Genome-wide, as opposed to local, antisilencing is mediated redundantly by the euchromatic factors Set1 and H2A.Z

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In *Saccharomyces cerevisiae*, several nonessential mechanisms including histone variant H2A.Z deposition and transcription-associated histone H3 methylation antagonize the local spread of Sir-dependent silent chromatin into adjacent euchromatic regions. However, it is unclear how and where these factors cooperate. To probe this question, we performed systematic genetic array screens for gene deletions that cause a synthetic growth defect in an *htz1Δ* mutant but not in an *htz1Δ sir3Δ* double mutant. Of the four genes identified, three, *SET1*, *SWD1*, and *SWD3*, encode components of the Set1 complex, which catalyzes the methylation of histone H3 on lysine 4 (H3-K4), a highly conserved modification that occurs in the coding sequences of transcribed genes. Using microarray-based transcriptional profiling, we find that H2A.Z and Set1 cooperate to prevent Sir-dependent repression of a large number of genes located across the genome, rather than the local effects reported previously for the individual mechanisms. This global, redundant function appears to be direct: using a DamID chromatin profiling method, we demonstrate ectopic association of Sir3 and Sir4 in *htz1Δ set1Δ* mutants at loci distant from silent chromatin domains. Antisilencing mechanisms may therefore cooperate to play a considerably broader role in regulating genome-wide transcription than previously thought.

histone methylation | silencing | Sir2 | histone variant

Eukaryotic genomes are organized into transcriptionally permissive euchromatin and silent heterochromatin. In *Saccharomyces cerevisiae*, heterochromatin is formed at telomeres and the silent mating-type cassettes through the action of the Sir2–Sir3–Sir4 complex (reviewed in ref. 1). Sir2 is a histone deacetylase; Sir3 and Sir4 are histone-binding proteins that preferentially bind the deacetylated N-terminal tails of histones H3 and H4. Sir proteins are recruited to chromatin by DNA sequences called silencer elements. From these sites, the Sir complex is thought to spread progressively along the chromatin fiber by a mechanism involving rounds of histone deacetylation by Sir2 and binding of the deacetylated histone tails by Sir3 and Sir4.

A number of highly conserved antisilencing factors have recently been defined, including H2A.Z, Sas2, and Dot1 (2–5). These proteins antagonize the "local spread" of Sir-mediated silencing from telomeric heterochromatin to neighboring euchromatic genes. The histone variant H2A.Z (encoded by *HTZ1*) is selectively deposited at the promoters of genes within euchromatin (6–9). The histone acetyltransferase Sas2 and histone methyltransferase Dot1 generate euchromatin-specific modifications at H4-K16 and H3-K79, respectively (3–5). In cells lacking either H2A.Z or Sas2, Sir proteins spread from telomeres, silencing genes located within 20–30 kb of telomeres (2–4). Similarly, loss of Dot1 results in increased binding of Sir proteins at subtelomeric Y′ elements with a concomitant decrease within telomeres (5).

Disruption of any one of the above mechanisms has only limited consequences on gene expression, raising the possibility that these antisilencing factors have redundant roles. Presumably, if local spread of Sir proteins resulted in the silencing of even a single essential gene or sufficiently reduced transcription of multiple essential genes, then loss of H2A.Z, Sas2, or Dot1 should be lethal. However, *htz1Δ*, *sas2Δ*, and *dot1Δ* cells are viable and have only a mild growth defect (2, 10, 11). In this article, we describe our analysis of double-mutant phenotypes, which has revealed a genome-wide, as opposed to local, antisilencing function that is shared by H2A.Z and the histone methyltransferase Set1. In contrast to the local spread of Sir2–4 in *htz1Δ* cells, *set1Δ htz1Δ* cells exhibit a global redistribution of Sir proteins from telomeres to genes located across the genome. This results in widespread ectopic repression at sites >100 kb from heterochromatin, a phenomenon that has not been described previously. These results suggest that antisilencing mechanisms have a very broad scope, operating not only in the vicinity of heterochromatin, but also throughout euchromatin.

