

Conserved Histone Variant H2A.Z Protects Euchromatin from the Ectopic Spread of Silent Heterochromatin

Marc D. Meneghini, Michelle Wu,
and Hiten D. Madhani*

Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

Summary

Boundary elements hinder the spread of heterochromatin, yet these sites do not fully account for the preservation of adjacent euchromatin. Histone variant H2A.Z (Htz1 in yeast) replaces conventional H2A in many nucleosomes. Microarray analysis revealed that *HTZ1*-activated genes cluster near telomeres. The reduced expression of most of these genes in *htz1Δ* cells was reversed by the deletion of *SIR2* (*sir2Δ*) suggesting that H2A.Z antagonizes telomeric silencing. Other Htz1-activated genes flank the silent *HMR* mating-type locus. Their requirement for Htz1 can be bypassed by *sir2Δ* or by a deletion encompassing the silencing nucleation sites in *HMR*. In *htz1Δ* cells, Sir2 and Sir3 spread into flanking euchromatic regions, producing changes in histone H4 acetylation and H3 4-methylation indicative of ectopic heterochromatin formation. Htz1 is enriched in these euchromatic regions and acts synergistically with a boundary element to prevent the spread of heterochromatin. Thus, euchromatin and heterochromatin each contains components that antagonize switching to the opposite chromatin state.

Introduction

Eukaryotic genomes are organized into two distinct yet interconvertible states, euchromatin and heterochromatin. While originally termed for their cytological appearances, it has become clear that each chromatin state comprises a series of different structures and that each can be thought of more broadly as being associated with a set of distinct functional and biochemical properties (reviewed by Richards and Elgin, 2002). Heterochromatin spreads from nucleation sites (silencers) across chromosomal regions, consuming euchromatin in the process, and converting it into a transcriptionally silent state. Integral to this process are alterations in the patterns of posttranslational modifications of histone H3 and H4 N-terminal tails, which permit the binding of heterochromatin-specific histone binding proteins required for silencing (reviewed by Jenuwein and Allis, 2001; Moazed, 2001).

Since heterochromatin formation resembles a nucleation-polymerization process, crucial regulatory mechanisms must exist that prevent the silencing machinery from exhausting its euchromatic substrate. However, little is known about such inhibitory mechanisms. Deletion of DNA sequences that lie at the boundaries of

heterochromatic regions can result in further spread of heterochromatin. However, the precise molecular mechanisms by which such boundary elements function have been elusive (reviewed by Bell et al., 2001; Oki and Kamakaka, 2002). Because deletion of some boundary elements results in only a limited ectopic spread of heterochromatin (Noma et al., 2001), there must be undiscovered mechanisms which negatively regulate the process.

In the budding yeast, *S. cerevisiae*, silencing of the silent mating-type cassettes *HMR* and *HML* by the Sir proteins (Sir1–4) involves changes in chromosome structure characteristic of heterochromatin formation; these include decreases in accessibility to enzymatic probes and acetylation levels of histone tails (Braunstein et al., 1993; Moazed, 2001; Singh and Klar, 1992). Similarly, telomeric regions are silenced via the action of Sir2–4 and are also rendered into a heterochromatin-like state (Aparicio et al., 1991; Braunstein et al., 1993; Gottschling, 1992; Gottschling et al., 1990). In both instances, the silent mating-type cassettes and telomeric regions Sir2–4 are intrinsic components of silent heterochromatin (Hecht et al., 1996; Strahl-Bolsinger et al., 1997). Supporting the notion that histone tail modifications are critical to the specification of chromatin type in this system, purified Sir2 displays an NAD-dependent histone deacetylase activity that is required for silencing (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Moreover, analysis of mutants suggests that deacetylation of acetyl-lysine 16 of histone H4, a preferred in vitro target of Sir2, is important for silencing (Imai et al., 2000; Johnson et al., 1990). Current evidence is consistent with a sequential assembly model for Sir heterochromatin in which Sir2–4 is recruited to sites where silencing is initiated via interactions with sequence-specific DNA binding proteins (Hoppe et al., 2002; Luo et al., 2002; Rusche et al., 2002). Deacetylation of the tails of nearby histones in adjacent nucleosomes may promote the binding of Sir3 and Sir4, which directly bind histone H3 and H4 N-terminal tails with a strong preference for the underacetylated form of the H4 tail (Carmen et al., 2002). It has been proposed that this allows local recruitment of additional Sir2 via a protein-protein interaction with Sir4. Repeated cycles of deacetylation, Sir3/Sir4 binding, and recruitment Sir2 offers a plausible mechanistic framework for understanding the propagation of Sir heterochromatin (Moazed, 2001).

As in other eukaryotes, chromatin boundary elements have been identified in *S. cerevisiae*, which flank the silenced loci *HMR* and *HML*; others have been identified near telomeres (Bi and Broach, 2001). One example is a tRNA^{Thr} gene that occurs to the right of *HMR* (Donze et al., 1999). Although it is known that transcription of this gene by RNA polymerase III is required for its boundary function (Donze and Kamakaka, 2001), the molecular mechanism by which it inhibits silencing is incompletely understood.

Histone H2A.Z is a universally conserved, intrinsic component of eukaryotic chromatin that replaces the conventional H2A protein in a significant fraction of

*Correspondence: hiten@biochem.ucsf.edu

nucleosomes (reviewed by Redon et al., 2002). Previous studies have indicated a role for H2A.Z in transcriptional activation. For example, in the ciliate *Tetrahymena thermophila* H2A.Z is associated with the transcriptionally active macronucleus but not the quiescent micronucleus (Allis et al., 1980). Additionally, in *Saccharomyces cerevisiae* the deletion of *HTZ1* results in a defective induction of the *GAL1* and *PHO5* genes in response to their respective inducing signals, particularly in the absence of the SWI/SNF ATP-dependent nucleosome remodeling complex (Santisteban et al., 2000). However, H2A.Z has also been shown to promote gene silencing at reporter genes integrated at *HMR* and at a telomeric locus, leading to the proposal that it functions both in gene activation and gene silencing (Dhillon and Kamakaka, 2000).

We have utilized whole-genome microarray hybridization to identify genes that require Htz1 for their normal expression. We find that Htz1-activated genes are highly enriched near telomeres and often cluster together in small chromosomal domains. We show that Htz1 functions to protect genes from Sir-dependent silencing at these telomeric locations and in regions flanking the *HMR* silent cassette. High-resolution chromatin immunoprecipitation experiments revealed that, in the absence of Htz1, Sir2 and Sir3 spread outside of their normal boundaries at *HMR* concomitant with the appearance of histone tail acetylation and methylation modification patterns indicative of heterochromatin formation. The euchromatic regions that flank *HMR* are enriched for Htz1, whereas the region within *HMR* is relatively depleted. Genetic tests indicate that Htz1 antagonizes heterochromatin spread as part of a pathway, which is separate from that of a characterized boundary element. Thus, Htz1 is an intrinsic component of euchromatin that functions to antagonize the formation of Sir heterochromatin via a boundary element-independent pathway.

Results

Genes Requiring Htz1 for Normal Expression Are Enriched near Telomeres

To identify the genes regulated by *HTZ1* in yeast, we compared global transcript levels in *htz1* Δ mutant cells to wild-type cells by hybridization of differentially labeled cDNAs to whole-genome yeast microarrays (DeRisi et al., 1997). Four replicate experiments were performed using independent cultures, and genes positively and negatively regulated by *HTZ1* were identified using the significance analysis of microarrays (SAM) package (Tusher et al., 2001). This analysis yielded 214 genes that are significantly activated by *HTZ1* and 107 that are repressed by *HTZ1*. Visual inspection of the list of *HTZ1*-activated genes revealed that many were near telomeres. As shown in Figure 1, Htz1-activated genes are highly enriched within 30–40 kb of chromosome ends. This enrichment is not due to an increase in overall gene density at telomeres: analysis of the fraction of genes activated by Htz1 in 10 kb windows from chromosome ends reveals that a significant portion of genes within approximately 30 kb of telomeres are activated by Htz1 (Table 1). For example, 30% of genes between 10–20 kb

of telomeres are activated by Htz1 (Table 1). In contrast, genes greater than 60 kb from telomeres are significantly depleted for Htz1-activated genes (Table 1). We note that genes repressed by Htz1 appear to be randomly distributed throughout the genome (data not shown).

