DOI: 10.1534/genetics.104.026674

Redundant Roles for Histone H3 N-Terminal Lysine Residues in Subtelomeric Gene Repression in *Saccharomyces cerevisiae*

Amy M. Martin, Derek J. Pouchnik, Jennifer L. Walker and John J. Wyrick[1]

School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-4660

Manuscript received January 21, 2004
Accepted for publication April 2, 2004

ABSTRACT

The transcription of genes located in subtelomeric regions of yeast chromosomes is repressed relative to the rest of the genome. This repression requires wild-type nucleosome levels but not the telomere silencing factors Sir2, Sir3, Sir4, and Rap1. Subtelomeric heterochromatin is characterized by the absence of acetylation or methylation of histone H3 lysine residues, but it is not known whether histone H3 hypoacetylation or hypomethylation is a prerequisite for the establishment of subtelomeric heterochromatin. We have systematically mutated the N-terminal tails of histone H3 and H4 in *Saccharomyces cerevisiae* and characterized the effects each mutant has on genome-wide expression. Our results show that subtelomeric transcriptional repression is dependent on the histone H3 N-terminal domain, but not the histone H4 N-terminal domain. Mutating lysine-4, lysine-9, lysine-14, lysine-18, lysine-23, and lysine-27 to glycine in histone H3 is also sufficient to significantly reduce subtelomeric gene repression. Individual histone H3 lysine mutations, however, have little effect on subtelomeric gene repression or genome-wide expression, indicating that these six lysine residues have redundant functions. We propose that acetylation and methylation of histone H3 N-terminal lysine residues act as redundant mechanisms to demarcate regions of euchromatin from heterochromatin.

THE packaging of DNA with histones into nucleosomes and higher-order chromatin structures has profound effects on transcription initiation (Horn and Peterson 2002; Narlikar *et al.* 2002; Grewal and Moazed 2003). Genomic chromatin structure is organized into regions of heterochromatin and euchromatin by a cadre of chromatin-modifying enzymes. Heterochromatin is characterized by a compact chromatin structure that is inaccessible to DNA-binding proteins (Grewal 2000), including components of the transcription machinery. Genes located in heterochromatin are transcriptionally silenced, irrespective of their own promoter sequences (Moazed 2001). Heterochromatin is also distinguished by distinct patterns of post-translational histone modifications (Jenuwein and Allis 2001; Richards and Elgin 2002). Each of the four core histone proteins (H2A, H2B, H3, and H4) is post-translationally modified at multiple residues *in vivo*. These modifications include acetylation of lysine residues, methylation of arginine and lysine residues, phosphorylation of serine residues, and ubiquitination of lysine residues (Iizuka and Smith 2003).

The link between histone modifications and heterochromatin has been best established at yeast telomeres. Sir2, Sir3, and Sir4 are recruited to telomeres by the telomere-binding proteins Rap1 and yeast Ku (Lustig 1998; Luo *et al.* 2002). Sir2 is a NAD-dependent histone deacetylase (HDAC) that deacetylates lysine-16 of histone H4, among other residues (Imai *et al.* 2000; Landry *et al.* 2000). Since Sir3 binds preferentially to unacetylated H4 lysine-16 (Carmen *et al.* 2002), the Sir2 HDAC activity allows the Sir complex to spread along chromatin up to 6–8 kb away from the telomere (Kimura *et al.* 2002; Suka *et al.* 2002). Sir complex spreading is opposed by the Sas2 and, to a lesser extent, the Esa1 histone acetyltransferases (HATs; Kimura *et al.* 2002; Suka *et al.* 2002). These HATs acetylate lysine-16 of H4 in euchromatin, so as to make Sir3 binding unfavorable.

Recent functional genomics studies have identified a novel heterochromatin domain adjacent to telomeres in *Saccharomyces cerevisiae*. These subtelomeric or HAST (Hda1-affected subtelomeric) domains extend 10–25 kb from the telomere end (Robyr *et al.* 2002). Genes located in these domains are transcriptionally repressed when yeast are grown in standard glucose media. DNA microarray analysis has revealed that the repression of subtelomeric genes is dependent upon local nucleosome structure, but is independent of Sir2, Sir3, Sir4, or Rap1 function (Wyrick *et al.* 1999). Acetylation microarray experiments have determined that these subtelomeric domains are characterized by hypoacetylation of histone H3 residues through the activity of the Hda1 HDAC (Bernstein *et al.* 2002; Robyr *et al.* 2002). However, deletion of *HDA1* has a small but significant effect on subtelomeric gene repression (Bernstein *et al.* 2000;

[1]*Corresponding author:* School of Molecular Biosciences, Washington State University, Fulmer Hall 675, Pullman, WA 99164-4660. E-mail: jwyrick@wsu.edu

TABLE 1

List of yeast strains and genotypes used in array experiments

No.	Strain	Experiment	Genotype
1	RMY430	Histone H3 Δ(4-30)	Isogenic to RMY200, plus pRM430 *(CEN TRP1 HHF2 hht2Δ4-30)*
2	RMY200	Wild type for no. 1	*MAT***a** *ade2-101 his3Δ200 lys2-801 trp1Δ901 ura3-52 hht1,hhf1::LEU2 hht2,hhf2::HIS3* plus pRM200 *(CEN TRP1 HHF2 HHT2)*
3	WY122	Histone H3 K9,14,18,23G	Isogenic to WY121, plus pJL002 *(CEN URA3 HHF2 hht2-K9,14,18,23G)*
4	WY123	Histone H3 K27G	Isogenic to WY121, plus pJL003 *(CEN URA3 HHF2 hht2-K27G)*
5	WY124	Histone H3 K9,14,18,23,27G	Isogenic to WY121, plus pJL004 *(CEN URA3 HHF2 hht2-K9,14,18,23,27G)*
6	WY128	Histone H3 K4,9,14,18,23,27G	Isogenic to WY121, plus pJL008 *(CEN URA3 HHF2 hht2-K4,9,14,18,23,27G)*
7	WY121	Wild type for nos. 3–6	Isogenic to RMY200, plus pJL001 *(CEN URA3 HHF2 HHT2)*
8	WY140	Histone H3 K4G	Isogenic to WY121, plus pJW029 *(CEN ADE2 HHF2 hht2-K4G)*
9	WY141	Histone H3 K4,9,14,18,23G	Isogenic to WY121, plus pJW030 *(CEN, ADE2, HHF2, hht2-K4,9,14,18,23G)*
10	WY139	Wild type for nos. 8 and 9	Isogenic to WY121, plus pJW028 *(CEN ADE2 HHF2 HHT2)*
11	PKY503	Histone H4 K5,8,12,16G	Isogenic to PKY501 plus *(CEN URA3 hhf2-K5,8,12,16G)*
12	PKY501	Wild type for no. 10	*MAT***a** *ade2-101 his3-201 leu2-3,112 lys2-801 trp1-901 ura3-52 hhf1::HIS3 hhf2::LEU2* plus pPK301 *(CEN URA3 HHF2)*

Hughes *et al.* 2000), so it is unclear if there is a functional requirement for histone H3 hypoacetylation in subtelomeric heterochromatin.

