2138-16B	*MATahir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS1-10A	*MATahir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS24-8A	*MATahir2-1ura3-52*	This study
PS208-3C	*MATαhir2-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52ade2*	This study
PS24-10D	*MATαhir2-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52ade2*	This study
PS2-7A	*MATahir2-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS2438-2A	*MATαhir2-1his4-912δlys2-128δura3-52*	This study
PSMC-26A	*MATαhir2-1his4-912δlys2-128δura3-52*	This study
PS3037-12B	*MATαhir3-1his4-912δlys2-128δura3-52*	This study
PS30-2A	*MATαhir3-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	This study
PS3-11A	*MATαhir3-1his4-912δlys2-128δtrp1Δ1ura3-52*	This study
PS2D38-6A	*MATαhir3-1his4-912δlys2-128δura3-52*	This study
PSEM13	*MATαhir2-1pet17his4-912δade6arg4leu2-3,112lys2-1trp1*	This study
PSMC-38C	*MATαhis4-912δlys2-128δura3-52*	This study
FW1237	*MATahis4-912δlys2-128δura3-52*	CLARK-ADAMS *et al.* (1988)
FW1238	*MATαhis4-912δlys2-128δura3-52*	CLARK-ADAMS *et al.* (1988)
CC204	*MATαhis4-912δlys2-128δhtb1-5::Tn10LUK*	CLARK-ADAMS *et al.* (1988)
CC268	*MATαhis4-912δlys2-128δΔhta1-htb1::URA3ura3-52*	CLARK-ADAMS *et al.* (1988)
DN106	*MATαΔhta1-htb1::URA3his3ura3-52*	NORRIS and OSLEY (1987)
8499-9C	*MATaspt1-1his4-912leu2-3*	G. FINK
FW236	*MATaspt2-150his4-912δleu2-3lys1-1ura3-52*	WINSTON *et al.* (1984)
FW506	*MATαspt3-101his4-917δleu2-3,112ura3-52*	F. WINSTON
FW251	*MATαspt4-3his4-912leu2-3lys1-1*	F. WINSTON
FW225	*MATaspt5-194his4-912δleu2-3ura3-52ade2-1c^s*	F. WINSTON
FW247	*MATaspt6-140his4-912δleu2-3*	F. WINSTON
FW1113	*MATaspt7-217his4-917δlys2-173R2ura3-52*	F. WINSTON
FW1136	*MATaspt8-113his4-917leu2-1lys2-173R2ura3-52*	F. WINSTON
L210	*MATaspt10-118his4-917lys2-128δtrp1Δ1leu2-3,112*	F. WINSTON
FW1764	*MATαspt10::TRP1his4-912δlys2-128δsuc2ΔUASleu2Δ1trp1ura3-52*	F. WINSTON
JF277	*MATαspt11-120lys2-128δleu2-3trp1Δ1*	J. FASSLER
FW1349	*MATaspt12-19his4-917lys2-128δleu2-3ura3-52*	J. FASSLER
JF916	*MATaspt13-101his4-917lys2-128δleu2ura3-52*	J. FASSLER
L215	*MATaspt14-1his4-917lys2-128δleu2-3,112trp1Δ1*	J. FASSLER
FW1476	*MATaspt15-21his4-917δlys2-173R2leu2-3,112trp1Δ1ura3-52*	J. FASSLER
FW1619	*MATaspt21-1his4-917lys2-128δleu2ura3-52*	J. FASSLER
K381-10A	*MATαpet17ura3-1ade6arg4aro7-1asp5met14lys2-1trp1*	KLAPHOLZ and ESPOSITO (1982)
PSD1	*MATαhir1-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52* / *MATahis4-912δlys2-128δura3-52*	This study
PSD2	*MATαhir2-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52ade2* / *MATahis4-912δlys2-128δura3-52*	This study
PSD3	*MATαhir3-1HTA1-HTB1::HTA1-lacZ::LEU2ura3-52* / *MATahis4-912δlys2-128δura3-52*	This study
PSD4	*MATahir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52* / *MATαhis4-912δlys2-128δura3-52*	This study
PSD5	*MATahir2-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52* / *MATαhis4-912δlys2-128δura3-52*	This study
PSD6	*MATαhir3-1his4-912δlys2-128δtrp1Δ1ura3-52* / *MATahis4-912δlys2-128δura3-52*	This study

TABLE 1—Continued

Yeast Strains

Strain	Genotype	Source
PSD7	*MATαhir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52* *MATaspt1-1his4-912leu2-3*	This study
PSD8	*MATαhir2-1his4-912δlys2-128δura3-52* *MATaspt1-1his4-912leu2-3*	This study
PSD9	*MATαhir3-1his4-912δlys2-128δtrp1Δ1ura3-52* *MATaspt1-1his4-912leu2-3*	This study
PSD10	*MATαhir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52* *MATaspt10-118his4-917lys2-128δtrp1Δ1leu2-3,112*	This study
PSD11	*MATαhir2-1his4-912δlys2-128δura3-52* *MATaspt10-118his4-917lys2-128δtrp1Δ1leu2-3,112*	This study
PSD12	*MATαhir3-1his4-912δlys2-128δtrp1Δ1ura3-52* *MATaspt10-118his4-917lys2-128δtrp1Δ1leu2-3,112*	This study
PSD13	*MATαhir1-1his4-912δlys2-128δHTA1-HTB1::HTA1-lacZ::LEU2ura3-52* *MATaspt21-1his4-917lys2-128δleu2ura3-52*	This study
PSD14	*MATαhir2-1his4-912δlys2-128δura3-52* *MATaspt21-1his4-917lys2-128δleu2ura3-52*	This study
PSD15	*MATαhir3-1his4-912δlys2-128δtrp1Δ1ura3-52* *MATaspt21-1his4-917lys2-128δleu2ura3-52*	This study

HTA-HTB copy number (MORAN, NORRIS and OSLEY 1990).