A second striking characteristic of the set of Htz1-activated genes is that they tend to occur in small clusters along the chromosome, termed HZADs for Htz1-activated domains (Supplemental Table S1 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>). Eighteen such clusters can be discerned that contain three or more Htz1-activated genes occurring within 15 kb of each other. Of the 18 HZADs, 15 occur within 35 kb of a telomere (and 14 within 20 kb). For example, HZAD 15, which occurs on the right arm of chromosome XIV, is a cluster of five adjacent genes (*YNR071C*, *HXT17*, *YNR073C*, *YNR074C*, and *COS10*), that lie between 4.3 and 12.8 kb from the telomere (Supplemental Table S1 available at above website). Of the 134 Htz1-activated genes that are not near telomeres, only 10 form clusters (Supplemental Table S1 available at above website; HZADs 1, 7, and 8).

Htz1 Protects a Set of Telomere-Proximal Genes from Sir2-Dependent Silencing

One explanation for the enrichment of Htz1-activated genes near chromosome ends would be that Htz1 protects genes from Sir-dependent telomeric silencing. We term such an activity “anti-silencing.” This hypothesis predicts that the expression defect of telomeric proximal genes in *htz1* Δ cells should be reversed by the removal of the silencing machinery. To test this prediction, we used microarray hybridization to examine the global transcript levels of an *htz1* Δ *sir2* Δ double mutant. Analysis of the data revealed that of 81 Htz1-activated genes within 35 kb of telomeres, the gene expression defect of 46 (57%) genes in *htz1* Δ strains was significantly reversed by deletion of *SIR2* (Figure 2A). Thus, for the majority of telomeric Htz1-activated genes, Htz1 indirectly or directly protects them from being silenced by Sir2. The defect in gene expression in *htz1* Δ cells for the remaining 35 genes either showed no effect upon deletion of *SIR2* or displayed a more enhanced defect in gene expression in the *htz1* Δ *sir2* Δ double mutant (Figure 2B). We suspect that these genes are also anti-silenced by Htz1, but for a Sir2-independent repression system (see Discussion). We note that of the 134 non-telomeric Htz1-activated genes, the expression defect for a fraction (24%) can be partially reversed by deletion of *SIR2*; however, suppression is considerably weaker than that observed for the telomeric Htz1-activated genes (see <http://madhanilab.ucsf.edu/public/htz1/>).

While our microarray analysis best supports the conclusion that Htz1 protects genes from Sir-dependent silencing, a trivial explanation for these results might be that silencing factor levels are increased in *htz1* Δ mutants. Although the observation that silencing of an artificial reporter inserted adjacent to a telomere is reduced in an *htz1* Δ mutant (Dhillon and Kamakaka, 2000) makes this possibility unlikely, we examined the levels of an integrated HA-tagged version of *SIR3* by immunoblotting and found no difference in wild-type versus *htz1* Δ cells (Supplemental Figure S1 available at <http://>

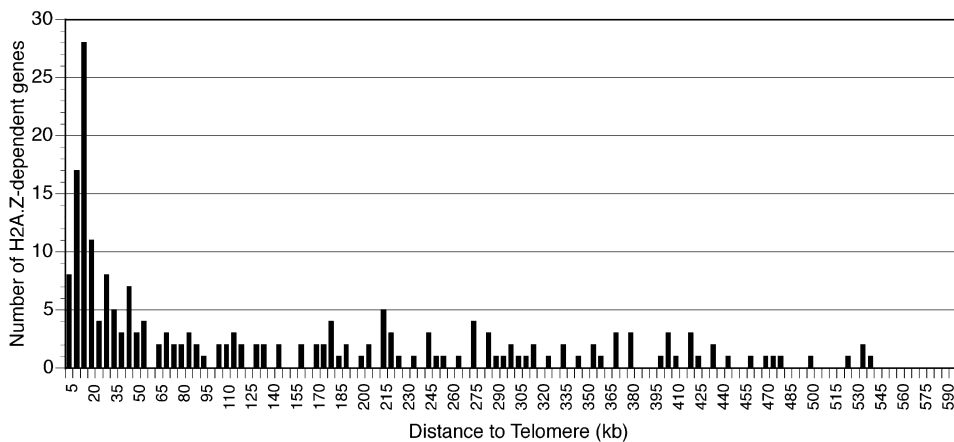


Figure 1. Chromosomal Distribution of Genes that Require *HTZ1* for Normal Expression

Shown is a histogram of *Htz1*-activated genes identified by microarray analysis plotted as a function of their distance from a chromosomal end.

www.cell.com/cgi/content/full/112/5/725/DC1). Also, inspection of our microarray data revealed no increases in the mRNA levels for genes encoding known silencing factors such as *SIR1-4*, *RAP1*, *ABF1*, and *ORC* genes (see <http://madhanilab.ucsf.edu/public/htz1/>).

Htz1 Protects Genes Flanking the *HMR* Silent Cassette from Sir2-Dependent Silencing Initiated at *HMR*

A prominent HZAD (HZAD 2) flanks the silent mating-type cassette *HMR* (Figure 3A) and corresponds to the gene *YCR095C* to the left of *HMR* and the genes *GIT1*, *YCR099C*, *YCR100C*, *YCR101C*, *YCR105W*, and *YCR106W*, which lie to the right of *HMR*. The latter set spans the region between *HMR* and the right telomere of chromosome III. (Note that sequences corresponding to *YCR102W*, *YCR103W*, and *YCR104W* are repeated on other chromosomes; thus, these genes may also be activated by *HTZ1*, but this effect would be masked by cross-hybridization to the duplicated sequences). The proximity of these genes to *HMR*, a site where silencing initiates and then spreads to its left and right boundaries, raised the possibility that in *htz1* Δ cells, silencing initiated at *HMR* spreads ectopically to silence HZAD 2. To test this hypothesis, we used microarray hybridization to determine whether the defect in the expression of *HMR*-proximal genes in *htz1* Δ cells could be suppressed by a deletion of *HMR* that eliminates the known nucleation sites for silencing, termed E and I. Indeed, such a deletion of *HMR* or deletion of *SIR2* significantly re-

versed the gene expression defect for each of the *HTZ1*-activated genes within HZAD 2 (Figure 3B). Suppression of the defect of *htz1* Δ cells by *hmr* Δ is highly specific for these seven loci (Supplemental Figure S2 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>) indicating that the effects of the *HMR* deletion are specific to the region around *HMR*. It is notable that several of the *HMR*-proximal loci display repression by Sir2 in an *HTZ1*⁺ genetic background (e.g., *YCR106W* in Figure 3B), suggesting that a locus can be partially protected from silencing by Htz1; that is, in some cases, Htz1 may reduce but not eliminate heterochromatin formation.

Ectopic Spread of Heterochromatin-Associated Histone Modification Patterns in Cells Lacking *HTZ1*

If Htz1 protects genomic regions from Sir-mediated silencing, then these regions should acquire histone modification patterns characteristic of Sir heterochromatin in the *htz1* Δ mutant. We used quantitative chromatin immunoprecipitation (ChIP) analysis to test this prediction (see Experimental Procedures). We prepared chromatin immunoprecipitation (ChIP) DNA pellets using antibodies specific for modified histones and performed quantitative PCR reactions to determine the relative enrichments of these chromatin markers in wild-type and *htz1* Δ strains. Using 20 PCR primers that amplify fragments corresponding to intergenic regions, we focused our analysis on two genomic locations: the *HMR*-proximal loci discussed above and another region on chromosome XIV that contains a strongly anti-silenced gene, *YNR074C*, which lies 6.7 kb from the telomere (Figure 3A; Supplemental Table S3 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>).

As Sir2-mediated silencing involves the deacetylation of N-terminal tails of histones, we first compared the histone acetylation landscape of the regions cited above in wild-type and *htz1* Δ cells to determine if loss of *HTZ1* produced the predicted reduction in acetylation levels. To do this, we performed ChIP analysis using an antibody specific for tetra-acetylated histone H4 (Ac-H4). Representative gels are shown in Figure 4. In agreement with recent data, we observe a relative paucity of Ac-

Table 1. Genes Requiring Htz1 for Normal Expression Are Enriched near Telomeres

Interval	Fraction Requiring Htz1	χ^2	P
0–10 kb	0.203	102.3	P < 0.001
10–20 kb	0.298	264.8	P < 0.001
20–30 kb	0.090	11.9	P < 0.1
30–40 kb	0.065	3.8	NS
40–50 kb	0.061	3.5	NS
50–60 kb	0.029	0.1	NS
>60 kb	0.021	27.0	P < 0.001