Global analysis of histone H3 lysine-4 methylation patterns has shown that yeast telomeric and subtelomeric heterochromatin is also preferentially hypomethylated relative to euchromatin (Bernstein *et al.* 2002). Deletion of the Set1 histone methyltransferase, which is required for methylation of histone H3 lysine-4 (Briggs *et al.* 2001; Roguev *et al.* 2001), results in loss of silencing at the rDNA locus and at telomeres (Nislow *et al.* 1997; Briggs *et al.* 2001; Bryk *et al.* 2002; Krogan *et al.* 2002). However, a direct role for lysine-4 methylation in telomeric, subtelomeric, or rDNA silencing has yet to be established (Bernstein *et al.* 2002).

To determine the functional role of histone hypoacetylation and hypomethylation in subtelomeric gene repression, we have profiled the genome-wide expression changes due to mutations in the histone H3 and H4 N-terminal tails. We have chosen this strategy because genome-wide expression analysis is the best available assay of subtelomeric gene expression and because it has proven to be highly successful in advancing our knowledge of how transcription is regulated by histones (Wyrick *et al.* 1999) and other factors (Holstege *et al.* 1998; Bernstein *et al.* 2000; Lee *et al.* 2000; Wyrick and Young 2002). Our results indicate that acetylated and methylated lysine residues in histone H3 have redundant functions in repressing the transcription of both subtelomeric genes and genes located elsewhere in the genome.

MATERIALS AND METHODS

Strains, media, and growth conditions: A complete list of yeast strains is given in Table 1. For each genome-wide expression experiment, two mutant and two wild-type yeast cultures were grown in parallel to a final OD_{600} of 0.5–0.7 in yeast extract/peptone/dextrose (YPD) media and then harvested as described previously (Holstege *et al.* 1998).

Plasmid construction and site-directed mutagenesis: The wild-type histone H3 (*HHT2*) and H4 (*HHF2*) genes were PCR amplified from plasmid pRM200 (Mann and Grunstein 1992) using primers OJLW17For (AAACTCGAGGGATTGC TAGTAAAAACAACTGG) and OJLW17Rev (AAATCTAGAC AGTGACTCAAAATTCAAACG). The resulting 2.1-kb PCR fragment was digested with the *Xho*I and *Xba*I restriction enzymes (New England Biolabs, Beverly, MA) and cloned into the pRS316 shuttle vector (Sikorski and Hieter 1989) to give the plasmid pJL001. The wild-type histone H4 and mutant histone H3 (K9,14,18,23G) genes were PCR amplified using the same primers and cloned into the pRS316 vector to give plasmid pJL002.

The QuikChange kit (Stratagene, La Jolla, CA) was used to mutate histone H3 lysine-4 to glycine and lysine-27 to glycine in plasmids pJL001 and pJL002. Oligos OJLW1 (CTCCACAAT GGCCAGAACTGGACAAACAGCTAGAAAATCC) and OJLW7 (GGATTTTCTAGCTGTTTGTCCAGTTCTGGCCATTGTG GAG) were used to mutate lysine-4 to glycine. Oligos OJLW1C (CCAAGGCTGCCAGAGGATCCGCCCCATCTAC) and OJLW8 (GTAGATGGGGCGGATCCTCTGGCAGCCTTGG) were used to mutate lysine-27 to glycine in pJL001; oligos OJLW18 (CCG GTGCTGCCAGAGGATCCGCCCCATCTA) and OJLW9 (TAG ATGGGGCGGATCCTCTGGCAGCACCGG) were used to mutate lysine-27 to glycine in pJL002. Each mutation was confirmed by DNA sequencing using primers OJLW2 (GTTCTTTTATA TAGGACC) and OJLW3 (AGATTGAAATCTCAAGTC). A subset of the mutant histone H3 alleles were cloned into the pRS412 shuttle vector (Brachmann *et al.* 1998) using the *Xho*I and *Not*I restriction enzymes.

Genome-wide expression profiling: Total RNA was isolated from each yeast culture and used to prepare complementary DNA and biotin-cRNA following standard protocols (Affymetrix, Santa Clara, CA; Holstege *et al.* 1998). For the histone H3 Δ(4-30) and histone H4 K5,8,12,16G mutant and wild-type strains, the cRNA was hybridized to a set of four Ye6100 oligonucleotide arrays (Affymetrix) and scanned as described elsewhere (Wodicka *et al.* 1997). The cRNA from all other strains was hybridized to a single S98 genome oligonucleotide array and scanned following standard protocols. Intensities were captured using GeneChip software (Affymetrix) and a single raw expression level for each gene was determined. Complete data sets are available at http://wyrick.sbs.wsu.edu/histoneH3/.

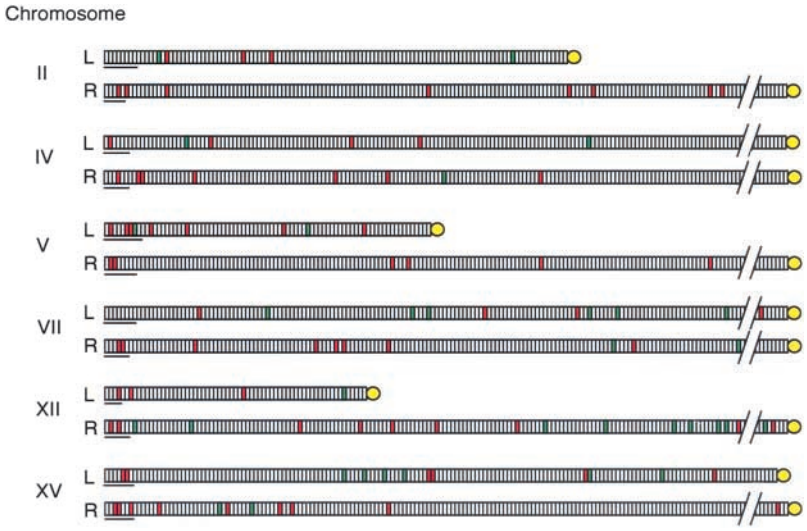

FIGURE 1.—Chromosome display reveals that upregulated genes in the histone H3 Δ(4-30) mutant are clustered in subtelomeric regions. A representative set of six yeast chromosomes is shown. Each box represents a gene. Upregulated genes are shown in red; downregulated genes are shown in green. The genes are ordered according to their normal positions along each chromosome in half chromosome segments, beginning at the telomere of the chromosome (left or right) and ending at the centromere (yellow circle). Genes located within 20 kb of a telomere end are underlined.

Data analysis: For the Ye6100 arrays, the data from each chip were normalized and corrected as described previously (HOLSTEGE *et al.* 1998). A change in mRNA levels was deemed significant on the basis of the following criteria: (1) the average fold change (up or down) was >3-fold, (2) the fold change (up or down) in each replicate experiment was >1.5-fold, and (3) the change in absolute intensity values was above background values in both comparisons. See WYRICK *et al.* (1999) for more details.