Results

Identification of the Set1 Complex in a Genetic Screen for Antisilencing Factors That Act Redundantly with H2A.Z. To identify pathways that operate in parallel with H2A.Z to inhibit Sir-mediated silencing, we used the synthetic genetic array (SGA) method (12) to perform a systematic screen for gene deletions that have a synthetic growth defect in an *htz1Δ* mutant but not in an *htz1Δ sir3Δ* double mutant (see *Materials and Methods*). Of the 45 synthetic interactions with the *HTZ1* deletion [supporting information (SI) Table 1], four were Sir3-dependent (Fig. 1*A*), one of which is between *HTZ1* and *SIF2*, which encodes a Sir4-interacting protein that antagonizes telomeric silencing (13). Notably, the remaining three interactions involved genes encoding members of the highly conserved Set1 complex, which methylates histone H3 on lysine 4 in euchromatin (14–20). The growth defect of the *set1Δ htz1Δ* mutant was also suppressed by deletion of *SIR2* or *SIR4* (Fig. 1*B*). This suppression is not due to differences in mating-type identity caused by derepression of the silent mating-type cassettes in a *sir* mutant (SI Fig. 6). These genetic data are consistent with a model in which the histone methyltransferase Set1 and H2A.Z play redundant roles in antagonizing Sir-dependent silencing.

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Abbreviations: DamID, dam identification; SGA, synthetic genetic analysis.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE4826).

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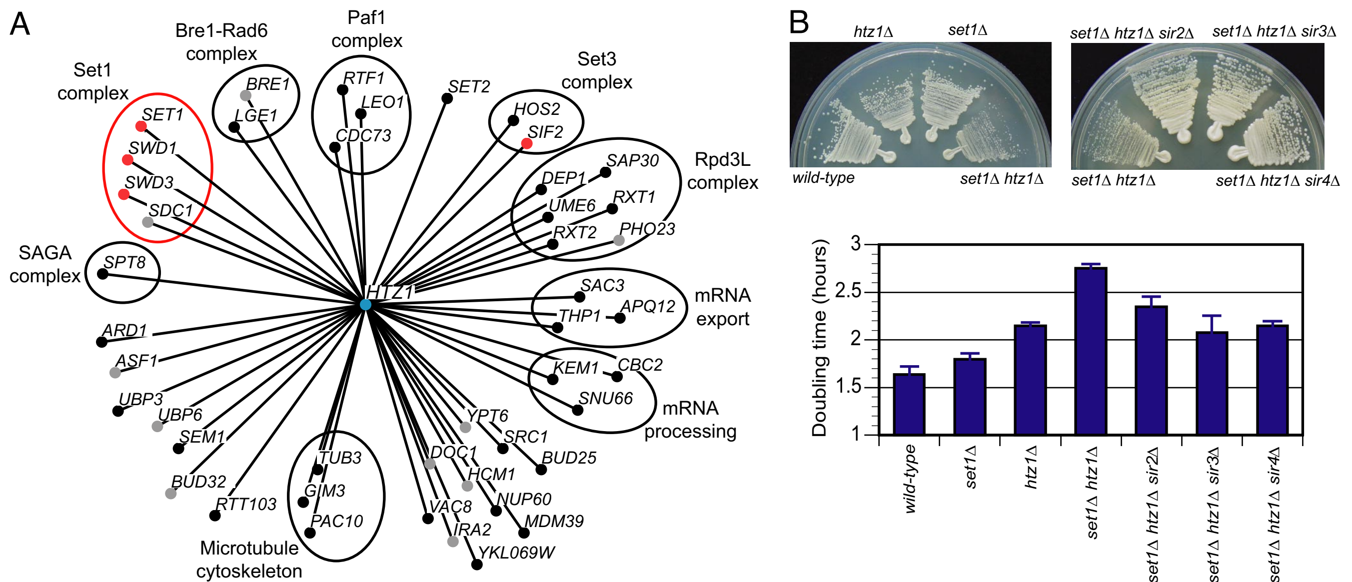

Fig. 1. Identification of the Set1 complex in a screen for antisilencing factors that cooperate with H2A.Z. (*A*) Results of an SGA screen for factors that act redundantly with H2A.Z (Htz1) to antagonize Sir3 activity. Each node represents a gene, and each line represents a synthetic genetic interaction between two genes. Forty-five gene deletions have a synthetic growth defect with a deletion of *HTZ1*. These genes are grouped according to membership in a complex or common function. The nodes are colored according to whether suppression of the corresponding genetic interaction by deletion of *SIR3* was observed (red), not observed (black), or not tested (gray). Of the four interactions that are *SIR3*-dependent, three represent members of the Set1 complex (circled in red). (*B*) Deletion of *SIR2*, *SIR3*, or *SIR4* rescues the slow-growth phenotype of a *set1*Δ *htz1*Δ mutant as seen by growth on plates (*Upper*) and in liquid cultures (graph, *Lower*).