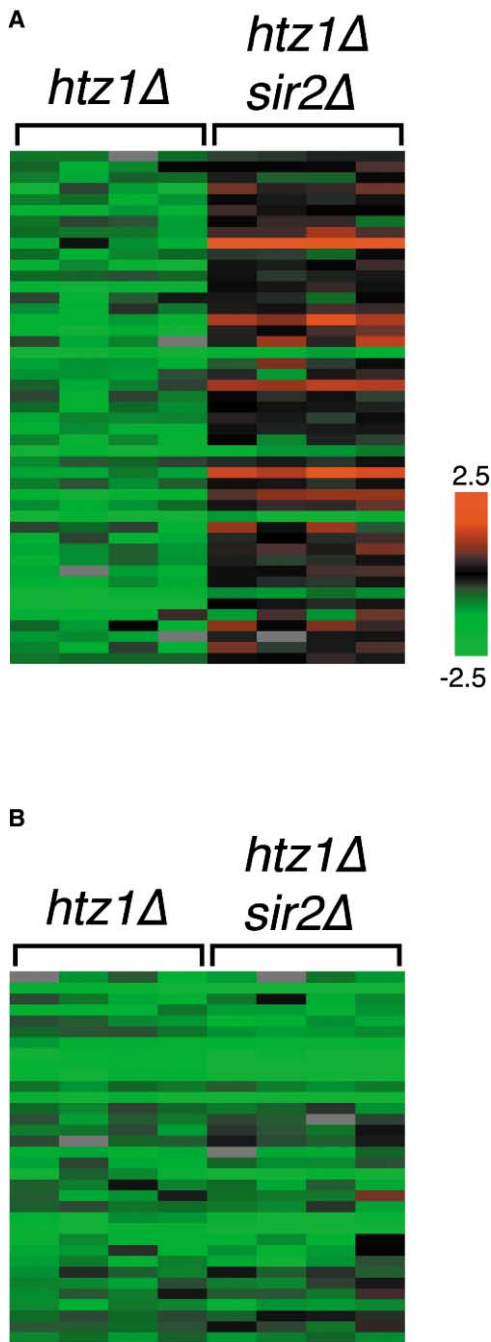


Figure 2. Suppression of the *htz1Δ* Transcription Defect near Telomeres by Deletion of *SIR2*

(A) Telomeric genes whose defect in *htz1Δ* is significantly suppressed by *sir2Δ*. Color representation of gene expression values of genes within 35 kb of a chromosome end whose expression defect in *htz1Δ* cells is suppressed by deletion of *SIR2*. Four replicate experiments are shown for each genotype. Color scale is shown for log₂ of the mutant:wild-type gene expression ratios.

(B) Telomeric genes whose defect in *htz1Δ* is not significantly suppressed by *sir2Δ*. Color representation of gene expression values of genes within 35 kb of a chromosome end whose expression defect in *htz1Δ* cells is not suppressed by deletion of *SIR2*. Four replicate experiments are shown for each genotype. Scale is as in (A).

H4 within *HMR* compared to the flanking euchromatic regions (Rusche et al., 2002). At regions upstream of genes just outside of *HMR* and near the right telomere chromosome XIV, we observed from ~6.5- (*YCR095C*) to 17.5- (*YCR106W*) fold increases in abundance of Ac-H4 relative to the levels within *HMR* in wild-type cells. In cells lacking *HTZ1*, the relative enrichments of Ac-H4 are significantly reduced at all Htz1-activated genes, supporting the view that Htz1 protects these genes from Sir-mediated silencing (Figure 5A and 5B).

Set1-mediated methylation of histone H3 on lysine 4 (K4Me-H3) has been shown to be enriched outside of telomeric heterochromatin in *S. cerevisiae*, consistent with evidence from other fungal and metazoan systems that K4-MeH3 is a marker for euchromatin (Bernstein et al., 2002). To investigate this euchromatic marker in anti-silenced regions we used ChIP to compare K4Me-H3 levels in wild-type and *htz1Δ* strains (see Experimental Procedures). In wild-type cells, we detected a pattern that is similar to that of Ac-H4, namely a striking paucity of K4Me-H3 within *HMR* and an ~4- to 13-fold relative enrichment of K4Me-H3 at anti-silenced regions outside of *HMR* and near *YNR074C* on chromosome XIV (Figures 5C and 5D). Again as with Ac-H4, cells lacking *HTZ1* have reduced K4Me-H3 at the promoters of every gene protected from silencing by Htz1 with the exception of the region upstream of *YCR095C* (Figures 5C and 5D). Recent work indicates that K4Me-H3 is more highly enriched in ORFs over promoters (Bernstein et al., 2002). Because our analysis was restricted to intergenic regions, we may have therefore underestimated the differences in K4Me-H3 levels in wild-type compared with *htz1Δ* at *YCR095C* and elsewhere. Nevertheless, these data show that in cells lacking *HTZ1*, anti-silenced regions acquire modification patterns (reduced Ac-H4 and K4Me-H3) consistent with the formation of ectopic heterochromatin.

Ectopic Spread of Sir2 and Sir3 in Cells Lacking *HTZ1*

To determine if the ectopic Sir-dependent silencing and changes in histone modification patterns in *htz1Δ* mutants is caused by the ectopic spread of components of the Sir complex, we used ChIP to compare the patterns of Sir2 and Sir3 association in wild-type and *htz1Δ* strains. In *HTZ1* strains, we observed large enrichments of both Sir3 (58- to 68-fold) and Sir2 (19- to 40-fold) within the silenced region of *HMR* (Figures 6A and 6C), and much reduced levels of Sir2 and Sir3 outside of the boundaries of *HMR*. In *htz1Δ* cells, the relative enrichment of Sir3 is significantly increased at all anti-silenced regions both proximal to *HMR* and on chromosome XIV (Figures 6A and 6B). For example, the strongly anti-silenced genes *GIT1* and *YNR074C* have average Sir3 ChIP association values of approximately 1.2 and 2.3 in wild-type cells and these values increase to 3.2 and 12.4, respectively in *htz1Δ* mutants (Figure 6A). Similar results were obtained for Sir2 (Figures 6C and 6D), with increases ranging from 1.2- to 3.4-fold observed in the *HMR*-proximal region and 4.7-fold at *YNR074C*. We conclude that Htz1 functions to prevent the Sir complex from spreading into the euchromatic regions that are adjacent to silenced regions.

