SAS4 and *SAS5* Are Locus-Specific Regulators of Silencing in *Saccharomyces cerevisiae*

Eugenia Y. Xu, Susan Kim and David H. Rivier

Department of Cell and Structural Biology and Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Manuscript received October 5, 1998
Accepted for publication May 4, 1999

ABSTRACT

Sir2p, Sir3p, Sir4p, and the core histones form a repressive chromatin structure that silences transcription in the regions near telomeres and at the *HML* and *HMR* cryptic mating-type loci in *Saccharomyces cerevisiae*. Null alleles of *SAS4* and *SAS5* suppress silencing defects at *HMR*; therefore, *SAS4* and *SAS5* are negative regulators of silencing at *HMR*. This study revealed that *SAS4* and *SAS5* contribute to silencing at *HML* and the telomeres, indicating that *SAS4* and *SAS5* are positive regulators of silencing at these loci. These paradoxical locus-specific phenotypes are shared with null alleles of *SAS2* and are unique among phenotypes of mutations in other known regulators of silencing. This work also determined that these *SAS* genes play roles that are redundant with *SIR1* at *HML*, yet distinct from *SIR1* at *HMR*. Furthermore, these *SAS* genes are not redundant with each other in silencing *HML*. Collectively, these data suggest that *SAS2*, *SAS4*, and *SAS5* constitute a novel class of regulators of silencing and reveal fundamental differences in the regulation of silencing at *HML* and *HMR*. We provide evidence for a model that accounts for the observation that these *SAS* genes are both positive and negative regulators of silencing.

THREE regions of the yeast genome, the *HML* and *HMR* cryptic mating-type loci and the regions adjacent to the telomeres, are each assembled into a heterochromatic structure that inactivates transcription. Inactivation of transcription at *HML* and *HMR* is referred to as silencing, whereas inactivation of transcription in the telomeric regions is typically referred to as the telomeric position effect or TPE. Silencing and TPE depend on histone H3, histone H4, and on Sir2p, Sir3p, and Sir4p, which associate with each other to form heterochromatin in the silent regions (reviewed in Grunstein 1997, 1998; Lustig 1998). Furthermore, silencing and TPE are mitotically stable forms of gene inactivation; once a gene is silenced it remains silent through many rounds of cell division (Pillus and Rine 1989; Gottschling *et al.* 1990). The initial inactivation of the gene, establishment, corresponds to the assembly of heterochromatin, whereas the clonal propagation of silencing, inheritance, presumably results from the duplication of heterochromatin during DNA replication and mitosis. A related form of silencing also occurs at the *RDN1* locus, the region of the yeast genome that contains approximately 200 repeated copies of the ribosomal DNA (rDNA; Bryk *et al.* 1997; Smith and Boeke 1997).

Silencing at *HML* and *HMR* requires DNA elements known as silencers (Abraham *et al.* 1983; Feldman *et al.* 1984; Brand *et al.* 1985). The two silencers that flank *HML* are known as *HML-E* and *HML-I*, and the two that flank *HMR* are known as *HMR-E* and *HMR-I*. The silencers bind combinations of three proteins: ORC, the replication initiator protein, and two transcriptional activators, Rap1p and Abf1p (reviewed in Laurenson and Rine 1992; Loo and Rine 1995).

The establishment of silencing and assembly of heterochromatin in the silent regions is thought to occur in two steps. The first step, nucleation, involves the initial recruitment of Sir3p and Sir4p to the silent regions. The second step involves the subsequent spreading or polymerization of heterochromatin throughout the region. At least one role of the silencers, telomeres, and their associated proteins is to nucleate the formation of heterochromatin. In particular, Rap1p binds the silencers and telomeres and recruits Sir3p and Sir4p to the loci, and Sir3p and Sir4p, in turn, nucleate the assembly of heterochromatin (Moretti *et al.* 1994; Lustig *et al.* 1996; Marcand *et al.* 1996). Similarly, Sir1p binds to ORC, recruits Sir4p, and plays a central role in nucleating silencing (Chien *et al.* 1993; Triolo and Sternglanz 1996; Gardner *et al.* 1999).

Each of the silenced regions is differentially sensitive to mutations in the genes that contribute to, but are not required for, silencing. For instance, *NAT1* and *ARD1* encode subunits of an N-terminal acetyl transferase that positively regulates silencing (Whiteway *et al.* 1987; Mullen *et al.* 1989; Aparicio *et al.* 1991; Park and Szostak 1992). Mutations in *NAT1* or *ARD1* result in a loss of TPE and a partial loss of silencing at *HML* but do not result in a loss of silencing at *HMR* (Whiteway *et al.* 1987; Mullen *et al.* 1989; Aparicio *et al.* 1991). However, mutation of *NAT1* or *ARD1* results in a sub-

Corresponding author: David H. Rivier, Department of Cell and Structural Biology, University of Illinois, 601 S. Goodwin Ave., Urbana, IL 61801. E-mail: rivier@uiuc.edu

stantial loss of silencing at *HMR* in combination with a mutation in *SIR1* (Whiteway *et al.* 1987; Stone *et al.* 1991). Consequently, it has been proposed that a hierarchy of silencing exists in which silencing at the telomeres is less efficient than silencing at *HML*, which is less efficient than silencing at *HMR* (Aparicio *et al.* 1991).

The differential efficiency of silencing among the silent loci is due, at least in part, to the locus-specific action of Sir1p. Deletion of *SIR1* results in a partial loss of silencing at *HML* and *HMR* but does not result in a defect in TPE (Aparicio *et al.* 1991). Thus, Sir1p contributes to silencing at *HML* and *HMR* but does not contribute to TPE. Consequently, the increased efficiency of silencing at *HML* and *HMR* relative to TPE is likely due, at least in part, to the action of Sir1p at *HML* and *HMR* but not at the telomeres. The basis for the greater efficiency of silencing at *HMR* relative to *HML* is not known.

The efficiency of silencing in a particular region can also be influenced indirectly by perturbations that alter the physical distribution of the protein components of heterochromatin within the nucleus. For instance, deletion of *SIR4* results in increased silencing at the *RDN1* locus (Smith and Boeke 1997). In contrast to HM silencing and TPE, *SIR4* is not a direct regulator of silencing at *RDN1* (J. S. Smith *et al.* 1998). However, *SIR2* is required for rDNA silencing, and furthermore, the endogenous level of Sir2p is limiting for silencing within the rDNA. It has been proposed that deletion of *SIR4* results in a loss of TPE and a failure of Sir2p to sequester at the telomeres, thereby increasing the effective concentration of free Sir2p and resulting in increased silencing in the rDNA (J. S. Smith *et al.* 1998). Therefore, deletion of *SIR4* is thought to increase silencing in the rDNA as an indirect consequence of disruption of TPE.