***set1*Δ and *H3K4A* Cells Exhibit Increased Expression of Genes Near Telomeres.** How Set1 regulates Sir-mediated silencing remains unclear. *set1*Δ mutants exhibit decreased Sir3 association (21) and reduced gene silencing at telomeres (16, 22), suggesting that Set1 acts within heterochromatin to promote silencing. However, Set1-mediated H3-K4 methylation is enriched outside heterochromatin. One explanation for this discrepancy would be that Set1 promotes silencing through methylation of a novel heterochromatic target. If this were the case, robust silencing would require Set1 but not H3-K4 methylation. To test this hypothesis, we compared the expression of genes near telomeres in *set1*Δ and *H3K4A* mutants using spotted cDNA microarrays (see *Materials and Methods*). We observed increased expression for 200 and 157 genes in the *set1*Δ and *H3K4A* mutants, respectively; very few genes decreased in expression in either mutant. Genes up-regulated in a *set1*Δ mutant were enriched near telomeres, consistent with prior observations of the silencing defect (Fig. 2*A*). We observed a similar enrichment in an *H3-K4A* mutant (Fig. 2*B*), indicating that the role of Set1 in regulating silencing is mediated through H3-K4 methylation. This role is likely to be indirect, given that H3-K4 methylation is depleted from silent regions. The results described below are consistent with this role being an indirect consequence of the antisilencing function of Set1 across euchromatin (see *Discussion*).

***set1*Δ *htz1*Δ Cells Display a Genome-Wide Transcription Defect That Is Suppressed by Deletion of *SIR2*.** Our genetic data suggested that Set1 and H2A.Z have redundant roles in antagonizing Sir2 activity (Fig. 1*B*). To elucidate these roles, we profiled genome-wide transcription in *set1*Δ *htz1*Δ and *set1*Δ *htz1*Δ *sir2*Δ mutants. One thousand one hundred thirty-three genes are up-regulated

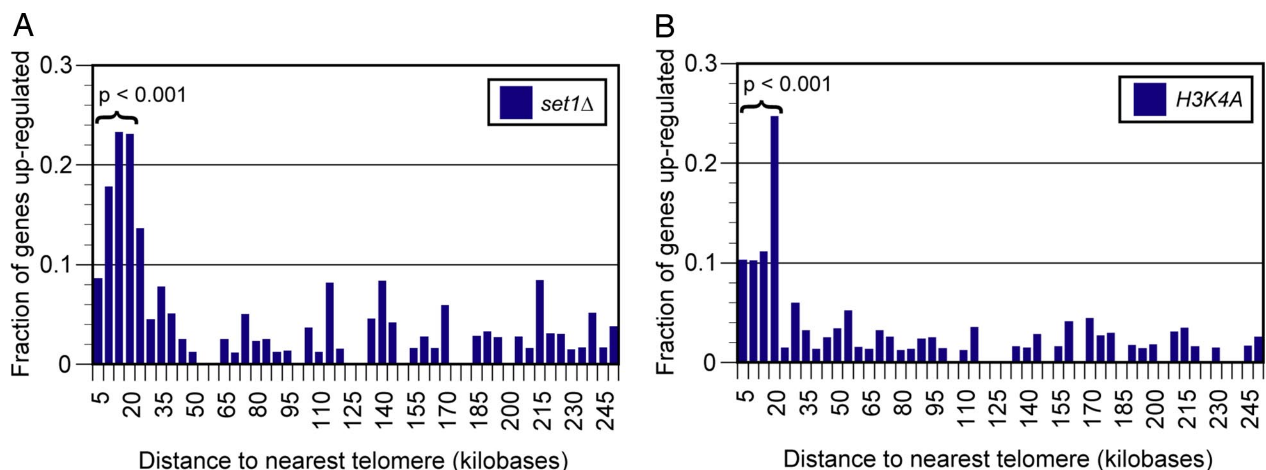

Fig. 2. Genes up-regulated in *set1*Δ and *H3K4A* cells are enriched near telomeres. Histograms, showing the fraction of genes in 5-kb intervals that are up-regulated in *set1*Δ and *H3K4A* mutants, are plotted as a function of their distance to the nearest telomere. The *P* values were calculated by using a χ^2 test.