The data from each S98 array were normalized using the GeneChip software (Affymetrix), since no global changes in mRNA levels were detected in any of the experiments. The same criteria as above were used to identify genes with significant changes, except that the average fold change (up or down) was required to be more than twofold instead of threefold. The data from the S98 arrays were of a higher quality and reliability than the data from the Ye6100 arrays, allowing us to use this less stringent threshold. Analysis of S98 array data from four wild-type strains using these criteria revealed that only 1 gene of 6064 was identified as significantly changed, indicating a very low false-positive rate.

Telomere-proximal gene analysis and statistics: Genes within 40 kb of a telomere were pooled and ordered according to their distance from a telomere. For the Ye6100 array data [histone H3 Δ(4-30) and histone H4 K5,8,12,16G], genes with Affymetrix '_f' calls were excluded from the analysis, as described previously (WYRICK *et al.* 1999). A sliding 50-gene window was moved along the ordered gene list in 10-gene steps. The fraction of genes upregulated in each mutant and the average distance from the telomere were plotted for each 50-gene window. The statistical significance of the enrichment of upregulated genes in subtelomeric regions (10–20 kb from a telomere end) for each data set was calculated using the hypergeometric probability distribution.

Statistical analysis of comparisons of array data: A hypergeometric probability model was employed to determine the statistical significance of the overlap between the lists of upregulated genes in the various histone H3 mutants. The resultant *P*-values were numerically calculated using the Mathematica software package (Wolfram Research, Champaign, IL).

RESULTS

Histone H3 N-terminal domain is required for the repression of subtelomeric genes: Whole-genome Affymetrix oligonucleotide arrays were used to investigate whether the N-terminal domain of histone H3 was required for the repression of subtelomeric gene transcription in *S. cerevisiae*. Analysis of replicate samples revealed that the mRNA levels of 275 genes were upregulated and the mRNA levels of 185 genes were downregulated in the histone H3 Δ(4-30) mutant strain relative to wild type. Chromosome display of these data revealed that many of the upregulated genes clustered in subtelomeric chromosomal regions. Several representative chromosomes are shown in Figure 1; the effects on all chromosomes can be seen at http://wyrick.sbs.wsu.edu/histoneH3/. About 17% of genes located in subtelomeric regions showed increased mRNA levels in the histone H3 Δ(4-30) strain, compared to a genome-wide average of only 4% ($P = 5.0 \times 10^{-6}$). Subtelomeric regions were defined by a location of 10–20 kb from each telomere end. This region was chosen on the basis of previous studies that had shown that gene expression of this region was repressed by histones but was independent of Sir function (WYRICK *et al.* 1999). The choice of different boundaries for the subtelomeric region (*e.g.*, 8–20 kb or 1–25 kb) did not significantly change the results (data not shown).

The pattern of subtelomeric gene expression in the histone H3 Δ(4-30) mutant strain was compared to that found in a *sir2*Δ or a nucleosome-depleted strain (WYRICK *et al.* 1999). In Figure 2A, the fraction of genes that showed increased mRNA levels in each mutant strain was plotted *vs.* distance from a telomere end. The histone H3 Δ(4-30) mutant appears to have significant effects on the expression of genes up to 20 kb from a telomere end, similar to the effects seen in the nucleosome-depleted strain, although smaller in magnitude. Our analysis of the published data for the histone H3 Δ(1-28) mutant strain (SABET *et al.* 2003) showed similar results (A. MARTIN and J. WYRICK, unpublished data).

As a comparison, the pattern of subtelomeric gene expression in a histone H4 mutant strain (K5,8,12,16G) was analyzed in a similar manner in Figure 2B. The

