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Spt10 and Spt21 Are Required for Transcriptional Silencing in *Saccharomyces cerevisiae*▽†

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In *Saccharomyces cerevisiae*, transcriptional silencing occurs at three classes of genomic regions: near the telomeres, at the silent mating type loci, and within the ribosomal DNA (rDNA) repeats. In all three cases, silencing depends upon several factors, including specific types of histone modifications. In this work we have investigated the roles in silencing for Spt10 and Spt21, two proteins previously shown to control transcription of particular histone genes. Building on a recent study showing that Spt10 is required for telomeric silencing, our results show that in both *spt10* and *spt21* mutants, silencing is reduced near telomeres and at *HML*α, while it is increased at the rDNA. Both *spt10* and *spt21* mutations cause modest effects on Sir protein recruitment and histone modifications at telomeric regions, and they cause significant changes in chromatin structure, as judged by its accessibility to dam methylase. These silencing and chromatin changes are not seen upon deletion of *HTA2-HTB2*, the primary histone locus regulated by Spt10 and Spt21. These results suggest that Spt10 and Spt21 control silencing in *S. cerevisiae* by altering chromatin structure through roles beyond the control of histone gene expression.

Changes in chromatin state modulate gene expression. These changes can involve alterations in the composition of bound proteins, the positions or presence of nucleosomes, the array of covalent modifications on histones, or higher-order chromatin structure (reviewed in references 11, 41, and 60). In *Saccharomyces cerevisiae*, one form of specialized chromatin structure exists in silenced regions, where genomic regions are packaged into a repressive state characterized by hypoacetylated histone proteins (reviewed in references 17 and 59). In these regions, gene expression is silenced as a consequence of location, largely independent of promoter composition (59).

In *S. cerevisiae*, silencing occurs at three loci: subtelomeric regions (telomere position effect [TPE]), the silent mating type loci *HMR***a** and *HML*α, and the ribosomal DNA (rDNA) repeats (reviewed in references 7 and 59). Silencing at telomeres and silencing at the mating type loci show many similarities, including a requirement for the silent information regulator (Sir) proteins Sir2, Sir3, and Sir4. At the silent mating type loci, Sir1 plays an additional role in facilitating recruitment of the other Sir proteins, though it is not essential for silencing (54, 78). Silencing at the rDNA locus is unique in that Sir2 is the only Sir protein required for silencing. Furthermore, at the rDNA, *sir4*Δ mutants show an increase in silencing (66).

Silencing occurs in three steps: nucleation, spreading, and limitation of spreading. Nucleation is seeded by sequence-specific DNA binding complexes, including Rap1 at telomeres (45, 49) and Rap1, Abf1, and ORC at the silent mating type loci (44, 49, 78). These complexes recruit Sir2, Sir3, and Sir4. Sir complex binding is facilitated by hypoacetylation of the histone H3 and H4 tails, which is accomplished by the NAD^+-dependent histone deacetylase activity of Sir2 (32, 38, 39, 65). Once nucleated, the silenced region spreads through the iterative deacetylation of histones and recruitment of additional SIR complexes, leading to the formation of broad regions of silenced chromatin (reviewed in reference 59). *O*-Acetyl-ADP-ribose, a product of Sir2-mediated deacetylation, is thought to further stabilize Sir binding (42, 75, 76). Boundary elements, as well as the interplay between Sir binding and histone modifications, function to limit the spread of silenced chromatin (14, 58, 73). Recent studies have reconstituted silencing *in vitro*, demonstrating the direct roles of Sir proteins and histone modifications in silencing (33, 46).

Recent evidence suggests that silencing in *S. cerevisiae* requires not only the recruitment and spreading of Sir proteins but also the proper formation of a higher-order chromatin structure. For example, when lysine 56 of histone H3 is changed to glycine, glutamine, or arginine, telomeric silencing is abolished without detectable changes in Sir binding (84). However, in these mutants subtelomeric chromatin shows a greater accessibility to *Escherichia coli* dam methylase, suggesting that the K56 amino acid changes interfere with silencing by interfering with formation of a higher-order chromatin structure (84). Similarly, when the N-terminal tail of histone H3 is deleted, silencing is lost without alterations to the Sir binding profile, yet the chromatin is more sensitive to dam methylation (69). The structures of this higher-order chromatin and other factors that control it remain unclear.

Recently, the putative histone acetyltransferase Spt10 has been implicated in silencing. Using a *URA3* reporter inserted near a telomere, Braun et al. (6) found that *spt10*Δ mutants are defective for silencing in subtelomeric regions. Consistent with this result, we found evidence that *spt10*Δ mutants may also be defective in mating type silencing, as *spt10*Δ mutants do not undergo a G_1 arrest upon exposure to the mating pheromone α-factor (unpublished observations).

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Spt10 was first identified in selections for mutations that suppress the transcription defects caused by Ty1 insertions (Spt$^-$ phenotype [16, 50]), as well as other types of transcription defects (12, 85). It possesses a zinc finger domain through which it binds cooperatively as a dimer to a consensus sequence found at all four histone gene promoters (15, 47, 48). Spt10 also possesses a putative histone acetyltransferase domain (52). Although no acetyltransferase activity has been detected for Spt10 *in vitro* (D. Hess and F. Winston, unpublished results; R. Sternglanz, personal communication), mutations changing putative catalytic residues do cause an Spt$^-$ phenotype (28).

Several results suggest that Spt10 is functionally related to another protein, Spt21. Mutations in either gene cause the unusual phenotype of permitting transcription to initiate from the 3′ long terminal repeat (LTR) of Ty1 elements (50). In addition, *spt10* and *spt21* mutations affect histone gene transcription similarly, with *spt10*Δ and *spt21*Δ mutants each showing a 20-fold decrease in transcript levels for the histone gene pair *HTA2-HTB2*, one of the two *S. cerevisiae* loci that encode histones H2A and H2B, and a much more modest decrease in transcript levels for *HHF2* (13, 15, 26, 28). Spt10 and Spt21 proteins can also physically interact, and particular alleles of *SPT10* can suppress a deletion of *SPT21* (28). However, *spt10* and *spt21* mutants do show some differences. Deletion of *SPT10* causes a severe growth defect, while deletion of *SPT21* causes only a mild growth phenotype (51). In addition, *SPT21* mRNA and protein levels are cell cycle regulated, while *SPT10* levels are not (9, 68). Finally, *spt21* mutants have been identified in screens for mutants with altered telomere length (3, 20). Taken together, these studies suggest that Spt10 may play a broad role in transcription and chromatin structure and that Spt21 plays a role in a subset of these processes.

Given the observed silencing defect in *spt10*Δ mutants, as well as the functional connections between Spt10 and Spt21, we have investigated the roles of both Spt10 and Spt21 in silencing. Our results show that both *spt10* and *spt21* mutations impair silencing at subtelomeric regions and the silent mating locus *HML*α, while strengthening silencing at the rDNA. In both *spt10* and *spt21* mutants, Sir protein recruitment and histone modifications are modestly altered. Furthermore, both mutants have clearly increased accessibility of chromatin to dam methylation, suggesting possible effects on higher-order chromatin structure. Additional results suggest that these phenotypes are not the consequence of altered histone gene expression, indicating an additional role for Spt10 and Spt21 in controlling chromatin structure.

MATERIALS AND METHODS

Yeast strains and plasmids. The yeast strains used in this study are listed in Table 1. The *GAL1pr-SPT10* allele was generated by PCR amplification of a cassette containing *KanMX* and the *GAL1* promoter from a pFA6a-*KanMX6-PGAL1* plasmid (43), which was then integrated just upstream of the *SPT10* ATG at its native genomic location. The *spt21*Δ::*HIS3*, *sir1*Δ::*NatMX*, *sir2*Δ::*NatMX*, *sir3*Δ::*KanMX*, and *sir4*Δ::*NatMX* deletion mutants were generated by replacing the entire open reading frames with the indicated auxotrophic or drug resistance marker (21, 62). The *SPT10-13MYC* and *SPT21-13MYC* alleles, created using a previously described method (43), were confirmed by Western blotting. Strains containing these tagged alleles were tested for both growth and Spt$^-$ phenotypes and behaved the same as wild-type strains. The *telV-R* reporter strains derive from crosses with UCC35 (2), and the *mURA3* reporter strains derive from crosses with JS215-10 (64). Strains bearing the *E. coli* dam methylase gene contain the methylase gene inserted at the *LYS2* genomic locus (22). Media, basic yeast techniques, mating, sporulation, and tetrad dissection were performed as previously described (57). Plasmids used in this study include pRS425 and pRS426 (10); pLP891 (2μ-*SIR2 HIS3*), pLP1047 (2μ-*SIR3 HIS3*), pLP304 (2μ-*SIR3 LEU2*), and pLP2206 (2μ-*SIR4 HIS3*), all generously provided by Lorraine Pillus; pFW217 (2μ-*SPT10-URA3*) (51); and pPK128 (2μ-*HTA1-HTB1 HHT1-HHF1 LEU2*) (35). Because *sir2*Δ, *sir3*Δ, and *sir4*Δ strains are nonmating, their crosses were facilitated by transformation with a high-copy-number plasmid encoding the corresponding wild-type gene (pLP891, pLP1047, and pLP2206, respectively).

Spot tests. For all spot tests, cultures were grown to saturation (1×10^8 to 2×10^8 cells/ml) overnight, and then 5-fold serial dilutions were spotted onto the indicated media and incubated at 30°C for 2 to 5 days. For Fig. 1C, to minimize the acquisition of spontaneous suppressors of the *spt10*Δ slow-growth phenotype, the *spt10*Δ strain was first grown with pFW217 (2μ-*SPT10-URA3*) and then grown on 5-fluoroorotic acid (5-FOA) medium to select for cells that had lost the plasmid. Strains were then patched onto yeast extract-peptone-dextrose (YPD) medium, grown 1 to 2 days, resuspended in water to 1×10^7 cells/ml, and subjected to 5-fold serial dilutions.

