Copyright © 1997 by the Genetics Society of America

The Role of Sas2, an Acetyltransferase Homologue of *Saccharomyces cerevisiae*, in Silencing and ORC Function

Ann E. Ehrenhofer-Murray, David H. Rivier[1] and Jasper Rine

Division of Genetics, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720

Manuscript received August 27, 1996
Accepted for publication January 10, 1997

ABSTRACT

Silencing at the cryptic mating-type loci *HML* and *HMR* of *Saccharomyces cerevisiae* requires regulatory sites called silencers. Mutations in the Rap1 and Abf1 binding sites of the *HMR*-E silencer (*HMR*a-e**) cause the silencer to be nonfunctional, and hence, cause derepression of *HMR*. Here, we have isolated and characterized mutations in *SAS2* as second-site suppressors of the silencing defect of *HMR*a-e**. Silencing conferred by the removal of *SAS2* (*sas2*Δ) depended upon the integrity of the ARS consensus sequence of the *HMR*-E silencer, thus arguing for an involvement of the origin recognition complex (ORC). Restoration of silencing by *sas2*Δ required *ORC2* and *ORC5*, but not *SIR1* or *RAP1*. Furthermore, *sas2*Δ suppressed the temperature sensitivity, but not the silencing defect of *orc2-1* and *orc5-1*. Moreover, *sas2*Δ had opposing effects on silencing of *HML* and *HMR*. The putative Sas2 protein bears similarities to known protein acetyltransferases. Several models for the role of Sas2 in silencing are discussed.

EUKARYOTIC genomes are organized into functionally distinct domains of gene expression. The heritable inactivation, or silencing, of particular regions of the genome is a widespread phenomenon. Silencing and related processes play a fundamental role in the control of segment determination and differentiation in Drosophila (MULLER 1995) and in *X*-chromosome inactivation, which is essential in female mammals to compensate for the higher dosage of the *X* chromosome (RASTAN 1994). Silenced genomic regions are also found at centromeres (LE *et al.* 1995) and telomeres of many organisms (BLACKBURN 1994). In the yeast *Saccharomyces cerevisiae*, *HML* and *HMR*, which are used as donors of *MAT* alleles during mating-type interconversion, are silenced throughout the cell cycle, although they contain promoters of identical sequence to those at the expressed *MAT* locus (LOO and RINE 1995). Also, genes that are moved close to the telomeres become subject to a type of silencing that shares many similarities with that at *HML* and *HMR* (APARICIO and GOTTSCHLING 1994).

Silencing at *HML* and *HMR* is controlled by sequence elements known as silencers that flank both loci. The silencers contain a combination of binding sites for the transcription factors Rap1 and Abf1 (BRAND *et al.* 1987; KIMMERLY *et al.* 1988) as well as an ARS consensus sequence, which is the binding site of the origin recognition complex (ORC) (BELL *et al.* 1993). Natural silencers appear to be functionally redundant in that a mutation in any one binding site alone has little effect on repression. The proteins Orc2 (FOSS *et al.* 1993; MICKLEM *et al.* 1993), Orc5 (LOO *et al.* 1995a), Rap1 (SUSSEL *et al.* 1993) and Abf1 (LOO *et al.* 1995b) function in silencing by binding directly to the silencer. The role of the Sir proteins in silencing remains unclear, but they are good candidates for being structural components of silenced chromatin.

A link has been found between the transcriptional state of genes and the modification of the nucleoproteins in which their DNA is packaged (JEPPESEN and TURNER 1993). The charged amino-terminal tails of histones H3 and H4 are subject to ε-N-acetylation at up to four different lysine residues. Generally, the nucleosomes in inactive chromatin appear to be hypoacetylated. In most cases, it has been difficult to establish whether hypoacetylation is a cause or effect of inactive chromatin, but in the case of silencing in *S. cerevisiae*, there is growing evidence that changes in acetylation cause changes in gene expression. For instance, mutations in histones H3 (THOMPSON *et al.* 1994) and H4 (MEGEE *et al.* 1990; PARK and SZOSTAK 1990; JOHNSON *et al.* 1992) that alter the acetylation of lysine residues in the amino-terminal domain cause silencing defects at *HML*, *HMR*, and at telomeres. Also, silencing of *HMR* is associated with hypoacetylation (BRAUNSTEIN *et al.* 1993). Furthermore, the deletion of *NAT1* and *ARD1*, which encode subunits of a N-terminal acetyltransferase, causes silencing defects in yeast (MULLEN *et al.* 1989). Although N-terminal acetylation is a modification distinct from the acetylation of lysine residues, this discovery establishes the importance of at least one type of protein acetylation in silencing. Moreover, the yeast transcriptional regulator Rpd3 is homologous to the

Corresponding author: Jasper Rine, Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720. E-mail: jasper@mendel.berkeley.edu

[1]*Present address:* Department of Cell and Structural Biology and Department of Microbiology, University of Illinois, Urbana, IL 61801.

catalytic subunit of a human histone deacetylase, implying that the dynamic acetylation of chromatin components may regulate gene expression (TAUNTON *et al.* 1996). This report identifies a potential yeast acetyltransferase, Sas2, by the ability of mutations in *SAS2* to suppress silencing defects at *HMR* and explores the role of Sas2 in silencing and in the function of ORC, the replication initiator.

MATERIALS AND METHODS

The genotype of the yeast strains and the plasmids used in this study are presented in Tables 1 and 2. Standard yeast media, genetic and recombinant DNA methods were used as described (SHERMAN 1991). Yeast transformations were performed according to KLEBE *et al.* (1983).

Construction of *sas2-Δ1::TRP1*: The *SAS2* deletion allele was obtained by inserting a 839-bp *Bgl*II-*Stu*I fragment of *TRP1* into pJR1372 (*Bgl*II-*Stu*I) to create pJR1642. A *Xho*I-*Bam*HI fragment of pJR1642 was used to transform a wild-type diploid strain to tryptophan prototrophy, and the correct integration of *sas2-Δ1::TRP1* was confirmed by DNA blot hybridization (SAMBROOK *et al.* 1989).

Strain constructions: Strains containing a mutant *HMR* allele and *sas2Δ* were obtained by crossing a *MATα* strain containing the respective silencer allele to a *MAT***a** *hmr::URA3* *sas2-Δ1::TRP1* strain (JRY5076). Even with poorly mating parent strains, selection was strong enough to produce diploids. To ensure that no silencer mutations had been selected for in the diploids, the mating ability of wild-type *MATα* segregants from the cross was analyzed. Subsequently, segregants were chosen that were Trp^+ and Ura^-. One representative was selected for quantitative mating analysis.

Quantitative mating assays: Cells were grown into logarithmic growth phase in rich medium (YPD), and serial dilutions were mixed with 2.5×10^7 cells of the *MAT***a** mating-type tester strain (JRY2726). The suspensions were plated onto selective medium (YM) and grown at 30 or 23°, as appropriate. Dilutions were also plated onto rich medium to determine the number of viable cells. Mating efficiencies were calculated as the number of diploids formed per viable cell. All mating efficiencies presented are the average of two independent determinations and are normalized to the wild-type strain JRY3009.