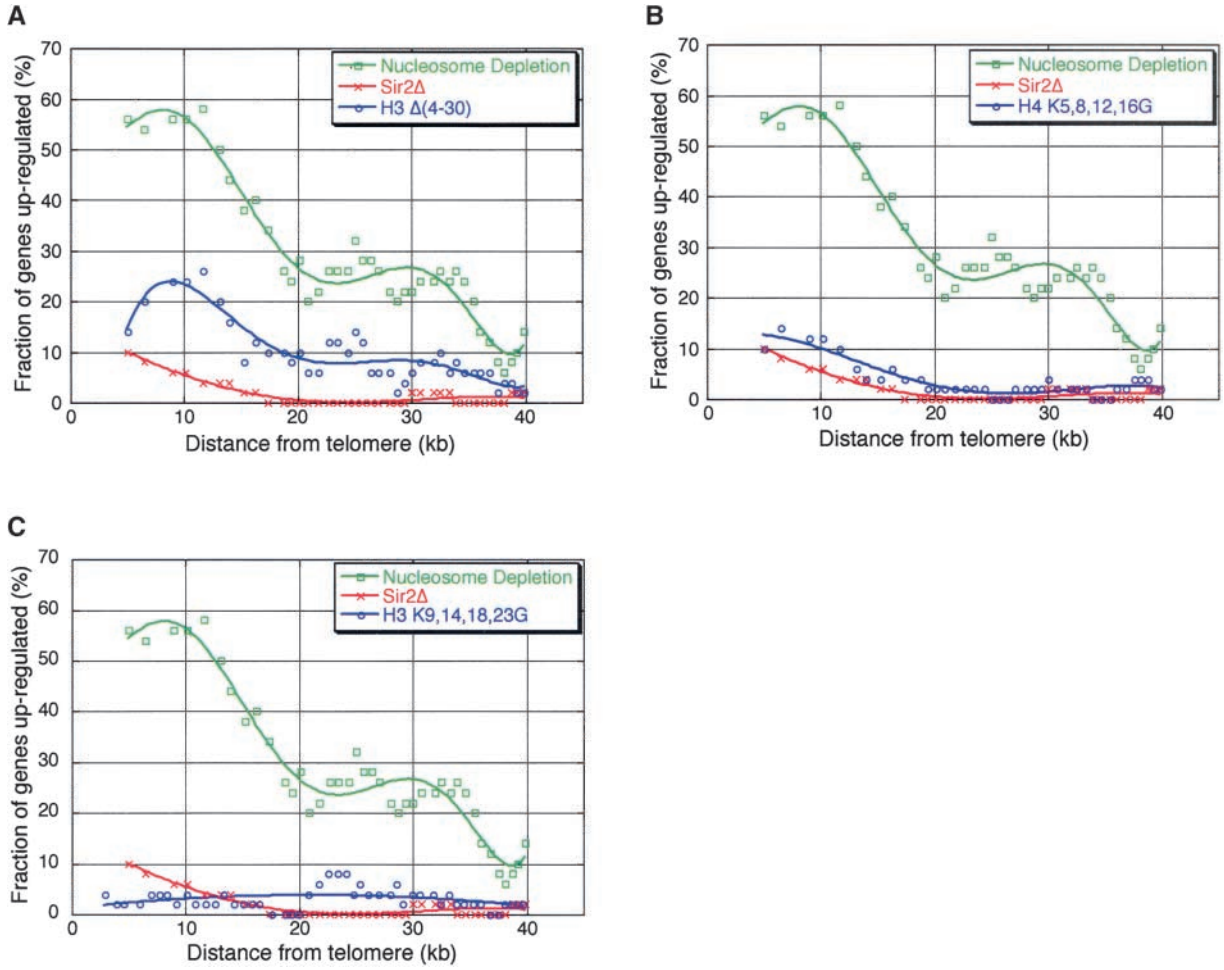

FIGURE 2.—Telomere distance plots of genome expression data for the (A) histone H3 Δ(4-30); (B) histone H4 K5,8,12,16G; and (C) histone H3 K9,14,18,23G mutant strains. Genes within 40 kb of a telomere were ordered as a function of their distance from a telomere. The fraction of genes that were upregulated in the mutant strain relative to wild type and the average distance from a telomere were plotted for each sliding 50-gene window. The genome expression data for the nucleosome depletion and the *sir2*Δ mutant strains (WYRICK *et al.* 1999) were included for comparison. Nucleosome depletion upregulates the expression of telomere-proximal and subtelomeric genes; *sir2*Δ upregulates the expression of telomere-proximal genes but not subtelomeric genes.

histone H4 mutant strain appears to have major effects on the expression of genes within 10 kb of a telomere end, similar to the effects seen in the *sir2*Δ strain. This result is consistent with the current model that Sir2 acts to silence telomere-proximal genes by deacetylating lysine-16 in histone H4. The histone H4 mutant did not have significant effects on subtelomeric gene expression.

These results indicate that subtelomeric gene repression requires the histone H3 N-terminal domain, but does not require the four acetylated lysine residues in the histone H4 N-terminal domain. Intriguingly, other studies have shown that lysine-9, lysine-14, and lysine-18 in the histone H3 N-terminal domain are preferentially hypoacetylated in subtelomeric heterochromatin (BERNSTEIN *et al.* 2002; ROBYR *et al.* 2002). This correlation suggests that hypoacetylated lysine residues in the N-terminal domain of histone H3 may be required for subtelomeric gene repression.

Genome-wide expression analysis of histone H3 lysine mutants: To test this hypothesis, oligonucleotide arrays were used to profile the genome expression changes in a histone H3 K9,14,18,23G mutant. Each of these lysine residues is acetylated in yeast (ROTH *et al.* 2001; SUKA *et al.* 2001), and mutation of all four lysine residues to glycine leads to loss of telomeric silencing in *S. cerevisiae* (THOMPSON *et al.* 1994). Analysis of replicate samples revealed that the mRNA levels of 44 genes were upregulated and the mRNA levels of 14 genes were downregulated in the histone H3 K9,14,18,23G mutant strain relative to wild type. Only two of the upregulated genes were located in the subtelomeric region. The overall pattern of subtelomeric gene expression was analyzed in Figure 2C. This analysis shows that the histone H3 K9,14,18,23G mutant does not have significant effects on subtelomeric gene expression, indicating that these four lysine residues alone are not required for the repression of subtelomeric genes.

Two other modified lysine residues are present in the histone H3 N-terminal tail. Lysine-27 has recently been shown to be acetylated by Gcn5 and Elp3 (SUKA *et al.*

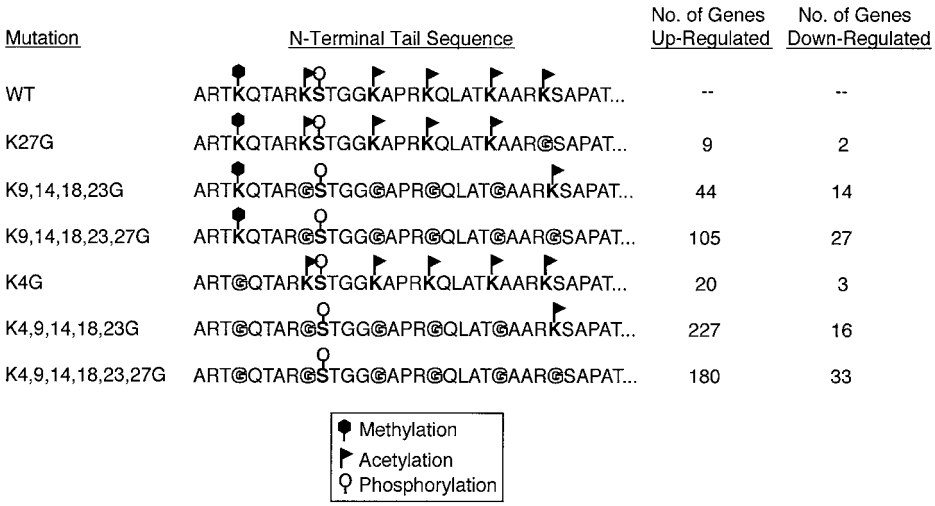

FIGURE 3.—Summary of genome-wide expression changes in each histone H3 mutant strain. Methylation, acetylation, and phosphorylation are indicated by hexagons, triangles, and circles, respectively. Modified residues are shown in boldface type and mutated residues are shown in outline type.

2001; KRISTJUHAN *et al.* 2003), and lysine-4 is methylated by Set1 (BRIGGS *et al.* 2001; ROGUEV *et al.* 2001). Whole-genome oligonucleotide arrays were used to examine the effects of mutating lysine-4 and lysine-27 to glycine, both singly and in combination with the K9,14,18,23G mutation. Figure 3 shows the complete list of mutations examined and the numbers of genes up- and downregulated in each mutant strain. To estimate the false-positive error rate in these data sets, data sets from four wild-type strains were analyzed in the same manner as the experimental data sets. Only 1 of 6064 genes represented on the oligonucleotide array was identified as changing significantly in the wild-type to wild-type comparisons, indicating a very low false-positive rate.

Overall, 312 genes were upregulated in at least one of the six mutant strains listed in Figure 3, and 62 genes were downregulated in at least one of the six mutant strains. The list of upregulated genes showed significant enrichment of genes involved in vitamin metabolism ($P = 2.1 \times 10^{-9}$), carbohydrate metabolism ($P = 1.7 \times 10^{-7}$), and carboxylic acid metabolism ($P = 7.4 \times 10^{-7}$). The list of downregulated genes showed significant enrichment of genes involved in pheromone response ($P = 2.0 \times 10^{-6}$).

Effects of histone H3 lysine mutants on subtelomeric gene expression: To determine what effect these mutations had on subtelomeric gene transcription, we calculated what fraction of genes located 10–20 kb from a telomere end were upregulated in each mutant strain. As shown in Table 2, genes upregulated by the histone H3 K4,9,14,18,23,27G are highly enriched in subtelomeric regions ($P = 8.1 \times 10^{-9}$), similar to the enrichment seen in the histone H3 Δ(4-30) mutant. The histone H3 K27G, K9,14,18,23,27G, and K4,9,14,18,23G mutants also showed enrichment in subtelomeric regions, although to a lesser extent. The histone H3 K4G and K9,14,18,23G mutants showed no enrichment for genes upregulated in subtelomeric regions.