Quantitative mating assays. Quantitative mating assays were performed as previously described (24). Briefly, cultures of a query strain and a wild-type strain of the opposite mating type were grown to 1×10^7 to 2×10^7 cells/ml, and then 10^6 cells of the query strain and 10^7 cells of the wild-type strain were filtered together onto a 25-mm-diameter glass microfiber disc (0.45-μm pore size; Whatman). The disc was placed onto a YPD plate and incubated at 30°C for 5 h. The cells were eluted into 1 ml of 1 M sorbitol and sonicated briefly. Dilutions were plated onto solid medium that selects for diploids (mated) and for total query cells (mated plus unmated), and after 2 to 3 days colonies were counted. The percentage of mated cells is expressed as a ratio of diploids to total query cells.

Northern hybridization analysis. RNA isolation and Northern blotting were performed as previously described (4, 74). ^{32}P-labeled probes were generated by random priming using PCR products amplified from genomic DNA using the oligonucleotides listed in Table S1 in the supplemental material. For analysis of **a**2 and α*2* mRNAs, the two transcripts were probed simultaneously, as the entire coding region of **a***2* is identical to a region of α*2*.

Western blotting. Whole-cell extracts were prepared by trichloroacetic acid (TCA) precipitation (S. P. Bell, unpublished). Western blotting was performed using SDS-PAGE (15% polyacrylamide for gels to visualize histones and 7% polyacrylamide for all others), as previously described (19). Antibodies used were anti-Sir2 (1:5,000) (70), anti-Sir3 (1:5,000) (40), anti-Sir4 (1:5,000) (76), anti-histone H3 (1:5,000) (Abcam), anti-histone H3 K79me2 (1:5,000) (Abcam), anti-acetylated histone H4 (recognizing acetylation at K5/K8/K12/K16; 1:5,000) (Upstate), anti-Myc (1:5,000) (9E10; Santa Cruz Biotechnology), and anti-Pgk1 (1:5,000) (Molecular Probes). All antibody dilutions were made in 5% milk in Tris-buffered saline–Tween 20 (TBST).

ChIP. Chromatin immunoprecipitations (ChIPs) were performed as previously described (31). Exponentially growing cultures were harvested, cross-linked with formaldehyde (1% final concentration) for 30 min, and quenched with glycine (115 mM final concentration). After pelleting, cells were lysed by bead beating and then sonicated with six 30-s pulses, with 90 s between pulses, using a Bioruptor machine (Diagenode). One microliter of antibody was added to 625 μg of soluble lysate in 1 ml of lysis buffer and incubated for 14 h at 4°C. Fifty microliters of a 50% protein G-Sepharose slurry (protein G-Sepharose; GE Healthcare) was added and incubated for another 4.5 h. After washing, samples were eluted, and cross-linking was reversed at 65°C overnight. Samples were subjected to proteinase K digestion and phenol-chloroform extraction, and the DNA was then precipitated. For input samples, cross-linking was reversed for 15 μg of soluble lysate in parallel with the IP samples described above. The antibodies used are described in the previous section, except for anti-Sir2, which was previously described (76).

Real-time PCR. Real-time PCR was performed using SYBR green chemistry (Brilliant II SYBR green master mix; Stratagene) and a Stratagene MX3000P machine. Primer sequences are listed in Table S1 in the supplemental material. The annealing temperature was 55°C for all primer pairs, and all reactions were performed in triplicate. For chromatin immunoprecipitation experiments, data are normalized to binding at either *ACT1* or a region of chromosome V lacking open reading frames (37), relative to input DNA. for Fig. 7, values were further normalized to ChIP of total histone H3.

Dam methylase assay and Southern blotting. Genomic DNA was isolated from 1×10^9 to 2×10^9 cells in exponential growth as previously described (30). The DNA was digested with RNase, precipitated, and digested with 160 units of NdeI (New England Biolabs) at 37°C for 8 h. An additional 50 units of NdeI was

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype
FY2200	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0*
FY1856	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0*
FY2813	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 GAL1pr-SPT10*::*KanMX*
FY2814	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 GAL1pr-SPT10*::*KanMX*
FY2815	*MAT*α *his3*Δ*200 ura3*Δ*0 leu2*Δ*0*
FY2816	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 spt21*Δ::*HIS3*
FY2817	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 spt21*Δ::*HIS3*
FY2818	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir2*Δ::*NatMX*
FY2819	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir2*Δ::*NatMX*
FY2820	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 GAL1pr-SPT10*::*KanMX spt21*Δ::*HIS3*
FY2821	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 GAL1pr-SPT10*::*KanMX spt21*Δ::*HIS3*
FY605	*MAT***a** *ura3-52 his3*Δ*200 leu2*Δ*1 trp1*Δ*63*(*hta2-htb2*)Δ::*TRP1*
FY2822	*MAT*α *lys2-128*δ *his3*Δ*200 ura3-52* (*leu2*Δ*0* or *leu2*Δ*1*) *trp1*Δ*63*(*hta2-htb2*)Δ::*TRP1*
FY2823	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX*
FY2824	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX*
FY2825	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX GAL1pr-SPT10*::*KanMX*
FY2826	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX GAL1pr-SPT10*::*KanMX*
FY2827	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX spt21*Δ::*HIS3*
FY2828	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX spt21*Δ::*HIS3*
FY2829	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX GAL1pr-SPT10*::*KanMX spt21*Δ::*HIS3*
FY2830	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir1*Δ::*NatMX GAL1pr-SPT10*::*KanMX spt21*Δ::*HIS3*
FY2831	*MAT***a** *lys2-128*δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *trp1*Δ*63 sir1*Δ::*NatMX* (*hta2-htb2*)Δ::*TRP1*
FY2832	*MAT*α *lys2-128*δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *trp1*Δ*63 sir1*Δ::*NatMX* (*hta2-htb2*)Δ::*TRP1*
FY2833	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 SPT10-13*×*MYC*:*KanMX*
FY2834	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 SPT10-13*×*MYC*:*KanMX*
FY2835	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 SPT21-13*×*MYC*:*KanMX*
FY2836	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 SPT21-13*×*MYC*:*KanMX*
FY2837	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir3*Δ::*KanMX*
FY2838	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 sir4*Δ::*KanMX*
FY636	*MAT*α *his4-912*δ *ura3-52 leu2*Δ*1 lys2*Δ*202*::*LYS2-dam*
L1134	*MAT*α *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 telV-R*::*URA3*
L1135	*MAT*α *lys2-128*δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *telV-R*::*URA3 sir2*Δ::*NatMX*
L1136	*MAT*α *ura3*Δ*0 leu2*Δ*0 telV-R*::*URA3 GAL1pr-SPT10*::*KanMX*
L1137	*MAT*α (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *telV-R*::*URA3 spt21*Δ::*HIS3*
L1138	*MAT*α *lys2-128*δ *his3*Δ*200* (*ura3-52* or *ura3*Δ*0*) (*leu2*Δ*1* or *leu2*Δ*0*) *trp1*Δ*63 telV-R*::*URA3* (*hta2-htb2*)Δ::*TRP1*
L1139	*MAT***a** *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *rDNA*::*mURA3-LEU2*
L1140	*MAT*α *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *rDNA*::*mURA3-LEU2 sir2*Δ::*NatMX*
L1141	*MAT***a** *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *rDNA*::*mURA3-LEU2 sir4*Δ::*NatMX*
L1142	*MAT***a** *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *rDNA*::*mURA3-LEU2 GAL1pr-SPT10*:*KanMX*
L1143	*MAT***a** *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *rDNA*::*mURA3-LEU2 spt21*Δ::*HIS3*
L1144	*MAT***a** *lys2-128*Δ *his3*Δ*200* (*ura3*Δ*0* or *ura3-167*) (*leu2*Δ*0* or *leu2*Δ*1*) *trp1*Δ*63 rDNA*::*mURA3-LEU2* (*hta2-htb2*)Δ::*TRP1*
L1145	*MAT***a** *lys2-128*Δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 telV-R*::*URA3*
L1146	*MAT***a** *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 telV-R*::*URA3 GAL1pr-SPT10*:*KanMX*
L1147	*MAT***a** *lys2-128*δ *his3*Δ*200 ura3*Δ*0 leu2*Δ*0 telV-R*::*URA3 spt21*Δ::*HIS3*
L1148	*MAT***a** (*his3*Δ*200* or *his4-912*δ) (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *lys2*Δ*202*::*LYS2-dam*
L1149	*MAT?* (*his3*Δ*200* or *HIS3*) (*his4-912*δ or *HIS4*) (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *lys2*Δ*202*::*LYS2-dam sir2*Δ::*NatMX*
L1150	*MAT***a** (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *lys2*Δ*202*::*LYS2-dam GAL1pr-SPT10*:*KanMX*
L1151	*MAT*α (*his3*Δ*200* or *HIS3*) (*ura3*Δ*0* or *ura3-52*) (*leu2*Δ*0* or *leu2*Δ*1*) *lys2*Δ*202*::*LYS2-dam spt21*Δ::*HIS3*

added, and samples were incubated for an additional 4 h. NdeI was inactivated for 20 min at 65°C, and the DNA was aliquoted into four tubes. To each tube was added either 20 units of DpnI (digests methylated DNA), MboI (digests unmethylated DNA), or Sau3AI (digests methylated + unmethylated DNA) or a no-enzyme control and incubated for 5.5 h. Samples were separated on a 1.2% agarose gel and blotted as previously described (57). ^{32}P-labeled probes were generated by random priming using PCR product templates. The primers are listed in Table S1 in the supplemental material. A fragment within *MSN5* is 350 bp from a telomere and serves as a loading control, with no dam methylation sites between two NdeI sites.