Taken together, these observations suggest that silencing is regulated by three classes of genes: (1) genes that encode components of heterochromatin or direct regulators of silencing at *HML*, *HMR*, and the telomeres; (2) genes that encode locus-specific regulators of silencing; and (3) genes that encode proteins that indirectly effect silencing by altering the distribution of components of the silencing machinery.

Deletion of *SAS2* causes silencing defects at *HML* and telomeres but suppresses silencing defects at *HMR* (Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997). Therefore, *SAS2* behaves as a positive regulator of TPE and silencing at *HML* and a negative regulator of silencing at *HMR*. These opposite phenotypes at *HML* and *HMR* are unique among mutations known to effect silencing, suggesting that an understanding of the basis for these locus-specific phenotypes will likely lead to new insights into the regulation of silencing. We recently identified two genes, *SAS4* and *SAS5*, that, when mutated, are capable of restoring silencing at *HMR* in the presence of a partially defective *HMR-E* silencer (Xu *et al.* 1999). Thus, *SAS4* and *SAS5*, like *SAS2*, are formally negative regulators of silencing at *HMR*. In this report we investigated whether the *SAS4* and *SAS5* genes had the same set of unique locus-specific regulatory properties as *SAS2*. Furthermore, we investigated a possible mechanism by which *SAS2* acts as a positive regulator of silencing at *HML* and a negative regulator of silencing at *HMR*.

MATERIALS AND METHODS

Strain construction: The entire coding regions of the *SAS4* and *SAS5* genes were deleted by PCR-mediated gene disruption (Baudin *et al.* 1993) as described previously (Xu *et al.* 1999). *SAS4* was deleted from the haploid strains UCC1001 and DRY439 to generate DRY1371 and DRY1364, respectively. All gene disruptions were confirmed by DNA blot analysis. All additional W303-derived strains containing the *sas4*Δ*::kanMX4* allele were derived from crosses of DRY1322 to standard laboratory strains, as described below (see Table 1). *SAS5* was deleted from haploid strains UCC1001, UCC1003, and JRY5273, resulting in DRY1372, DRY1392, and DRY1314, respectively. All additional W303-derived strains containing the *sas5*Δ*::HIS3* allele were derived from crosses of DRY1314 to standard laboratory strains, as described below. *sas2*Δ*-1::TRP1* strains were similarly derived from crosses with JRY5071 (*MAT*α *sas2-*Δ*1::TRP1*; Ehrenhofer-Murray *et al.* 1997).

A series of strains (DRY1655–1657, DRY1661–1664, and DRY1697–1699) containing various combinations of null alleles of the *SAS* genes with wild-type *HMR* were segregants derived from a diploid formed from a cross between JRY5071 and DRY1345 (W303-1a; *hmr*Δ*::URA3 sas4*Δ*::kanMX4 sas5*Δ*:: HIS3*).

Strains containing combinations of null alleles of the *SAS* genes together with a null allele of *SIR1* were generated from two crosses. DRY1658 and DRY1800 were segregants from a cross between JRY4622 and DRY1805 (W303-1a; *MAT*α *sas2-*Δ*1::TRP1*). DRY1659, DRY1660, DRY1801, and DRY1802 were segregants from a cross between JRY4622 and DRY1806 (W303-1a; *MAT*α *sas4*Δ*::kanMX4 sas5*Δ*::HIS3*). DRY1805 and DRY1806 were segregants from the cross between JRY5071 and DRY1345 described above.

DRY1399 (*HMR***a***-e*** *sir1*Δ*::LEU2*) was a segregant derived from a cross between JRY4622 (*sir1*Δ*::LEU2*) and DRY1314 (*HMR***a***-e*** *sas5*Δ*::HIS3*). DRY1424 (*HMR-SS* Δ*I sas5*Δ*::HIS3*) was a segregant from a cross between DRY439 (*HMR-SS* Δ*I*) and DRY1316 (W303-1a; *MAT***a** *HMR-ssabf1::ADE2 sas5*Δ*:: HIS3*).

PCR protocol: PCR reactions for gene disruption were carried out using the high-fidelity Elongase kit (GIBCO, Grand Island, NY) under the conditions recommended by the manufacturer.

Plasmid construction: pDR590 (pRS426-*SIR3*) was constructed by cloning a 4.5-kb *Sal*I fragment containing the *SIR3* gene from pJR508 (provided by J. Rine) into *Sal*I cleaved pRS426 (Christianson *et al.* 1992). pDR583 (pRS426-*SIR4*) was constructed in two steps. A 6.8-kb *Eco*RI-*Sst*II fragment of *SIR4* derived from pJR368 (provided by J. Rine) was inserted into pBluescript cleaved with *Eco*RI and *Sst*II resulting in pDR304. The *Xho*I-*Sst*II *SIR4*-containing fragment of pDR304 was inserted into *Xho*I-*Sst*II-cleaved pRS246 resulting in pDR583.

Quantitative and patch mating assays: Quantitative matings were performed as described previously (Xu *et al.* 1999). For patch mating analysis, test strains were patched onto solid rich medium, grown overnight, replica plated onto a lawn of $\sim 1.2 \times 10^7$ *MAT***a** cells (JRY2726) or *MAT*α cells (JRY2728) on YM plates supplemented with adenine, and grown for 1–2 days at 30°. Strains containing pRS426-derived plasmids were