Construction of a W303-isogenic *MATα HMR*a-e strain:** The *HMR***a**-e** *Sac*II-*Eco*RI fragment from pJR891 was inserted into a *Sac*II-*Eco*RI linearized integrating *URA3* vector (pRS306) to produce pJR1749. Subsequently, pJR1749 was linearized within *HMR* with *Mlu*I and used to transform a *MATα sir4Δ::LEU2* strain (JRY4582) to uracil-prototrophy. Integration of pJR1749 at *HMR* was verified by DNA blot hybridization. The resulting strain was streaked onto 5-fluoroorotic acid-containing medium to select for Ura^- recombinants, and candidates were subjected to DNA blot hybridization to determine whether they had retained the *HMR***a**-e** allele. A representative candidate was transformed with a *URA3-SIR4* plasmid (pJR368) to complement the mating defect, and mated to a *MAT***a** *hmr::URA3* strain (JRY5078), which allowed for following the segregation of the *HMR***a**-e** allele in the cross. Diploids were selected by their phenotype and sporulated, and tetrads were analyzed. A *MATα HMR***a**-e** strain (JRY5273) was identified on the basis of its auxotrophies and its weak α-mating phenotype.

Construction of the plasmid-borne *HMR*a-e allele:** The *HMR***a**-e** allele (from JRY2635) was cloned onto a plasmid by gap repair. For this aim, plasmid pRO3-1, which is a derivative of pRS316 that contains a 10-kB genomic *HMR* fragment, was cut with *Mlu*I and *Pac*I, which resulted in a plasmid with a 3-kb gap at *HMR*. The linearized plasmid was used to transform JRY2635 to uracil prototrophy. Ura^+ transformants could arise only if the plasmid was circularized, either by a recombination event between the plasmid ends or by repair of the plasmid by the homologous sequence from the chromosome. The plasmids containing *HMRα*-e** were introduced into a *mat***a***Δp HMR***a** strain (JRY4077), to which they conferred the α-mating phenotype, thus confirming that α genes were expressed from the derepressed *HMR* locus.

RESULTS

A screen for suppressors of silencing defects: Screens for genes involved in silencing have primarily concentrated on identifying proteins and sites that are required for silencing. In the predecessor to this study, suppressor analysis was used to learn more about proteins that participate in silencing by the ability of mutations in the genes encoding these proteins to restore silencing (AXELROD and RINE 1991). This screen made use of an allele of the *HMR*-E silencer (designated *HMR***a**-e**), which contains point mutations in two of the three silencer domains: a point mutation in the Rap1 site and a single base-pair insertion in the Abf1 binding site (KIMMERLY *et al.* 1988). The ARS consensus sequence, which is the binding site for the ORC (BELL *et al.* 1993), remained intact. Due to the two point mutations in the silencer, **a**1 information is expressed at *HMR***a**-e**, resulting in a nonmating phenotype in a *MATα* strain. A strain containing the *HMR***a**-e** allele was used to search for mutations that suppressed its silencing defect. These mutants, which were termed *sas* (for something about silencing), have been placed into five complementation groups (D. H. RIVIER and J. RINE, unpublished results). *sas1-1*, the only temperature-sensitive mutant from this screen, has been described elsewhere (AXELROD and RINE 1991) and is an allele of *CDC7*, which encodes a protein kinase acting at the G_1/S-phase transition (PATTERSON *et al.* 1986). Other *SAS* genes have been isolated based upon homology to *SAS2* (REIFSNYDER *et al.* 1996). Here, we report the characterization of a second gene, *SAS2*, which suppressed the *HMR***a**-e** allele.

Identification of *SAS2*: *SAS2* was isolated as a low-copy suppressor of the mating ability of a *MATα* *HMR***a**-e** strain containing an uncharacterized *sas* mutation (JRY5356). A plasmid was isolated from a *CEN* *LEU2* yeast genomic library (SPENCER *et al.* 1990) that was capable of complementing the *sas* mutation. Subcloning various fragments of the yeast insert from this plasmid into the *LEU2 CEN* vector pRS315 revealed that a 3-kb *Hin*dIII fragment (pJR1382) was sufficient for complementation. The DNA sequence of this insert (GenBank accession No. U14548) contained one complete open reading frame (ORF, nucleotides 1435–2448; Figure 1) and a second, incomplete ORF on the opposite strand (1–868). A subclone of the 3-kb *Hin*dIII fragment containing the large ORF (nucleotides 1375–

TABLE 1

Yeast strains used in this study

Strain[a]	Genotype	Reference[b]
JRY2334	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100* (=W303-1A)	THOMAS and ROTHSTEIN (1989)
JRY3009	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100* (=W303-1B)	
JRY2635	*mat***a**1 *HMRα*-e** *ade2 leu2-3,113 ura3*	AXELROD and RINE (1991)
JRY3937	JRY3009 *HMR-SS rap*$^-$ *+I*	
JRY4014	JRY2334 × JRY4473	
JRY4077	JRY3009 *mat***a***Δp*	
JRY4473	JRY3009 *HMR-SS ΔI*	
JRY4475	JRY3009 *HMR-SS ΔI orc2-1*	
JRY4476	JRY3009 *HMR-SS ΔIorc5-1*	
JRY4527	JRY3009 *HMR-SS ars*$^-$ *+I*	
JRY4531	JRY3009 *HMR-SS (Gal4*$_{bs}$*-RAP-ABF)*[c] *ΔI*	
JRY4581	JRY2334 *sir4Δ::LEU2 ADE2 lys2Δ*	
JRY4582	JRY3009 *sir4Δ::LEU2 ADE2 lys2Δ*	
JRY4621	JRY3009 *sir1Δ::LEU2 ADE2 lys2Δ*	
JRY4889	JRY3009 *HMR-SS abf1*$^-$ *ΔI*	
JRY5068	JRY2334 *sas2-Δ1::TRP1*	
JRY5070	JRY3009 *HMR-SS ΔI sas2-Δ1::TRP1*	
JRY5071	JRY3009 *sas2-Δ1::TRP1*	
JRY5076	JRY2334 *hmr::URA3 ADE2 lys2Δ*	
JRY5078	JRY2334 *hmr::URA3*	
JRY5110	JRY3009 *HMR-SS abf1*$^-$ *ΔI sas2-Δ1::TRP1*	
JRY5273	JRY3009 *HMR***a**-e**	
JRY5274	JRY3009 *HMR***a**-e** *sas2-Δ1::TRP1*	
JRY5275	JRY2334 *sir1Δ::LEU2 sas2-Δ1::TRP1*	
JRY5276	JRY3009 *sir1Δ::LEU2*	
JRY5277	JRY3009 *sir1Δ::LEU2 sas2-Δ1::TRP1*	
JRY5278	JRY3009 *HMR-SS (ACS-Gal4*$_{bs}$*-ABF)*[d] *ΔI*	
JRY5279	JRY3009 *HMR-SS (ACS-Gal4*$_{bs}$*-ABF)*[d] *ΔI sas2-Δ1::TRP1*	
JRY5280	JRY3009 *HMR-SS rap*$^-$ *+I sas2-Δ1::TRP1*	
JRY5281	JRY3009*HMR***a**-e** *rap1-12::LEU2*	
JRY5282	JRY3009 *HMR***a**-e** *rap1-12::LEU2 sas2-Δ1::TRP1*	
JRY5283	JRY3009 *HMR-SS (Gal4*$_{bs}$*-RAP-ABF)*[c] *ΔI sas2-Δ1::TRP1*	
JRY5284	JRY3009 *HMR-SS ars*$^-$ *+I sas2-Δ1::TRP1*	
JRY5285	JRY3009 *HMR***a**-e** *sir1Δ::LEU2*	
JRY5286	JRY3009 *HMR***a**-e** *sir1::LEU2 sas2-Δ1::TRP1*	
JRY5289	JRY3009 *HMR-SS ΔI sir1Δ::LEU2*	
JRY5290	JRY3009 *HMR-SS ΔI sir1Δ::LEU2 sas2-Δ1::TRP1*	
JRY5291	JRY3009 *HMR***a**-e** *orc2-1*	
JRY5292	JRY3009 *HMR***a**-e** *orc2-1 sas2-Δ1::TRP1*	
JRY5293	JRY3009 *HMR***a**-e** *orc5-1*	
JRY5294	JRY3009 *HMR***a**-e** *orc5-1 sas2-Δ1::TRP1*	
JRY5295	JRY3009 *HMR-SS ΔI orc2-1 sas2-Δ1::TRP1*	
JRY5296	JRY3009 *HMR-SS ΔI orc5-1 sas2-Δ1::TRP1*	
JRY5356	*MATα HMR***a**-e** *sas ade2-1 his lys2 tyr1 ura3-52*	