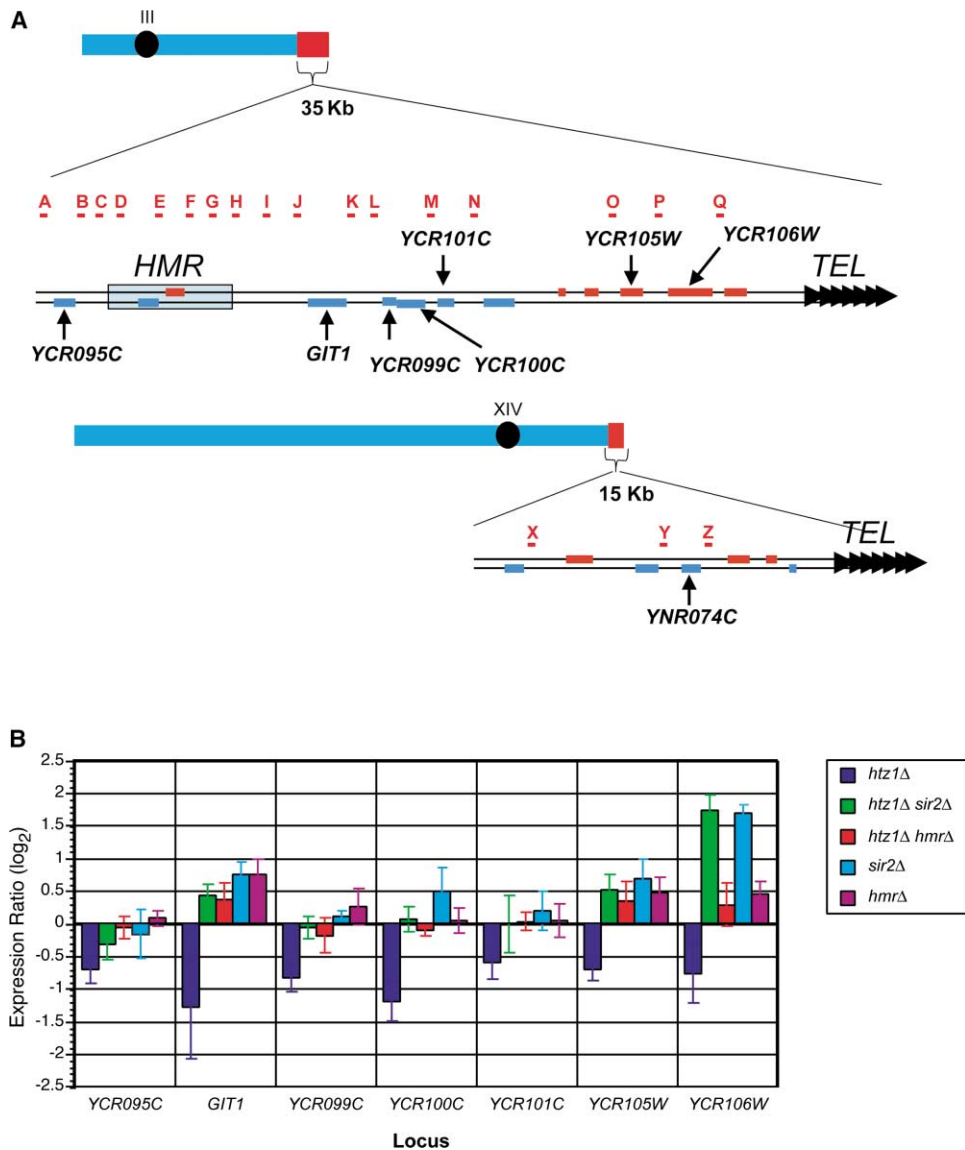


Figure 3. Geography of Anti-Silencing

(A) Diagram of two prominent anti-silenced domains. Shown is a region of chromosome III containing the *HMR* silent mating-type cassette and HZAD 2 and a chromosomal region near the right telomere of XIV corresponding to part of HZAD 15. Depicted are the locations of open reading frames found in these regions. Those subject to anti-silencing by Htz1 are indicated with arrows. Also indicated are the locations and designations of PCR primers used in this study for ChIP analysis.

(B) Effect of deletion of *SIR2* or *HMR* on the expression of *HMR*-proximal genes. Shown are the average expression ratios derived from microarray hybridization relative to wild-type of the indicated genotypes at genes near *HMR* that require *HTZ1* for normal expression. Plotted are means and standard deviations for four independent experiments performed on each genotype.

In addition to the increase in Sir association in euchromatic regions in cells lacking *HTZ1*, we detected a statistically significant decrease of Sir3 association within *HMR* in *htz1* Δ mutants (Figures 6A and 6B). Accordingly, *HMR* acquired slightly more euchromatin-specific histone modification patterns in *htz1* Δ mutants, with increased levels of Ac-H4 and K4Me-H3 (Figures 5B and 5D). These results are consistent with the observation that an *ADE2* reporter gene integrated at *HMR* is mildly derepressed in *htz1* Δ mutants (Dhillon and Kamakaka, 2000).

Htz1 Is Enriched in Euchromatic Regions Outside of *HMR*

In principle, Htz1 could perform its anti-silencing function in one of three different regions: (1) Htz1 could act at silenced regions to tether Sir proteins to heterochromatin, thus preventing them from spreading ectopically; (2) Htz1 may function as a component of boundary elements that block the spread of heterochromatin beyond their normal bounds; or (3) Htz1 could manifest its protective function at the anti-silenced genes themselves.

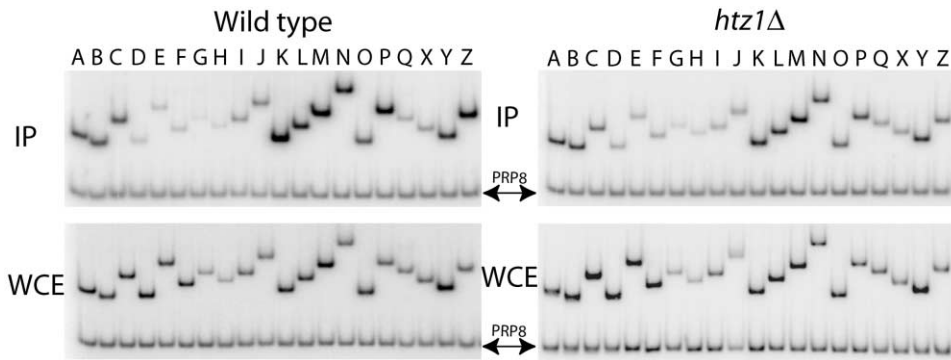


Figure 4. Quantitative PCR Analysis of Tetra-Ac-H4 ChIP Pellets

Quantitative PCR with ³²P-dATP incorporation was performed on Ac-H4 ChIP pellets and electrophoresed on polyacrylamide gels (see Experimental Procedures). Shown are representative phosphorimager scans of reactions performed on immunoprecipitation (IP) and input whole-cell extract (WCE) DNA from paired wild-type and mutant strains. Primer pair names are indicated above the gels. The genomic positions of these primers are shown in Figure 3A.

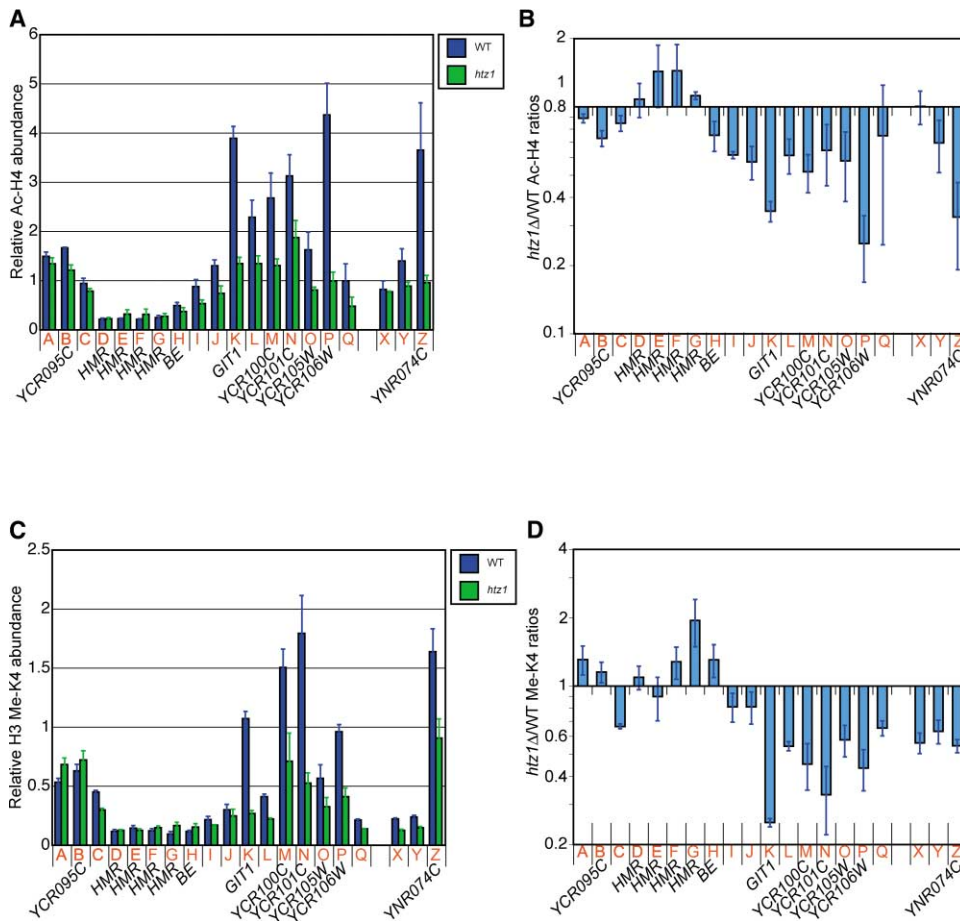


Figure 5. ChIP Analysis of Euchromatic Markers in Wild-Type and *htz1Δ*

(A and C) Average relative Ac-H4 (A) and K4Me-H3 (C) enrichments for wild-type and *htz1Δ* are shown for each primer set with its amplified region denoted as indicated in Figures 3A and 4. Standard error of the mean (SEM) error bars are shown.

(B and D) The degree of change of Ac-H4 or H3 Me-K4 enrichments in *htz1Δ* versus wild-type for each locus was determined by dividing the *htz1Δ* enrichment value of each locus by the correlating enrichment value from a paired wild-type experiment. Shown are average *htz1Δ*/WT Ac-H4 (B) and H3 Me-K4 (D) ratios for each locus with SEM error bars plotted on logarithmic scales. Values that are less than one indicate there is less enrichment at that locus in *htz1Δ* compared to wild-type.