RESULTS

Use of the *GAL1pr-SPT10* allele to decrease Spt10 levels without causing a severe growth defect. Deletion of *SPT10* causes a severe slow-growth defect, and spontaneous suppressors of this defect arise at a high rate (8, 50). These suppressor mutations occur in many different genes, causing a variety of effects on *spt10*Δ growth and other phenotypes (8). Out of concern that the slow growth and suppressors would complicate our analysis of *spt10*Δ silencing phenotypes, we constructed a repressible *SPT10* allele in which *SPT10* is regulated by the *GAL1* promoter. When strains with this allele are grown in glucose-containing media, *SPT10* mRNA levels are reduced but not abolished, presumably because repression of the *GAL1pr-SPT10* allele is leaky (Fig. 1A). The decrease in expression confers an Spt$^-$ phenotype (albeit not as severe as for *spt10*Δ strains) (Fig. 1C), yet the strains grow significantly better than *spt10*Δ strains (Fig. 1B). Unless otherwise noted, all experiments with the *GAL1pr-SPT10* allele in this study were conducted under repressive conditions (2% glucose and no galactose).

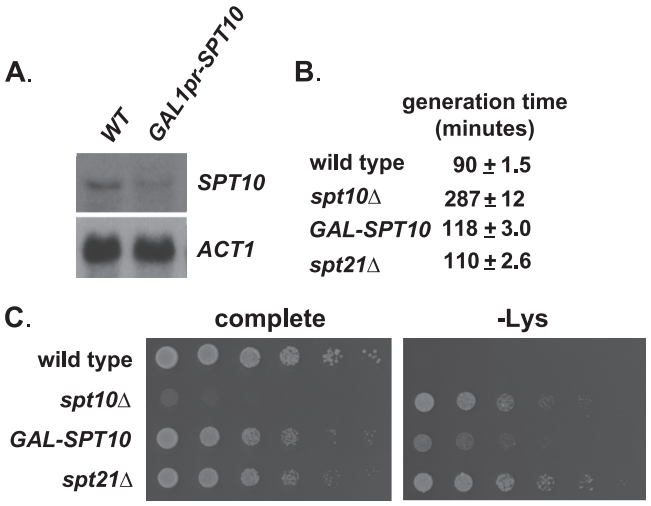

FIG. 1. Use of the *GAL1pr-SPT10* allele to deplete Spt10. (A) Northern blot of wild type (WT) and *GAL1pr-SPT10* strains grown on medium containing 2% glucose and no galactose. RNA was isolated from exponentially growing cultures and subjected to Northern blotting. *ACT1* serves as a loading control. The slight migration difference for *SPT10* in the *GAL1pr-SPT10* lane is due to the replacement of the *SPT10* 5′ untranslated region (UTR) with the 5′ UTR of *GAL1*. (B) Generation times of wild-type, *spt10*Δ, *GAL1pr-SPT10*, and *spt21*Δ strains. Exponentially growing cultures were diluted to 100 cells/ml and then grown to a density of 1×10^7 to 2×10^7 cells/ml. The doubling time was calculated as time of incubation ÷ $\log_2$(final density/initial density). Shown are the means ± standard errors of the means (SEM) for at least eight independent cultures. (C) Growth and Spt$^-$ phenotypes of the *GAL1pr-SPT10* mutant. Cultures were grown as described in Materials and Methods, subjected to 5-fold serial dilutions, and spotted onto the indicated media, which contain 2% glucose and no galactose. Plates were scanned after 2 days (SC) or 3 days (−Lys) of incubation at 30°C. Overall growth is shown on complete medium, and growth on SC without lysine (−Lys) indicates an Spt$^-$ phenotype (suppression of *lys2-128*δ).

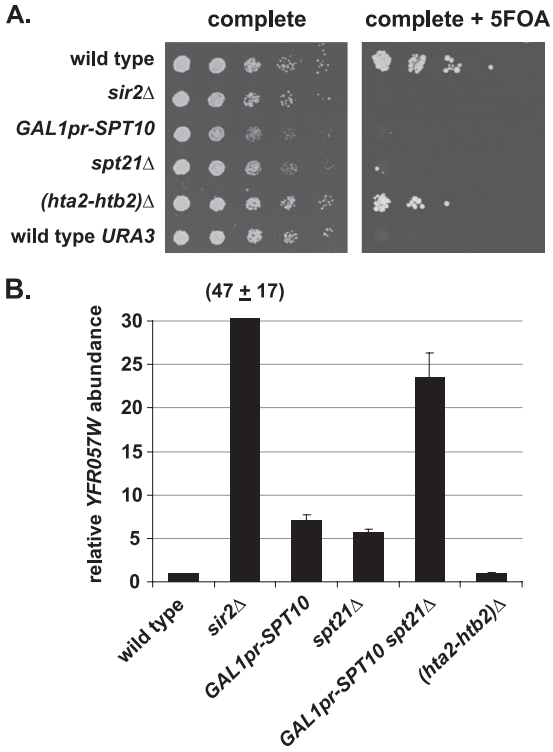

FIG. 2. *SPT10* and *SPT21* are required for telomere position effect. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. (A) Fivefold dilution spot assays with strains carrying the *telV-R*::*URA3* reporter. Cultures were grown in YPD overnight to saturation, subjected to 5-fold serial dilutions, and spotted onto the indicated media. All strains have the *telV-R*::*URA3* reporter, except for the wild-type *URA3* strain, which has the wild-type *URA3* gene at the endogenous locus, where it is not silenced. The mild growth defect of *GAL1pr-SPT10* and *spt21*Δ mutants can be seen on the complete plate. (B) Real-time reverse transcription-PCR (RT-PCR) analysis of silencing of *YFR057W*. The measurement of *YFR057W* transcript levels was normalized to *ACT1*. Values are expressed relative to the wild-type level, which was assigned a value of 1. Shown are the means ± SEM for at least three independent experiments. The *y* axis was truncated to facilitate comparison; the value for *sir2*Δ is indicated above the corresponding bar.

Characterization of *GAL1pr-SPT10* and *spt21*Δ defects in telomere position effect. To begin our characterization of possible silencing defects, we assayed silencing near telomeres in both *GAL1pr-SPT10* and *spt21*Δ strains. To do this, we utilized a *telV-R*::*URA3* reporter (2), in which the *URA3* gene is placed near the telomere on the right arm of chromosome V, where it is normally subject to silencing. Growth on 5-FOA medium reflects intact silencing (see Materials and Methods). Using this reporter, we found that silencing near the telomere is defective in both *GAL1pr-SPT10* and *spt21*Δ mutants (Fig. 2A). *GAL1pr-SPT10* and *spt21*Δ strains are able to grow well on 5-FOA medium in a *ura3*Δ background without the telomeric reporter (data not shown), indicating that 5-FOA is not toxic to the mutants. Given that the best-characterized function of Spt10 and Spt21 is to activate expression of the *HTA2-HTB2* histone genes (13, 15, 28), we also tested whether a (*hta2-htb2*)Δ mutant is also impaired for telomeric silencing using the *telV-R*::*URA3* reporter system. Our results show that this mutant does not have a telomeric silencing defect, suggesting that the decreased *HTA2-HTB2* transcription in *spt10* and *spt21* mutants is not sufficient to cause defective telomeric silencing. Taken together, our results extend the previous results of Braun et al. (6) to show that both *SPT10* and *SPT21* are required for telomeric silencing.

To determine whether silencing defects also occur at a natural telomere-proximal gene, we measured the mRNA levels of *YFR057W*, an endogenous subtelomeric gene located approximately 0.6 kb from the telomere on the right arm of chromosome VI and subject to silencing (81). Using reverse transcription followed by real-time PCR quantitation, we found that in *GAL1pr-SPT10* and *spt21*Δ mutants, *YFR057W* mRNA levels are elevated 5- to 7-fold over wild-type levels (Fig. 2B), indicative of a silencing defect. The derepression is comparable between the two mutants, although less than we observed for a *sir2*Δ mutant. Silencing of *YFR057W* is intact in the (*hta2-htb2*)Δ mutant (Fig. 2B), furthering the evidence that reduced transcription of these genes in *spt10* and *spt21* mutants in insufficient to cause defective silencing. We also found that expression of *IMD1*, a different endogenous gene subject to telomeric silencing on chromosome I (5), is derepressed in *GAL1pr-SPT10* and *spt21*Δ mutants but not (*hta2-htb2*)Δ mutants (data not shown). These results demonstrate that Spt10

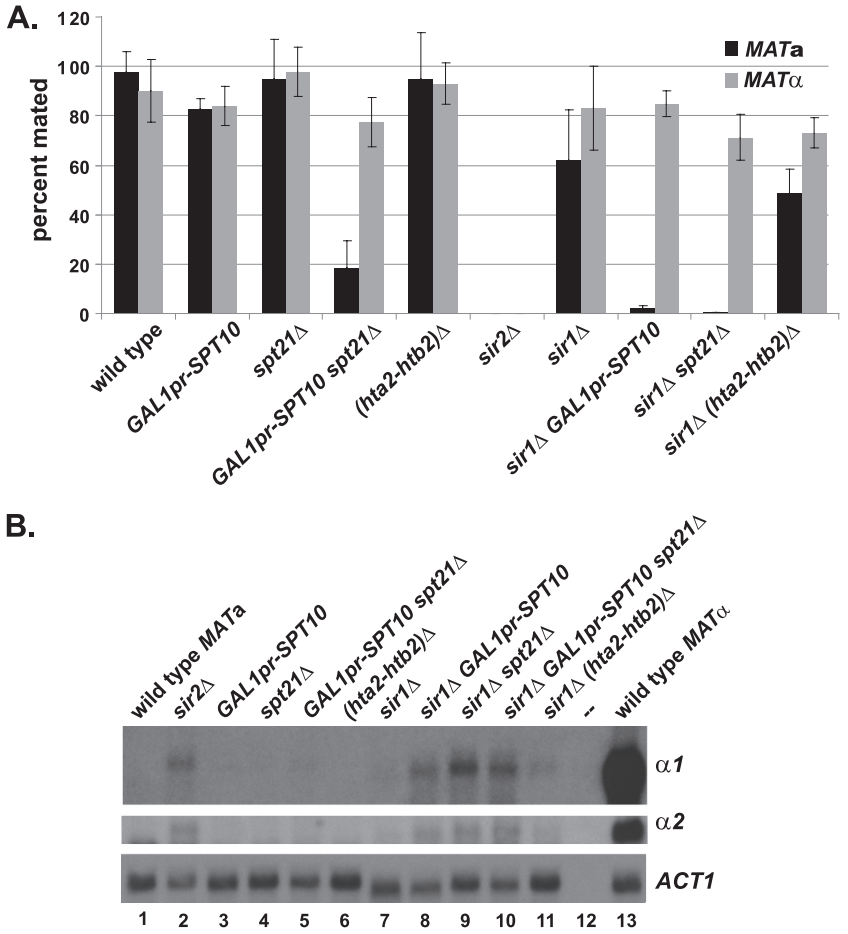

FIG. 3. *GAL1pr-SPT10* and *spt21*Δ show mating type silencing defects in a *MAT***a** background. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. (A) Quantitative mating assays with *MAT***a** and *MAT*α haploid strains. Cells of each query strain were incubated for 5 h with a wild-type lawn of the opposite mating type, and the percentage of mated query cells was calculated (see Materials and Methods). Shown are the means ± standard deviations for at least three independent experiments. (B) Northern blot analysis of silencing at *HML*α. The strains in lanes 1 to 11 are *MAT***a**, and the strain in lane 13 is *MAT*α. The α*1* and α*2* transcripts are silenced in wild-type *MAT***a** haploids and expressed in wild-type *MAT*α haploids.

and Spt21 are necessary for proper silencing in a native telomeric context.