TABLE 1

Strains used in this study

Strain		Source
UCC1001[a]	*MAT***a** *TEL(VIII) adh4::URA3 ade2-101*^{oc} *his3-Δ200 leu2-Δ1 lys2-801*^{am} *trp1-Δ1 ura3-52*	D. Gottschling
UCC1003	*MAT***a** *adh4::URA3*	D. Gottschling
W303-1a[b]	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	R. Rothstein
DRY439	*MATα HMR***a***-SSΔI*	
DRY1235	*MATα sir4Δ::URA3 lys2Δ::hisG*	
DRY1236	*MAT***a** *sir4Δ::URA3 lys2Δ::hisG*	
DRY1264	*MATα sir3Δ::LEU2*	
DRY1314	*MATα HMR***a***-e** sas5ΔHIS3*	
DRY1322	*MATα HMR***a***-e** sas4Δ::kanMX4 ade2::HIS3*	
DRY1338	*MAT***a***/α HMR***a***-e**/HMR***a***-ssb::ADE2 ade2::HIS3/ade2::Leu2*	
DRY1361	DRY1338 *sas4Δ::kanMX4/SAS4*	
DRY1364	*MATα HMR***a***-SSΔI sas4Δ::kanMX4*	
DRY1371	UCC1001 *sas4Δ::kanMX4*	
DRY1372	UCC1001 *sas5Δ::HIS3*	
DRY1391	*MAT***a** *HMR***a***-e** sas5Δ::HIS3*	
DRY1392	UCC1003 *sas5Δ::HIS3*	
DRY1399	*MATα HMR***a***-e** sir1Δ::LEU2 lys2::hisG*	
DRY1424	*MATα HMR***a***-SSΔI sas5Δ::HIS3*	
DRY1448	*MATα* [pRS426]	
DRY1452	JRY5273 [pRS426]	
DRY1456	*MATα HMR***a***-e** sas5Δ HIS3* [pRS426]	
DRY1460	JRY5273 [pDR583 (pRS426-*SIR4*)]	
DRY1464	JRY5273 [pDR590 (pRS426-*SIR3*)]	
DRY1655	*MAT***a** *sas2-Δ1::TRP1 ADE2*	
DRY1656	*MAT***a** *sas4Δ::kan ADE2*	
DRY1657	*MAT***a** *sas5Δ::HIS3 ADE2*	
DRY1658	*MAT***a** *sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2*	
DRY1659	*MAT***a** *sir1Δ::LEU2 sas4Δ::kanMX4 ADE2*	
DRY1660	*MAT***a** *sir1Δ::LEU2 sas5Δ::HIS3 ADE2*	
DRY1661	*MAT***a** *sas2-Δ1::TRP1 sas5Δ::HIS3 ADE2*	
DRY1662	*MAT***a** *sas2Δ1::TRP1 sas4Δ::kanMX4 ADE2*	
DRY1663	*MAT***a** *sas4Δ::kan sas5Δ::HIS3 ADE2*	
DRY1664	*MAT***a** *sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2*	
DRY1797	*MATα sas2-Δ1::TRP1 ADE2*	
DRY1798	*MATα sas4Δ::kanMX4 ADE2*	
DRY1799	*MATα sas5Δ::HIS3 ADE2*	
DRY1800	*MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2 lys2Δ::hisG*	
DRY1801	*MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2*	
DRY1802	*MATα sir1Δ::LEU2 sas5Δ::HIS3 ADE2*	
JRY3009	*MATα*	J. Rine
JRY4621	*MATα sir1Δ::LEU2 ADE2 lys2Δ::hisG*	J. Rine
JRY4622	*MAT***a** *sir1Δ::LEU2 lys2::hisG*	J. Rine
JRY4624	*MATα HMR***a***-SSΔI sir1Δ::HIS3*	J. Rine
JRY5071	*MATα sas2-Δ1::TRP1*	J. Rine
JRY5273	*MATα HMR***a***-e***	J. Rine
JRY2726[c]	*MAT***a** *his4*	J. Rine
JRY2728[c]	*MATα his4*	J. Rine

[a] Strains below are isogenic with UCC1001 except as noted.
[b] Strains below are isogenic with W303-1a except as noted.
[c] JRY2726 and JRY2728 are lawn strains for mating assays.

patched onto solid minimal medium lacking uracil, incubated for 2 days at 30°, and replica plated onto mating lawns as described above.

Assay for TPE: Silencing of the *TEL(VIII) adh4::URA3* gene (Gottschling *et al.* 1990) was measured as a function of growth on medium containing 5-fluoroorotic acid (5-FOA; Guthrie and Fink 1991). Aliquots (5 μl) of 10-fold serial dilutions containing from 10^6 to 10^2 cells per aliquot were spotted onto solid minimal medium containing 5-FOA and incubated for 2–3 days at 30°. As a control for cell viability, 5-μl aliquots of the serial dilutions were also spotted onto solid rich medium and onto minimal medium supplemented with uracil.

Media and genetic manipulations: Rich medium (YPD) and minimal medium (YM) were as described (Sherman 1991). Medium containing 5-FOA was as described (Guthrie and Fink 1991). Transformation was by a modified lithium-acetate method (Gietz and Schiestl 1991).

RESULTS

SAS4* and *SAS5* are required for TPE:** The *HMR-E* silencer is composed of an ARS consensus sequence (ACS) element, which is the binding site for ORC, and one binding site each for Rap1p and Abf1p (Brand *et al.* 1987; Kimmerly *et al.* 1988; McNally and Rine 1991). *SAS2*, *SAS4*, and *SAS5* were identified by recessive mutations that restored silencing to an allele of *HMR* that contained the defective *HMRa**-*e*** silencer (Axelrod and Rine 1991; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1999). This silencer contains a point mutation in the Rap1 binding site and a 1-bp insertion in the Abf1 binding site and is almost completely defective in silencing. Null mutations in *SAS2*, *SAS4*, or *SAS5* restore silencing to *HMR***a**-*e*** (Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1999).

To further characterize the role of *SAS4* and *SAS5* in silencing, we tested whether these genes were required for TPE. Yeast strains that transcribe *URA3* are sensitive to the drug 5-FOA, whereas strains that do not transcribe *URA3* are resistant to 5-FOA (Guthrie and Fink 1991). Strains that contain *URA3* inserted into the *ADH4* locus adjacent to an artificial telomere [*TEL(VIIL) adh4::URA3*] display a variegated phenotype of *URA3* expression (Gottschling *et al.* 1990). In approximately half the cells in the *TEL(VIIL) adh4::URA3* population, heterochromatin spreads from the telomere to the *URA3* gene and silences it, resulting in 5-FOA resistance. In the other half of the cells in the population, *URA3* is not silenced, resulting in 5-FOA sensitivity. To test the possible role of *SAS4* and *SAS5* in TPE, these genes were individually deleted from a strain carrying the *TEL(VIIL) adh4::URA3* allele and silencing was monitored by 5-FOA sensitivity. The proportion of cells sensitive to 5-FOA increased by at least five orders of magnitude as a result of deletion of either *SAS4* (DRY1371) or *SAS5* (DRY1372; Figure 1). In contrast, deletion of *SAS4* or *SAS5* did not alter the proportion of cells sensitive to 5-FOA in strains containing a mutant allele of *URA3* (DRY1391) or a copy of *URA3* that was not adjacent to a telomere (DRY1392) and was not subject to TPE (Figure 1 and data not shown). Thus, *SAS4* and *SAS5* are required for TPE. Furthermore, these results indicate that Sas4p and Sas5p can play an essential role in silencing independent of Sir1p, since, as described above, Sir1p does not play a role in TPE.