In this study, we have examined the effects of *h*istone *r*egulatory (*hir*) mutations (OSLEY and LYCAN 1987) on the transcription of two well characterized genes that contain insertions of solo δ elements: *his4-912δ* (FARBAUGH and FINK 1980; ROEDER and FINK 1980) and *lys2-128δ* (CLARK-ADAMS and WINSTON 1987). The effect of the δ insertions in these genes is to alter the site at which transcription is initiated or terminated; in the *his4-912δ* allele, transcription initiates exclusively within the upstream δ element (SILVERMAN and FINK 1984), and in the *lys2-128δ* allele, transcription initiated at the normal *LYS2* start site prematurely terminates within the δ element (CLARK-ADAMS and WINSTON 1987). In both cases, the consequences of the transcriptional alterations are to make cells phenotypically His^- or Lys^-.

Mutations in numerous *trans*-acting *SPT* (*s*uppressor of *T*y) genes that suppress both Ty and solo δ insertion alleles of *HIS4* and *LYS2* have been characterized (WINSTON *et al.* 1984, 1987; FASSLER and WINSTON 1988). Many of the *SPT* genes encode products with genetically or biochemically defined roles as transcription factors. One of these genes has a general role in transcription (*SPT15* [encoding TFIID], EISENMANN, DOLLARD and WINSTON 1989; HAHN *et al.* 1989), while others have more specific roles in the transcription of several different loci (*GAL11/SPT13*, FASSLER and WINSTON 1989; NISHIZAWA *et al.* 1990; *SIN1/SPT2*, ROEDER *et al.* 1985; W. KRUGER and I. HERSKOWITZ, personal communication; *CRE1/SPT10*, FASSLER and WINSTON 1988; DENIS and MALVAR 1990; *SSN20/CRE2/SPT6*, NEIGEBORN, RUBIN and CARLSON 1984; NEIGEBORN, CALENZA and CARLSON 1987; CLARK-ADAMS and WINSTON 1987; DENIS and MALVAR 1990). Another class of *SPT* genes encodes integral chromosomal proteins. This class was first identified in a screen for high copy number suppressors of the *his4-912δ* and *lys2-128δ* mutations (CLARK-ADAMS *et al.* 1988). One such suppressor contained two genes previously identified by the mutations *spt11* and *spt12* (FASSLER and WINSTON 1988). Subsequent analysis established that *SPT11* and *SPT12* were the *HTA1* and *HTB1* histone genes, and that another high copy number suppressor contained the intact *HTA2-HTB2* locus (CLARK-ADAMS *et al.* 1988). Other changes in histone gene dosage, including deletion of the *HTA1-HTB1* locus or expression of any one of the four histone gene loci on a high copy number plasmid, were also found to suppress δ insertion mutations (CLARK-ADAMS *et al.* 1988).

The observation that δ insertion alleles are suppressed by changes in histone gene dosage suggested that the same mutations might be suppressed by *trans*-acting mutations that altered the level of histone gene expression. The results of this study establish that mutations in three *HIR* genes can suppress both the *his4-912δ* and *lys2-128δ* mutations by altering the site of transcription initiation. In addition, we demonstrate that mutations in five *SPT* genes have effects on histone gene regulation.

MATERIALS AND METHODS

Yeast strains and genetic methods: The yeast strains used in this study are listed in Table 1. Standard yeast genetic procedures and media were used (SHERMAN, FINK and HICKS 1982). The Spt phenotype of strains was determined by replica plating onto SD complete media lacking histidine or lysine followed by incubation at 23° for 3–5 days (WINSTON *et al.* 1984). Occasional ambiguity in determining Spt phenotypes due to background growth associated with replica plating was resolved by spotting serial dilutions of cells onto SD complete media lacking histidine or lysine, followed by incubation at 23° for 3–6 days.

Histone gene regulation assay: The Hir phenotype of strains was determined as previously described (OSLEY and LYCAN 1987), except that YPD medium was used in place of YM-1 medium in some instances, and treatment of cultures with sodium azide prior to freezing was omitted. Cultures (50 ml) were grown at 23° or 30° to a density of 5×10^6 cells/ml. The cultures were split, hydroxyurea (Sigma) was added to one half of each culture to a concentration of 0.2 M, and incubation was continued for an additional 30 min. Cells were harvested by centrifugation and cell pellets were frozen at −80° prior to RNA isolation. Total RNA was isolated (TRECO 1989) and 10 μg were analyzed by formaldehyde-agarose gel electrophoresis and Northern blotting (SAMBROOK, FRITSCH and MANIATIS 1989). The probe used to detect *HTA1* transcripts was a 2.4-kb *Sac*I fragment from the *HTA1-HTB1* locus (HEREFORD *et al.* 1979). This fragment contains the entire *HTA1* gene, as well as a region of the constituitively transcribed *AKY2* (*PRT1*) gene (HEREFORD, BROMLEY and OSLEY 1982; OECHSNER *et al.* 1988), which serves as an internal control for the amount of RNA loaded. This probe does not detect the *HTA2* transcript.