[a] All strains were *HMLα HMR***a** unless indicated otherwise and, except for JRY2635 and JRY5356, were derivatives of W303.
[b] All strains were from the laboratory collection or constructed during the course of this work.
[c] The ARS consensus sequence of the synthetic *HMR*-E silencer was replaced by a Gal4 binding site.
[d] The Rap1 binding site of the synthetic *HMR*-E silencer was replaced by a Gal4 binding site.

3033) complemented the *sas* mutation, whereas a subclone containing the partial ORF (nucleotides 1–1754) did not. Comparison of this sequence to the yeast genome database showed that it was located on the right arm of chromosome *XIII*.

An allelism test with the disruption allele of the complementing ORF (*sas2Δ*, see below) and the uncharacterized *sas* mutation revealed that the cloned gene was not allelic to the *sas* mutation (data not shown). Hence, *SAS2* constituted a low-copy suppressor of the mutation. Findings described below led us to name the cloned gene *SAS2* and to study its role in silencing.

Disruption of *SAS2* suppressed silencing defects: We constructed a strain in which the majority of the *SAS2* open reading frame was replaced by *TRP1* and tested whether this deletion (*sas2Δ*) displayed a silencing phenotype. A *sas2-Δ1::TRP1* allele was integrated into a wild-type diploid strain, the resulting diploid was spor-

TABLE 2

Plasmids used in this study

Plasmid	Description	Reference
pRS306	*ARSH4/URA3*	Sikorski and Hieter (1989)
pRS315	*CEN6/ARSH4/LEU2*	Sikorski and Hieter (1989)
pRS316	*CEN6/ARSH4/URA3*	Sikorski and Hieter (1989)
pRS404	*ARSH4/TRP1*	Sikorski and Hieter (1989)
YCp50	*CEN4/ARS1/URA3*	Rose *et al.* (1987)
pJR157	YCp50;*MATα*	
pJR368	YCp50;*SIR4*	
pJR882	pRS316;*HMRα*	
pJR891	pRS316;*HMR***a**-e**	
pJR1382	pRS315;*SAS2*	
pJR1642	pRS315;*sas2-Δ1::TRP1*	
pJR1749	pRS306;*HMR***a**-e**	
pJR1792	pRS316;*HMRα*-e**	
pRO3-1	pRS316;*HMR***a**	R. Kamakaka, unpublished results

ulated, and tetrads were dissected and grown for 3 days at 30°. Of the 32 tetrads dissected, 30 produced four viable spores, demonstrating that *SAS2* was not an essential gene. The *sas2Δ* strains displayed no detectable conditional phenotype, including temperature sensitivity, cold sensitivity, sensitivity to hydroxyurea or caffeine, or growth defects on acetate-containing medium. Also, a *sas2Δ* strain had no growth advantage nor disadvantage over a wild-type strain in a short-term cocultivation experiment (data not shown).

To determine whether the deletion of *SAS2* restored the α-mating ability of a *MATα HMR***a**-e**, a *MAT***a** *sas2-Δ1::TRP1* strain in which *HMR* was replaced by *URA3* (*hmr::URA3*, JRY5076) was crossed to an isogenic *MATα HMR***a**-e** strain (JRY5273). Several *MATα sas2-Δ1::TRP1* segregants that were Ura$^-$ and therefore, by inference, contained *HMR***a**-e**, were analyzed for their mating phenotype. All such segregants were mating-proficient (Figure 2), whereas the *MATα SAS2* Ura$^-$ segregants were mating deficient. In a quantitative mating assay, the *MATα HMR***a**-e** strain mated with an efficiency of 4.3×10^{-5} relative to an isogenic *MATα* strain. In contrast, the *MATα HMR***a**-e** *sas2Δ* strain mated with an efficiency of 3.9×10^{-2}, approximately a 1000-fold increase over the isogenic *SAS2* strain (compare Figure 6A, lane 3). This showed that the deletion of *SAS2* restored mating in a *MATα HMR***a**-e** strain. Since *sas2Δ* shared this phenotype with the other *sas* mutations, it constituted a class of *HMR***a**-e** suppressors of its own.

```
  1  MARSLSQSLT ATTQKLKGKK NGGKGKNKPS AKIKKTQKEM
 41  LYGILNERNI RQIQFGLNKK FSTWYGSAVY FDPETKRLGC
 81  SETKGQLSSV SNSQYWLDTL FVCEYCFKYT DDQTRFVGHV
121  ASCPFQYRVP GKIKYKSPEY TIRRVKGSKY QLFCQCLCLF
161  TKLYLDNKSM YFKVDHYEFY IVYETGSTKP MGFFSKDLVS
201  YQQNNLACIL IFPPYQRRGL GLLLIEFSYK LSQLEGVISG
241  PEVPLSPFGL IGYLKYWSQI LCWHLIEGDL AHYDKVTLED
281  LSIVTGMRVN DVILTLKHLN CIGENNQIYL QSLNSWLKLH
321  GTKRNWFKLK DEYLLIDD
```

FIGURE 1.—The deduced amino acid sequence of *SAS2*. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