Previous work has suggested that while Spt10 and Spt21 share many roles, they do have some nonoverlapping roles. Therefore, we asked whether Spt10 and Spt21 might have nonoverlapping roles in regulating silencing near the telomere. By analysis of *YFR057W* mRNA levels, we found that the *GAL1pr-SPT10 spt21*Δ double mutant shows a more substantial derepression of *YFR057W* transcript levels than either single mutant (Fig. 2B). The stronger phenotype of the *GAL1pr-SPT10 spt21*Δ double mutant suggests that Spt10 and Spt21 are not completely redundant in their roles in silencing.

Spt10 and Spt21 play a role in silencing at *HML*α. Given that both Spt10 and Spt21 are required for telomeric silencing, we tested whether they are also required for silencing of the silent mating type cassettes, *HMR***a** and *HML*α. We began by assessing mating ability, which is decreased in silencing mutants due to the expression of both sets of mating type information. Quantitative mating assays (see Materials and Methods) show that while the *GAL1pr-SPT10* and *spt21*Δ single mutants show no significant defect, the *GAL1pr-SPT10 spt21*Δ double mutant shows a substantial decrease in mating in a *MAT***a** background, to approximately 20% of wild-type levels, while showing no significant defect in a *MAT*α background (Fig. 3A). Mating type-specific mating defects have been observed previously in mutants with intermediate effects on silencing, as silencing is believed to be more easily disrupted at *HML* than at *HMR* (83). Our results suggest that Spt10 and Spt21 are both required for silencing of *HML*α.

We also tested whether either *GAL1pr-SPT10* or *spt21*Δ exhibits a mating defect when combined with *sir1*Δ. The rationale for testing this possibility came from a screen for mutations in the nonessential gene deletion set that impair growth when combined with *spt10*Δ (8), using methods previously described (77). The results of that screen suggested that a *sir1*Δ *spt10*Δ double mutant is inviable; however, when we dissected tetrads after sporulation of an *spt10*Δ/*SPT10 sir1*Δ/*SIR1* heterozygote, we readily obtained *sir1*Δ *spt10*Δ double mutants at the expected frequency, with no apparent growth defects. This result was reminiscent of an earlier study (80) in which a *sir1*Δ *dot1*Δ double mutant appeared to be inviable in the same type of screen, but the apparent inviability was actually the result of

a defect in silencing of the *HML*α locus, as mating must be intact in order for the screen to work properly. Thus, our screen results raised the possibility that *spt10*Δ *sir1*Δ double mutants are defective for silencing of *HML*α.

To assess potential silencing defects for *GAL1pr-SPT10* and *spt21*Δ in combination with *sir1*Δ, we performed quantitative mating assays with *sir1*Δ *GAL1pr-SPT10* and *sir1*Δ *spt21*Δ strains. In a *MAT***a** background, both *sir1*Δ *GAL1pr-SPT10* and *sir1*Δ *spt21*Δ strains show a severe defect in mating ability (Fig. 3A). In contrast, in a *MAT*α background, neither strain shows a mating defect beyond that seen in a *sir1*Δ single mutant. Analysis of a *sir1*Δ (*hta2-htb2*)Δ double mutant suggests that decreased *HTA2-HTB2* transcript levels are insufficient to explain the *GAL1pr-SPT10* and *spt21*Δ mating defects (Fig. 3A). The synergistic mating defects with *sir1*Δ classify *GAL1pr-SPT10* and *spt21*Δ as Eso (enhancer of sir one) mutants, many of which also have effects limited to the *MAT***a** mating type (55).

To directly examine silencing at *HMR***a** and *HML*α, we measured the levels of transcripts by Northern hybridization analysis. To do this, we tested *MAT***a** strains for transcription of *HML*α and *MAT*α strains for transcription of *HMR***a**. Our analysis showed that transcription at *HML*α and *HMR***a** correlates with the mating defects we observed. First, in *GAL1pr-SPT10* and *spt21*Δ single mutants of either mating type, no aberrant mating transcripts could be detected. However, in a *MAT***a** background, both α1 and α2 transcripts are present at significantly greater levels in *sir1*Δ *GAL1pr-SPT10* and *sir1*Δ *spt21*Δ mutants than in a *sir1*Δ single mutant (Fig. 3B). In contrast, in a *MAT*α background, *sir1*Δ *GAL1pr-SPT10* and *sir1*Δ *spt21*Δ double mutants expressed both **a**1 and **a**2 transcripts at a level comparable to that for a *sir1*Δ single mutant (data not shown). These results are consistent with a synergistic silencing defect between *sir1*Δ and *GAL1pr-SPT10* or *spt21*Δ in a *MAT***a** background. No increased derepression was seen in *sir1*Δ (*hta2-htb2*)Δ mutants, again suggesting that the *GAL1pr-SPT10* and *spt21*Δ phenotypes cannot be explained by decreased *HTA2-HTB2* transcription alone. Taken together, the mating and transcriptional defects suggest that Spt10 and Spt21 contribute to silencing at *HML*α.

rDNA silencing is strengthened in *GAL1pr-SPT10* and *spt21*Δ mutants. Given that *GAL1pr-SPT10* and *spt21*Δ show defects in silencing at the telomere and the silent mating type loci, we investigated whether they show defects in rDNA silencing as well. We utilized a reporter containing a modified *URA3* gene inserted in a single copy at the rDNA locus (see Materials and Methods). Growth on SC medium without uracil reflects the ability to silence *URA3* expression, with slower growth reflecting a greater degree of silencing. Consistent with previous observations, the *sir2*Δ mutant shows more rapid growth on SC medium without uracil, reflecting impaired silencing, and the *sir4*Δ mutant shows slower growth, reflecting an increased level of silencing (64). Our results indicate that the *GAL1pr-SPT10* and *spt21*Δ mutations, despite causing decreased silencing at other regions, cause increased silencing at the rDNA locus, similar to the case for *sir4*Δ (Fig. 4). The increased rDNA silencing (Irs$^-$) phenotype of *sir4*Δ mutants has been attributed to an excess of free Sir2, which is then able to bind and silence the rDNA (66, 67). Our results raise the possibility that free Sir2 levels are also increased in *GAL1pr-SPT10* and *spt21*Δ mutants.

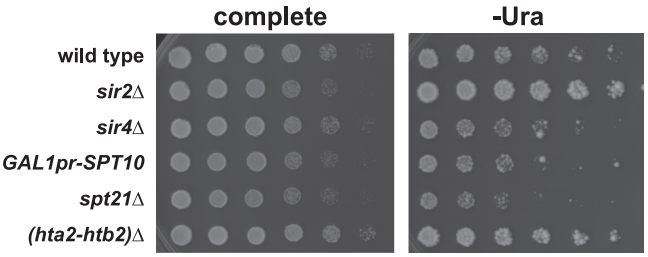

FIG. 4. Levels of rDNA silencing are increased in *GAL1pr-SPT10* and *spt21*Δ mutants. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. Fivefold dilution spot tests were done on the indicated strains, all of which carry an *mURA3* reporter in a single copy within the rDNA. Growth on medium without uracil (−Ura) assesses the degree of reporter silencing, and complete medium controls for growth. Papillation reflects the stochastic nature of silencing changes.

Spt10 and Spt21 do not detectably associate with a silenced region. To test whether Spt10 and Spt21 affect silencing by direct association with silenced loci, we performed chromatin immunoprecipitation (ChIP) of Spt10 and Spt21 using strains in which a 13Myc epitope tag was fused to the C terminus of either protein (see Materials and Methods). In order to test single-copy DNA, we focused our analysis on the subtelomeric region on the right arm of chromosome VI (*telVI-R*), which is silenced in an Spt10- and Spt21-dependent fashion (Fig. 2). We assayed for Spt10 and Spt21 binding by ChIP at 0.6 kb from the telomere, a position that overlaps the *YFR057W* gene, and at 2.8 kb from the same telomere, where Sir binding and silencing also occur, albeit at lower levels (25, 72). Our results do not detect significant binding of either Spt10 or Spt21 at either location (see Fig. S1 in the supplemental material), a result consistent with genome-wide ChIP analysis using microarrays (8, 15). As a positive control, both Spt10 and Spt21 did show significant binding at the *HTA2-HTB2* promoter, which is consistent with previous findings (28). Although this is a negative result, and it remains possible that Spt10 and Spt21 are physically present at this silenced region, our results suggest that Spt10 and Spt21 may influence the telomere position effect without direct binding at the telomere.