SAS4* and *SAS5* are positive regulators of silencing at *HML*:** To determine whether *SAS4* or *SAS5* is required for silencing at *HML*, we used a quantitative mating-type assay to monitor expression of the *HML*α genes. Wild-type *MATa** strains display the **a**-mating phenotype, whereas *MAT***a** strains in which silencing at *HML* is disrupted display the nonmating phenotype. Similar to deletion of *SIR1* (JRY4622), deletion of either *SAS4* (DRY1656) or *SAS5* (DRY1657) results in a modest reduction in silencing at *HML* as indicated by quantitative mating analysis (Figure 2A). Therefore, both *SAS4* and *SAS5* contribute to the efficient silencing of *HML* but neither is required for silencing of *HML*.

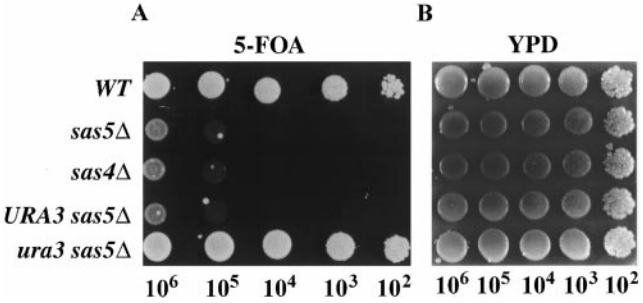

Figure 1.—*SAS4* and *SAS5* are required for TPE. (A) Deletion of *SAS4* or *SAS5* disrupts telomeric silencing and results in sensitivity to 5-FOA. A dilution series of isogenic strains plated on solid medium containing 5-FOA is shown. The number of cells plated per dilution is indicated at the bottom. The strains shown are UCC1001 (WT, *TEL-VIIL adh4::URA3*), DRY 1372 (*sas5*Δ, *TEL-VIIL adh4::URA3*), DRY 1371 (*sas4*Δ, *TEL-VIIL adh4::URA3*), DRY 1392 (*sas5*Δ *URA3*), and DRY1391 (*sas5*Δ *ura3*). (B) Viability of strains on solid rich medium. Aliquots of the serial dilutions from A were plated onto rich medium to control for cell viability.

As described above, *SIR1* plays a role in the nucleation of heterochromatin and the establishment of silencing. However, deletion of *SIR1* results in only a modest silencing defect at *HML* (Pillus and Rine 1989). Thus, Sir1p is redundant with other molecules that contribute to the establishment of silencing, or Sir1p acts in collaboration with other molecules, such as Rap1p, to collectively nucleate silencing. In contrast, strains lacking *SIR1* do not appear to be defective in the clonal propagation of silencing through mitosis. Thus the role of *SIR1* in silencing may be limited to the initial formation of heterochromatin (Pillus and Rine 1989). As described above, deletion of both *SIR1* and *SAS2* causes a much more severe silencing defect at *HML* than deletion of either gene alone (Reifsnyder *et al.* 1996). Thus, *SAS2* and *SIR1* appear to play redundant roles in silencing *HML*.

To explore the possibility that either *SAS4* or *SAS5* plays a role in silencing *HML* that is redundant with *SIR1*, we analyzed the mating phenotype of strains harboring a null allele of *SIR1* in combination with a null allele of either *SAS4* or *SAS5*. The α-mating phenotype of a *sas4*Δ *sir1*Δ strain (DRY1659) and a *sas5*Δ *sir1*Δ strain (DRY1660) was four orders of magnitude less than that of the wild-type strain or the singly mutated *sir1*Δ (JRY4622), *sas4*Δ (DRY1656), or *sas5*Δ (DRY1657) strains (Figure 2B). Thus, both *SAS4* and *SAS5* are required in combination with *SIR1* for efficient silencing at *HML*.

The observation that the role of *SAS4* and *SAS5* in silencing *HML* is redundant with that of *SIR1* raised the possibility that *SAS4* and *SAS5* provide redundant functions with each other in silencing *HML*. Similarly,

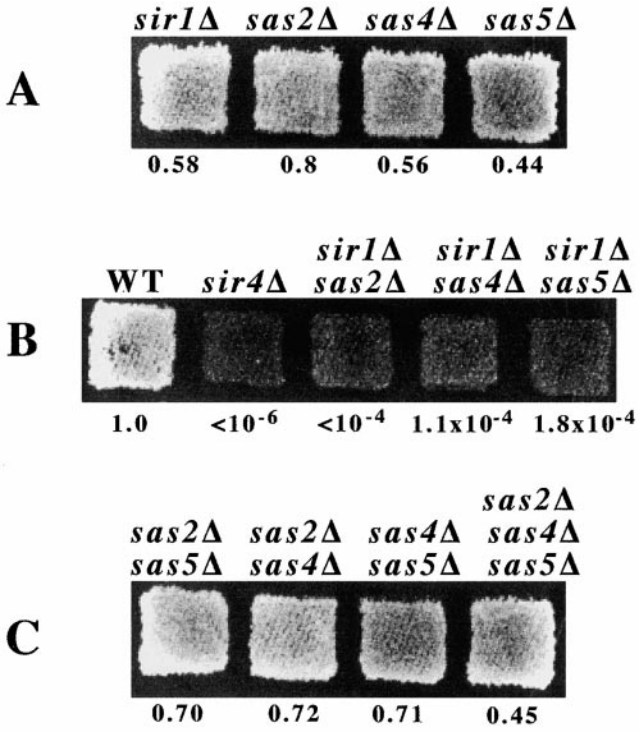

Figure 2.—Contribution of *SAS2*, *SAS4*, and *SAS5* to silencing at *HML*. Strains shown are isogenic with W303-1a. Qualitative patch mating assays are shown in the panels with quantitative mating analysis given below and genotypes above. (A) **a**-Mating phenotype of *MAT***a** *HML*α strains mutant in individual genes. Strains shown are JRY4622 (*sir1*Δ), DRY1655 (*sas2*Δ), DRY1656 (*sas4*Δ), and DRY1657 (*sas5*Δ). (B) **a**-Mating phenotype of strains mutant in *SIR1* and individual *SAS* genes. Strains shown are W303-1a (WT), DRY1236 (*sir4*Δ), DRY1658 (*sir1*Δ *sas2*Δ), DRY1659 (*sir1*Δ *sas4*Δ), and DRY1660 (*sir1*Δ *sas5*Δ). (C) **a**-Mating phenotype of strains mutant in combinations of *SAS* genes. Strains shown are DRY1661 (*sas2*Δ *sas5*Δ), DRY1662 (*sas2*Δ *sas4*Δ), DRY1663 (*sas4*Δ *sas5*Δ), and DRY1664 (*sas2*Δ *sas4*Δ *sas5*Δ).