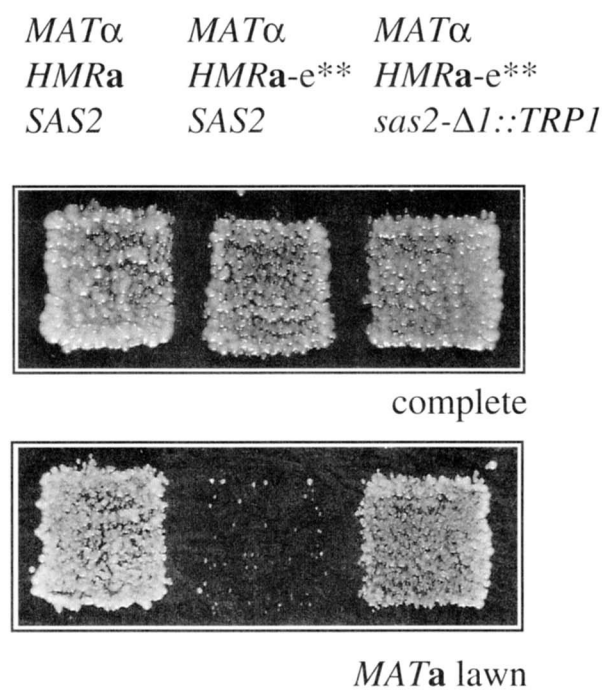

FIGURE 2.—Deletion of *SAS2* suppressed the silencing defect of *HMR***a**-e**. The strains JRY3009, JRY5273 and JRY5274 (from left to right) were patched on complete medium, grown for 12 hours at 30° and replica plated onto complete medium and onto minimal medium spread with a lawn of *MAT***a** mating tester. The plates were incubated at 30° for 2 days.

Silencing by *sas2Δ* was *SIR*-dependent: In theory, *sas2Δ* could be restoring the α-mating ability of the *MATα HMR***a**-e** strain by mechanisms other than restoring repression at *HMR*, such as interfering with splicing of the **a**1 transcript from *HMR* or destabilization of **a**1 message. As a simple test of whether the restoration of mating was caused by a restoration of silencing or by other indirect means, we tested whether the presumptive silencing that was brought about by *sas2Δ* at *HMR***a**-e** was *SIR*-dependent. For this purpose, we determined the mating phenotype of *MATα HMR***a**-e** *sas2Δ sir4Δ* strains. A *MATα HMR***a**-e** *sas2-Δ1::TRP1* strain (JRY5274) was crossed to a *MAT***a** *HMR***a** *sir4Δ::LEU2* strain (JRY4581) carrying the *SIR4*-containing plasmid pJR368. The plasmid, pJR368, which was present to allow mating, was lost by counterselection in the diploid before sporulation. All 37 *sas2Δ sir4Δ* segregants from 37 complete tetrads were non-

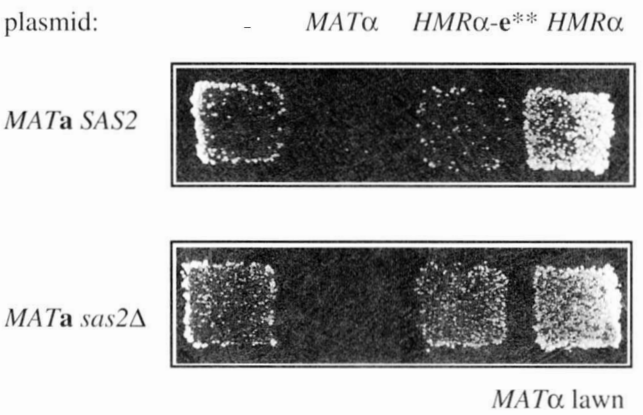

FIGURE 3.—Deletion of *SAS2* was capable of suppressing the α genes at *HMR***α**-e**. The **a** mating ability of JRY2334 (top) and JRY5068 (bottom) transformed with pRS316 (−), pJR157 (*MATα*), pJR1792 (*HMRα*-e**) or pJR882 (*HMRα*) is shown.

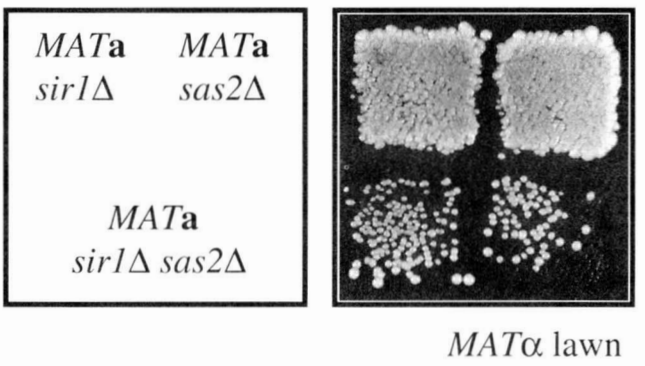

FIGURE 4.—*HMLα* was derepressed in *sir1*Δ *sas2*Δ strains. The **a** mating ability of JRY4621 (top left), JRY5068 (top right) and two isolates of JRY5275 is shown.

maters, indicating that the *sas2*Δ phenotype was indeed dependent on *SIR4*. Based upon Mendelian inheritance and 40% recombination between *MAT* and *HMR*, 11 of the 37 *sas2*Δ *sir4*Δ strains were expected to be *MATα* *HMR***a**-e**. Also, *sir4*Δ::*LEU2* cosegregated with *MATα* in 10 tetrads analyzed from this cross, thus allowing 10 haploids to be positively identified as *MATα* *sir4*Δ *sas2*Δ. DNA blot hybridization showed that six of these carried the *HMR***a**-e** allele, all of which were non-maters. This indicated that the mating ability of a *MATα* *HMR***a**-e** *sas2*Δ was lost by the deletion the *SIR4* gene. Thus, *sas2*Δ established bona fide SIR-dependent silencing at the mutant *HMR* allele.

sas2*Δ suppressed silencing defects at *HMR* in a gene-independent manner:** A hallmark of silencing is the ability to block the expression of a variety of different genes when inserted at or near *HML* and *HMR*. As an independent test of whether *sas2*Δ restored silencing, we asked whether *sas2*Δ could suppress genes other than the **a** mating-type genes when placed near the *HMRa**-e** silencer. For this aim, an allele of *HMR***a**-e** was created in which the **a** genes were replaced by the α mating-type genes (*HMRα*-e**). The α1 and α2 genes of *HMRα* do not require splicing of their mRNAs and the promoters of these genes bind a different transcription factor than that bound by the **a**1 promoter. If *HMRα*-e** were silenced in the *sas2*Δ, but not the wild-type background, then the mating ability of a *MAT***a** *sas2*Δ strain carrying an *HMRα*-e** allele would be better than that of a *MAT***a** *SAS2* strain carrying the same allele. To test this hypothesis, a plasmid containing *HMRα*-e** (pJR1792) and, as controls, a *MATα* (pJR157) and a *HMRα* plasmid (pJR882), were each introduced into a *MAT***a** and a *MAT***a** *sas2*Δ strain (JRY2334 and JRY5068, respectively), and the mating ability of the transformants was assayed (Figure 3). As expected, both strains became non-maters when carrying the *MATα* plasmid due to the co-expression of the **a** and α genes, demonstrating that *sas2*Δ was unable to silence *MAT*. Furthermore, the strains retained their **a**-mating ability when transformed with the *HMRα* carrying plasmid, suggesting that a wild-type *HMRα* was silenced in the wild-type as well as in the *sas2*Δ strain. However, when transformed with the *HMRα*-e** plasmid, the two strains displayed different mating abilities. The *sas2*Δ strain mated better than the *SAS2* strain, indicating that the α genes of *HMRα*-e** were more repressed in the *sas2*Δ strain. This effect was less pronounced than the difference observed between *SAS2* and *sas2*Δ in the *MATα* *HMR***a**-e** strain (Figure 2), presumably because the mating assays in Figure 3 were performed with strains pregrown on selective minimal medium in order to maintain the plasmids carrying the *HMR* alleles. Nonetheless, this result demonstrated that *sas2*Δ was capable of silencing the α genes at *HMRα*-e** but not when the same genes were at *MAT*. Combined with the preceding result, these data established that the repression of genes at *HMR* by *sas2* mutations reflected bona fide silencing.