Fig. 3. Transcriptional profiling of *set1*Δ *htz1*Δ and *set1*Δ *htz1*Δ *sir2*Δ mutants. (*A*) Telomere-proximal genes are preferentially up-regulated in a *set1*Δ *htz1*Δ mutant. Histogram of the fraction of genes up-regulated in a *set1*Δ *htz1*Δ mutant as a function of distance to the nearest telomere. (*B*) Deletion of *SIR2* suppresses the transcription defect of *set1*Δ *htz1*Δ cells. Shown is a color representation of the expression of 585 genes (≈10% of the genome) in *set1*Δ, *htz1*Δ, *set1*Δ *htz1*Δ, and *set1*Δ *htz1*Δ *sir2*Δ mutants relative to wild type. Each row indicates the change in expression of a single gene in each of the above mutants (columns). The colors indicate the average change in expression across four independent replicate experiments according to the scale shown. Hierarchical clustering of genes was performed by using Cluster 3.0, and the results were visualized by using Java Treeview software. These genes exhibit a reproducible expression defect in *set1*Δ *htz1*Δ cells but not in either *set1*Δ or *htz1*Δ cells. The expression of these genes is significantly higher in *set1*Δ *htz1*Δ *sir2*Δ cells compared with the *set1*Δ *htz1*Δ cells, indicating suppression of the *set1*Δ *htz1*Δ expression defect by deletion of *SIR2*.

in a *set1*Δ *htz1*Δ double mutant, with a strong enrichment for telomere-proximal genes (Fig. 3*A*). This phenotype is similar to that of a *set1*Δ single mutant, but much more severe, encompassing nearly half of all genes within 20 kb of telomeres. However, unlike *set1*Δ or *htz1*Δ single mutants (2), the *set1*Δ *htz1*Δ double mutant also exhibited a widespread, albeit quantitatively subtle, transcription defect, with 1,446 genes (24%) being down-regulated 1.4-fold on average. These changes in expression were consistent across four independent replicate experiments (Fig. 3*B*), controlled by using the Significance Analysis of Microarrays (SAM) statistical tool for false discovery (see *Materials and Methods*). Of these 1,446 genes, the expression level of 585 (40%) was significantly higher in *set1*Δ *htz1*Δ *sir2*Δ cells compared with *set1*Δ *htz1*Δ cells, indicating that deletion of *SIR2* suppresses a large part of the transcription defect in *set1*Δ *htz1*Δ cells (Fig. 3*B*). We observed no enrichment of these genes near the telomeres. Thus, in the absence of Set1 and H2A.Z, we observed Sir2-dependent repression of 10% of telomere-distal euchromatic genes. If this repression were direct, it might be expected to occur at clusters of adjacent genes, reflecting the regional nature of Sir-mediated silencing. This prediction was verified; using the Pyxis algorithm (23), we found statistically significant clusters distributed across the genome (SI Fig. 7).

The down-regulation of the remaining 60% (861) of genes in *set1*Δ *htz1*Δ cells appears not to be Sir2-dependent, suggesting additional redundant roles for these chromatin modifications in gene expression that remain to be explored. Nonetheless, the suppression of the *set1*Δ *htz1*Δ growth defect by genetic removal of the Sir complex from cells together with the microarray data makes it clear that promiscuous Sir-dependent repression is a prominent phenotype of cells that lack both H3-K4 methylation and H2A.Z.

Chromatin Association of Sir3 and Sir4 Is Increased at Telomere-Distal Euchromatic Genes in *set1*Δ *htz1*Δ Cells. Transcriptional profiling experiments suggested increased binding of the Sir complex at ectopic sites within euchromatin in the absence of Set1 and H2A.Z. To test this hypothesis, we measured the *in vivo* association of Sir3 and Sir4 to chromatin in wild-type and *set1*Δ *htz1*Δ cells. By using ChIP as described (2), Sir3 association at several telomere-distal euchromatic genes appeared to be at background levels, consistent with a previous report (21) and likely reflecting weak or transient Sir3 binding and/or inefficient cross-linking of Sir3 to DNA at telomere-distal loci.

To overcome this potential limitation of ChIP, we modified the DamID methodology established in *Drosophila melanogaster* to profile chromatin-associated proteins (24). We fused Sir3 and Sir4 to *Escherichia coli* dam (DNA adenine methyltransferase) in wild-type and *set1*Δ *htz1*Δ strains, resulting in dam-mediated DNA methylation at sites where Sir3 or Sir4 bind to chromatin (Fig. 4*A*). With no endogenous DNA methylation in *S. cerevisiae*, the percentage of cells methylated at a particular locus provides a read-out of the chromatin association of Sir3 or Sir4. We accounted for variations in chromatin accessibility using paired strains bearing fused and unfused dam (Fig. 4*A*) (24). A comparison of Sir3 enrichment values obtained by ChIP and DamID at *HMR***a**, the adjoining gene *GIT1*, and two telomere-distal loci suggests that DamID can be more sensitive than ChIP (see *Materials and Methods* and SI Fig. 8).