***GAL1pr-SPT10* and *spt21*Δ silencing phenotypes can be partially rescued by overexpression of histone genes or *SIR3*.** Previous studies have shown that overexpression of histone genes can suppress some *spt10* and *spt21* mutant phenotypes. In one case, overexpression of *HTA1-HTB1*, encoding histones H2A and H2B, was able to suppress the Spt$^-$ phenotypes of both *spt10* and *spt21* mutants (61), while in another case, overexpression of two loci, *HTA1-HTB1* and *HHT1-HHF1*, encoding the four core histones, was able to suppress the poor-growth phenotype caused by *spt10*Δ (15). To determine if overexpression of histone genes can also suppress the *GAL1pr-SPT10* and *spt21*Δ silencing phenotypes, we tested whether a high-copy-number plasmid containing *HTA1-HTB1* and *HHT1-HHF1* caused any effect on telomere position effect, using the *telV-R*::*URA3* reporter. Our results show that this high-copy-number histone plasmid moderately suppresses the *GAL1pr-SPT10* silencing defect and strongly suppresses the

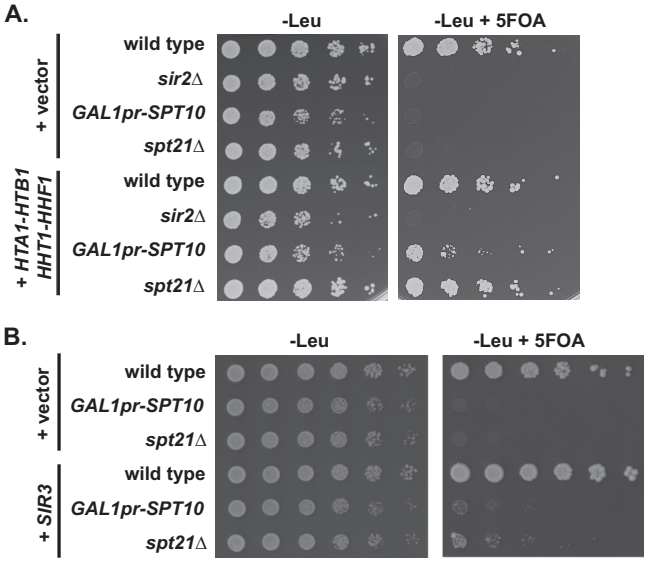

FIG. 5. (A) A high-copy-number plasmid encoding all four histones can partially suppress *spt21*Δ and *GAL1pr-SPT10* silencing phenotypes. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. The indicated strains carry either an empty vector (2μ *LEU2*) or a high-copy-number plasmid encoding the first copy of each histone protein (2μ *LEU2 HTA1-HTB1 HHT1-HHF1*). Fivefold dilution spot tests were grown on either medium without leucine (−Leu), which selects for plasmid maintenance, or on −Leu plus 5-FOA. Cells are able to grow in the presence of 5-FOA only when the telomere position effect is intact, and they are able to silence *URA3* expression. (B) A high-copy-number plasmid with *SIR3* can partially suppress the silencing defects of *GAL1pr-SPT10* and *spt21*Δ. Fivefold dilution spot tests of telomere position effect were performed on the indicated strains carrying either an empty vector (2μ-*LEU2*) or a high-copy-number plasmid with *SIR3* (2μ-*LEU2-SIR3*). All strains have the *telV-R*::*URA3* reporter.

*spt21*Δ silencing defect (Fig. 5A). Furthermore, this plasmid suppressed the Eso$^-$ phenotype weakly for *GAL1pr-SPT10* and strongly for *spt21*Δ (data not shown). However, neither *HTA1-HTB1* nor *HHT1-HHF1* alone conferred any suppression of the Eso$^-$ phenotypes (data not shown). These results show that additional copies of the histone gene pairs *HTA1-HTB1* and *HHT1-HHF1* can strongly suppress the silencing defects of *spt21*Δ mutants while having a smaller effect on *GAL1pr-SPT10* mutants.

We then asked whether overexpression of any *SIR* genes can rescue the *GAL1pr-SPT10* and *spt21*Δ silencing defects. The Eso$^-$ mating phenotypes of *GAL1pr-SPT10* and *spt21*Δ mutants suggest that Spt10 and Spt21 have some roles in silencing complementary to Sir1, and overexpressing *SIR1* might compensate for the *GAL1pr-SPT10* and *spt21*Δ silencing defects near the telomere. In addition, overproduction of Sir3 has been shown to enhance the spreading of silencing near the telomere of a wild-type cell (56), raising the possibility that its overproduction can also improve silencing in *GAL1pr-SPT10* and *spt21*Δ mutants. Our results show that a high-copy-number plasmid carrying *SIR3* can weakly suppress the defects in telomere position effect in both *GAL1pr-SPT10* and *spt21*Δ mutants in a *telV-R*::*URA3* reporter strain, with suppression stronger in the *spt21*Δ mutant (Fig. 5B). This plasmid could also partially suppress the Eso$^-$ mating phenotypes of both mutants (data not shown). Among the four *SIR* genes tested, only the *SIR3* plasmid conferred any suppression for either type of silencing (data not shown). These results indicate that increased Sir3 levels can partially compensate for decreases in Spt10 and Spt21 levels.

Effect of *GAL1pr-SPT10* and *spt21*Δ on Sir protein levels and binding. Because Sir3 overexpression can partially suppress the *GAL1pr-SPT10* and *spt21*Δ silencing defects, it is possible that Spt10 and Spt21 control silencing by influencing the levels or localization of Sir proteins. To measure the levels of Sir2, Sir3, and Sir4, we performed Western blotting using antibodies specific to each of these proteins. Our results showed that Sir2, Sir3, and Sir4 levels are not significantly altered in *GAL1pr-SPT10* or *spt21*Δ mutants or in the *GAL1pr-SPT10 spt21*Δ double mutant (Fig. 6A).

To test whether *GAL1pr-SPT10* and *spt21*Δ might affect Sir complex recruitment to silenced regions, we used ChIP to measure the level of association of Sir2 and Sir3 at the right telomere of chromosome VI to the same regions 0.6 and 2.8 kb from the telomere. Our results show that in a *GAL1pr-SPT10* mutant, Sir2 binding occurs at approximately 80% of wild-type levels in both regions, while Sir3 binding is nearly indistinguishable from wild type (Fig. 6B and C). In an *spt21*Δ mutant the levels of Sir2 and Sir3 binding show a slightly greater decrease in both regions, to 60 to 70% of wild-type levels at 0.6 kb from the telomere and to 40 to 50% of wild-type levels at 2.8 kb. For both mutants, the level of Sir association is much greater than in a *sir2*Δ control strain. Because *spt10* and *spt21* mutants have decreased expression of histone genes (13), we considered whether the modest decrease in Sir2 and Sir3 association in this mutant could be accounted for by decreased nucleosome density. To test this, we also used ChIP to measure histone H3 association at the same regions near telomere VI-R. We found that the level of histone H3 associated with these regions was unaffected in both the *GAL1pr-SPT10* and *spt21*Δ mutants (Fig. 6D). In conclusion, our ChIP assays show that *GAL1pr-SPT10* and *spt21*Δ mutants have modestly decreased levels of Sir2 and Sir3 binding.

Mutant effects on histone modifications at silenced loci. Given the silencing defects in *GAL1pr-SPT10* and *spt21*Δ mutants, we asked whether these mutants show changes in the levels of histone modifications associated with silenced chromatin. We focused our analysis on two modifications previously shown to affect silencing: methylation of histone H3 on lysine 79 (H3 K79me) (18, 79) and acetylation of the N-terminal tail of histone H4 (H4-Ac) (34). To analyze these histone modifications at a silenced region in *GAL1pr-SPT10* and *spt21*Δ strains, we performed ChIP, again assaying the regions at 0.6 kb and 2.8 kb from the right telomere of chromosome VI. Our results show that in both *GAL1pr-SPT10* and *spt21*Δ mutants, the levels of the two histone modifications were nearly indistinguishable from wild type levels at 0.6 kb from the telomere; however, the levels of both histone modifications are increased in both mutants at 2.8 kb from the telomere (Fig. 7A and B). By Western analysis, neither *GAL1pr-SPT10* nor *spt21*Δ mutations altered the overall level of these modified histones (Fig. 7C). These results indicate that *GAL1pr-SPT10* and *spt21*Δ mutants have significant alterations in the pattern of histone modifications, especially at greater distances from the telomere.

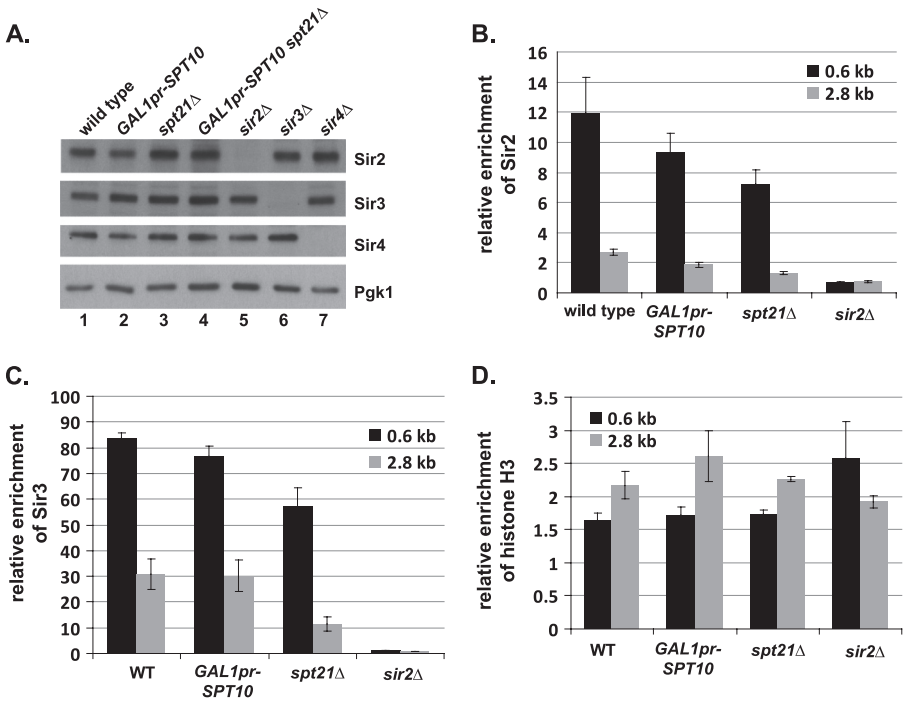

FIG. 6. Measurement of Sir protein levels and Sir protein association with silenced chromatin in *GAL1pr-SPT10* and *spt21*Δ mutants. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. (A) Measurement of Sir protein levels. Total protein was isolated from the indicated strains and analyzed by Western analysis. Pgk1 is a glycolytic enzyme and serves as a loading control. (B) Chromatin immunoprecipitation (ChIP) of Sir2 on chromosome VI. Antibodies specific to Sir2 were used to immunoprecipitate the proteins in sonicated chromatin extracts from wild-type, *GAL1pr-SPT10*, and *spt21*Δ strains. Following reversal of cross-linking, the amount of immunoprecipitated DNA was quantitated using real-time PCR with primers specific to regions 0.6 kb and 2.8 kb from the right telomere of chromosome VI. Values were calculated relative to binding at the *ACT1* gene, which is not subjected to silencing, and normalized to input DNA. Shown is the relative enrichment of Sir2 binding at the two subtelomeric locations. Plotted are the means ± SEM for four independent experiments. (C) ChIP of Sir3 was performed as described for Sir2, using an anti-Sir3 antiserum. (D) ChIP of histone H3 was conducted as described for Sir2, using an antiserum specific to histone H3.