To further test the observation that the histone H3 K4,9,14,18,23,27G mutant and the histone H3 Δ(4-30) mutant had similar effects on subtelomeric gene expression, the genome expression data for each of these mutants were compared using a telomere distance plot. As shown in Figure 4, the profile of upregulated genes as a function of distance from the telomere is similar for the histone H3 Δ(4-30) and K4,9,14,18,23,27G mutants. This analysis confirms the observation that mutating lysine-4 and lysine-27 in combination with lysine-9, lysine-14, lysine-18, and lysine-23 in histone H3 leads to an induction of subtelomeric gene expression that is similar to the effect of deleting amino acids 4–30 in histone H3.

Comparison of genome-wide expression profiles for histone H3 lysine mutants: To determine the contributions of the H3 lysine residues to genome-wide expression, we compared the sets of genes upregulated in each histone H3 mutant strain. As shown in Figure 5A, the sets of genes upregulated in the histone H3 K4G and K9,14,18,23G mutants are small subsets of the set of genes upregulated in the combined histone H3 K4,9,14,18,23G mutant. This analysis indicates that lysine-4 and one or more of lysine-9, -14, -18, and -23 have redundant roles in repressing the transcription of 181 genes. A similar pattern is seen in Figure 5B. The sets of genes upregulated in the histone

TABLE 2

Effects of histone H3 mutations on subtelomeric gene expression

	Fraction of genes upregulated (%)		
Histone H3 mutation	Subtelomeric[a]	Genome	*P*-value
K27G	2.5	0.1	5.5×10^{-4}
K9,14,18,23G	1.7	0.7	NS[b]
K9,14,18,23,27G	7.6	1.7	1.8×10^{-4}
K4G	0.0	0.3	NS
K4,9,14,18,23G	13.6	3.7	6.8×10^{-6}
K4,9,14,18,23,27G	15.3	3.0	8.1×10^{-9}

[a] Genes located 10–20 kb from a telomere end.
[b] NS, not significant ($P > 0.05$).

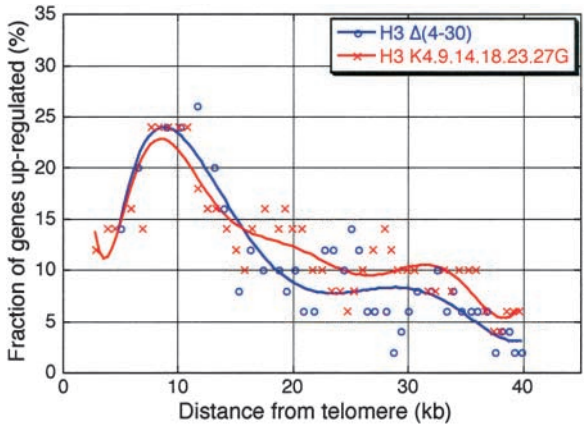

FIGURE 4.—The histone H3 K4,9,14,18,23,27G mutation and the histone H3 Δ(4-30) mutation have similar effects on subtelomeric gene expression. The fraction of genes upregulated in each mutant strain relative to wild type and the average distance of the genes from the telomere was plotted for each sliding 50-gene window.

H3 K27G and K9,14,18,23G mutants are primarily subsets of the genes upregulated in the combined histone H3 K9,14,18,23,27G mutant, indicating that lysine-27 and one or more of lysine-9, -14, -18, and -23 have redundant functions in repressing the transcription of 68 genes.

As shown in Figure 5C, there is a large degree of overlap between the genes upregulated in the histone H3 K4,9,14,18,23,27 and H3 K4,9,14,18,23G mutants. A total of 57% of the genes upregulated in the K4,9,14,18,23G mutant overlap with 72% of the genes upregulated in the K4,9,14,18,23,27G mutant ($P = 2.1 \times 10^{-161}$). The effect of mutating lysine-4 in combination with the other two sets of mutations is examined in Figure 5D. There is a highly significant degree of overlap ($P = 7.6 \times 10^{-75}$) between the sets of genes upregulated by the histone H3 K9,14,18,23,27G mutant and the histone H3 K4,9,14,18,23,27G mutant (64 genes). An additional 116 genes that are not affected in the histone H3 K9,14,18,23,27G mutant are upregulated in the histone H3 K4,9,14,18,23,27G mutant, showing (similar to Figure 5A) that lysine-4 and one or more of lysine-9, -14, -18, -23, and -27 have redundant functions in repressing the transcription of >100 genes.

DISCUSSION

Previous studies had demonstrated that lysine residues in the N-terminal domain of histone H3 are preferentially hypomethylated and hypoacetylated in subtelomeric heterochromatin (BERNSTEIN *et al.* 2002; ROBYR *et al.* 2002). We have now characterized the functional role of histone H3 lysine residues in regulating subtelomeric heterochromatin and genome-wide expression. First, we have shown that the histone H3 N-terminal domain is uniquely required for subtelomeric gene repression. Second, mutating H3 lysine-4, -9, -14, -18, -23, and -27 to glycine mimics the effect of the H3 N-terminal deletion on subtelomeric gene repression. Third, H3 lysine-4, -9, -14, -18, -23, and -27 have redundant functions in repressing the transcription of genes located in subtelomeric regions and elsewhere in the genome. These findings suggest a model for the role of histone H3 lysine residues in subtelomeric transcriptional repression, which is discussed later in this section.

Distinct roles for histone H3 and H4 in yeast heterochromatin: We find that the mRNA levels of telomere-proximal genes are induced by N-terminal mutations