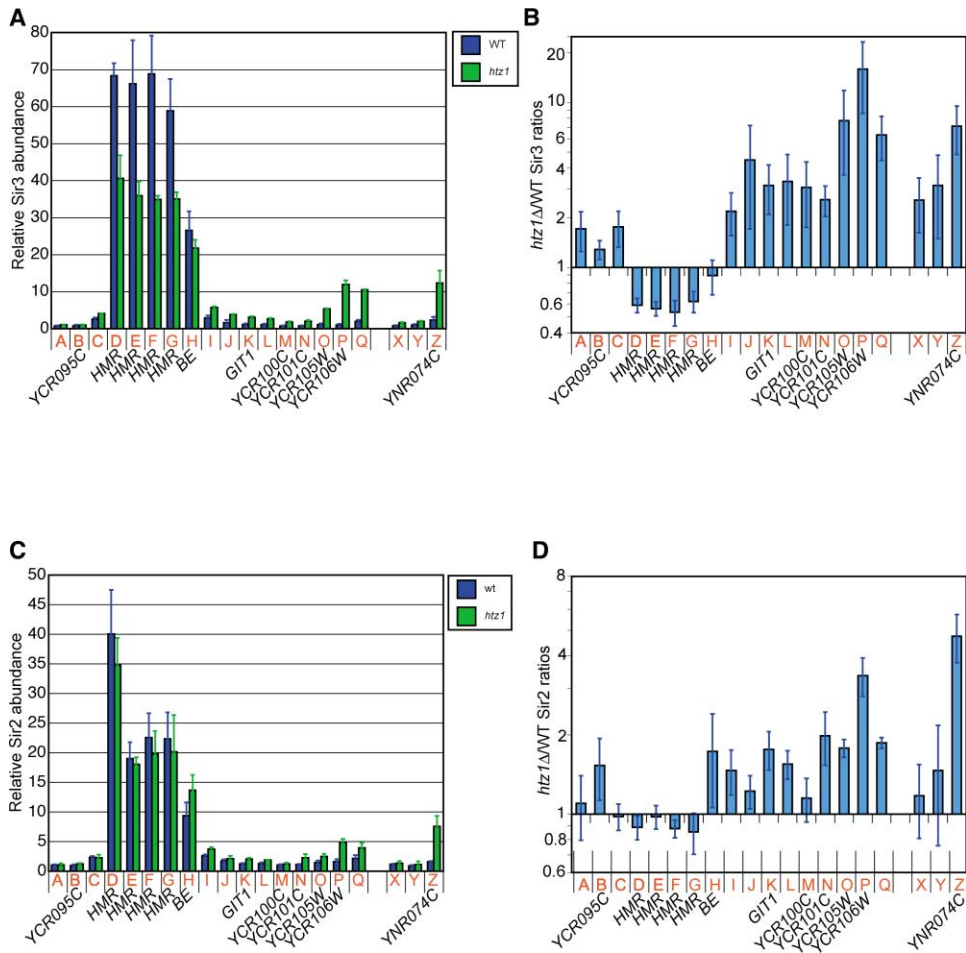


Figure 6. ChIP Analysis of Sir2 and Sir3 Enrichment in Wild-Type and *htz1* Δ

(A and C) Average Sir3 (A) and Sir2 (C) enrichments for wild-type and *htz1* Δ are shown as for Figures 5A and 5C.

(B and D) The degree of change in Sir3 (B) and Sir2 (D) enrichments in *htz1* Δ versus wild-type for each locus was determined as for Figures 5B and 5D. Average *htz1* Δ /WT ratios are shown plotted on logarithmic scales with SEM error bars. Values that are greater than one indicate that there is more enrichment at that locus in *htz1* Δ compared with wild-type.

To help distinguish between these possibilities, we used ChIP to determine the distribution of Htz1 along chromosomes (see Experimental Procedures). We found that Htz1 is enriched at the promoters of anti-silenced genes relative to *HMR* and the known boundary element that flanks *HMR* on the right (Figure 7A). For example, the promoters of *GIT1* and *YNR074C* contain amongst the most abundant levels of Htz1 (~7-fold higher than within *HMR*) and these genes are strongly anti-silenced by Htz1. While we cannot exclude the possibility that Htz1 is present at *HMR* and/or the boundary element at low levels and functions there, the enrichment of Htz1 at the promoters of genes protected from silencing by Htz1 is most consistent with it functioning in anti-silencing directly at these regions.

Because Htz1 has previously been shown to be present at the promoters of the non-telomeric genes *PHO5* and *GAL1* (Santisteban et al., 2000), we asked whether Htz1 is particularly enriched in euchromatic regions flanking heterochromatin or whether the levels of Htz1 in these anti-silenced regions are comparable to its levels at other euchromatic regions. We used ChIP to exam-

ine the levels of Htz1 at several well-characterized loci that are distant to telomeres (*GPD1*, *CYC1*, *GAL1*, *PHO5*, *ADH1*, and *ACT1*) and compared them to levels found at *HMR* (primer D) and *GIT1* (Supplemental Figure S3 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>). In general we found that Htz1 was enriched at these regions relative to *HMR*, though not as much so as at *GIT1*. Htz1 was shown previously to be enriched in the promoters versus coding sequences of the *GAL1* and *PHO5* genes, consistent with a role in transcriptional initiation (Santisteban et al., 2000). We confirmed that Htz1 is more enriched in the promoters of *GAL1* and *PHO5* compared with their respective ORFs and observed a similar pattern for *GPD1*. Interestingly however, for *CYC1* at least as much Htz1 was present in the ORF as at the promoter and for *ADH1* and *ACT1*, Htz1 was more abundant in the respective ORF regions than the promoters. We also examined the levels of Htz1 in two additional heterochromatic regions, the protosilencers contained within the repetitive subtelomeric elements core X and Y' and found that, like within *HMR*, Htz1 is depleted from these regions (Supplemental Figure S3

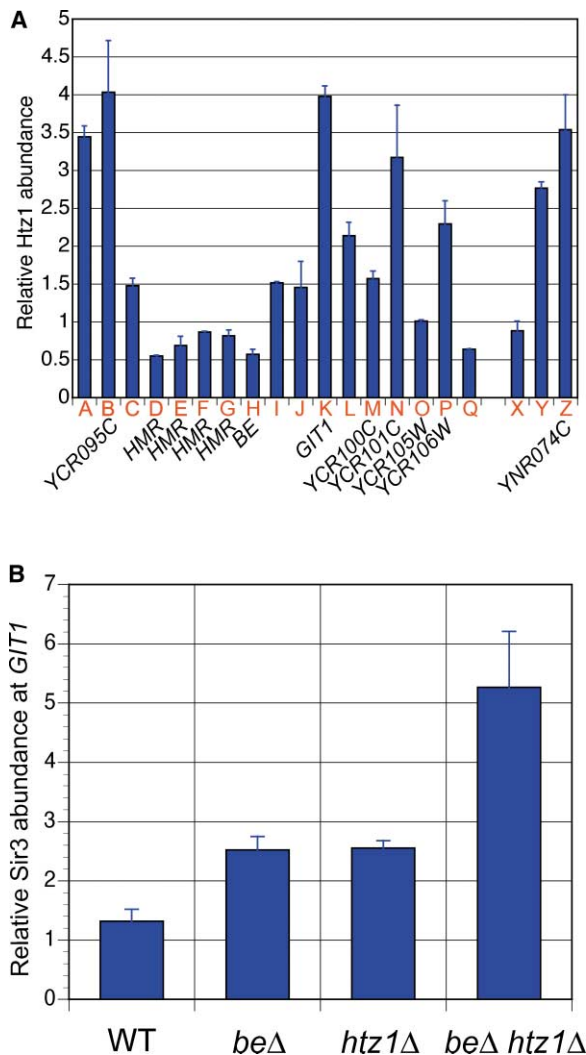


Figure 7. Htz1 Localization and Relationship to the *HMR* Right Boundary Element (*BE*)

(A) Htz1 is enriched in euchromatic regions and relatively depleted in heterochromatic regions. Shown is a bar graph with average enrichment values of Htz1-HA₃ with SEM error bars given. The loci are indicated on the bottom with primer sets as designated in Figure 3A.

(B) Genetic relationship between *HTZ1* and *BE*. Shown is the relative enrichment of Sir3 determined by ChIP at the *GIT1* promoter in strains of the indicated genotypes. Means are plotted with SEM error bars.

available at above website). Thus, Htz1 appears to be generally enriched in euchromatic regions, but the previously noted correlation with Htz1 deposition and promoters appears not to be general.