Silenced chromatin is improperly assembled at telomeres in *GAL1pr-SPT10* and *spt21*Δ mutants. Previous studies showed that chromatin isolated from *spt10* mutants is hypersensitive to micrococcal nuclease digestion *in vitro* compared to chromatin from a wild-type strain (15). To examine chromatin structure *in vivo* for both *spt10* and *spt21* mutants, we assayed the accessibility of chromatin to bacterial dam methylase, which was previously shown to detect changes in chromatin structure in silenced regions (22, 29, 63). To do this, we examined the same region of telomere VR as described above, using previously described methods (69, 84) employing the methylation-specific enzymes DpnI, MboI, and Sau3AI, which cut methylated, unmethylated, and all GATC sequences, respectively (diagrammed in Fig. 8A). When we examined a region 1.3 kb from telomere VI-R, our results showed that DNA from the *GAL1pr-SPT10* and *spt21*Δ mutants is more readily cleaved by DpnI and less readily cleaved by MboI than is wild-type DNA (Fig. 8B, top panel). This pattern is similar to that seen in a *sir2*Δ mutant and indicates that the chromatin has increased accessibility for dam methylation, consistent with the silencing defect seen in these mutants. At 20 kb from the same telomere, dam methylase accessibility in the *sir2*Δ mutant more closely parallels that of the wild type, suggesting that chromatin structure is not substantially perturbed at that location (Fig. 8B, middle panel). However, in both *GAL1pr-SPT10* and *spt21*Δ mutants the chromatin is less accessible to MboI cleavage (Fig. 8B; compare band D to bands E and F), suggesting that it remains predominantly methylated, reflecting a more accessible chromatin structure. Differences from the wild type are less apparent with DpnI digestion at this locus, potentially because the enzyme digests both fully methylated DNA, which is inaccessible to dam methylation, and hemimethylated DNA, which is partially accessible. Taken together, these results suggest that the chromatin in *GALpr-SPT10* and *spt21*Δ mutants is more accessible than that in the wild type to dam methylase at both 1.3 kb and 20 kb from the telomere, reflecting an altered chromatin structure.

DISCUSSION

Our analysis of *spt10* and *spt21* mutations has shown that both Spt10 and Spt21 play a role in silencing in *S. cerevisiae.* Both *spt10* and *spt21* mutations impair silencing near telomeres and at the *HML*α mating type locus, while strengthening silencing at the rDNA. The most well-characterized role for Spt10 and Spt21 is in activating the transcription of histone genes, primarily *HTA2-HTB2* (13, 15, 27). However, deletion of this histone gene pair does not cause silencing defects, suggesting that Spt10 and Spt21 control silencing, at

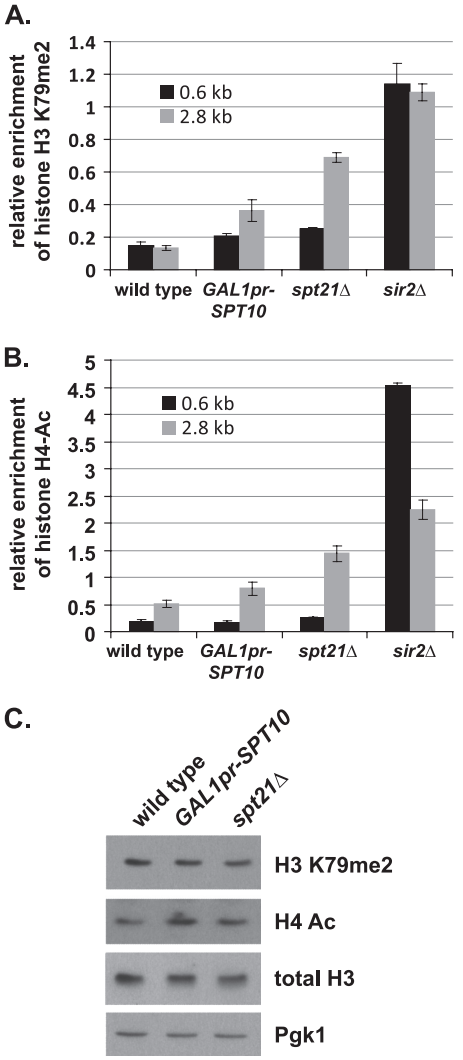

FIG. 7. Measurement of histone H3 K79 dimethylation and histone H4 acetylation levels at telomere VI-R in *GAL1pr-SPT10* and *spt21*Δ mutants. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele. (A) ChIP of histone H3 K79 dimethylation was performed using the same strains, primers, and calculations as for Fig. 6, using antibodies specific to dimethyl histone H3 K79. Values are shown relative to total histone H3 ChIP. (B) ChIP of acetylated histone H4. (C) Western blot to measure total cellular levels of dimethylated histone H3 K79, acetylated histone H4, total histone H3, and Pgk1 as a loading control.

least in part, by a mechanism that is independent of their roles in regulating *HTA2-HTB2* transcription.

Previous work has shown that mutations in other genes cause a profile of silencing phenotypes similar to that caused by *spt10* and *spt21* mutations, including a silencing defect at *HML*α but not *HMR***a**, an Eso$^-$ phenotype, and a telomeric silencing defect. The factors identified by these mutations, all of which play roles in Sir3 localization, include the NatA acetyltransferase complex subunits Nat1 and Ard1 (2, 71, 83), the histone H3 K79 methyltransferase Dot1 (18, 79), and the N-terminal BAH domain of Sir3 (71). These proteins have been found to regulate silencing through a common genetic pathway (80). The NatA complex acetylates Sir3 within its N-terminal BAH domain (80, 82), which results in increased binding specificity to the unmethylated histone H3 K79 characteristic of silenced regions (80). To prevent inappropriate Sir3 binding, Dot1 methylates histone H3 K79 residues in regions that are not silenced (1, 53, 79). Together, mutations in Nat1, Ard1, Dot1, and the Sir3 BAH domain are thought to disrupt silencing by interfering with Sir3 localization to silenced regions. The parallels between the silencing phenotypes of the above-described Sir3 regulatory mutants and *spt10* and *spt21* mutants raise the possibility that Spt10 and Spt21 may regulate silencing at telomeres and silent mating loci, at least in part, by modulating Sir3 function.

One possibility is that the silencing defect in *spt10* and *spt21* mutants is caused by multiple effects of the mutations. The modest decrease in the level of Sir proteins recruited to *telV-R* in *spt10* and *spt21* mutants, along with the significantly increased accessibility to *E. coli* dam methylase, suggests that a significant contribution to the silencing defect in *spt10* and *spt21* mutants occurs at a step subsequent to Sir protein recruitment. These phenotypes place *spt10* and *spt21* mutants in the same category as two other classes of silencing mutants recently described: those that alter H3 K56 (84) and those that delete the H3 amino-terminal tail (69). These histone mutants and other evidence (36, 86, 87) strongly suggest that the presence of Sir proteins, while necessary, is not sufficient to confer silencing. The silencing defects seen in *spt10* and *spt21* mutants are similar to those for H3 tail deletion in that effects are strongest at telomeres and are only seen at *HM* loci in the presence of a second mutation (69). Thus, as in the case of the H3 tail deletion mutant, the *spt10* and *spt21* mutations may impair higher-order chromatin structure. The *spt10* and *spt21* defects appear to be distinct from those that result from alteration of H3 K56 in that *spt10* and *spt21* mutations strengthen rDNA silencing, while the changes at H3 K56 were reported to weaken it (84). In common to all of these mutants, however, is the lack of a strong defect in the recruitment of Sir proteins, yet with greatly increased accessibility to *E. coli* dam methylase (69, 84). Perhaps changes in chromatin structure, as revealed by the increased accessibility to *E. coli* dam methylase, alter the ability of Sir proteins to function.