For this analysis, we chose 38 genes that displayed reduced transcription in the *set1*Δ *htz1*Δ double mutant in a *SIR2*-dependent manner. Strikingly, at 11 of these genes, DamID experiments revealed increased association of Sir3 and Sir4 in *set1*Δ *htz1*Δ cells compared with wild-type cells (Fig. 4 *B* and *C*). We also probed 39 control genes that exhibit no detectable Set1-, H2A.Z- or Sir2-dependent transcriptional regulation. To avoid artifacts due to saturation effects (see below), we restricted our comparison to 17 of the 39 control genes that had methylation levels within the range of the above 11 genes. In the *set1*Δ *htz1*Δ mutant, Sir3 association was increased at these 17 genes; in contrast, the changes in Sir4 association were significantly lower compared with the 11 Sir2-repressed genes (SI Fig. 9). The broader distribution of Sir3 compared with Sir4 is reminiscent of an earlier observation of an extended subtelomeric domain of Sir3, but not Sir4, association in cells overexpressing *SIR3* (25). The correlation between concomitant binding of both Sir3 and Sir4 at euchromatic genes in *set1*Δ *htz1*Δ cells and ectopic Sir2-mediated repression argues that this repression is direct.

At some of the above 11 genes, the chromatin association of Sir3 or Sir4 was also elevated in *set1*Δ or *htz1*Δ single mutants (Fig. 4 *B* and *C*), suggesting that ectopic Sir binding, but not repression, can occur in the absence of either Set1 or H2A.Z

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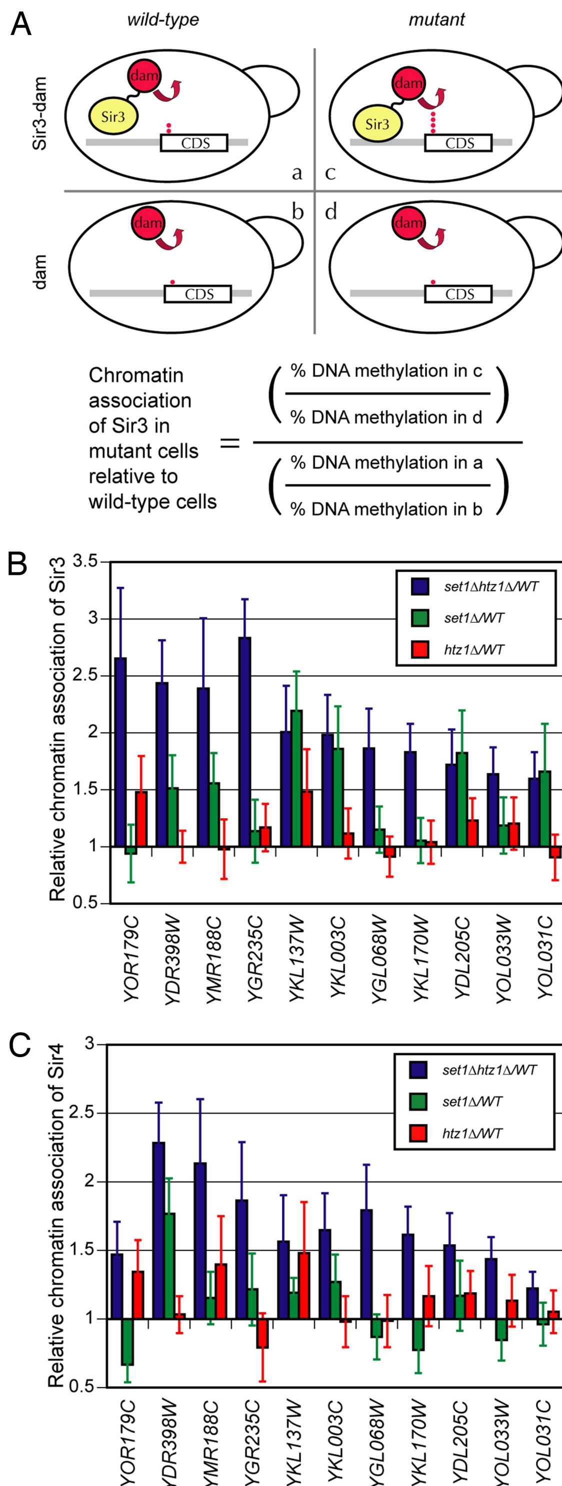