sas2*Δ caused a silencing defect at *HML*:** During the course of genetic experiments with *sas2*Δ (see below), we observed that the phenotype of *sas2*Δ was dramatically different at *HML* and *HMR*. Specifically, *sas2*Δ caused silencing defects at *HMLα*. In a cross of a *sas2*Δ strain (JRY5068) to a *sir1*Δ strain (JRY4621), all nine *MATa** *sir1*Δ *sas2*Δ haploids of the 32 complete tetrads investigated were unable to mate efficiently, although either mutation alone had little effect on silencing (Figure 4), suggesting that *HMLα* was derepressed in these strains. In contrast, *HMR* was not derepressed in these strains since none of the 18 *MATα* *sir1*Δ *sas2*Δ haploids from this cross had a mating deficiency. In a quantitative mating assay, a *MATα* *sir1*Δ strain (JRY5276) mated with an efficiency of 0.09 as compared with 1 of a wild-type *MATα* strain (JRY3009). A *MATα* *sas2*Δ strain (JRY5071) mated with an efficiency of 1.83, whereas a *MATα* *sir1*Δ *sas2*Δ strain (JRY5277) mated with an efficiency of 0.37 (compare Figure 6A, lanes 1 and 2). These results indicated that *sas2*Δ enhanced the silenc-

ing defects of *sir1*Δ at *HML*, but not *HMR*. Similar observations have also been made by L. PILLUS and colleagues (REIFSNYDER *et al.* 1996).

The *sas2*Δ phenotype depended upon the ACS of the silencer: The *HMR*a-e** allele, at which *sas2*Δ was able to restore repression, is a version of the natural silencer that contains mutated Rap1 and Abf1 binding sites (KIMMERLY *et al.* 1988). Therefore, the ability of *sas2*Δ to restore silencing did not depend upon the Rap1 or Abf1 binding sites of the silencer. If the effect of *sas2*Δ was mediated by the mutant silencer, *sas2*Δ should require the remaining intact element, the ACS, to restore silencing. However, there are other possible explanations, such as the *HMR*-I silencer may become a stronger silencer in a *sas2*Δ mutant. If this were the case, then *sas2*Δ would not require any particular silencer element to restore repression, but would restore repression at any mutant silencer. To test this hypothesis, we tested the ability of *sas2*Δ to restore repression at mutant versions of the synthetic *HMR*-E silencer. In contrast to the natural silencer, the synthetic silencer is a minimal silencer in which much of the functional redundancy of the natural *HMR*-E silencer is missing (MCNALLY and RINE 1991). In strains lacking the *HMR*-I silencer, each element of the synthetic silencer is indispensable for complete silencing and therefore allowed a more precise determination of which, if any, silencer element was required for *sas2*Δ to restore silencing.

A *MAT*α strain carrying the synthetic silencer mated with an efficiency six- to sevenfold lower than that of a wild-type strain. Deletion of *SAS2* in this strain restored the mating efficiency of this strain to approximately wild-type levels (Figure 5, lane 1), indicating that wild-type *SAS2* limited silencing even in the absence of a mutation in the silencer. We first investigated the effect of *sas2*Δ on silencing mediated by a *HMR*-E silencer lacking the Abf1 binding site in a strain lacking the *HMR*-I silencer (*HMR-SS abf1*$^-$ Δ*I*). The ABF1 site made a minor contribution to silencing at the synthetic silencer, since introduction of the Abf1 mutation reduced the mating frequency of a *MAT*α strain only by about fourfold. However, *sas2*Δ restored the mating ability of this strain by ~14-fold (Figure 5, lane 2) and thus fully compensated for the loss of the Abf1 site. Therefore, *sas2*Δ was capable of restoring repression in the absence of the Abf1 binding site, as expected, because the *HMR*a-e** allele also lacks this site.

We next investigated the requirement of *sas2*Δ for an intact Rap1 binding site. Removal of the Rap1 binding site (*HMR-SS rap*$^-$ Δ*I*) reduced the mating efficiency of the strain by four to five orders of magnitude. However, *sas2*Δ restored the mating ability by ~100-fold (Figure 5, lane 3). Thus, *sas2*Δ was capable of partially restoring repression in the absence of the Rap1 binding site, which was also expected because *HMR*a-e** lacks a Rap1 binding site. However, this degree of restoration of repression by *sas2*Δ in cells with the Rap1 site mutation in the synthetic

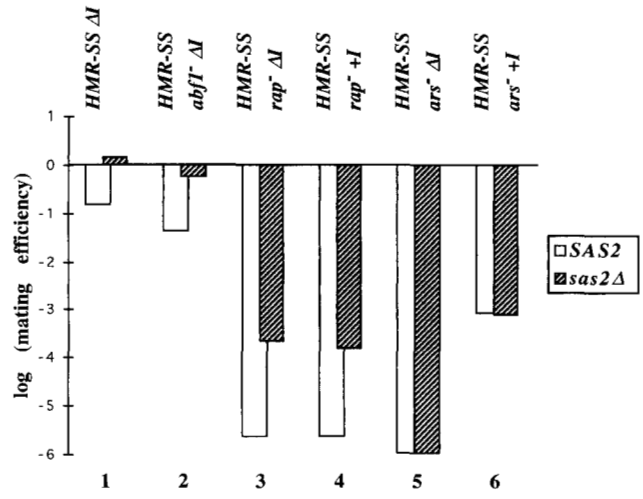

FIGURE 5.—Effect of the deletion of *SAS2* on repression at the synthetic *HMR*-E silencer and at mutant silencer versions. The logarithmic values of the mating efficiencies of *MAT*α strains carrying the indicated *HMR* alleles are given. Each value is the average of at least two independent determinations. Empty bars show *SAS2* strains, striped bars show *sas2*Δ strains. The strains used were JRY4473 and JRY5070 (lane1), JRY4889 and JRY5110 (lane 2), JRY5278 and JRYJRY5279 (lane 3), JRY3937 and JRY5280 (lane 4), JRY4531 and JRY5283 (lane 5) and JRY4527 and JRY5284 (lane 6).

silencer was ~10-fold lower than the 1000-fold effect observed in cells with *HMR*a-e**. One explanation for the difference in the extent of repression at *HMR*a-e** relative to *HMR-SS rap*$^-$ Δ*I* was the presence or absence of the *HMR*-I silencer. However, *sas2*Δ restored repression in a strain with both *HMR-SS rap*$^-$ and *HMR*-I to approximately the same extent as in a strain lacking *HMR*-I (Figure 5, lanes 3 and 4). Therefore, *HMR*-I did not significantly influence the effect of *sas2*Δ on silencing at *HMR*a-e**.