Quantitative S1 nuclease protection assay: S1 nuclease protection assays were carried out using conditions previously described (OSLEY *et al.* 1986). A 1.18-kb *Pvu*II-*Cla*I fragment from the *H1S4* gene (−650 to +533) and a 580-bp *Sal*I-*Ava*II fragment from the *RP51A* gene (spanning 340 bp of intron and 240 bp of exon sequences, TEEM and ROSBASH 1983) were end-labeled using [γ-^{32}P]ATP and polynucleotide kinase (New England Biolabs). Ten nanograms of each probe were hybridized to 20 μg of total RNA isolated from cells grown at 23° in SD complete medium. Hybridization reactions contained 80% formamide, 0.04% PIPES (pH 6.8), 0.4 M NaCl, 0.1 M EDTA, and were carried out for 12–16 hr at 47°. Following digestion with 1200 units of S1 nuclease per ml (Bethesda Research Laboratories), protected fragments were separated by 8 M urea-4% polyacrylamide gel electrophoresis, and visualized by autoradiography.

RESULTS

Mutations in *HIR* genes suppress δ insertion alleles: Changes in histone gene dosage in a wild-type strain suppress δ insertion mutations in the *HIS4* and *LYS2* loci presumably by altering the stoichiometric balance of the four core histones (CLARK-ADAMS *et al.* 1988). We reasoned that *trans*-acting mutations which derepress transcription of only three of the four histone loci might also alter the stoichiometric balance of histones and thus suppress the same insertion alleles. We therefore crossed *HIS4LYS2* strains that contained a mutation in the *HIR1*, *HIR2* or *HIR3* gene to a *HIR*$^+$*his4-912δlys2-128δ* strain. In each of these crosses, we found that the His and Lys phenotypes did not always segregate 2$^+$: 2$^-$ and that an excess of His$^+$ and Lys$^+$ spore colonies arose. Some of the segregants showed fast growth on media lacking histidine or lysine and were presumed to carry the wild-type *HIS4* or *LYS2* gene. Other His$^+$ or Lys$^+$ segregants were slow growing and were predicted to result from suppression of the *his4-912δ* or *lys2-128δ* allele. His$^+$ or Lys$^+$ segregants in this latter class were also Hir$^-$ as determined by their failure to repress *HTA1* transcription in response to the inhibition of DNA replication. These results suggested that each of the hir mutations tested (*hir1-1*, *hir2-1* and *hir3-1*) could suppress the two δ insertion alleles.

Segregants that were *hir*$^-$*his4-912δlys2-128δ* were isolated and backcrossed to the original *HIR*$^+$*his4-912δlys2-128δ* strain to constuct diploids homozygous for the suppressible δ insertion alleles. These diploids were phenotypically Hir$^+$His$^-$Lys$^-$, indicating that both the Hir$^-$ and Spt$^-$ phenotypes of the *hir* strains were the result of a recessive mutation. Tetrad analysis was then performed to confirm that suppression was due exclusively to the presence of a particular *hir* mutation. The phenotypes of two representative tetrads resulting from the sporulation of a *hir2-1his4-912δlys2-128δ/HIR2*$^+$*his4-912δlys2-128δ* diploid are shown in Figure 1. In this cross, the His, Lys and Hir phenotypes segregated 2$^+$:2$^-$. In addition, every His$^+$Lys$^+$ segregant was Hir$^-$, and all His$^-$Lys$^-$ segregants were Hir$^+$ (n = 11), consistent with the prediction that the *hir2* mutation could suppress both insertion alleles. Table 2 summarizes more extensive results obtained from backcrosses with *hir1-1*, *hir2-1* and *hir3-1* mutant strains. From these data, we conclude that the *hir1* and *hir2* mutations can suppress both the *his4-912δ* and the *lys2-128δ* alleles. The results with the *hir3* mutant are more complex, and suggest that the original *hir3* strain harbored a second mutation that affected the Spt phenotypes since a significant number of Hir$^+$ segregants were Lys$^+$ (15) or His$^+$ (3). Moreover, the *hir3-1* mutation appeared to suppress the *his4-912δ* and *lys2-128δ* alleles differentially; many Hir$^-$His$^+$ segregants were Lys$^-$ (14 of 32). Both conclusions have been confirmed by further backcrosses of *hir3his4-912δlys2-128δ* strains to a *HIR*$^+$*his4-912δlys2-128δ* strain. When the putative second mutation was segregated away from the *hir3-1* mutation, the His phenotype now segregated 2His$^+$:2His$^-$ in all tetrads (n = 14). Only ten segregants were Lys$^+$, but the Lys$^+$ phenotype always segregated with the His$^+$ phenotype (data not shown). These results suggest that while suppression of *his4-912δ* in *hir3* mutants is fully penetrant, suppression of *lys2-128δ* in *hir3* mutants is partially impenetrant.