Htz1 and the Right *HMR* Boundary Element Act Synergistically to Antagonize the Ectopic Spread of Sir3

Htz1 is not enriched at the boundaries of *HMR* suggesting that it does not contribute to boundary element function at these locations. To test more rigorously whether Htz1 contributes to boundary element function, we performed a genetic test to determine if *htz1Δ* shows synergistic interactions with a deletion of the well-char-

acterized boundary element (*BE*) that flanks the right side of *HMR*. If *HTZ1* and *BE* function in separate anti-silencing pathways, then the *htz1Δ beΔ* double mutant should display a more severe phenotype than either *htz1Δ* or *beΔ* single mutant. We constructed isogenic strains of the following genotypes: *beΔ*, *htz1Δ*, *beΔ htz1Δ*, or wild-type for these two loci. We then used ChIP with antibodies to Sir3 to examine the ectopic spread of Sir3 in these strains. We focused our analysis on *GIT1*, the most proximal gene to the *HMR* right boundary element. As shown in Figure 7B, both *beΔ* and *htz1Δ* strains show an increase in the association of Sir3 at *GIT1* relative to wild-type (~2-fold increase). The *htz1Δ beΔ* double mutant shows a significant further increase in Sir3 association at *GIT1* compared with either of the single mutants (~4-fold increase compared with WT), consistent with Htz1 acting independently of this boundary element to antagonize the ectopic spread of Sir3 to the *GIT1* locus.

Discussion

Recent studies have begun to reveal the mechanisms by which silent heterochromatin is initiated and propagated. In both yeast and mammals, silencing initiates at a specific site and then spreads across a chromosome converting euchromatin into heterochromatin via alterations in histone modification states that are in turn recognized by proteins required for heterochromatin formation (reviewed by Jenuwein and Allis, 2001; Moazed, 2001). For instance, during mammalian X chromosome inactivation, silencing and heterochromatin formation initiates at the X-inactivation center and spreads outward, coating the chromosome with the Xist non-coding RNA, which is itself required for the initiation and maintenance of the process (reviewed by Plath et al., 2002). Likewise, in yeast, silencing at the *HMR* silent mating-type cassette initiates at the E and I silencers and spreads, coating that chromosomal region with the Sir complex (reviewed by Moazed, 2001). These observations have led to the general concept that euchromatin can be converted to the heterochromatic state through the action of a specialized silencing machinery.

H2A.Z Antagonizes Sir-Dependent Silencing and Heterochromatin Formation

In this paper, we have described evidence that in budding yeast, the universally conserved histone variant H2A.Z functions to antagonize Sir-dependent gene silencing and heterochromatin formation. Global gene expression measurements revealed that genes located near chromosomal domains subject to silencing, namely telomeres and the silent mating type cassette *HMR*, require Htz1 for their full expression. For the majority of these genes, the defect in gene expression in *htz1Δ* cells can be reversed by deletion of *SIR2*, indicating that H2A.Z inhibits Sir2-dependent silencing. Supporting this conclusion, the gene expression defects in *htz1Δ* cells of the genes flanking *HMR* were specifically reversed by a deletion that removes both the E and I silencers of *HMR*, which are the nucleation sites for the formation of Sir heterochromatin at this site. Our observation that some telomeric genes require *HTZ1* for a reason unre-

lated to Sir-dependent silencing may be explained by a second type of Sir-like telomeric repression recently described that is mediated by the Hda1 histone deacetylase together with the Tup1/Ssn6 corepressor rather than by the Sir complex (Robyr et al., 2002). Indeed, several of the HZADs (HZAD 5,12,13,16–18) contain genes that occur within previously reported Hda1-repressed (HAST) domains (Supplemental Table S1 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>). Thus, Htz1 may function to protect some telomeric genes from Hda1-dependent or an as yet undiscovered mode of Sir-independent telomeric repression (Wyrick et al., 1999).

Using chromatin immunoprecipitation, we observed the ectopic spread of Sir3 and Sir2 in *htz1*Δ mutants beyond their normal boundaries at *HMR*. These data are in agreement with our microarray-based genetic suppression experiments, which indicate that Htz1 protects these genes from Sir-mediated silencing. Our results show that Htz1 acts to inhibit silencing upstream of the physical spread of the Sir complex as opposed to a step that occurs after the association of the Sir complex. We also identified a similar increase in Sir protein association near the right telomere of chromosome XIV, particularly at the *YNR074C* locus, which is strongly dependent on *HTZ1* for its expression. Concomitant with the spread of Sir proteins in *htz1*Δ cells, ChIP analysis revealed a decrease in both acetylated histone H4 and lysine 4-methylated histone H3 at anti-silenced loci (Figures 4 and 5). Methyl-lysine 4 of H3 has been proposed as a euchromatin-specific “mark” in other eukaryotes, but whether it serves this purpose in *S. cerevisiae* has been controversial (Braunstein et al., 1993; Briggs et al., 2001; Bryk et al., 2002; Nislow et al., 1997; Turner, 2002). Our observation that K4-methylated H3 is enriched in the euchromatic regions outside of *HMR* is more consistent with the view that this modification is a conserved marker for euchromatin (Bernstein et al., 2002). Moreover, an association between H3 K4 trimethylation and gene activity has recently been reported (Santos-Rosa et al., 2002).

While our global microarray and ChIP analyses show that Htz1 antagonizes silencing, a previous study concluded that Htz1 promotes silencing. This conclusion was based largely on the observations that the silencing of an *ADE2* reporter gene integrated within *HMR* and a *URA3* reporter integrated adjacent to a telomere show decreased silencing in *htz1*Δ cells and that the overexpression of *HTZ1* was found to suppress the silencing defect of some hypomorphic alleles of *SIR1* (Dhillon and Kamakaka, 2000). One way to reconcile these observations relies on the finding that Sir protein pools are limiting for silencing (Maillet et al., 1996; Smith et al., 1998). We observe a small but significant decrease in Sir3 association and correlating increases in euchromatic histone modifications within *HMR* in *htz1*Δ mutants, suggesting that association of Sir with ectopic sites in *htz1*Δ mutants results in a depletion of the pool available to nucleate silencing elsewhere. The observed decreases in silencing of the two aforementioned reporter genes in *htz1*Δ could thus be due to a redistribution of silencing factors. Likewise, the ability of *HTZ1* overexpression to suppress *sir1* mutant alleles could reflect increased availability of Sir proteins caused by improved anti-

silencing outside of *HMR* and elsewhere. Other factors previously thought to be involved in promoting silencing might actually be anti-silencing factors whose loss results in a redistribution of Sir proteins to sites. In particular, our observation that K4-MeH3 is highly enriched in euchromatic regions flanking heterochromatin suggests that the previously described defects in silencing observed in cells lacking the H3 4-methylase Set1 may be indirect.

To summarize, Sir proteins spread beyond their normal bounds in *htz1*Δ cells and this spread is accompanied by ectopic transcriptional silencing that is accompanied by the formation of chromatin with a heterochromatic histone tail modification pattern. While the simplest model is that Sir proteins spread along the chromosome in *htz1*Δ cells, we cannot rule out the possibility that Htz1 functions by preventing the activation of unidentified cryptic protosilencers similar to those identified in the subtelomeric elements core X and Y' (reviewed by Fourel et al., 2002). We note that our examination of Htz1 levels at core X and Y' corresponded to the sites of previously defined protosilencers; however, no enrichment of Htz1 at these sites was apparent making it less likely that it acts there (Supplemental Figure S2 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>).

How Does Htz1 Mediate Anti-Silencing?

To begin to determine how Htz1 mediates anti-silencing, we first determined the relative enrichments of Htz1 at silenced regions compared with the anti-silenced regions (Figure 7A and Supplemental Figure S2 available at above website). We observed a relative enrichment of Htz1 at anti-silenced genes and a paucity of Htz1 within silenced regions. This observation supports the model that Htz1 acts directly at anti-silenced genes to protect them from silencing that is initiated at nearby silencers. We also addressed the possibility that Htz1 contributes to the function of a known boundary element, which flanks *HMR* (*BE*). Our results indicate that *BE* and *HTZ1* define separate parallel mechanisms for antagonizing the spread of silencing: (1) Htz1 is relatively depleted at *BE* (Figure 7A), and (2) *be*Δ *htz1*Δ double mutants exhibit synergistic anti-silencing defects (Figure 7B). As the spread of heterochromatin across chromosomes seems likely to be a highly cooperative process, it may have been necessary for cells to evolve multiple redundant mechanisms to antagonize its consumption of euchromatin.

The anti-silencing activity we have described for Htz1 may be an intrinsic property of chromatin harboring this molecule: studies of purified nucleosome arrays containing H2A.Z versus H2A have found that H2A.Z-containing chromatin is inherently resistant to condensation, at least in vitro (Abbott et al., 2001; Fan et al., 2002). Being an integral component of nucleosomes, Htz1 may thus antagonize silencing due to its effects on the biophysical properties of chromatin. That is, Htz1 nucleosomes may be refractory to the mechanisms by which the Sir complex spreads through and condenses chromatin. Ultimately, high-resolution structural analysis of silent chromatin may yield insights into what aspect of heterochromatin formation is disrupted by H2A substitution by H2A.Z.