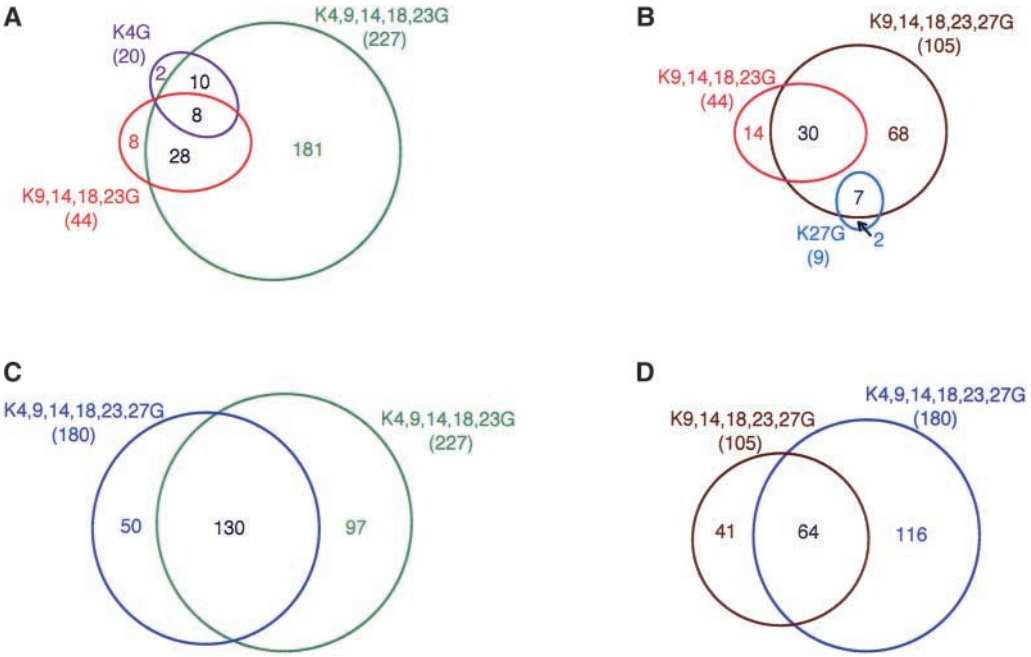

FIGURE 5.—Functionally redundant roles for histone H3 lysine residues in transcriptional regulation. Venn diagrams were used to compare the sets of upregulated genes among the following mutants: (A) histone H3 K4G; K9,14,18,23G; and K4,9,14,18,23G; (B) histone H3 K27G; K9,14,18,23G; and K9,14,18,23,27G; (C) histone H3 K4,9,14,18,23G and K4,9,14,18,23,27G; and (D) histone H3 K9,14,18,23,27 and K4,9,14,18,23,27G. The size of the circles is proportionate to the number of genes upregulated in each mutant strain. The degree of overlap between the two circles is proportionate to the number of genes that are upregulated in both mutant strains.

in histone H3 or H4, in accord with previous studies (Aparicio *et al.* 1991; Thompson *et al.* 1994). In contrast, we find that mRNA levels of subtelomeric genes are induced by N-terminal mutations in histone H3 but not by N-terminal mutations in histone H4. These findings indicate that the histone H3 N-terminal domain plays a unique role in the repression of genes located in subtelomeric heterochromatin.

These results, taken together with previous studies of the silent mating loci, reveal that *S. cerevisiae* employs a surprising diversity of mechanisms to silence the transcription of genes in heterochromatin. Transcriptional repression of the silent mating loci requires the N-terminal domain of histone H4 but not histone H3 (Kayne *et al.* 1988; Morgan *et al.* 1991; Mann and Grunstein 1992); repression of subtelomeric genes requires the N-terminal domain of histone H3 but not histone H4; and repression of telomere-proximal genes requires both histone H3 and H4, although histone H4 appears to play a more important role. Each of these distinct heterochromatin domains appears to require a specific pattern of histone modifications for regulating gene expression, as specified by the histone code hypothesis.

We have also examined the role of the N-terminal tails of histone H2A and H2B in subtelomeric gene repression. Our results indicate that deletion of the histone H2A N-terminal tail [H2A Δ(4-20)] leads to a small but significant effect on subtelomeric repression, while deletion of the histone H2B N-terminal tail [H2B Δ(3-32)] has no effect on subtelomeric repression (M. Parra and J. Wyrick, unpublished data). Interestingly, these results appear to mirror the effects seen on telomeric silencing for each of these N-terminal deletions (Thompson *et al.* 1994; Wyatt *et al.* 2003).

Genome-wide analysis of histone H3 lysine mutations: Our results indicate that mutating lysine-4, -9, -14, -18, -23, and -27 to glycine is sufficient to significantly reduce subtelomeric gene repression. The H3 K4,9,14,18,23,27G mutation also leads to the derepression of telomere-proximal genes, although not to the same extent as that seen for subtelomeric genes. Since the H3 K4,9,14,18,23,27G mutation does not affect the expression of any known silencing genes, it is likely that the effect on subtelomeric gene expression is a direct consequence of the histone H3 N-terminal mutations. Overall, the histone H3 K4,9,14,18,23,27G mutation resulted in an increase in the mRNA levels for 180 genes and a decrease in the mRNA levels for 33 genes, indicating that the six lysine residues in the histone H3 N-terminal domain function primarily to repress transcription. This finding is consistent with previously published microarray data for a histone H3 Δ(1-28) mutant strain (Sabet *et al.* 2003).

Lysine-4 appears to play a key role in subtelomeric gene repression. Only mutants that contained the K4G mutation showed maximal induction of subtelomeric gene expression and maximal induction of genome-wide expression. The histone H3 K4G mutation on its own, however, had no affect on subtelomeric gene expression and little affect on genome-wide expression. Only when lysine-4 was mutated in combination with lysine-9, -14, -18, and -23 was a significant effect seen on subtelomeric gene expression. These results indicate that lysine-4 and lysine-9, -14, -18, and -23 have redundant functions in repressing the transcription of genes located in subtelomeric regions and elsewhere in the genome.

Our data indicate that lysine-27 also plays an important role in subtelomeric heterochromatin. The histone H3 K27G mutation had a small but significant effect on subtelomeric gene expression. While the histone H3 lysine-27 residue is acetylated in yeast, in higher eukaryotes it is also methylated. Intriguingly, lysine-27 methylation has been shown to play a key role in X inactivation and heterochromatin formation in mammalian cells (Plath *et al.* 2003).

The histone H3 K9,14,18,23G mutation alone had little affect on subtelomeric or telomeric gene repression. This finding is at odds with a previous study that indicated that the H3 K9,14,18,23G mutation resulted in a substantial loss of telomeric silencing, as measured using a 5-fluoroorotic acid sensitivity assay (Thompson *et al.* 1994). Since the observed effect on telomeric silencing in the H3 K9,14,18,23G mutant strain was significantly less than the effects seen in a Sir2 deletion or histone H4 mutation (Thompson *et al.* 1994), it is possible that this silencing defect was not significant enough to be detected in the DNA array assay. In addition, the previous study measured the silencing effects for a single, artificially constructed telomere, while our results measured the effects on all 32 wild-type telomeres.

Functional redundancy between histone H3 lysine residues: Our data demonstrate that individual lysine-to-glycine mutations (such as K4G and K27G) had little effect on genome-wide expression, while combinations of lysine-to-glycine mutations (such as K4,9,14,18,23G and K9,14,18,23,27G) had large and synergistic effects on genome-wide expression. This observation is reminiscent of the signaling network model of chromatin (Schreiber and Bernstein 2002).