Another explanation for the difference in the degree of repression at *HMR*a-e** and *HMR-SS rap*$^-$ +*I* could be that the Rap1 protein still contributed to silencing at *HMR*a-e**, but not at *HMR-SS rap*$^-$ +*I*, even though its binding site was absent, perhaps by binding to other proteins bound at the silencer. To test this hypothesis, we determined the contribution of the Rap1 protein to silencing at *HMR*a-e**. The silencing-specific *rap1-12* mutation (SUSSEL *et al.* 1993) was introduced into *HMR*a-e** strains that were *SAS2* or *sas2*Δ, and their mating ability was assayed. The *rap1-12* mutation in a *MAT*α *HMR*a-e** strain caused a decrease in mating ability by 16-fold. *sas2*Δ restored the mating ability of this strain by ~600-fold (Figure 6A, lane 4). Thus, the Rap1 protein still contributed to silencing at *HMR*a-e**, even though the Rap1 binding site was mutated. However, the ability of *sas2*Δ to restore silencing was still higher at *HMR*a-e**, even in the absence of Rap1, than at the synthetic silencer with the Rap1 site mutation. Thus, *sas2*Δ restored silencing in the absence of either a functional Rap1 or Abf1 binding site.

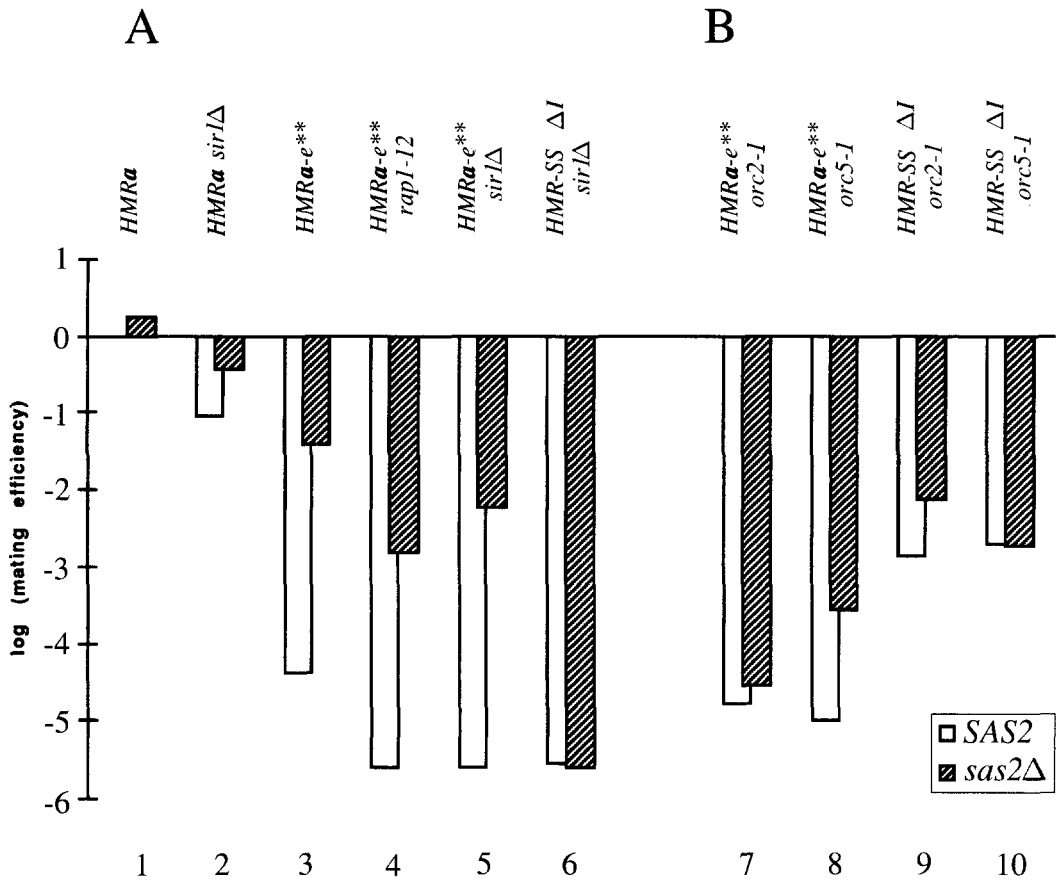

FIGURE 6.—(A) Sir1 and Rap1 are not required for repression by *sas2*Δ. (B) Orc2 and Orc5 are required for repression by *sas2*Δ. The data are presented as in Figure 5. The strains used were JRY3009 and JRY5071 (lane 1), JRY5276 and JRY5277 (lane 2), JRY5273 and JRY5274 (lane 3), JRY5281 and JRY5282 (lane 4), JRY5285 and JRY5286 (lane 5), JRY5289 and JRY5290 (lane 6), JRY5291 and JRY5292 (lane 7), JRY5293 and JRY5294 (lane 8), JRY4475 and JRY5295 (lane 9), JRY4476 and JRY5296 (lane 10).

We next determined whether *sas2*Δ could restore silencing in the absence of a functional ACS, which is the only known functional domain of the *HMR*-E silencer remaining in *HMR***a**-e**. Removal of the ACS in the synthetic silencer (*HMR-SS ars*$^-$ Δ*I*) reduced the mating efficiency of a *MAT***a** strain by ~8000-fold. Interestingly, introduction of *sas2*Δ did not rescue the mating deficiency of this strain in either the presence or absence of *HMR*-I (Figure 5, lanes 5 and 6). Moreover, the silencing defect of an ACS mutation in the presence of *HMR*-I is less severe than the silencing defect of the strain with the Rap1 site mutation. Thus, the inability of *sas2*Δ to restore silencing in the ACS mutant strain cannot be explained by an insensitivity of the assay.

In summary, these findings suggested that *sas2*Δ required the ACS, but not the Rap1 and Abf1 binding sites of the *HMR*-E silencer, to restore repression at *HMR*. Furthermore, these data suggested that the function of wild-type *SAS2* was inhibitory to the role of the ACS in silencing. Because the ACS is the binding site for ORC, we inferred that *SAS2* might inhibit the role of ORC in silencing.

sas2*Δ required *ORC2* and *ORC5* for suppression:** Because ORC, the yeast DNA replication initiator, is the protein that binds the ACS, the ACS dependence of *sas2*Δ-mediated silencing was likely to reflect a dependence on ORC. We tested this prediction by determining whether *sas2*Δ was capable of suppressing silencing defects in *orc2-1* and *orc5-1* strains. As described above, *sas2*Δ suppressed the mating deficiency of a *MAT*α *HMRa**-e** strain by 1000-fold. A *MAT*α *HMR***a**-e** strain that carried either *orc2-1* or *orc5-1* had a mating efficiency approximately threefold lower than the corresponding Orc$^+$ strain. In contrast to Orc$^+$ strains, introduction of *sas2*Δ into the *MAT***α** *HMR***a**-e** *orc2-1* strain increased the mating efficiency of the strain by only twofold, and introduction into the *orc5-1* strain increased the mating efficiency by ~30-fold (Figure 6B, lanes 7 and 8) at the permissive temperature. Thus, efficient restoration of silencing at *HMR***a**-e** by *sas2*Δ required both *ORC2* and *ORC5*. In a parallel study, we investigated whether *sas2*Δ was capable of suppressing the silencing defects of *orc2-1* and *orc5-1* at the synthetic *HMR* silencer. Either *orc2-1* or *orc5-1* caused an ~100-fold loss in mating efficiency of a *MAT*α *HMR-SS* Δ*I* strain. Introduction of *sas2*Δ into these strains left their mating frequency unaltered (Figure 6B, lanes 9 and 10), showing that *sas2*Δ required both *ORC2* and *ORC5*