Some *spt* mutations suppress not only solo δ inser-

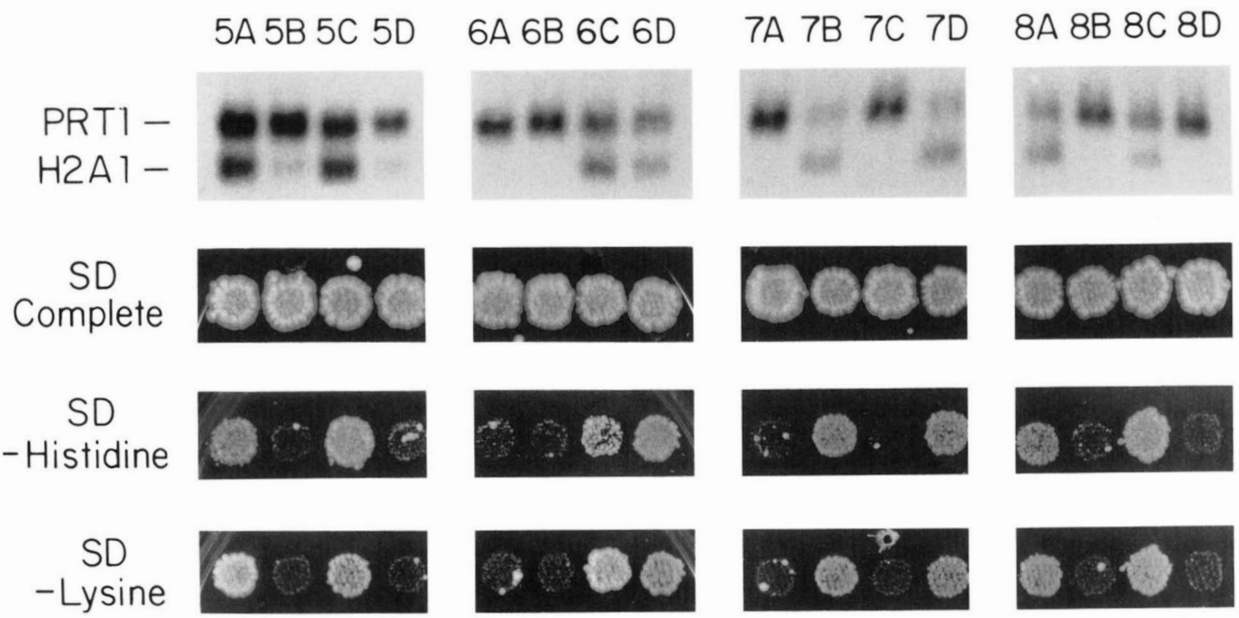

FIGURE 1.—Suppression of *his4-912δ* and *lys2-128δ* mutations in *hir2* mutants. *Top row:* segregants from four tetrads of a cross between a *HIR*$^+$*his4-912δlys2-128δ* strain (FW1238) and a *hir2-1his4-912δlys2-128δ* strain (PS2-7A) were treated with hydroxyurea and analyzed by Northern blot analysis to determine their Hir phenotype as described in MATERIALS AND METHODS. The H2A1 and PRT1 transcripts were identified by hybridization with a 2.4-kb *Sac*I fragment that contains the *HTA1* and *AKY2* genes. A Hir$^-$ phenotype is manifest as the persistence of the H2A1 transcript following a block in chromosome replication. *Bottom three rows:* the same segregants (aligned in columns) from the cross described above were replica printed to SD complete, SD-histidine and SD-lysine media to determine suppression of the *his4-912δ* and *lys2-128δ* alleles as described in MATERIALS AND METHODS. A Spt$^-$ phenotype is seen as growth on all three media.

tions in *HIS4* or *LYS2*, but also insertions of an entire Ty element (see below). We tested whether one *hir* mutation, *hir2-1*, could suppress a mutation in *HIS4* (*his4-917*; ROEDER *et al.* 1980) that is caused by insertion of a Ty element in an orientation opposite to that of *his4-912* (from which the solo δ insertion allele *his4-912δ* was derived). Suppression of *his4-917* by *hir2-1* was not detected (data not shown).

Suppression of *his4-912δ* by mutations in *HIR* genes occurs at the level of transcription: In nonsuppressing (*SPT*$^+$) strains that contain the *his4-912δ* allele, transcription of the *HIS4* gene initiates within the δ element (upstream of the normal *HIS4* start site), giving rise to a longer, nonfunctional transcript (see Figure 2) (SILVERMAN and FINK 1984). In all cases that have been examined, suppression of δ insertions occurs at the transcriptional level (SILVERMAN and FINK 1984; WINSTON, DURBIN and FINK 1984; CLARK-ADAMS and WINSTON 1987; FASSLER and WINSTON 1988; CLARK-ADAMS *et al.* 1988; HIRSCHMAN, DURBIN and WINSTON 1988). These studies have shown that the suppressors of the *his4-912δ* mutation fall into two general classes with respect to their pattern of transcription (CLARK-ADAMS and WINSTON 1987). In one class, transcription that initiates within the δ element is abolished, and instead initiates exclusively from the normal *HIS4* start site. Mutations in the *SPT3*, *SPT7* and *SPT8* genes–which strongly suppress Ty insertions in addition to solo δ insertions–represent this first class of suppressors. In a second class, transcription initiating within the δ element continues to occur, but transcription initiating from the normal *HIS4*$^+$ start site now reappears. Mutations in this class–represented by alleles of *SPT4*, *SPT5* and *SPT6*–strongly suppress solo δ insertions, but in general do not suppress Ty insertions. Deletion of the *HTA1-HTB1* locus or expression of any one of the four histone gene loci on a high copy number plasmid suppress *his4-912δ* by a mechanism consistent with this second class of suppressors (CLARK-ADAMS *et al.* 1988). It was therefore of interest to determine how the *hir1*, *hir2* and *hir3* mutations suppress the *his4-912δ* mutation at the molecular level.

Total RNA was isolated from *HIR*$^+$ and *hir*$^-$ strains that contained either a wild-type *HIS4* gene or the *his4-912δ* allele, and transcription of the *HIS4* locus was examined by a quantitative S1 nuclease protection assay (Figure 2). In a *HIR*$^+$*his4-912δ* strain, most transcription initiated within the δ element (Figure 2, lane 1). The small amount of transcription initiating from the normal *HIS4* start site in this strain is insufficient to support growth at 23° on media lacking histidine. In all *hir*$^-$*his4-912δ* strains, a significantly greater level of transcription initiated from the normal *HIS4* start site, and transcription still continued to initiate within the δ element, although at a somewhat reduced level (Figure 2, lanes 3, 5 and 7). This phenotype was identical to that of a *Δhta1-htb1his4-912δ* strain (Figure 2, lane 9). The *hir* mutations also did not significantly affect the levels of *HIS4*$^+$ transcripts in *hir*$^-$*HIS4* strains (Figure 2, lanes 4, 6 and 8).