The signaling network model of chromatin argues that the behavior of post-translational histone modifications resembles that of receptor tyrosine kinase signaling (Schreiber and Bernstein 2002). A prediction of this model is that individual histone modifications would have redundant effects on transcription initiation. This prediction is based in part on a study by Grunstein and colleagues (Durrin *et al.* 1991), which showed that single lysine mutations in the N-terminal domain of histone H4 had little effect on *GAL1* transcription, while mutating three or all four acetylated lysine residues in histone H4 resulted in a significant decrease in *GAL1* transcription. Our data indicate that lysine residues in the histone H3 N-terminal tail act redundantly to repress gene tran-

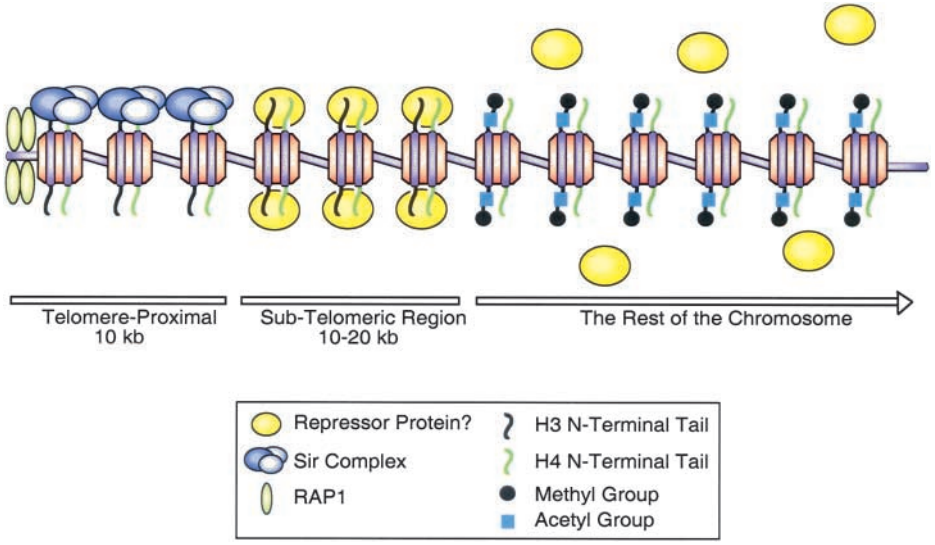

FIGURE 6.—Model for the regulation of subtelomeric gene expression. We propose that a repressor protein binds specifically to unmethylated and unacetylated lysine residues in the N-terminal tail of histone H3. Repressor binding is thereby restricted to subtelomeric regions and other chromosomal regions where histone H3 is both hypoacetylated and hypomethylated. The solid circle represents lysine-4 methylation and the blue box represents lysine-9, -14, -18, -23, and -27 acetylation.

scription and provide evidence in support of the signaling network model of chromatin.

A model for subtelomeric gene repression: In higher eukaryotes, methylation of lysine-9 and -27 in histone H3 has been linked to heterochromatin formation (LACHNER and JENUWEIN 2002; PLATH *et al.* 2003). *S. cerevisiae*, however, lacks these heterochromatin specific post-translational modifications. Our data indicate that lysine-4, -9, -14, -18, -23, and -27 in histone H3 are required for subtelomeric gene repression, yet none of these lysine residues is frequently post-translationally modified in subtelomeric heterochromatin. Lysine-4 is hypomethylated in subtelomeric heterochromatin and is hypermethylated in the coding regions of active genes (BERNSTEIN *et al.* 2002). The other histone H3 lysine residues are hypoacetylated in subtelomeric heterochromatin (BERNSTEIN *et al.* 2002; ROBYR *et al.* 2002), but are hyperacetylated in euchromatin, particularly in promoter regions (BERNSTEIN *et al.* 2002).

We propose that methylation and acetylation of histone H3 act as redundant mechanisms to demarcate regions of euchromatin from heterochromatin in *S. cerevisiae*. In this model, hypomethylated and hypoacetylated histone H3 lysine residues play a direct role in establishing repressive subtelomeric heterochromatin. While the mechanism for this repression is unknown, in Figure 6 we hypothesize that unacetylated and unmethylated lysine residues may function as binding sites for one or more repressor proteins. Methylation or acetylation of the target lysine residues would disrupt their interaction with the repressor proteins, just as acetylation of lysine-16 in histone H4 disrupts binding of Sir3 in telomeric heterochromatin (CARMEN *et al.* 2002; KIMURA *et al.* 2002; SUKA *et al.* 2002). For this reason, the repressor protein would be restricted to chromosomal regions in which histone H3 is hypomethylated and hypoacetylated (*e.g.*, subtelomeric regions). A similar model has been proposed to explain the role of histone H3 lysine-79 methylation in telomeric silencing (VAN LEEUWEN and GOTTSCHLING 2002).

Mutating lysine residues to glycine completely removes the lysine side-chain and thus should eliminate any potential interaction with a repressor protein. However, since each of the unacetylated and unmethylated lysine residues in the histone H3 N-terminal tail can serve as a potential binding site for the repressor protein, all of these lysine residues would need to be mutated to glycine to eliminate subtelomeric gene repression. This prediction of the model fits nicely with our array data, since maximal loss of subtelomeric repression is seen only when we mutate H3 lysine-4, -9, -14, -18, -23, and -27 to glycine, and single mutations, such as K4G, have no effect on subtelomeric repression.

This model also predicts that deletion of the Set1 histone methyltransferase would lead to the derepression of subtelomeric genes. In a *SET1* deletion strain, unmethylated lysine-4 would be present in euchromatin and would serve as a potential binding site for the repressor protein. According to the model, this would result in a redistribution of the repressor protein from subtelomeric regions to euchromatin, leading to the upregulation of subtelomeric genes, as has been reported (BERNSTEIN *et al.* 2002).

An alternative model is that the histone H3 K4,9,14, 18,23,27G mutation might disrupt subtelomeric heterochromatin by altering the electrostatic charge of the histone H3 N-terminal tail. Previous studies have suggested that positively charged lysine residues may play an important role in histone-DNA and histone-histone interactions (WOLFFE and HAYES 1999). Hence, disruption of these interactions through mutation of histone H3 lysine residues could directly disrupt subtelomeric heterochromatin. This model cannot completely ex-

plain our results since lysine-4 (which is methylated) is also required for depression of subtelomeric genes. Future studies will allow us to determine which of these models is correct and to determine the identity of the postulated repressor proteins. It will also be interesting to examine whether lysine-36 and lysine-79 in histone H3 play a role in subtelomeric gene repression.

We are grateful to Lisa Gloss, Michael Parra, Ray Reeves, and Michael Smerdon for helpful discussions and comments on the manuscript. We thank Michael Grunstein for the generous gift of yeast strains and plasmids. We thank Randall Morse for sharing data prior to publication. This project originated in Richard Young's laboratory and we thank him for his support, encouragement, and helpful suggestions. We thank R. Trace Sinclair and Monique Kohagura for software and web support. This work was supported by Research Scholar grant RSG-03-181-01-GMC from the American Cancer Society and by funds from an American Cancer Society Institutional Research grant.

LITERATURE CITED

Aparicio, O. M., B. L. Billington and D. E. Gottschling, 1991 Modifiers of position effect are shared between telomeric and silent mating-type loci in S. cerevisiae. Cell **66:** 1279–1287.