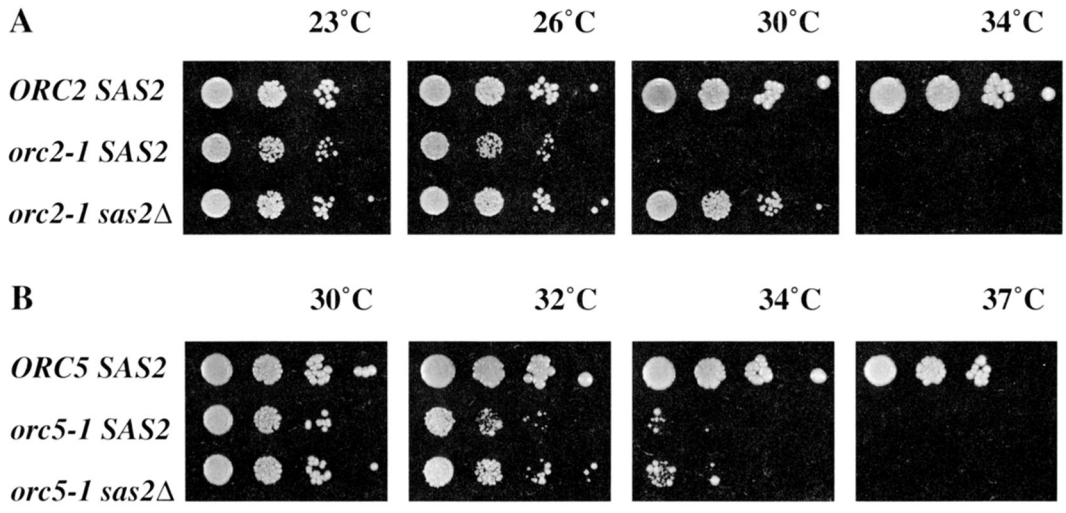

FIGURE 7.—The loss of *SAS2* partially suppressed the temperature sensitivity of *orc2-1* (A) and *orc5-1* (B). The strains to be tested were grown to late logarithmic phase in complete medium and diluted to $\sim 10^6$ cells/ml. This and 1:10, 1:100 and 1:1000 dilutions thereof were spotted onto complete medium that was supplemented with additional tryptophan to compensate for the Trp^- phenotpye of the wild-type *SAS2* strains. The plates were incubated for 2–3 days at the indicated temperatures. The strains used were (A, from top to bottom) JRY4473, JRY4475 transformed with *PmlI* linearized pRS404 and JRY5070; (B, from top to bottom) JRY5273, JRY5293 and JRY5294.

for restoring silencing in a strain containing a synthetic silencer.

***sas2*Δ partially suppressed the temperature sensitivity of *orc2-1* and *orc5-1*:** The observation that *sas2*Δ required the ACS to restore silencing suggested that the Sas2 protein was a negative regulator of ORC. We therefore investigated whether *SAS2* had an effect on the role of ORC in replication initiation. For this aim, the growth of *orc2-1* or *orc5-1* strains that were either *SAS2* or *sas2*Δ was compared at several temperatures. The growth of an *orc2-1* strain (JRY4475) was slightly impaired at 26°, and the strain was unable to grow at temperatures ≥30°. However, an *orc2-1 sas2*Δ strain (JRY5295) grew at both 26 and 30°, while being temperature sensitive at 34° (Figure 7A). Thus, because the loss of Sas2 function increased residual ORC function in an *orc2* mutant, formally *SAS2* was an inhibitor of ORC. Deletion of *SAS2* had a barely discernible ability to increase ORC function in an *orc5-1* mutant. The reason for the difference between the effect of *sas2*Δ on *orc2-1* and on *orc5-1* was unclear.

sas2*Δ suppressed silencing defects in the absence of Sir1:** The genetic experiments described above suggested that *SAS2* inhibited ORC's role in silencing. One model for the role of ORC is, in combination with Rap1, to provide a surface to recruit the Sir1 protein to the silencer, which then promotes the assembly of silenced chromatin (FOX *et al.* 1996; TRIOLO and STERNGLANZ 1996). Perhaps, the Sas2 protein directly or indirectly inhibited an interaction between ORC and Sir1. Thus, in the absence of Sas2, the ORC-Sir1 interaction may be stronger and thus no longer require the adjacent Rap1 protein. This model predicted that the restoration of silencing by *sas2*Δ would be *SIR1*-dependent. In strains with the wild-type silencer, *sir1*Δ caused a decrease in mating ability of ~10-fold. Deletion of *SAS2* in this strain restored the mating efficiency to approximately wild-type levels (Figure 6A, lane 1). Similarly, *sir1*Δ caused a decrease in mating ability of a *MATα HMRa**-e** strain by 17-fold. However, deletion of *SAS2* in this strain restored its mating ability by 2000-fold (Figure 6A, lane 5), indicating that *SIR1* was not required for *sas2*Δ-mediated silencing at *HMR***a**-e**. By this analysis, the ability of *sas2*Δ to restore silencing was *SIR1* independent.

However, a rather different picture emerged for the role of *SIR1* in *sas2*Δ-mediated silencing at the synthetic silencer. The loss of *SIR1* in strains with a synthetic *HMR*-E silencer reduced the mating efficiency of a *MATα* strain by approximately six orders of magnitude, indicating that the Sir1 protein was absolutely required for silencing by the synthetic silencer, but not the natural silencer. Interestingly, deletion of *SAS2* in a *MATα HMR-SS ΔI sir1*Δ strain did not restore silencing (Figure 6A, lane 6), indicating that *sas2*Δ was unable to bypass the role of *SIR1* in this context. Thus, if Sas2 inhibits an interaction between ORC and Sir1 at the natural *HMR*-E silencer, then factors other than Sas2 must limit the recruitment of Sir1 to the synthetic silencer.