TABLE 2

Suppression of *his4-912δ* and *lys2-128δ* in *hir* mutants

Strain[a]	Parental genotype	No. of tetrads: His⁺:His⁻ 0:4	1:3	2:2	3:1	4:0	Lys⁺:Lys⁻ 0:4	1:3	2:2	3:1	4:0
PSD4	*hir1-1his4-912δlys2-128δ* / *HIR1his4-912δlys2-128δ*	0	0	10	0	0	0	1	9	0	0
PSD5	*hir2-1his4-912δlys2-128δ* / *HIR2his4-912δlys2-128δ*	0	0	11	0	0	0	0	11	0	0
PSD6	*hir3-1his4-912δlys2-128δ* / *HIR3his4-912δlys2-128δ*	0	1	11	4	0	2	9	5	0	0

		No. of segregants: Hir⁻His⁺	Hir⁻His⁻	Hir⁺His⁺	Hir⁺His⁻
PSD4	*hir1-1his4-912δlys2-128δ* / *HIR1his4-912δlys2-128δ*	20	0	0	20
PSD5	*hir2-1his4-912δlys2-128δ* / *HIR2his4-912δlys2-128δ*	22	0	0	22
PSD6	*hir3-1his4-912δlys2-128δ* / *HIR3his4-912δlys2-128δ*	28	0	3	33

		No. of segregants: Hir⁻Lys⁺	Hir⁻Lys⁻	Hir⁺Lys⁺	Hir⁺Lys⁻
PSD4	*hir1-1his4-912δlys2-128δ* / *HIR1his4-912δlys2-128δ*	18	1	1	20
PSD5	*hir2-1his4-912δlys2-128δ* / *HIR2his4-912δlys2-128δ*	22	0	0	20
PSD6	*hir3-1his4-912δlys2-128δ* / *HIR3his4-912δlys2-128δ*	16	2	15	31

The His and Lys phenotypes were determined by replica printing onto SD complete and SD-histidine or SD-lysine media, followed by incubation at 23°. The Hir phenotype was determined by analyzing the transcription of the *HTA1* gene following a block in DNA replication as described in MATERIALS AND METHODS.

[a] The diploid strains used in this analysis are listed in Table 1.

In addition to analyzing the effects of *hir* mutations on transcription of the solo δ insertion allele, *his4-912δ*, we tested whether these mutations altered the transcription of intact Ty1 elements. Transcription of Ty1 elements normally initiates in the 5′δ element and terminates within the 3′δ element, giving rise to a 5.7-kb δ-δ transcript (ELDER *et al.* 1983). Both the length and level of Ty1 transcripts are unaffected in *spt4*, *spt5* and *spt6* mutants (CLARK-ADAMS and WINSTON 1987). In *spt3*, *spt7* and *spt8* strains, however, full length δ-δ transcription is abolished and a novel 4.9-kb transcript is produced at a low level (WINSTON *et al.* 1987). Northern blot analysis of RNA extracted from *hir1-1*, *hir2-1* and *hir3-1* mutants indicated that both the length and level of δ-δ transcripts were unaffected in these strains (data not shown). Together with the results shown in Figure 2, these data suggest that the *hir1*, *hir2* and *hir3* mutations suppress *his4-912δ* by a mechanism similar to that conferred by mutations in *SPT4*, *SPT5* and *SPT6* or by altered histone gene dosage (class 2 above).

Five *spt* strains have a Hir⁻ phenotype: The observation that mutations in three *HIR* genes conferred an Spt⁻ phenotype raised the possibility that some *spt* mutations suppress δ insertion alleles because of their effects on histone gene expression. To test this possibility, we determined the Hir phenotype of 15 different *spt* strains (see Table 1). Five mutants, *spt1-1*, *spt10::TRP1*, *spt11-120*, *spt12-19* and *spt21-1*, were found to have a Hir⁻ phenotype (Figure 3). The *spt11* and *spt12* mutations (FASSLER and WINSTON 1988) have been previously mapped to the *HTA1-HTB1* locus (CLARK-ADAMS *et al.* 1988). Repression of the *HTA1-HTB1* locus after a replication block has been shown to be at least partially mediated by the autogenous regulation of this locus by histones H2A and H2B (MORAN, NORRIS and OSLEY 1990). It was therefore not unexpected that mutations within the locus itself might abolish this form of transcriptional repression.

We next asked whether the *spt1*, *spt10* or *spt21* mutations were alleles of any of the three *HIR* genes.