Fig. 4. DamID chromatin profiling of Sir3 and Sir4 in the *set1*Δ *htz1*Δ mutant. (*A*) Schematic showing the use of DamID to measure relative chromatin association of Sir3 in *set1*Δ *htz1*Δ cells compared with wild type. Expression of a functional fusion of Sir3 to *E. coli* dam results in local DNA methylation (red dots) at genes (rectangle), in a manner proportional to the extent of Sir3 binding to chromatin (gray). The percentage of cells methylated at a particular site in wild-type or mutant strains bearing Sir3-dam (or Sir4-dam) was normalized to that in corresponding strains bearing unfused dam to account for differences in chromatin accessibility. (*B* and *C*) Increased Sir3 and Sir4 binding at 11 telomere-distal euchromatic loci in the *set1*Δ *htz1*Δ mutant. The values on the *y* axis indicate the ratio of normalized DNA methylation in *set1*Δ *htz1*Δ cells to that in wild-type cells (average ± SEM, $n = 3$). Also shown is the relative chromatin association of Sir3 and Sir4 in *set1*Δ and *htz1*Δ cells.

alone. We did not detect increased Sir3 or Sir4 binding by DamID at 27 of the above 38 genes; however, these 27 genes consistently displayed a higher level of DNA methylation in wild-type strains bearing Sir3-dam, Sir4-dam, or unfused dam (SI Fig. 10 *A–D*). We observed a similar trend among the 39 control genes (SI Fig. 10 *E* and *F*). Our data suggest that, for genes with high levels of DNA methylation, DamID may not be sensitive to increased Sir3 or Sir4 binding due to saturation effects, consistent with the observations of Greil *et al.* (26). Therefore, we accounted for levels of DNA methylation in comparing Sir3 and Sir4 association at experimental and control genes, as described above. In summary, the above data indicate that, in the absence of both Set1 and H2A.Z, Sir3 and Sir4 bind to euchromatic genes far removed from telomeric heterochromatin (Fig. 5).

Discussion

In this article, we present evidence for a genome-wide antisilencing function shared by Set1 and H2A.Z. We identified the Set1 complex in an unbiased genetic screen for factors that act redundantly with histone variant H2A.Z to antagonize the activity of the Sir2–4 complex. We show that Set1 and H2A.Z function in parallel to prevent the global redistribution of silencing factors from telomeric heterochromatin to sites across euchromatin and this redundant function is important for cellular fitness.

Set1 and H2A.Z Act Redundantly to Antagonize the Global Redistribution of Sir2–4. Loss of both Set1 and H2A.Z (but not either alone) results in Sir2-dependent repression of nearly 10% of all genes (Fig. 3*B*), some of which occur within clusters across euchromatin (SI Fig. 7), consistent with the regional nature of Sir-mediated silencing. We observed a concomitant increase in Sir3 and Sir4 binding to a subset of these genes (Fig. 4 *B* and *C*). These changes are not due to increased expression of the Sir proteins; the levels of Sir3 and Sir4 protein are unaffected in *set1*Δ *htz1*Δ cells; Sir2 protein levels are decreased 3-fold (SI Fig. 11). Together, these results indicate that Set1 and H2A.Z share a genome-wide antisilencing function that prevents ectopic silencing of genes across euchromatin. In contrast, ectopic silencing in *htz1*Δ cells is localized near telomeres and the silent mating cassette *HMR***a** (2). Similarly, *set1*Δ cells have been shown to display silencing of two genes adjacent to telomeres (21). Using published data (9, 14), we determined that genes antisilenced redundantly by Set1 and H2A.Z are associated with slightly higher (10%) H3-K4 methylation in wild-type cells (SI Table 2). Although these genes do exhibit modestly higher levels of H2A.Z, the same is true for genes down-regulated in *set1*Δ *htz1*Δ cells in a Sir2-independent manner. Thus the levels of these chromatin modifications do not appear to be strong predictors of sites of global antisilencing.