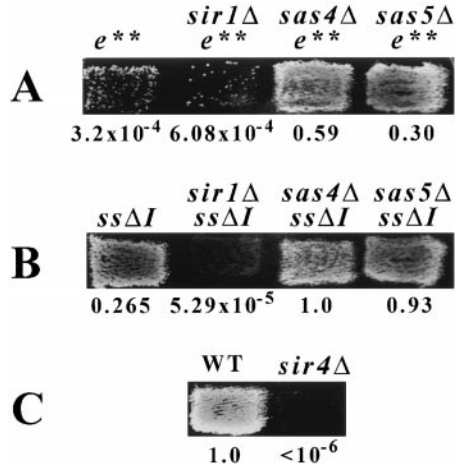

Figure 3.—*sas4*Δ and *sas5*Δ have opposite phenotypes to *sir1*Δ at *HMR* as revealed by mutant alleles of the *HMR-E* silencer. Qualitative patch mating assays of isogenic strains are shown in the panels with quantitative mating analysis given below and genotypes above. (A) α-Mating phenotype of *MAT*α *HMR***a**-*e*** strains mutant in *SIR1*, *SAS4*, or *SAS5*. Strains shown are JRY5273 (*HMR***a**-*e***), DRY1399 (*HMR***a**-*e*** *sir1*Δ), DRY1322 (*HMR***a**-*e*** *sas4*Δ), and DRY1314 (*HMR***a**-*e*** *sas5*Δ). (B) α-Mating phenotype of strains mutant in *SIR1*, *SAS4*, or *SAS5*. Strains shown are DRY439 (*HMR-SS* Δ*I*), JRY4624 (*HMR-SS* Δ*I sir1*Δ), DRY1364 (*HMR-SS* Δ*I sas4*Δ), and DRY1424 (*HMR-SS* Δ*I sas5*Δ). (C) α-Mating phenotype of wild-type and *sir4*Δ control strains. Strains shown are JRY3009 (WT) and DRY1235 (*sir4*Δ).

since the role of *SAS2* in silencing *HML* is redundant with *SIR1*, it is possible that *SAS2*, *SAS4*, and *SAS5* provide redundant functions with each other. Alternatively, *SAS2*, *SAS4*, and *SAS5* may act collectively to provide a single function in silencing. To determine whether *SAS2*, *SAS4*, and *SAS5* provided silencing functions that were redundant with each other, we quantitated the extent of silencing at *HML* in strains that contained combinations of null alleles of *SAS2*, *SAS4*, and *SAS5*. Deletion of both *SAS4* and *SAS5* (DRY1663) resulted in no greater silencing defect at *HML* than deletion of either gene alone (Figure 2). Similarly, strains containing null alleles of *SAS2* and *SAS4* (DRY1662), *SAS2* and *SAS5* (DRY1661), or *SAS2*, *SAS4*, and *SAS5* (DRY1664) were no more defective for *HML* silencing than any of the single mutant strains, indicating that the roles of *SAS2*, *SAS4*, and *SAS5* in silencing of *HML* were not redundant.

Null alleles of *SAS4* and *SAS5* have phenotypes at *HMR* opposite to that of a null allele of *SIR1*: The observation that the *SAS* genes were redundant with *SIR1* in silencing at *HML* suggests that there are fundamental differences in the regulation of *HML* and *HMR*. In particular, the *SAS* genes and *SIR1* do not appear to be redundant at *HMR* as they are at *HML*, since deletion of *SIR1* results in a silencing defect at *HMR*, whereas deletion of the *SAS* genes suppresses silencing defects at *HMR* (Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1999). However, these previous observations are not directly comparable since the phenotypes of null alleles of *SIR1* and null alleles of *SAS4* or *SAS5* were observed in strains containing different versions of the *HMR* silencers. To test directly whether *SAS4* or *SAS5* mutants display *HMR* phenotypes opposite to those of *SIR1* mutants, we compared the phenotypes of null mutations in *SAS4*, *SAS5*, and *SIR1* in two genetic backgrounds. One background contained the *HMR-SS* Δ*I* allele of *HMR*, which is composed of a synthetically constructed version of the *HMR-E* silencer in combination with a deletion of the *HMR-I* silencer. The *HMR-SS* Δ*I* (DRY439) allele is partially defective in silencing and mates with an efficiency of 0.265 relative to wild type (Figure 3). In this strain, deletion of *SIR1* (JRY4624) dramatically reduced silencing at *HMR*, whereas deletion of either *SAS4* or *SAS5* restored silencing to near wild-type levels (Figure 3). The other background contained the defective *HMR***a**-*e*** allele. Deletion of *SAS4* or *SAS5* in this background restored silencing, whereas

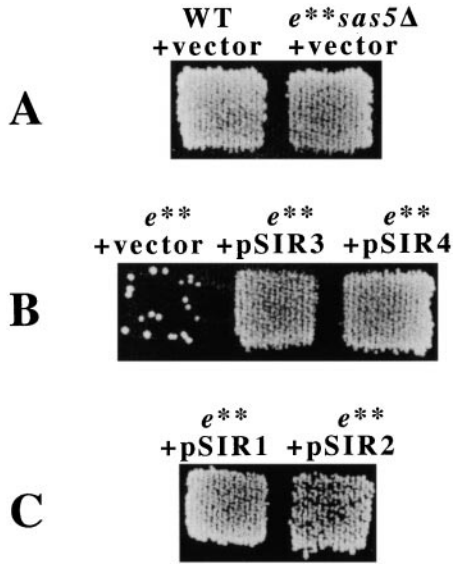

Figure 4.—Increased dosage of *SIR1*, *SIR2*, *SIR3*, or *SIR4* suppresses the *HMR***a**-*e*** silencing defect. (A) α-Mating phenotype of control strains transformed with the 2μ-based vector pRS426. Strains shown are DRY1448 [WT (pRS426)] and DRY1456 [*MAT*α *HMR***a**-*e*** *sas5*Δ (pRS426)]. (B) α-Mating phenotype of DRY1452 [*MAT*α *HMR***a**-*e*** (pRS426)], DRY1464 [*MAT*α *HMR***a**-*e*** (pRS426-*SIR3*)], and DRY1460 [*MAT*α *HMR***a**-*e*** (pRS426-*SIR4*)]. (C) α-Mating phenotype of DRY2107 [*MAT*α *HMR***a**-*e*** (pRS426-*SIR1*)] and DRY2108 [*MAT*α *HMR***a**-*e*** (pRS426-*SIR2*)].