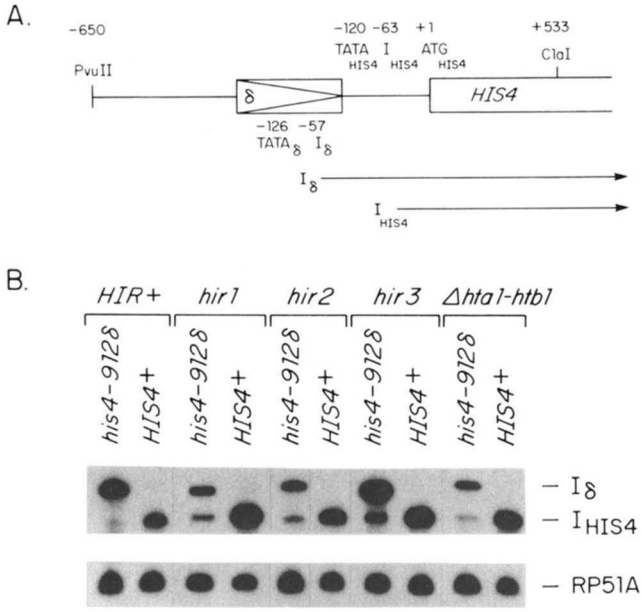

FIGURE 2.—Transcription of the *his4-912δ* locus in *hir* mutants. *A:* schematic diagram of the 5′ region of the *his4-912δ* locus, indicating the sites where transcription is initiated in the δ element (I_δ) and in a wild-type *HIS4* (I_{HIS4}) gene. A 1.18-kb *Cla*I (+533)-*Pvu*II (−650) fragment from the *HIS4*$^+$ gene was used to detect transcripts initiating from both the δ element and *HIS4*$^+$ initiation sites. *B:* S1 nuclease protection assay of total RNA from strains that contain the *his4-912δ* allele or *HIS4*$^+$ gene. Lane 1: *HIR*$^+$*his4-912δ* (FW1237); lane 2: *HIR*$^+$*HIS4*$^+$ (PS24-2A); lane 3: *hir1-1his4-912δ* (PS2138-6B); lane 4: *hir1-1H1S4*$^+$ (PS21-9A), lane 5: *hir2-1his4-912δ* (PS2–7A); lane 6: *hir2-1HIS4*$^+$ (PS24-8A); lane 7: *hir3-1his4-912δ* (PS3037-12B); lane 8: *hir3-1HIS4*$^+$ (PS30-2A); lane 9: *Δhta1-htb1his4-912δ* (CC268); lane 10: *Δhta1-htb1HIS4*$^+$ (DN106). Bands labeled I_δ and I_{HIS4} represent protected transcripts initiating from the δ element and wild-type *HIS4* transcription initiation site, respectively. The band labeled RP51A represents RNA produced by the *RP51A* gene (TEEM and ROSBASH 1983) and serves as a control for the amount of RNA analyzed in each assay.

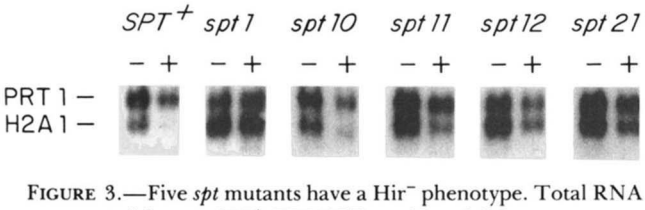

FIGURE 3.—Five *spt* mutants have a Hir$^-$ phenotype. Total RNA was extracted from a *SPT*$^+$ (FW1237) strain and five *spt* mutants–*spt1-1* (8499-9C), *spt10::TRP1* (FW1764), *spt11-120* (JF277), *spt12-19* (FW1349) and *spt21-1* (FW1619)–that were grown exponentially (−) or treated with hydroxyurea (+) to block DNA replication. RNA was analyzed by Northern blot analysis using a 2.4-kb *Sac*I fragment from the *HTA1-HTB1* locus as a hybridization probe. This fragment detects both H2A1 RNA and PRT1 RNA, which serves as an internal control. A Hir$^-$ phenotype is manifest as the continued production of H2A1 RNA in the presence of hydroxyurea.

Diploids were constructed between each *hir* and *spt* mutant strain and analyzed initially for their Spt phenotypes (Figure 4). The Spt$^-$ (His$^+$) phenotype of individual haploid strains was complemented in diploids formed from pairwise combinations of *spt10::TRP1* or *spt21-1* and *hir1-1*, *hir2-1* and *hir3-1* strains. In every case, the Hir$^-$ phenotype was also complemented (data not shown). *SPT10* and *SPT21* thus represent previously unidentified *HIR* genes. In contrast, the *spt1-1* mutation failed to complement either the Spt$^-$ (Figure 4) or Hir$^-$ phenotype (data not shown) of a *hir2-1* mutant, suggesting that the *spt1-1* mutation is an allele of *HIR2*. Our attempt to demonstrate allelism by analyzing segregants from crosses between *hir2-1* and *spt1-1* strains was confounded by an extremely low sporulation frequency of the diploid strain. Additional data, however, support the view that *HIR2* and *SPT1* are the same gene. First, all Spt$^-$ segregants from crosses between *spt1-1* and *SPT1*$^+$ strains were also Hir$^-$, and no Spt$^+$ segregants were Hir$^-$. Second, all Hir$^-$ segregants from crosses between *hir2-1* and *HIR*$^+$ strains were Spt$^-$, and no Hir$^+$ segregants were Spt$^-$. Finally, a cloned copy of the *HIR2* gene can complement the Spt$^-$ and Hir$^-$ phenotypes of both *spt1-1* and *hir2-1* strains (P. SHERWOOD, unpublished results).

The *HIR2* locus is centromere linked and maps near *PET17* on chromosome *XV*: The analysis of tetrads resulting from crosses in which both the *HIR2* and *TRP1* loci were heterozygous indicated that the *H1R2* locus was centromere linked (PD = 11, NPD = 16, T = 14). Yeast chromosomal blots hybridized with a fragment of the cloned *HIR2* gene localized *HIR2* to chromosome *XV* (data not shown). *HIR2* was subsequently mapped to within 10 cM of *PET17* on the right arm of chromosome *XV* (PD = 18, NPD = 0, T = 4).