The mechanism by which Spt10 and Spt21 control silencing is currently unclear. Based on previous results (15) and our ChIP experiments, neither protein appears to associate with chromatin at telomeres or anywhere other than histone gene promoters. While our results are consistent with a role for Spt10 and Spt21 in silencing outside their roles in histone gene regulation, until the mechanism is understood, we cannot rule out a contribution by the altered histone levels that exist in *spt10* and *spt21* mutants. Purification of Spt10 from *S. cerevisiae* has not provided any clues, as no interactions with other proteins were detected (8), though Spt10 has been shown to interact directly with Spt21 when they are both overexpressed in *E. coli* or when Spt21 is overexpressed in yeast (28). Given that Spt10 has sequence motifs that strongly suggest that it is an acetyltransferase (52), and given that genetic evidence has shown these motifs to be important for Spt10 function *in vivo* (28), one intriguing possibility is that Spt10 acetylates a nonhistone protein that

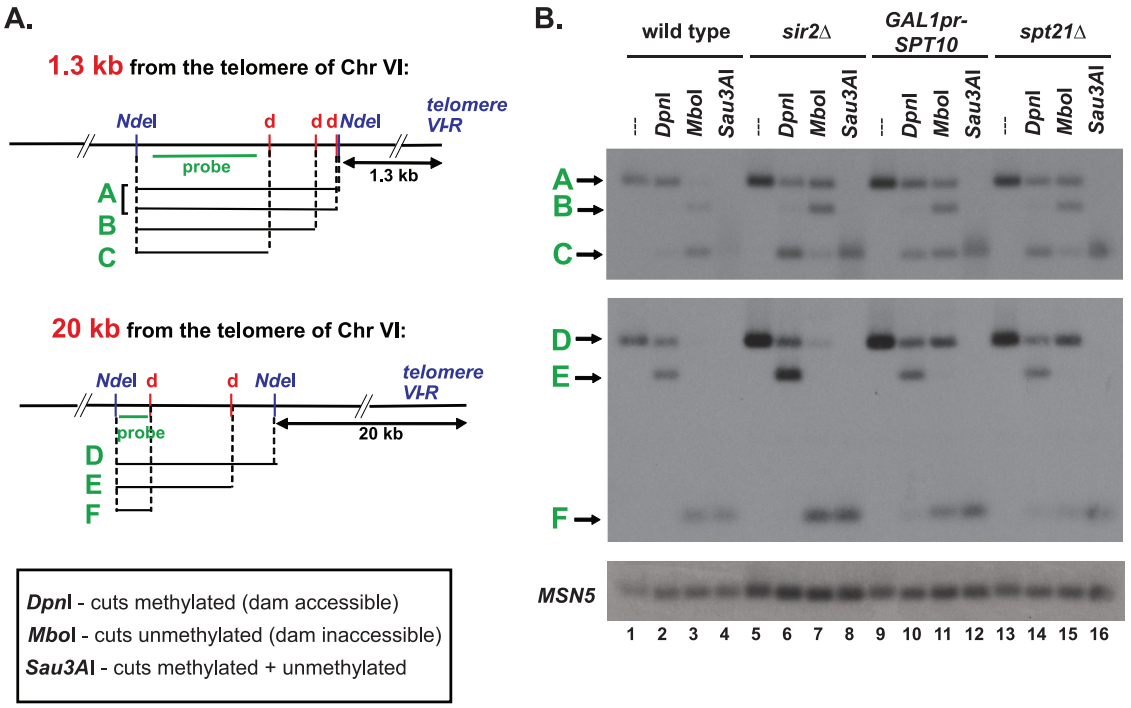

FIG. 8. Dam methylation assays to probe chromatin structure *in vivo*. (A) A restriction map of relevant sites within the regions probed. The region 1.3 kb from the right telomere of chromosome VI is subject to silencing, and the telomere-proximal end lies 200 bp from the sequences probed in ChIP and *YFR057W* RT-PCR (Fig. 2B, 6, and 7). The region approximately 20 kb from the same telomere is at a distance not expected to be subject to telomere position effect (23). In both cases, multiple dam methylation sites occur between NdeI digest sites, resulting in multiple bands. For the 1.3-kb region, a dam methylation site is present 16 bp from the NdeI site, and digestion does not result in a significant mobility shift (band A). A region within *MSN5*, where no dam methylation sites occur between two NdeI sites, serves as a loading control. (B) Southern blot analysis of genomic DNA digested with NdeI and the indicated methylation-specific enzymes, separated on an agarose gel and probed for the regions indicated in panel A. The same blot was probed in all three panels. All strains were grown in glucose, which represses expression of the *GAL1pr-SPT10* allele.

is required for silencing. Clearly, the elusive acetyltransferase activity of Spt10 must be understood in order to elucidate the functions of Spt10 and Spt21 in silencing and other activities in which they have been implicated.

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REFERENCES

1. **Altaf, M., R. T. Utley, N. Lacoste, S. Tan, S. D. Briggs, and J. Cote.** 2007. Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. Mol. Cell **28:**1002–1014.
2. **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.
3. **Askree, S. H., T. Yehuda, S. Smolikov, R. Gurevich, J. Hawk, C. Coker, A. Krauskopf, M. Kupiec, and M. J. McEachern.** 2004. A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length. Proc. Natl. Acad. Sci. U. S. A. **101:**8658–8663.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1988. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, NY.
5. **Barton, A. B., and D. B. Kaback.** 2006. Telomeric silencing of an open reading frame in *Saccharomyces cerevisiae*. Genetics **173:**1169–1173.
6. **Braun, M. A., P. J. Costa, E. M. Crisucci, and K. M. Arndt.** 2007. Identification of Rkr1, a nuclear RING domain protein with functional connections to chromatin modification in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **27:**2800–2811.
7. **Buhler, M., and S. M. Gasser.** 2009. Silent chromatin at the middle and ends: lessons from yeasts. EMBO J. **28:**2149–2161.
8. **Chang, J.** 2009. Ph.D. thesis. Harvard University, Cambridge, MA.
9. **Cho, R. J., M. J. Campbell, E. A. Winzeler, L. Steinmetz, A. Conway, L. Wodicka, T. G. Wolfsberg, A. E. Gabrielian, D. Landsman, D. J. Lockhart, and R. W. Davis.** 1998. A genome-wide transcriptional analysis of the mitotic cell cycle. Mol. Cell **2:**65–73.
10. **Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter.** 1992. Multifunctional yeast high-copy-number shuttle vectors. Gene **110:**119–122.
11. **Clapier, C. R., and B. R. Cairns.** 2009. The biology of chromatin remodeling complexes. Annu. Rev. Biochem. **78:**273–304.
12. **Denis, C. L., and T. Malvar.** 1990. The CCR4 gene from *Saccharomyces cerevisiae* is required for both nonfermentative and spt-mediated gene expression. Genetics **124:**283–291.
13. **Dollard, C., S. L. Ricupero-Hovasse, G. Natsoulis, J. D. Boeke, and F. Winston.** 1994. SPT10 and SPT21 are required for transcription of particular histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**5223–5228.
14. **Donze, D., C. R. Adams, J. Rine, and R. T. Kamakaka.** 1999. The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. Genes Dev. **13:**698–708.
15. **Eriksson, P. R., G. Mendiratta, N. B. McLaughlin, T. G. Wolfsberg, L. Marino-Ramirez, T. A. Pompa, M. Jainerin, D. Landsman, C. H. Shen, and D. J. Clark.** 2005. Global regulation by the yeast Spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. Mol. Cell. Biol. **25:**9127–9137.
16. **Fassler, J. S., and F. Winston.** 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. Genetics **118:**203–212.
17. **Fox, C. A., and K. H. McConnell.** 2005. Toward biochemical understanding of a transcriptionally silenced chromosomal domain in *Saccharomyces cerevisiae*. J. Biol. Chem. **280:**8629–8632.
18. **Frederiks, F., M. Tzouros, G. Oudgenoeg, T. van Welsem, M. Fornerod, J. Krijgsveld, and F. van Leeuwen.** 2008. Nonprocessive methylation by Dot1