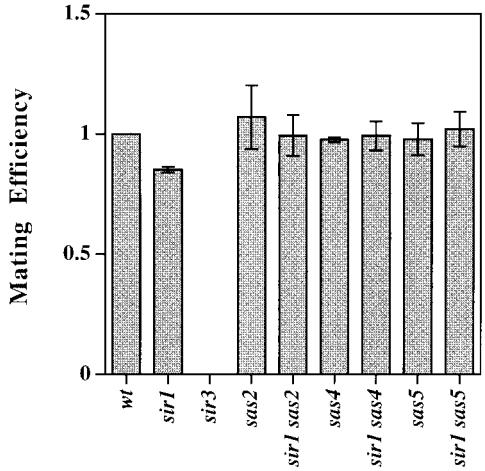

Figure 5.—*SAS4* and *SAS5* do not contribute to silencing at the wild-type *HMR* locus. Results of quantitative analysis of the α-mating phenotype of *MAT*α *HMR***a** strains are presented. The relevant genotype of each strain analyzed is given below the corresponding result. Strains analyzed were JRY3009 (WT), JRY4621 (*sir1*Δ), DRY1264 (*sir3*Δ), DRY1797 (*sas2*Δ), DRY1800 (*sir1*Δ *sas2*Δ), DRY1798 (*sas4*Δ), DRY1801 (*sir1*Δ *sas4*Δ), DRY1799 (*sas5*Δ), and DRY1802 (*sir1*Δ *sas5*Δ).

deletion of *SIR1* did not (Figure 3). These results confirm and extend the observation that deletion of *SAS4* (DRY1322) or *SAS5* (DRY1314) suppresses silencing defects at *HMR*. Furthermore, these results directly demonstrate that null alleles of *SAS4* and *SAS5* have *HMR* phenotypes opposite to a null allele of *SIR1*. Hence, in contrast to the redundant roles of the *SAS* genes with *SIR1* at *HML*, the *SAS* genes and *SIR1* have opposite roles in silencing at *HMR*.

Increased dosage of *SIR1, SIR2, SIR3,* or *SIR4* results in a *SAS* phenotype: How might mutations in *SAS2*, *SAS4*, and *SAS5* suppress the silencing defects of the *HMR***a**-*e*** silencer? In principle, deletion of the *SAS* genes could suppress silencing defects at *HMR* as an indirect consequence of disruption of silencing at the telomeres. In particular, as a result of disruption of TPE, Sir2p, Sir3p, and/or Sir4p could be released from the telomeres, effectively increasing the concentration of the pool of these proteins available for silencing at *HMR*. Since one role of the silencers is to nucleate silencing, it is possible that an increased concentration of the pool of the available Sir proteins could drive nucleation even in the presence of the defective *HMR***a**-*e*** silencer. A prediction of this model is that increasing the concentration of Sir2p, Sir3p, and/or Sir4p would suppress the defects of the *HMR***a**-*e*** silencer in an otherwise wild-type cell.

To test whether the *HMR***a**-*e*** silencing defects could be suppressed by an increased dosage of any of the Sir proteins, high copy number plasmids containing the individual *SIR1*, *SIR2*, *SIR3*, or *SIR4* genes were introduced into a strain harboring the *HMR***a**-*e*** allele. An increased dosage of either *SIR1* (DRY2107), *SIR2* (DRY2108), *SIR3* (DRY1464), or *SIR4* (DRY1460) suppressed the silencing defect caused by the *HMR***a**-*e*** allele (Figure 4). The simplest interpretation of these data is that mutations in *SAS2*, *SAS4*, or *SAS5* suppress defects in silencing at *HMR* as an indirect effect of disrupting telomeric silencing. By inference, these results suggest that the primary role of *SAS2*, *SAS4*, and *SAS5* is to bring about silencing at the telomeres and *HML*.

Deletion of *SAS4* or *SAS5* does not result in a silencing defect at *HMR*: The data presented above suggest that *SAS2*, *SAS4*, and *SAS5* are positive regulators of silencing at the telomeres and *HML* but not at *HMR*. However, the positive contribution of the *SAS* genes to silencing at *HML* was revealed by analysis of *HML* flanked by wild-type alleles of the *HML-E* and *HML-I* silencers, whereas the negative regulatory effect of the *SAS* genes on *HMR* was revealed by analysis of *HMR* flanked by mutant alleles of the *HMR-E* silencer. To assess more directly the role of the *SAS* genes at *HMR*, we tested whether *SAS2*, *SAS4*, or *SAS5* contributed to silencing of wild-type *HMR*. Deletion of *SAS2* (DRY1797), *SAS4* (DRY1798), or *SAS5* (DRY1799) did not result in a detectable reduction in silencing of *HMR* as measured by a quantitative mating assay (Figure 5). Thus, in contrast to *HML*, deletion of the *SAS* genes does not result in a silencing defect at *HMR*.

As described above, a null allele of *SIR1* in combina-

tion with a null allele in *SAS2*, *SAS4*, or *SAS5* resulted in a severe defect in silencing at *HML*, whereas deletion of any of these genes alone resulted in only a modest silencing defect. To explore further the possible role of the *SAS* genes in silencing wild-type *HMR*, we determined whether null alleles of the *SAS* genes caused a substantial defect in silencing at *HMR* in combination with a null allele of *SIR1*. Deletion of *SIR1* and *SAS2* (DRY1800), *SIR1* and *SAS4* (DRY1801), or *SIR1* and *SAS5* (DRY1802) did not result in a detectable silencing defect at *HMR* (Figure 5). In fact, deletion of *SAS2*, *SAS4*, or *SAS5* appeared to suppress the modest silencing defect that results from deletion of *SIR1* (JRY4621) alone (Figure 5). These results indicate that the locus-specific silencing phenotypes of null alleles of the *SAS* genes reflect the properties of the native *HML* and *HMR* silencers. Furthermore, these results suggest that the *SAS* genes do not normally contribute to silencing at *HMR* and that they are not redundant with *SIR1* function at *HMR* as they are at *HML*.