Genes up-regulated in *H3 K4A*, *set1*Δ, and *set1*Δ *htz1*Δ cells are enriched near telomeres and overlap significantly with genes derepressed in a *sir2*Δ mutant ($P < 1 \times 10^{-16}$, $P < 3 \times 10^{-12}$, and $P < 2 \times 10^{-22}$, respectively) (2). Moreover, previous reports indicate that *set1*Δ mutants exhibit reduced silencing of reporter genes placed adjacent to telomeres (16, 22). These observations indicate that, in addition to preventing ectopic silencing in euchromatin, Set1 and H3-K4 methylation are required for robust silencing in telomeric heterochromatin. Because the bulk of H3-K4 methylation is found outside heterochromatin (14), it is likely that Set1 functions in euchromatin to exclude Sir proteins rather than in heterochromatin to promote silencing, as proposed by Santos-Rosa *et al.* (21). Our analysis indicates that the scope of Sir-mediated silencing and Set1- and H2A.Z-mediated antisilencing is considerably wider than has been observed previously, extending >100 kb beyond the boundaries of native heterochromatin.

According to our model (Fig. 5), Sir proteins can bind to

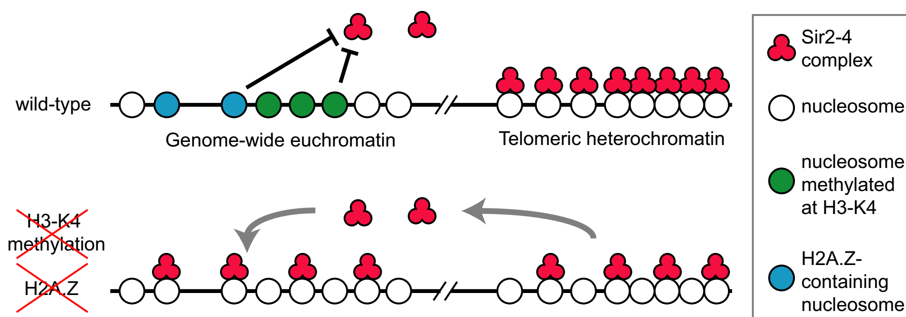

Fig. 5. Model of genome-wide antisilencing mediated redundantly by H2A.Z and Set1. In wild-type cells, nucleosomes containing H2A.Z (blue) and methylated on H3-K4 (green) by Set1 antagonize the binding of the Sir2–4 complex (red) across euchromatin. Disruption of both these antisilencing pathways in *set1*Δ *htz1*Δ cells results in redistribution of Sir proteins from telomeric heterochromatin to ectopic sites across the genome.

chromatin far removed from telomeres, although this binding appears to be much weaker than that within and adjacent to telomeres. Either H2A.Z or H3-K4 methylation alone is sufficient to prevent this weaker binding. Chromatin lacking both H3-K4 methylation and H2A.Z has a higher affinity for Sir3 and Sir4, resulting in ectopic binding of the Sir complex in *set1*Δ *htz1*Δ cells at sites across the genome. This global redistribution of a limited pool of Sir2–4 to 10% of euchromatin would be expected to result in (*i*) weak silencing at euchromatic loci, (*ii*) depletion of Sir proteins from telomeres, and (*iii*) dramatically reduced telomeric silencing. Indeed, the fact that Sir3 is undetectable at euchromatic loci by ChIP suggests that, compared with Sir protein association at sites near and within telomeres, ectopic Sir binding is much weaker and/or transient or occurs at a given gene only in a fraction of all cells. In addition, loss of Sir proteins from telomeres would explain the silencing defect in *set1*Δ and *set1*Δ *htz1*Δ mutants discussed above. This competition model is also consistent with observed competition between rDNA and telomeres for the limited pool of Sir2 protein (27).

There is evidence for multiple mechanisms by which H3-K4 methylation could inhibit the chromatin association of Sir3 and Sir4 *in vivo*. These include direct inhibition by the methylated lysine residue of binding between Sir3 and the H3 tail (21) and inhibition through recruitment of effector molecules such as the nucleosome-remodeling enzymes Chd1 (28) and Isw1 (29) and the histone acetyltransferase (HAT) complexes NuA3 (30, 31) and NuA4 (31). Additional support for histone acetylation as an effector mechanism comes from the parallels between *set1*Δ *htz1*Δ cells and *gcn5*Δ *elp3*Δ cells (32). The latter, lacking the HATs Gcn5 and Elp3, exhibit a Sir-dependent slow-growth phenotype and local spreading of Sir3 from telomeres into telomere-proximal chromatin.