Effects of a change in *HTA1-HTB1* copy number on suppression of δ insertion alleles in *hir* and *spt* strains: The introduction of any one of the four histone gene loci on a high copy number plasmid into a wild-type strain leads to suppression of δ insertion alleles of *HIS4* and *LYS2* (CLARK-ADAMS *et al.* 1988). Suppression is thought to occur because the production of histones has become unbalanced. It was therefore possible that the same insertion alleles are suppressed in the three *hir* mutants and in *spt10* and *spt21* strains through a similar imbalance in histones, which would be the consequence of an altered regulation of histone gene expression. Several lines of evidence suggest that there may be a net deficit of H2A and H2B in each of these mutants relative to a wild-type strain. The three *hir* mutations, for example, cause derepressed transcription of each of the histone loci except for the *HTA2-HTB2* locus (OSLEY and LYCAN 1987), and the *spt10* and *spt21* mutations decrease expression of the *HTA2-HTB2* locus (C. DOLLARD, G. NATSOULIS, J. BOEKE and F. WINSTON, personal communication). We reasoned that the Spt$^-$ phenotype of these strains might therefore be reversed by increasing expression of the *HTA-HTB* genes. We introduced a centromere plasmid that carried the *HTA1-HTB1* locus into the five mutant strains. Because transcrip-

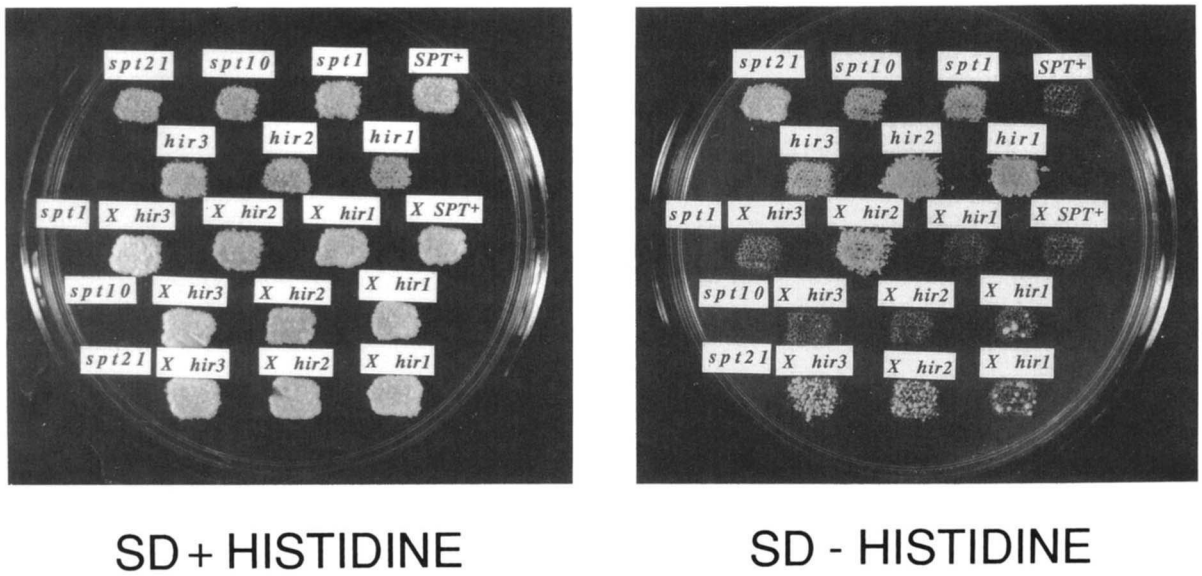

FIGURE 4.—Complementation analysis of *hir* and *spt* mutants. In each plate, the top two rows represent the haploid parents of the diploid strains shown in the bottom three rows. The His phenotype of these strains was determined by replica plating onto SD + Histidine and SD − Histidine media. A Spt⁻ phenotype is manifest as growth on both media. Strains left to right, top to bottom: *spt21-1* (FW1619); *spt10::TRP1* (FW1764); *spt1-1* (8499-9C); *SPT*⁺ (FW1237); *hir3-1* (PS3-11A); *hir2-1* (PS2–7A);. (PS2138-16B); *spt1-1* × *hir3-1* (PSD9); *spt1-1* × *hir2-1* (PSD8); *spt1-1* × *hir1-1* (PSD7); *spt1-1* × *SPT*⁺ (PSD16); *spt10::TRP1* × *hir3-1* (PSD12); *spt10::TRP1* × *hir2-1* (PSD11); *spt10::TRP1* × *hir1-1* (PSD10); *spt21-1* × *hir3-1* (PSD15); *spt21-1* × *hir2-1* (PSD14); *spt21-1* × *hir1-1* (PSD13). The complete genotypes of these strains are listed in Table 1.

tion of this locus is derepressed in each of these backgrounds (OSLEY and LYCAN 1987; Figure 3), we predicted that the mutants might now produce more wild-type levels of H2A and H2B. We found that the His⁺ phenotype of *hir2-1* and *spt10:TRP1* strains became very weak, and that the Lys⁺ phenotype of *spt10::TRP1* and *spt21-1* mutants was either severly reduced (*spt10*) or abolished (*spt21*). In contrast, the *HTA1-HTB1* plasmid had no effect on either the His⁺ or Lys⁺ phenotype of a *hir1-1* mutant or on the His⁺ phenotype of a *hir3-1* mutant. We noted in the latter mutant, however, that the Lys⁻ phenotype shown by some *hir3* strains (see Table 2) was now suppressed so that the cells became Lys⁺. These data suggest that the suppression of δ insertions in *HIS4* and *LYS2* by mutations in *HIR2*, *HIR3*, *SPT10* and *SPT21* may therefore be the direct consequence of the effects of these mutations on the regulation of histone gene expression. Suppression of these same insertion alleles by the *hir1-1* mutation, however, is either independent of the effects of this mutation on histone gene expression, or insensitive to the changes in histone gene dosage tested in this study.