Bernstein, B. E., J. K. Tong and S. L. Schreiber, 2000 Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA **97:** 13708–13713.

Bernstein, B. E., E. L. Humphrey, R. L. Erlich, R. Schneider, P. Bouman *et al.*, 2002 Methylation of histone H3 Lys 4 in coding regions of active genes. Proc. Natl. Acad. Sci. USA **99:** 8695–8700.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Briggs, S. D., M. Bryk, B. D. Strahl, W. L. Cheung, J. K. Davie *et al.*, 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., S. D. Briggs, B. D. Strahl, M. J. Curcio, C. D. Allis *et al.*, 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., L. Milne and M. Grunstein, 2002 Acetylation of the yeast histone H4 N terminus regulates its binding to heterochromatin protein SIR3. J. Biol. Chem. **277:** 4778–4781.

Durrin, L. K., R. K. Mann, P. S. Kayne and M. Grunstein, 1991 Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. Cell **65:** 1023–1031.

Grewal, S. I., 2000 Transcriptional silencing in fission yeast. J. Cell. Physiol. **184:** 311–318.

Grewal, S. I., and D. Moazed, 2003 Heterochromatin and epigenetic control of gene expression. Science **301:** 798–802.

Holstege, F. C., E. G. Jennings, J. J. Wyrick, T. I. Lee, C. J. Hengartner *et al.*, 1998 Dissecting the regulatory circuitry of a eukaryotic genome. Cell **95:** 717–728.

Horn, P. J., and C. L. Peterson, 2002 Molecular biology. Chromatin higher order folding—wrapping up transcription. Science **297:** 1824–1827.

Hughes, T. R., M. J. Marton, A. R. Jones, C. J. Roberts, R. Stoughton *et al.*, 2000 Functional discovery via a compendium of expression profiles. Cell **102:** 109–126.

Iizuka, M., and M. M. Smith, 2003 Functional consequences of histone modifications. Curr. Opin. Genet. Dev. **13:** 154–160.

Imai, S., C. M. Armstrong, M. Kaeberlein and L. Guarente, 2000 Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:** 795–800.

Jenuwein, T., and C. D. Allis, 2001 Translating the histone code. Science **293:** 1074–1080.

Kayne, P. S., U. J. Kim, M. Han, J. R. Mullen, F. Yoshizaki *et al.*, 1988 Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell **55:** 27–39.

Kimura, A., T. Umehara and M. Horikoshi, 2002 Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. Nat. Genet. **32:** 370–377.

Kristjuhan, A., B. O. Wittschieben, J. Walker, D. Roberts, B. R. Cairns *et al.*, 2003 Spreading of Sir3 protein in cells with severe histone H3 hypoacetylation. Proc. Natl. Acad. Sci. USA **100:** 7551–7556.

Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider *et al.*, 2002 COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. **277:** 10753–10755.

Lachner, M., and T. Jenuwein, 2002 The many faces of histone lysine methylation. Curr. Opin. Cell Biol. **14:** 286–298.

Landry, J., A. Sutton, S. T. Tafrov, R. C. Heller, J. Stebbins *et al.*, 2000 The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA **97:** 5807–5811.

Lee, T. I., H. C. Causton, F. C. Holstege, W. C. Shen, N. Hannett *et al.*, 2000 Redundant roles for the TFIID and SAGA complexes in global transcription. Nature **405:** 701–704.

Luo, K., M. A. Vega-Palas and M. Grunstein, 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.

Lustig, A. J., 1998 Mechanisms of silencing in Saccharomyces cerevisiae. Curr. Opin. Genet. Dev. **8:** 233–239.

Mann, R. K., and M. Grunstein, 1992 Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J. **11:** 3297–3306.

Moazed, D., 2001 Common themes in mechanisms of gene silencing. Mol. Cell **8:** 489–498.

Morgan, B. A., B. A. Mittman and M. M. Smith, 1991 The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. Mol. Cell. Biol. **11:** 4111–4120.

Narlikar, G. J., H. Y. Fan and R. E. Kingston, 2002 Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:** 475–487.

Nislow, C., E. Ray and L. Pillus, 1997 SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol. Biol. Cell **8:** 2421–2436.

Plath, K., J. Fang, S. K. Mlynarczyk-Evans, R. Cao, K. A. Worringer *et al.*, 2003 Role of histone H3 lysine 27 methylation in X inactivation. Science **300:** 131–135.

Richards, E. J., and S. C. Elgin, 2002 Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. Cell **108:** 489–500.

Robyr, D., Y. Suka, I. Xenarios, S. K. Kurdistani, A. Wang *et al.*, 2002 Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. Cell **109:** 437–446.

Roguev, A., D. Schaft, A. Shevchenko, W. W. Pijnappel, M. Wilm *et al.*, 2001 The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J. **20:** 7137–7148.

Roth, S. Y., J. M. Denu and C. D. Allis, 2001 Histone acetyltransferases. Annu. Rev. Biochem. **70:** 81–120.

Sabet, N., F. Tong, J. P. Madigan, S. Volo, M. M. Smith *et al.*, 2003 Global and specific transcriptional repression by the histone H3 amino terminus in yeast. Proc. Natl. Acad. Sci. USA **100:** 4084–4089.

Schreiber, S. L., and B. E. Bernstein, 2002 Signaling network model of chromatin. Cell **111:** 771–778.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:** 19–27.

Suka, N., Y. Suka, A. A. Carmen, J. Wu and M. Grunstein, 2001 Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. Mol. Cell **8:** 473–479.

Suka, N., K. Luo and M. Grunstein, 2002 Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. Nat. Genet. **32:** 378–383.

Thompson, J. S., X. Ling and M. Grunstein, 1994 Histone H3

amino terminus is required for telomeric and silent mating locus repression in yeast. Nature **369:** 245–247.

van Leeuwen, F., and D. E. Gottschling, 2002 Genome-wide histone modifications: gaining specificity by preventing promiscuity. Curr. Opin. Cell Biol. **14:** 756–762.

Wodicka, L., H. Dong, M. Mittmann, M. H. Ho and D. J. Lockhart, 1997 Genome-wide expression monitoring in Saccharomyces cerevisiae. Nat. Biotechnol. **15:** 1359–1367.

Wolffe, A. P., and J. J. Hayes, 1999 Chromatin disruption and modification. Nucleic Acids Res. **27:** 711–720.

Wyatt, H. R., H. Liaw, G. R. Green and A. J. Lustig, 2003 Multiple roles for *Saccharomyces cerevisiae* histone H2A in telomere position effect, Spt phenotypes and double-strand-break repair. Genetics **164:** 47–64.

Wyrick, J. J., and R. A. Young, 2002 Deciphering gene expression regulatory networks. Curr. Opin. Genet. Dev. **12:** 130–136.

Wyrick, J. J., F. C. Holstege, E. G. Jennings, H. C. Causton, D. Shore *et al.*, 1999 Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. Nature **402:** 418–421.

Communicating editor: F. Winston