Recently, Dot1 has been identified as a histone H3 lysine 79 methylase (Lacoste et al., 2002; Ng et al., 2002; van Leeuwen et al., 2002). It has been proposed that methylation at this site prevents the binding of Sir proteins and thereby serves to antagonize silencing (van Leeuwen et al., 2002). Others have proposed that this modification is required to tether Sir proteins at telomeric silenced sites to promote silencing (Ng et al., 2002). The observation that the fraction of H3 that is methylated (90%) is considerably larger than the small fraction of the genome that is near telomeres makes this second explanation less likely (van Leeuwen et al., 2002). Examination of the distribution of H3-K79 methylation across defined heterochromatin-euchromatin junctions, such as those that flank *HMR* may help to resolve this issue. In any case, comparison of the gene expression defects seen in *dot1Δ* cells (Hughes et al., 2000) to those of *htz1Δ* cells reveals no significant overlap (data not shown), suggesting that regardless of the precise function of H3-K79 methylation, it differs from that of Htz1.

Very recent observations indicate that the histone acetylase homolog Sas2 antagonizes silencing near telomeres in yeast by promoting the acetylation of lysine 16 of histone H4 (Kimura et al., 2002; Suka et al., 2002). Interestingly, *sas2Δ* and *htz1Δ* mutants show significant similarities in their gene expression defects (M.W. and H.D.M., unpublished data) suggesting that Htz1 and Sas2 may define a multi-step anti-silencing pathway. An intriguing possibility is that acetylation of lysine 16 directs the deposition of H2A.Z in euchromatin.

The SWI/SNF ATP-dependent remodeling complex and Htz1 function redundantly in the activation of the *GAL1* and *PHO5* genes, two well-studied inducible genes (Santisteban et al., 2000). This study indicated that, although Htz1 is required for the induction of these two genes, it associates with these promoters in the repressed state prior to induction and appears to be partially displaced from the genes upon induction (Santisteban et al., 2000). This novel behavior distinguishes Htz1 from conventional coactivators such as the SAGA complex, which is recruited by acid activators in the course of transcriptional induction (Bhaumik and Green, 2001; Larschan and Winston, 2001). These observations suggest that Htz1 is required very early in the pathway of transcriptional activation, consistent with the view that it functions to promote transcriptional competence prior to the action of conventional activators. Since transcriptional competence is a hallmark of euchromatin, the function of Htz1 in gene induction and in anti-silencing may be highly related or even the same: to antagonize the formation of condensed chromatin. Intriguingly, the SWI/SNF complex is particularly required for the expression of genes during mitosis, when chromatin is more condensed (Krebs et al., 2000). Similarly, the requirement for this complex in *htz1Δ* for the induction of the *GAL1* and *PHO5* genes may reflect a more condensed, heterochromatin-like state of these loci in *htz1Δ* cells. Consistent with this model, accessibility of the *PHO5* promoter to restriction endonuclease digestion is reduced in *htz1Δ* cells (Santisteban et al., 2000).

Thus, while our studies indicate a critical role for Htz1 for the expression of genes near silenced domains, the association with Htz1 with the promoters of *GAL1* and

PHO5 (neither lie near characterized silenced regions) suggests that Htz1 may have a broader, albeit redundant role in gene expression. Such a view would be consistent with our finding that Htz1 is enriched at many euchromatic loci. Alternatively, it could be that the primary function of Htz1 is to protect euchromatic genes from silencing, but that this effect is only apparent under standard laboratory conditions for genes near silent domains.

Is the Relationship between Euchromatin and Anti-Silencing Analogous to that between Heterochromatin and Silencing?

We have described a conserved, euchromatin-enriched protein, Htz1, that antagonizes the formation of the opposite chromatin state. Conceptually, Htz1 can be viewed as a euchromatic counterpart to a heterochromatic silencing protein that coats heterochromatin and promotes its formation. Euchromatin may not be merely a state of chromatin that is consumed by specialized silencing factors; rather, the situation may be more symmetric with each type of chromatin harboring factors that antagonize the switch to the opposite chromatin state. This view raises the speculative question of whether there exist mechanisms by which euchromatin formation is an active process that initiates at specific sites and spreads at the expense of heterochromatin to determine the epigenetic landscape of the genome.

Experimental Procedures

Yeast Strains

Strains used in this study are described in Supplemental Table S2 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>.

Microarray Hybridization Procedures

Exponentially growing cells in YPD media (Q-Biogene) supplemented with tryptophan and adenine were diluted to an O.D. 600 of 0.15 and grown to an O.D. of 0.7 at 30°C. For each mutant culture, a paired wild-type culture was grown in an adjacent slot in a floor shaker. Cultures were rapidly harvested by vacuum filtration onto a Millipore nitrocellulose filter, which was then immediately placed in a 15 ml polypropylene tube and flash-frozen in liquid nitrogen. Total RNA was isolated as described (www.microarrays.org) and mRNA was selected using biotinylated oligo-dT and magnetic streptavidin beads (Promega) using the manufacturer's protocol.

Microarrays were constructed as described (DeRisi et al., 1997). Yeast ORFs were amplified using a commercially available primers and yeast genomic DNA as a template (Research Genetics). Products were analyzed by gel electrophoresis, precipitated, resuspended in 3× SSC, and spotted onto poly-lysine coated microarray slides using a robot custom-built by Dr. Joseph DeRisi (UCSF). Detailed protocols are available at www.microarrays.org.

Cy3 and Cy5 labeled cDNAs were generated and hybridized to arrays for 6 hr at 63°C. For each experiment, four replicate experiments were performed, and the Cy3 and Cy5 labeling scheme was alternated to allow the subsequent removal of systematic biases arising from the dyes from the data by averaging. After washing and drying, arrays were scanned using a GenPix 4000 scanner (Axon, Inc). Following spot gridding and data extraction, the data were uploaded on an AMAD database (www.microarrays.org) running on an Intel-based PC.

Microarray Data Analysis

Genes significantly different from wild-type in *htz1Δ* cells were identified using the significance analysis of microarrays (SAM) package (Tusher et al., 2001). Missing values were estimated using the nearest 10 neighbors. Imputed datasets were analyzed using delta fac-

tors that yielded approximately 10% estimated false positives. This was found empirically to be a good trade-off between sensitivity (false negatives) and specificity (false positives), and yielded results similar to those obtained using arbitrary cut-offs to identify regulated genes. SAM analysis was also used to identify genes whose defect in *htz1Δ* could be reversed by deletion of *SIR2* or *HMR*. Htz1-activated domains (HZADs) were defined by three or more Htz1-activated genes occurring within a 15 kb interval (Supplemental Table S1 available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>). The full data set is available at <http://madhanilab.ucsf.edu/public/htz1/>.

Chromatin Immunoprecipitation (ChIP)

ChIP assays were performed essentially as described (Strahl-Bolsinger et al., 1997). For Htz1 ChIP, YM1730 (*htz1Δ*) was transformed with a plasmid that expresses an N terminally triple-HA tagged functional version of Htz1. Crosslinking times for the Htz1 ChIP were 60 min. For all other experiments, crosslinking times were 15 min. Pellet and whole-cell extract DNAs were analyzed by quantitative PCR performed in the linear range using ³²P-dATP incorporation, electrophoresis through 6% polyacrylamide gels in Tris-Borate-EDTA buffer, and phosphorimager quantitation of radioactive bands in the dried gels. Enrichments relative to the *PRP8* internal control were calculated as follows using the method of Noma et al. (2001). We normalized the enrichment values by using multiplex PCR and determined abundance ratios for each locus relative to a control PCR product corresponding to the middle of the open reading of *PRP8*, a gene for which there is no *HTZ1*-mediated regulation (our unpublished data). The pellet ratios were in turn normalized for amplification bias after division by the corresponding input whole-cell extract ratio to yield a value, which reflects relative abundance. For experiments shown in Supplemental Figure S2 (available at <http://www.cell.com/cgi/content/full/112/5/725/DC1>), quantitative PCR was performed using real-time PCR with SIBR green as a label. All of our ChIP experiments were performed in triplicate on paired wild-type and *htz1Δ* mutant strains where appropriate.

Acknowledgments

We thank Danesh Moazed and Jasper Rine for their generous gifts of anti-Sir antibodies, Mitchell Smith for an epitope-tagged Htz1 plasmid, and Michael Grunstein for yeast strain AYH2.45. We are especially grateful to Joe DeRisi for his enthusiastic advice on microarray experiments. We thank Sandy Johnson and Ira Herskowitz for critical reading of the manuscript and encouragement, N. Nguyen for excellent technical support, members of the Madhani lab for stimulating discussion, and Sandra Encalada for assistance with candidate cover art. M.D.M. is a Damon Runyon Fellow supported by the Damon Runyon Cancer Research foundation (DRG 1662). This work was supported the Packard Foundation, the Burroughs-Wellcome Fund, the Sandler Family Foundation, and UCSF School of Medicine Research Evaluation and Allocation Committee.