DISCUSSION

***SAS* genes define a new class of locus-specific regulators of silencing:** The analysis presented here established that *SAS4* and *SAS5*, like *SAS2*, are positive regulators of silencing at *HML* and the telomeres and are negative regulators of silencing at *HMR*. Specifically, each is required for TPE, each contributes a function in silencing at *HML* that is redundant with *SIR1* but is not redundant with the other *SAS* genes, and null alleles of each suppress silencing defects at *HMR*. These properties are unique among the genes known to regulate silencing, indicating that *SAS2*, *SAS4*, and *SAS5* define a novel class of locus-specific regulators of silencing. By inference, the functions of Sas2p, Sas4p, and Sas5p are likely to be intimately related.

Role of locus-specific regulators of silencing: As described above, the simplest interpretation of our data is that *SAS2*, *SAS4*, and *SAS5* are locus-specific regulators that bring about silencing at *HML* and the telomeres, but not at *HMR*. Similarly, previous analysis of *SIR1* suggests that it is also a locus-specific regulator of silencing that acts at *HML* and *HMR* but not at the telomeres. In this regard, the most informative clues to the role of the *SAS* genes may come from analysis of *HML*, where *SIR1* and the *SAS* genes appear to play redundant roles in silencing. This redundancy raises the possibility that the *SAS* genes, like *SIR1*, contribute to the establishment of silencing. In particular, the *SAS* genes could contribute to the nucleation of silencing at the telomeres at *HML*, as *SIR1* does at *HML* and *HMR*. By this model, the chromatin structures at *HML*, *HMR*, and in the telomeric regions would be predicted to be composed of identical components and differ only in the initial events that lead to their assembly. Furthermore, the differences in the efficiency of silencing in the different regions would be expected to result from differences in the efficiency of establishment.

What is the possible molecular role of the *SAS* genes in silencing at the telomeres and *HML*? Sas2p is a member of the MYST family of proteins (Borrow *et al.* 1996; Reifsnyder *et al.* 1996; E. R. Smith *et al.* 1998). The members of this family have similarity to protein acetylases, and two family members, Esa1p and Tip60, are histone acetylases (Yamamoto and Horikoshi 1997; E. R. Smith *et al.* 1998). One model of *SAS* gene function is that Sas2p regulates silencing through the acetylation of a component of the silencing machinery. Given the phenotypic similarities among mutations in *SAS2*, *SAS4*, and *SAS5*, it is possible that Sas4p and Sas5p are components of a Sas2p-dependent acetylase complex. Alternatively, Sas4p and/or Sas5p could be the targets of a Sas2p-dependent acetylase.

Role of the *SAS* genes in regulation of silencing at *HMR*: Three lines of evidence support a model in which null alleles of the *SAS* genes suppress silencing defects at *HMR* as an indirect consequence of disrupting TPE. First, *SAS4* and *SAS5* are required for TPE, as was previously shown for *SAS2* (Reifsnyder *et al.* 1996). Second, disruption of TPE can result in redistribution of the Sir proteins from the telomeres to other loci (Cockell *et al.* 1995; Gotta and Gasser 1996; Gotta *et al.* 1996, 1997; Kennedy *et al.* 1997). Third, increased dosage of *SIR1*, *SIR2*, *SIR3*, or *SIR4* was sufficient to suppress silencing defects at *HMR*. Collectively these observations support a model in which mutations in the *SAS* genes disrupt TPE, resulting in an increased concentration of the pool of free *SIR* proteins, which, in turn, can suppress silencing defects at *HMR*.

Differential regulation of *HML* and *HMR*: Our observations that null alleles of the *SAS* genes cause silencing defects at *HML* and suppress defects at *HMR* provide strong evidence that there are important differences in the regulation of silencing at these two loci. One possible explanation for this observation is that silencing at *HML* and *HMR* may differ qualitatively. As described above, the data presented here are consistent with a model in which the *SAS* genes are locus-specific regulators of silencing that normally act at *HML* and the telomeres but not at *HMR*.

If the regulation of silencing at *HML* and *HMR* differs qualitatively, it is likely that additional previously unidentified molecules or mechanisms account for the greater efficiency of silencing at *HMR* relative to *HML* and the telomeres. One way that *HMR* is known to differ from *HML* is that the silencers at *HMR* are origins of replication, whereas the silencers at *HML* are not (Dubey *et al.* 1991; Rivier and Rine 1992; Hurst and Rivier 1999; Rivier *et al.* 1999). It is possible that DNA replication, initiated at the *HMR* silencers, plays a role in the assembly or duplication of heterochromatin at *HMR* and that this function is lacking at *HML*. To date, a role for DNA replication in silencing at *HMR* has not

been revealed; however, it is possible that the efficiency of silencing at *HMR* and the redundancy that is inherent in the *HMR-E* silencer has masked a possible contribution of replication to silencing at this locus.

We thank K. Replogle for help with strain construction, J. Ekena for help with mating analysis presented in Figure 5, A. Belmont and C. Doe for discussions and comments on early versions of the manuscript, B. Cairns and S. Elgin for discussions, J. Rine for strains, and L. Pillus for discussion of unpublished work and for providing plasmids and strains. This work was supported by National Institutes of Health (NIH) grant GM-52103 (D.R.), by a Basil O'Connor Starter Scholar Research Award grant 5-FY96-0578 (D.R.), by the Charles M. Goodenberger Fund (D.R.), and by an NIH predoctoral training award 5T32-GM07283 (S.K.).

LITERATURE CITED

Abraham, J., J. Feldman, K. A. Nasmyth, J. N. Strathern, A. J. Klar *et al.*, 1983 Sites required for position-effect regulation of mating-type information in yeast. Cold Spring Harbor Symp. Quant. Biol. **47:** 989–998.

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.

Axelrod, A., and J. Rine, 1991 A role for *CDC7* in repression of transcription at the silent mating-type locus *HMR* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 1080–1091.

Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute and C. Cullin, 1993 A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:** 3329–3330.