leads to functional redundancy of histone H3K79 methylation states. Nat. Struct. Mol. Biol. **15:**550–557.
19. **Gallagher, S. R.** 2006. One-dimensional SDS gel electrophoresis of proteins. Curr. Protoc. Mol. Biol. **75:**8.4.1–8.4.37.
20. **Gatbonton, T., M. Imbesi, M. Nelson, J. M. Akey, D. M. Ruderfer, L. Kruglyak, J. A. Simon, and A. Bedalov.** 2006. Telomere length as a quantitative trait: genome-wide survey and genetic mapping of telomere length-control genes in yeast. PLoS Genet. **2:**e35.
21. **Goldstein, A. L., and J. H. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:**1541–1553.
22. **Gottschling, D. E.** 1992. Telomere-proximal DNA in *Saccharomyces cerevisiae* is refractory to methyltransferase activity in vivo. Proc. Natl. Acad. Sci. U. S. A. **89:**4062–4065.
23. **Gottschling, D. E., O. M. Aparicio, B. L. Billington, and V. A. Zakian.** 1990. Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell **63:**751–762.
24. **Guthrie, C., and G. R. Fink.** 1991. Guide to yeast genetics and molecular biology. Academic Press, San Diego, CA.
25. **Hecht, A., S. Strahl-Bolsinger, and M. Grunstein.** 1996. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. Nature **383:**92–96.
26. **Hereford, L., K. Fahrner, J. Woolford, Jr., M. Rosbash, and D. B. Kaback.** 1979. Isolation of yeast histone genes H2A and H2B. Cell **18:**1261–1271.
27. **Hess, D.** 2004. Ph.D. thesis. Harvard University, Cambridge, MA.
28. **Hess, D., B. Liu, N. R. Roan, R. Sternglanz, and F. Winston.** 2004. Spt10-dependent transcriptional activation in *Saccharomyces cerevisiae* requires both the Spt10 acetyltransferase domain and Spt21. Mol. Cell. Biol. **24:**135–143.
29. **Hoekstra, M. F., and R. E. Malone.** 1985. Expression of the *Escherichia coli dam* methylase in *Saccharomyces cerevisiae*: effect of in vivo adenine methylation on genetic recombination and mutation. Mol. Cell. Biol. **5:**610–618.
30. **Hoffman, C. S., and F. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. Gene **57:**267–272.
31. **Huang, J., and D. Moazed.** 2003. Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. Genes Dev. **17:**2162–2176.
32. **Imai, S., F. B. Johnson, R. A. Marciniak, M. McVey, P. U. Park, and L. Guarente.** 2000. Sir2: an NAD-dependent histone deacetylase that connects chromatin silencing, metabolism, and aging. Cold Spring Harbor Symp. Quant. Biol. **65:**297–302.
33. **Johnson, A., G. Li, T. W. Sikorski, S. Buratowski, C. L. Woodcock, and D. Moazed.** 2009. Reconstitution of heterochromatin-dependent transcriptional gene silencing. Mol. Cell **35:**769–781.
34. **Johnson, L. M., G. Fisher-Adams, and M. Grunstein.** 1992. Identification of a non-basic domain in the histone H4 N-terminus required for repression of the yeast silent mating loci. EMBO J. **11:**2201–2209.
35. **Kaufman, P. D., J. L. Cohen, and M. A. Osley.** 1998. Hir proteins are required for position-dependent gene silencing in *Saccharomyces cerevisiae* in the absence of chromatin assembly factor I. Mol. Cell. Biol. **18:**4793–4806.
36. **Kirchmaier, A. L., and J. Rine.** 2006. Cell cycle requirements in assembling silent chromatin in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **26:**852–862.
37. **Komarnitsky, P., E. J. Cho, and S. Buratowski.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. **14:**2452–2460.
38. **Landry, J., J. T. Slama, and R. Sternglanz.** 2000. Role of NAD(+) in the deacetylase activity of the SIR2-like proteins. Biochem. Biophys. Res. Commun. **278:**685–690.
39. **Landry, J., A. Sutton, S. T. Tafrov, R. C. Heller, J. Stebbins, L. Pillus, and R. Sternglanz.** 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. U. S. A. **97:**5807–5811.
40. **Lau, A., H. Blitzblau, and S. P. Bell.** 2002. Cell-cycle control of the establishment of mating-type silencing in *S. cerevisiae*. Genes Dev. **16:**2935–2945.
41. **Li, B., M. Carey, and J. L. Workman.** 2007. The role of chromatin during transcription. Cell **128:**707–719.
42. **Liou, G. G., J. C. Tanny, R. G. Kruger, T. Walz, and D. Moazed.** 2005. Assembly of the SIR complex and its regulation by O-acetyl-ADP-ribose, a product of NAD-dependent histone deacetylation. Cell **121:**515–527.
43. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:**953–961.
44. **Loo, S., P. Laurenson, M. Foss, A. Dillin, and J. Rine.** 1995. Roles of ABF1, NPL3, and YCL54 in silencing in *Saccharomyces cerevisiae*. Genetics **141:**889–902.
45. **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.
46. **Martino, F., S. Kueng, P. Robinson, M. Tsai-Pflugfelder, F. van Leeuwen, M. Ziegler, F. Cubizolles, M. M. Cockell, D. Rhodes, and S. M. Gasser.** 2009. Reconstitution of yeast silent chromatin: multiple contact sites and O-AADPR binding load SIR complexes onto nucleosomes in vitro. Mol. Cell **33:**323–334.
47. **Mendiratta, G., P. R. Eriksson, and D. J. Clark.** 2007. Cooperative binding of the yeast Spt10p activator to the histone upstream activating sequences is mediated through an N-terminal dimerization domain. Nucleic Acids Res. **35:**812–821.
48. **Mendiratta, G., P. R. Eriksson, C. H. Shen, and D. J. Clark.** 2006. The DNA-binding domain of the yeast Spt10p activator includes a zinc finger that is homologous to foamy virus integrase. J. Biol. Chem. **281:**7040–7048.
49. **Moretti, P., K. Freeman, L. Coodly, and D. Shore.** 1994. Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. Genes Dev. **8:**2257–2269.
50. **Natsoulis, G., C. Dollard, F. Winston, and J. D. Boeke.** 1991. The products of the SPT10 and SPT21 genes of *Saccharomyces cerevisiae* increase the amplitude of transcriptional regulation at a large number of unlinked loci. New Biol. **3:**1249–1259.
51. **Natsoulis, G., F. Winston, and J. D. Boeke.** 1994. The SPT10 and SPT21 genes of *Saccharomyces cerevisiae*. Genetics **136:**93–105.
52. **Neuwald, A. F., and D. Landsman.** 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem. Sci. **22:**154–155.
53. **Ng, H. H., D. N. Ciccone, K. B. Morshead, M. A. Oettinger, and K. Struhl.** 2003. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. Proc. Natl. Acad. Sci. U. S. A. **100:**1820–1825.
54. **Pillus, L., and J. Rine.** 1989. Epigenetic inheritance of transcriptional states in *S. cerevisiae*. Cell **59:**637–647.
55. **Reifsnyder, C., J. Lowell, A. Clarke, and L. Pillus.** 1996. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. **14:**42–49.
56. **Renauld, H., O. M. Aparicio, P. D. Zierath, B. L. Billington, S. K. Chhablani, and D. E. Gottschling.** 1993. Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by SIR3 dosage. Genes Dev. **7:**1133–1145.
57. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
58. **Rusche, L. N., A. L. Kirchmaier, and J. Rine.** 2002. Ordered nucleation and spreading of silenced chromatin in *Saccharomyces cerevisiae*. Mol. Biol. Cell **13:**2207–2222.
59. **Rusche, L. N., A. L. Kirchmaier, and J. Rine.** 2003. The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. Annu. Rev. Biochem. **72:**481–516.
60. **Ruthenburg, A. J., H. Li, D. J. Patel, and C. D. Allis.** 2007. Multivalent engagement of chromatin modifications by linked binding modules. Nat. Rev. Mol. Cell Biol. **8:**983–994.
61. **Sherwood, P. W., and M. A. Osley.** 1991. Histone regulatory (hir) mutations suppress delta insertion alleles in *Saccharomyces cerevisiae*. Genetics **128:**729–738.
62. **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.
63. **Singh, J., and A. J. Klar.** 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.
64. **Smith, J. S., and J. D. Boeke.** 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. **11:**241–254.
65. **Smith, J. S., C. B. Brachmann, I. Celic, M. A. Kenna, S. Muhammad, V. J. Starai, J. L. Avalos, J. C. Escalante-Semerena, C. Grubmeyer, C. Wolberger, and J. D. Boeke.** 2000. A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. Proc. Natl. Acad. Sci. U. S. A. **97:**6658–6663.
66. **Smith, J. S., C. B. Brachmann, L. Pillus, and J. D. Boeke.** 1998. Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. Genetics **149:**1205–1219.
67. **Smith, J. S., E. Caputo, and J. D. Boeke.** 1999. A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. Mol. Cell. Biol. **19:**3184–3197.
68. **Spellman, P. T., G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders, M. B. Eisen, P. O. Brown, D. Botstein, and B. Futcher.** 1998. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol. Biol. Cell **9:**3273–3297.
69. **Sperling, A. S., and M. Grunstein.** 2009. Histone H3 N-terminus regulates higher order structure of yeast heterochromatin. Proc. Natl. Acad. Sci. U. S. A. **106:**13153–13159.
70. **Stone, E. M., and L. Pillus.** 1996. Activation of an MAP kinase cascade leads to Sir3p hyperphosphorylation and strengthens transcriptional silencing. J. Cell Biol. **135:**571–583.
71. **Stone, E. M., C. Reifsnyder, M. McVey, B. Gazo, and L. Pillus.** 2000. Two

classes of sir3 mutants enhance the sir1 mutant mating defect and abolish telomeric silencing in *Saccharomyces cerevisiae*. Genetics **155:**509–522.
72. **Strahl-Bolsinger, S., A. Hecht, K. Luo, and M. Grunstein.** 1997. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. Genes Dev. **11:**83–93.
73. **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.
74. **Swanson, M. S., E. A. Malone, and F. Winston.** 1991. SPT5, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol. Cell. Biol. **11:** 3009–3019.
75. **Tanner, K. G., J. Landry, R. Sternglanz, and J. M. Denu.** 2000. Silent information regulator 2 family of NAD-dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose. Proc. Natl. Acad. Sci. U. S. A. **97:**14178–14182.
76. **Tanny, J. C., G. J. Dowd, J. Huang, H. Hilz, and D. Moazed.** 1999. An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell **99:**735–745.
77. **Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader, N. Page, M. Robinson, S. Raghibizadeh, C. W. Hogue, H. Bussey, B. Andrews, M. Tyers, and C. Boone.** 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:**2364–2368.
78. **Triolo, T., and R. Sternglanz.** 1996. Role of interactions between the origin recognition complex and SIR1 in transcriptional silencing. Nature **381:**251–253.
79. **van Leeuwen, F., P. R. Gafken, and D. E. Gottschling.** 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell **109:**745–756.
80. **van Welsem, T., F. Frederiks, K. F. Verzijlbergen, A. W. Faber, Z. W. Nelson, D. A. Egan, D. E. Gottschling, and F. van Leeuwen.** 2008. Synthetic lethal screens identify gene silencing processes in yeast and implicate the acetylated amino terminus of Sir3 in recognition of the nucleosome core. Mol. Cell. Biol. **28:**3861–3872.
81. **Vega-Palas, M. A., E. Martin-Figueroa, and F. J. Florencio.** 2000. Telomeric silencing of a natural subtelomeric gene. Mol. Gen. Genet. **263:**287–291.
82. **Wang, X., J. J. Connelly, C. L. Wang, and R. Sternglanz.** 2004. Importance of the Sir3 N terminus and its acetylation for yeast transcriptional silencing. Genetics **168:**547–551.
83. **Whiteway, M., R. Freedman, S. Van Arsdell, J. W. Szostak, and J. Thorner.** 1987. The yeast ARD1 gene product is required for repression of cryptic mating-type information at the HML locus. Mol. Cell. Biol. **7:**3713–3722.
84. **Xu, F., Q. Zhang, K. Zhang, W. Xie, and M. Grunstein.** 2007. Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. Mol. Cell **27:**890–900.
85. **Yamashita, I.** 1993. Isolation and characterization of the SUD1 gene, which encodes a global repressor of core promoter activity in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **241:**616–626.
86. **Yang, B., and A. L. Kirchmaier.** 2006. Bypassing the catalytic activity of SIR2 for SIR protein spreading in *Saccharomyces cerevisiae*. Mol. Biol. Cell **17:** 5287–5297.
87. **Yang, B., A. Miller, and A. L. Kirchmaier.** 2008. HST3/HST4-dependent deacetylation of lysine 56 of histone H3 in silent chromatin. Mol. Biol. Cell **19:**4993–5005.