Received: September 30, 2002

Revised: January 21, 2003

References

- Abbott, D.W., Ivanova, V.S., Wang, X., Bonner, W.M., and Ausio, J. (2001). Characterization of the stability and folding of H2A.Z chromatin particles: implications for transcriptional activation. *J. Biol. Chem.* 276, 41945–41949.
- Allis, C.D., Glover, C.V., Bowen, J.K., and Gorovsky, M.A. (1980). Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eucaryote, *Tetrahymena thermophila*. *Cell* 20, 609–617.
- Aparicio, O.M., Billington, B.L., and Gottschling, D.E. (1991). Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* 66, 1279–1287.
- Bell, A.C., West, A.G., and Felsenfeld, G. (2001). Insulators and boundaries: versatile regulatory elements in the eukaryotic. *Science* 291, 447–450.

- Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc. Natl. Acad. Sci. USA* 99, 8695–8700.
- Bhaumik, S.R., and Green, M.R. (2001). SAGA is an essential in vivo target of the yeast acidic activator Gal4p. *Genes Dev.* 15, 1935–1945.
- Bi, X., and Broach, J.R. (2001). Chromosomal boundaries in *S. cerevisiae*. *Curr. Opin. Genet. Dev.* 11, 199–204.
- Braunstein, M., Rose, A.B., Holmes, S.G., Allis, C.D., and Broach, J.R. (1993). Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.* 7, 592–604.
- Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y., Winston, F., and Allis, C.D. (2001). Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes Dev.* 15, 3286–3295.
- Bryk, M., Briggs, S.D., Strahl, B.D., Curcio, M.J., Allis, C.D., and Winston, F. (2002). Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in *S. cerevisiae* by a Sir2-independent mechanism. *Curr. Biol.* 12, 165–170.
- Carmen, A.A., Milne, L., and Grunstein, M. (2002). Acetylation of the yeast histone H4 N terminus regulates its binding to heterochromatin protein SIR3. *J. Biol. Chem.* 277, 4778–4781.
- DeRisi, J.L., Iyer, V.R., and Brown, P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278, 680–686.
- Dhillon, N., and Kamakaka, R.T. (2000). A histone variant, Htz1p, and a Sir1p-like protein, Esc2p, mediate silencing at HMR. *Mol. Cell* 6, 769–780.
- Donze, D., and Kamakaka, R.T. (2001). RNA polymerase III and RNA polymerase II promoter complexes are heterochromatin barriers in *Saccharomyces cerevisiae*. *EMBO J.* 20, 520–531.
- Donze, D., Adams, C.R., Rine, J., and Kamakaka, R.T. (1999). The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. *Genes Dev.* 13, 698–708.
- Fan, J.Y., Gordon, F., Luger, K., Hansen, J.C., and Tremethick, D.J. (2002). The essential histone variant H2A.Z regulates the equilibrium between different chromatin conformational states. *Nat. Struct. Biol.* 9, 172–176.
- Fourel, G., Lebrun, E., and Gilson, E. (2002). Protosilencers as building blocks for heterochromatin. *Bioessays* 24, 828–835.
- Gottschling, D.E. (1992). Telomere-proximal DNA in *Saccharomyces cerevisiae* is refractory to methyltransferase activity in vivo. *Proc. Natl. Acad. Sci. USA* 89, 4062–4065.
- Gottschling, D.E., Aparicio, O.M., Billington, B.L., and Zakian, V.A. (1990). Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* 63, 751–762.
- Hecht, A., Strahl-Bolsinger, S., and Grunstein, M. (1996). Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature* 383, 92–96.
- Hoppe, G.J., Tanny, J.C., Rudner, A.D., Gerber, S.A., Danaie, S., Gygi, S.P., and Moazed, D. (2002). Steps in assembly of silent chromatin in yeast: Sir3-independent binding of a Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. *Mol. Cell. Biol.* 22, 4167–4180.
- Hughes, T.R., Marton, M.J., Jones, A.R., Roberts, C.J., Stoughton, R., Armour, C.D., Bennett, H.A., Coffey, E., Dai, H., He, Y.D., et al. (2000). Functional discovery via a compendium of expression profiles. *Cell* 102, 109–126.
- Imai, S., Armstrong, C.M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403, 795–800.
- Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. *Science* 293, 1074–1080.
- Johnson, L.M., Kayne, P.S., Kahn, E.S., and Grunstein, M. (1990). Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 87, 6286–6290.
- Kimura, A., Umehara, T., and Horikoshi, M. (2002). Chromosomal

- gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* **32**, 370–377.
- Krebs, J.E., Fry, C.J., Samuels, M.L., and Peterson, C.L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. *Cell* **102**, 587–598.
- Lacoste, N., Utle, R.T., Hunter, J.M., Poirier, G.G., and Cote, J. (2002). Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. *J. Biol. Chem.* **277**, 30421–30424.
- Landry, J., Sutton, A., Tafrov, S.T., Heller, R.C., Stebbins, J., Pillus, L., and Sternglanz, R. (2000). The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc. Natl. Acad. Sci. USA* **97**, 5807–5811.
- Larschan, E., and Winston, F. (2001). The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. *Genes Dev.* **15**, 1946–1956.
- Luo, K., Vega-Palas, M.A., and Grunstein, M. (2002). Rap1-Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. *Genes Dev.* **16**, 1528–1539.
- Maillet, L., Boscheron, C., Gotta, M., Marcand, S., Gilson, E., and Gasser, S.M. (1996). Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev.* **10**, 1796–1811.
- Moazed, D. (2001). Common themes in mechanisms of gene silencing. *Mol. Cell* **8**, 489–498.
- Ng, H.H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y., and Struhl, K. (2002). Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* **16**, 1518–1527.
- Nislow, C., Ray, E., and Pillus, L. (1997). SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. *Mol. Biol. Cell* **8**, 2421–2436.
- Noma, K., Allis, C.D., and Grewal, S.I. (2001). Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. *Science* **293**, 1150–1155.
- Oki, M., and Kamakaka, R.T. (2002). Blockers and barriers to transcription: competing activities? *Curr. Opin. Cell Biol.* **14**, 299–304.
- Plath, K., Mlynarczyk-Evans, S., Nusinow, D.A., and Panning, B. (2002). Xist RNA and the mechanism of X chromosome inactivation. *Annu. Rev. Genet.* **36**, 233–278.
- Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., and Bonner, W. (2002). Histone H2A variants H2AX and H2AZ. *Curr. Opin. Genet. Dev.* **12**, 162–169.
- Richards, E.J., and Elgin, S.C. (2002). Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell* **108**, 489–500.
- Roby, D., Suka, Y., Xenarios, I., Kurdistani, S.K., Wang, A., Suka, N., and Grunstein, M. (2002). Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell* **109**, 437–446.
- Rusche, L.N., Kirchmaier, A.L., and Rine, J. (2002). Ordered nucleation and spreading of silenced chromatin in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **13**, 2207–2222.
- Santisteban, M.S., Kalashnikova, T., and Smith, M.M. (2000). Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. *Cell* **103**, 411–422.
- Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411.
- Singh, J., and Klar, A.J. (1992). Active genes in budding yeast display enhanced in vivo accessibility to foreign DNA methylases: a novel in vivo probe for chromatin structure of yeast. *Genes Dev.* **6**, 186–196.
- Smith, J.S., Brachmann, C.B., Pillus, L., and Boeke, J.D. (1998). Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. *Genetics* **149**, 1205–1219.
- Smith, J.S., Brachmann, C.B., Celic, I., Kenna, M.A., Muhammad, S., Starai, V.J., Avalos, J.L., Escalante-Semerena, J.C., Grubmeyer, C., Wolberger, C., and Boeke, J.D. (2000). A phylogenetically conserved NAD⁺-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl. Acad. Sci. USA* **97**, 6658–6663.
- Strahl-Bolsinger, S., Hecht, A., Luo, K., and Grunstein, M. (1997). SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev.* **11**, 83–93.
- Suka, N., Luo, K., and Grunstein, M. (2002). Sir2p and Sas2p oppositely regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* **32**, 378–383.
- Turner, B.M. (2002). Cellular memory and the histone code. *Cell* **111**, 285–291.
- Tusher, V.G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* **98**, 5116–5121.
- van Leeuwen, F., Gafken, P.R., and Gottschling, D.E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109**, 745–756.
- Wyrick, J.J., Holstege, F.C., Jennings, E.G., Causton, H.C., Shore, D., Grunstein, M., Lander, E.S., and Young, R.A. (1999). Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature* **402**, 418–421.