Borrow, J., V. P. Stanton, Jr., J. M. Andresen, R. Becher, F. G. Behm *et al.*, 1996 The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nat. Genet. **14:** 33–41.

Brand, A. H., L. Breeden, J. Abraham, R. Sternglanz and K. Nasmyth, 1985 Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41:** 41–48.

Brand, A. H., G. Micklem and K. Nasmyth, 1987 A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell **51:** 709–719.

Bryk, M., M. Banerjee, M. Murphy, K. E. Knudsen, D. J. Garfinkel *et al.*, 1997 Transcriptional silencing of Ty1 elements in the *RDN1* locus of yeast. Genes Dev. **11:** 255–269.

Chien, C. T., S. Buck, R. Sternglanz and D. Shore, 1993 Targeting of *SIR1* protein establishes transcriptional silencing at HM loci and telomeres in yeast. Cell **75:** 531–541.

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.

Cockell, M., F. Palladino, T. Laroche, G. Kyrion, C. Liu *et al.*, 1995 The carboxy termini of Sir4 and Rap1 affect Sir3 localization: evidence for a multicomponent complex required for yeast telomeric silencing. J. Cell Biol. **129:** 909–924.

Dubey, D. D., L. R. Davis, S. A. Greenfeder, L. Y. Ong, J. G. Zhu *et al.*, 1991 Evidence suggesting that the ARS elements associated with silencers of the yeast mating-type locus *HML* do not function as chromosomal DNA replication origins. Mol. Cell. Biol. **11:** 5346–5355.

Ehrenhofer-Murray, A. E., D. H. Rivier and J. Rine, 1997 The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. Genetics **145:** 923–934.

Feldman, J. B., J. B. Hicks and J. R. Broach, 1984 Identification of sites required for repression of a silent mating type locus in yeast. J. Mol. Biol. **178:** 815–834.

Gardner, K. A., J. Rine and C. A. Fox, 1999 A region of the Sir1 protein dedicated to recognition of a silencer and required for interaction with the orc1 protein in *Saccharomyces cerevisiae*. Genetics **151:** 31–44.

Gietz, R. D., and R. H. Schiestl, 1991 Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. Yeast **7:** 253–263.

Gotta, M., and S. M. Gasser, 1996 Nuclear organization and transcriptional silencing in yeast. Experientia **52:** 1136–1147.

Gotta, M., T. Laroche, A. Formenton, L. Maillet, H. Scherthan *et al.*, 1996 The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. **134:** 1349–1363.

Gotta, M., S. Strahl-Bolsinger, H. Renauld, T. Laroche, B. K. Kennedy *et al.*, 1997 Localization of Sir2p: the nucleolus as a compartment for silent information regulators. EMBO J. **16:** 3243–3255.

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.

Grunstein, M., 1997 Molecular model for telomeric heterochromatin in yeast. Curr. Opin. Cell Biol. **9:** 383–387.

Grunstein, M., 1998 Yeast heterochromatin: regulation of its assembly and inheritance by histones. Cell **93:** 325–328.

Guthrie, C., and G. R. Fink, 1991 *Guide to Yeast Genetics and Molecular Biology*. Academic Press, San Diego.

Hurst, S. T., and D. H. Rivier, 1999 Identification of a compound origin of replication at the *HMR*-E locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 4155–4159.

Kennedy, B. K., M. Gotta, D. A. Sinclair, K. Mills, D. S. McNabb *et al.*, 1997 Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. Cell **89:** 381–391.

Kimmerly, W., A. Buchman, R. Kornberg and J. Rine, 1988 Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J. **7:** 2241–2253.

Laurenson, P., and J. Rine, 1992 Silencers, silencing, and heritable transcriptional states. Microbiol. Rev. **56:** 543–560.

Loo, S., and J. Rine, 1995 Silencing and heritable domains of gene expression. Annu. Rev. Cell Dev. Biol. **11:** 519–548.

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

Lustig, A. J., C. Liu, C. Zhang and J. P. Hanish, 1996 Tethered Sir3p nucleates silencing at telomeres and internal loci in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2483–2495.

Marcand, S., S. W. Buck, P. Moretti, E. Gilson and D. Shore, 1996 Silencing of genes at nontelomeric sites in yeast is controlled by sequestration of silencing factors at telomeres by Rap 1 protein. Genes Dev. **10:** 1297–1309.

McNally, F. J., and J. Rine, 1991 A synthetic silencer mediates *SIR*-dependent functions in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 5648–5659.

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.

Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsunasawa, M. Gribskov *et al.*, 1989 Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. **8:** 2067–2075.

Park, E. C., and J. W. Szostak, 1992 ARD1 and NAT1 proteins form a complex that has N-terminal acetyltransferase activity. EMBO J. **11:** 2087–2093.

Pillus, L., and J. Rine, 1989 Epigenetic inheritance of transcriptional states in *S. cerevisiae*. Cell **59:** 637–647.

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.

Rivier, D. H., and J. Rine, 1992 An origin of DNA replication and a transcription silencer require a common element. Science **256:** 659–663.

Rivier, D. H., J. L. Ekena and J. Rine, 1999 *HMR*-I is an origin of replication and a silencer in *Saccharomyces cerevisiae*. Genetics **151:** 521–529.

Sherman, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 3–21.

Smith, E. R., A. Eisen, W. Gu, M. Sattah, A. Pannuti *et al.*, 1998 ESA1 is a histone acetyltransferase that is essential for growth in yeast. Proc. Natl. Acad. Sci. USA **95:** 3561–3565.

Smith, J. S., and J. D. Boeke, 1997 An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. **11:** 241–254.

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.

Stone, E. M., M. J. Swanson, A. M. Romeo, J. B. Hicks and R. Sternglanz, 1991 The *SIR1* gene of *Saccharomyces cerevisiae* and its role as an extragenic suppressor of several mating-defective mutants. Mol. Cell. Biol. **11:** 2253–2262.

Triolo, T., and R. Sternglanz, 1996 Role of interactions between the origin recognition complex and *SIR1* in transcriptional silencing. Nature **381:** 251–253.

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.

Xu, E., S. Kim, K. Replogle, J. Rine and D. H. Rivier, 1999 Identification of *SAS4* and *SAS5*, two genes that regulate silencing in *Saccharomyces cerevisiae*. Genetics **153:** 13–23.

Yamamoto, T., and M. Horikoshi, 1997 Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. J. Biol. Chem. **272:** 30595–30598.

Communicating editor: F. Winston