Why Have Redundant Antisilencing Mechanisms? The distribution of euchromatic modifications along chromatin is dynamic, especially during transcription and DNA replication. For instance, gene activation results in removal of H2A.Z from promoters and deposition of H3-K4 methylation within coding sequences. Were antisilencing to be mediated by one euchromatic mark alone, transient removal of this mark could render the gene more susceptible to deacetylation and silencing by the Sir complex. Redundancy between antisilencing mechanisms might, in this way, buffer gene transcription from the transient changes in the euchromatic landscape. Finally, given that the pools of Sir proteins are limiting, preventing their titration by ectopic binding sites could also promote efficient and stable silencing, which may be important for the fidelity of cellular processes that depend on robust silencing such as mating-type switching.

Materials and Methods

***S. cerevisiae* Strains.** Strains used in this study are listed in SI Table 3. For all experiments, cells were grown on YPD medium (Qbiogene, Irvine, CA) supplemented with tryptophan and adenine, solidified with agar for the plate assays.

SGA Analysis. An *htz1*Δ strain was mated to each of the ≈5,000 mutants in the *S. cerevisiae* gene-deletion library by using a colony-arraying robot (12). Upon diploid selection, sporulation, and germination, the double-mutant haploid progeny were selected by growth on appropriate media and scored for slow growth based on colony size. This screen was performed three times, yielding 67 genes that were scored positive in at least two of the three trials. We were able to confirm 45 of the 67 interactions by tetrad dissection. To test for suppression by *sir3*Δ, we deleted one copy of *SIR3* in the corresponding diploids, performed tetrad analysis, and compared the sizes of double- and triple-mutant colonies.

Transcriptional Profiling. Exponentially growing paired cultures of wild-type and mutant strains, grown in liquid YPD medium supplemented with tryptophan and adenine, were rapidly harvested at 0.7 OD_{600}. Total RNA was extracted by using the hot phenol method as described (http://derisilab.ucsf.edu/microarray/protocols.html), followed by mRNA purification using the OligoTex kit (Qiagen, Valencia, CA). mRNA (*set1*Δ, *set1*Δ *htz1*Δ, and *set1*Δ *htz1*Δ *sir2*Δ) or total RNA (*H3K4A*) was reverse-transcribed into cDNA, which was labeled with Cy3 or Cy5 dyes and hybridized to microarrays representing all annotated ORFs in *S. cerevisiae* (33). After washing, the fluorescent intensity of spots on the microarrays were quantitated by using an Axon Genepix 4000A/B scanner and Genepix 3.0 software. We removed spots of poor quality or low signal and normalized the mutant-to-wild type mRNA ratios, such that the average ratio of all genes in each experiment was 1. Thus, each ratio reflects the change (between mutant and wild-type cells) in the mRNA level of any given gene relative to the average of all genes. Statistical analysis was performed on data from four independent replicate experiments by using significance analysis of microarrays (SAM) software with a 10% false-detection rate. The data for these experiments have been deposited into the GEO database (accession no. GSE4826).

DamID Chromatin Profiling. Translational fusions of *E. coli* dam to the C termini of Sir3 and Sir4 were expressed from the chromosomal *SIR3* and *SIR4* loci under the control of the corresponding native promoters. We verified that the fusions did not disrupt Sir activity using two assays, a quantitative mating assay and growth rate measurements (SI Table 4). In addition, we expressed unfused dam from the chromosomal *SIR4* promoter.

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Wild-type and mutant strains bearing these constructs were grown in liquid YPD medium to 0.5–1.0 OD_{600}. Genomic DNA was prepared from 5 ml of yeast culture by mechanical disruption in 25 mM Tris (pH 8), 25 mM EDTA, 200 mM NaCl, and 0.4% SDS, followed by Proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation. One 50th of this DNA was digested overnight with 40 units of DpnII (NEB, Ipswich, MA). The percent undigested DNA (i.e., percent DNA methylation) was determined by real-time PCR quantitation of digested and undigested control DNA. For each gene, a single GATC site located in a 300-bp region centered on the start codon was probed by using the primers listed in SI Table 5. To account for variations in chromatin accessibility, we normalized the percent DNA methylation in strains bearing Sir3-dam (or Sir4-dam) by that in corresponding strains expressing unfused dam. Using this assay, we observed high levels of methylation within (*HMR***a**) and adjacent to (*GIT1*), a silenced region where high levels of Sir3 are detectable by ChIP (SI Fig. 8). On the other hand, we obtained lower, although above-background, levels of methylation at euchromatic loci, where Sir3 binding appears to be at background levels by ChIP. This suggests that DamID may be more sensitive than ChIP for profiling chromatin-associated proteins.

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