DISCUSSION

The results of this study demonstrate that mutations which alter the regulation of histone gene expression (*hir* mutations) are able to suppress mutations caused by the insertion of a δ element in the *H1S4* and *LYS2* loci. The *hir* mutations thus confer an Spt⁻ (Suppressor of Ty) phenotype (WINSTON *et al.* 1984). Suppression of the *his4-912δ* allele in *hir1*, *hir2* and *hir3* mutants occurs at the level of transcription, and by a mechanism similar to that observed in *spt4*, *spt5*, *spt6* and *Δhta1-htb1* mutants. In each of these strains, a new transcript is initiated from the normal *HIS4*⁺ start site while transcription continues to initiate within the δ element.

We have also found that five previously isolated *spt* mutants–*spt1*, *spt10*, *spt11*, *spt12* and *spt21*–have a Hir⁻ phenotype because they fail to repress transcription of the *HTA1-HTB1* locus when DNA replication is inhibited. The *spt11* and *spt12* mutations have been mapped to the *HTA1-HTB1* locus (CLARK-ADAMS *et al.* 1988). The *spt1-1* mutation is an allele of *HIR2*, while the *spt10* and *spt21* mutations are not in any previously identified *HIR* genes. Mutations in *SPT10* and *SPT21* result in reduced expression of the *HTA2-HTB2* locus (C. DOLLARD, G. NATSOULIS, J. BOEKE and F. WINSTON, personal communication), suggesting that the *SPT10* and *SPT21* gene products are involved in the activation of this histone gene locus. Since transcription of the *HTA1-HTB1* locus is partially dependent on the intracellular levels of H2A and H2B (MORAN, NORRIS and OSLEY 1990), the derepressed transcription of this locus in *spt10* and *spt21* mutants may therefore be an indirect consequence of an intracellular deficit in these histones.

The finding that mutations in *HIR1*, *HIR2(SPT1)*, *HIR3*, *SPT10*, *SPT11*, *SPT12* and *SPT21* all alter the

regulation of histone genes suggests that it is their effects on the expression of these genes which is the basis for their common suppression of δ insertion alleles. Several arguments indicate that as the result of such altered transcription, core histone stoichiometry may have become unbalanced. Mutations in *HIR1*, *HIR2* and *HIR3*, for example, derepress transcription of the *HTA1-HTB1*, *HHT1-HHF1* and *HHT2-HHF2* loci, but do not affect expression of the *HTA2-HTB2* locus (OSLEY and LYCAN 1987). The derepressed transcription of three of the four histone gene loci may therefore cause a net decrease in the level of H2A-H2B dimers relative to H3-H4 tetramers in these mutant backgrounds. By a similar line of reasoning, *spt10* and *spt21* mutants may also contain a deficit in H2A and H2B relative to H3 and H4 because of their decreased expression of the *HTA2-HTB2* locus. Both situations potentially mimic the deletion of the *HTA1-HTB1* locus or overexpression of the *HHT-HHF* loci in wild type cells, two conditions that produce the same pattern of suppression (CLARK-ADAMS *et al.* 1988). This view is consistent with the observation that the Spt⁻ phenotype of *hir2*, *spt10* and *spt21* strains can be reduced or abolished by increased expression of the *HTA1-HTB1* locus.

Numerous studies have shown that DNA-dependent processes as diverse as transcription (CLARK-ADAMS *et al.* 1988; HAN *et al.* 1988; HAN and GRUNSTEIN 1988; KAYNE *et al.* 1988; KIM *et al.* 1988) and mitotic chromosome transmission (MEEKS-WAGNER and HARTWELL 1986; HAN *et al.* 1987; KIM *et al.* 1988) can be influenced by alterations in core histone stoichiometry. Since the consequence of such altered stoichiometry is often a general perturbation in the structure of yeast chromatin (HAN *et al.* 1987; KIM *et al.* 1988; NORRIS, DUNN and OSLEY 1988), it is likely that the physiological effects are related to these structural changes. In one example, altered histone stoichiometry has been directly correlated with a specific effect on transcription; depletion of histone H4 causes a local loss of nucleosomes from the *PHO5* promoter and the concommitant activation of *PHO5* transcription (HAN *et al.* 1988). It is therefore probable that the role of unbalanced histone synthesis in the regulation of the Spt⁻ phenotype is also manifest through specific alteration of chromatin structure. There are two ways that altered chromatin structure might affect transcription of the *his4-912δ* allele. First, alteration of chromatin structure around a regulatory element in the promoter of this gene, *e.g.*, the TATA element, could allow utilization of the wild-type transcription initiation site. Alternatively, the expression of a gene that affects suppression of the δ insertion allele could be influenced by altered chromatin structure.

Is the transcription of other yeast genes also altered by the unbalanced production of histones? One well-characterized example of such an effect is the *HTA1-HTB1* locus, whose transcription is regulated by changes in the copy number of *HTA-HTB* genes (MORAN, NORRIS and OSLEY 1990). Other examples may include the heat shock gene, *UBI4*, whose transcription is derepressed when the *HTA1-HTB1* locus is deleted (NORRIS and OSLEY 1987), the *ADH2* gene, which is regulated in part by the *SPT10* (*CRE1*) gene (DENIS and MALVAR 1990), and the *STE6* and *PHO5* genes, whose transcription is altered in both *spt10* and *spt21* mutants (G. NATSOULSIS, personal communication).

Although the unbalanced production of histones in *hir* mutants has the potential to alter chromatin structure and result in specific effects on transcription, these alterations are not sufficient to cause gross alterations in cell physiology. Strains with a deletion of the *HTA1-HTB1* locus have several mutant phenotypes in addition to their suppression of δ insertion alleles, including cold sensitivity, slow growth, and constitutive induction of the stress response, but *hir* mutant strains are neither heat nor cold sensitive, nor do they exhibit any gross alterations in mitotic growth or mating efficiency.

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