Features and homologies of the Sas2 protein: The putative Sas2 protein was compared with the sequences in the database using the BLAST search tool (ALTSCHUL *et al.* 1990). This analysis revealed that Sas2 had several close homologues (Figure 8). The highest homology (31% identity, 57% similarity) was obtained with a *Homo sapiens* protein (GenBank accession No. U40989), Tip, that interacts with the viral transcriptional activator Tat

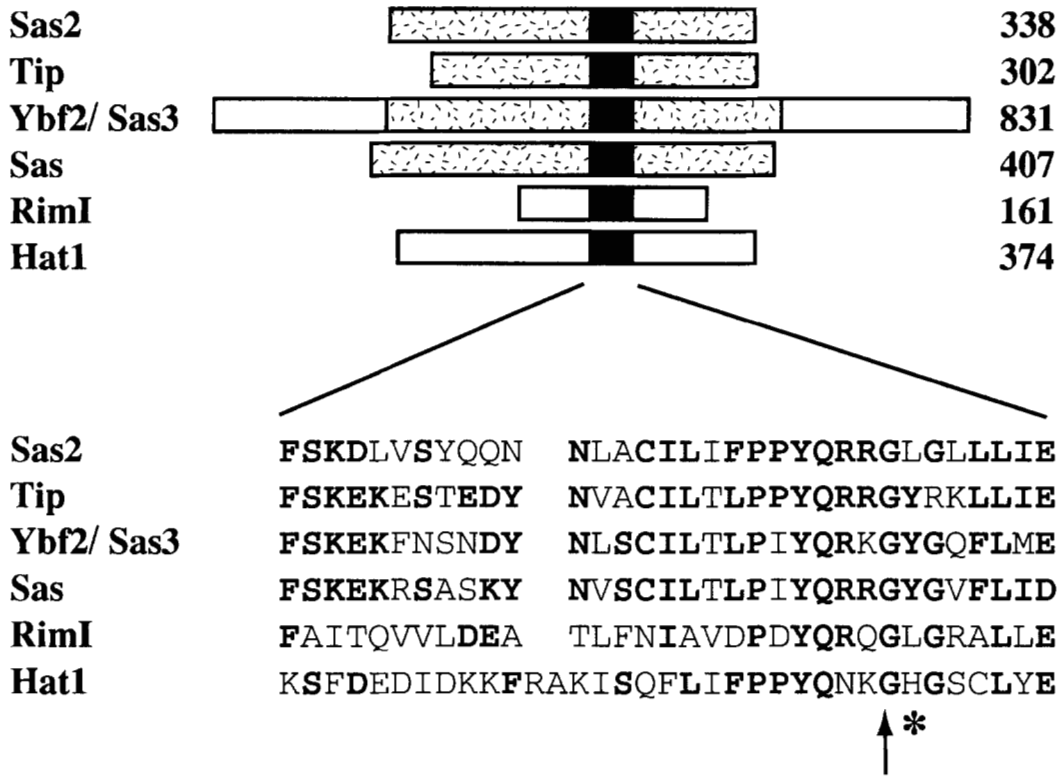

FIGURE 8.—Sas2 shares similarities with known acetyltransferases. Alignments are shown between *S. cerevisiae* Sas2, human Tat interacting protein (Tip), *S. cerevisiae* Sas3/Ybf2 , *S. pombe* Sas, *E. coli* RimI and *S. cerevisiae* Hat1 (top). The numbers indicate the length of the proteins. Stippled boxes denote domains of similarity between the proteins. Black boxes show the region of homology to the acetyltransferases that corresponds to part of a putative acetyl-coenzyme A binding site. The amino acid alignments in this region are shown below. The alignment starts at amino acid position 194 of Sas2. Bold letters indicate residues that are identical or similar between the proteins. The arrow marks a conserved glycine residue that, when mutated in the acetyltransferase Mak3, results in a loss of activity. The asterisk marks a histidine residue that is the active site at the equivalent position of the chloramphenicol acetyltransferase CATIII. The alignment was created using the Pileup program (Genetics Computer Group).

(KAMINE *et al.* 1996). The next closest homologue was a *Schizosaccharomyces pombe* protein of unknown function (Genbank accession No. Z69795). The search also revealed a *S. cerevisiae* Sas2 homologue, Ybf2/Sas3 (31% identity, 53% similarity), which, like Sas2, has a role in silencing (REIFSNYDER *et al.* 1996). Comparison of Sas2 with the DBEST database suggested the existence of a *Zea mays* homologue.

Interestingly, Sas2 showed homology to two known acetyltransferases, the *Escherichia coli* RimI protein, which is a N-terminal acetyltransferase for ribosomal S18 protein (YOSHIKAWA *et al.* 1987), and the yeast histone acetyltransferase Hat1 (KLEFF *et al.* 1995). However, the similarity was confined to one short region of high homology (Figure 8). Sas2 was homologous to RimI and Hat1 in a domain of the acetyltransferases that corresponds to region A of a bipartite coenzyme A binding domain (TERCERO *et al.* 1992), while region B of this domain was not found in Sas2. Furthermore, Sas2 was conserved at Gly^{219}, a residue that, when mutated at the corresponding position of the yeast Mak3 acetyltransferase, results in a loss of activity (Figure 8, →) (TERCERO *et al.* 1992). Moreover, Sas2 contained a leucine residue at position 220 rather than the conserved histidine, which has been proposed to constitute part of the active site of chloramphenicol acetyltransferases (Figure 8, *) (SHAW and LESLIE 1991). Thus, the structural similarities of Sas2 to known acetyltransferases are ambiguous as to whether Sas2 is an acetyltransferase or not. Indeed, as described below, we have not yet detected any acetyltransferase activity associated with Sas2.

The deduced Sas2 protein sequence contained one potential amidation site, one potential tyrosine phosphorylation site and many potential phosphorylation sites (1 for cAMP- and cGMP-dependent protein kinases, four for casein kinase II and nine for protein kinase C). The other noteworthy feature of Sas2 was a cluster of positively charged amino acids at the N-terminus of the protein. The PSORT program software (version 6.3) assigned a nuclear localization to Sas2.

Is Sas2 a histone acetyltransferase? Prompted by the similarity of a domain of Sas2 to known acetyltransferases, we explored whether Sas2 might be an acetyltransferase for specific histone residues that reportedly affect *HM* silencing. If Sas2 was a histone acetyltransferase,

then the deletion of *SAS2* would be expected to have the same phenotype as the mutation of its target residue(s) on a particular histone. There are four acetylatable lysine residues in both histone H3 (positions 9, 14, 18 and 23) and histone H4 (positions 5, 8, 12 and 16) that play a role in silencing. Genetic analysis of histone H4 has revealed that mutation of the acetylated lysine residue at position 16 (H4Q16) has the most pronounced effect on silencing (JOHNSON *et al.* 1992). However, in contrast to *sas2Δ*, this mutation causes strong derepression at *HML* on its own. Thus, if Sas2 is a histone acetyltransferase, it is not solely responsible for acetylation of this position. Furthermore, the combination of a N-terminal deletion of the residues 3–40 of histone H3 (H3Δ4–30) with H4Q16 results in strong derepression at *HMR*, while either mutation alone has little effect (THOMPSON *et al.* 1994). However, *sas2Δ* did not enhance the silencing defect of H4Q16 at *HMR* (data not shown). These findings suggested that neither residue 16 of H4 nor residues 3–40 of H3 were the target of a Sas2-dependent acetylation.

DISCUSSION

This report describes the discovery of the *SAS2* gene by virtue of its ability to suppress mutations in the *HMR-E* silencer of *S. cerevisiae*. *SAS2* thus joins *CDC7* as genes in which mutations that reduce or eliminate function bypass the requirement for a functional Rap1 and Abf1 binding site in the silencer (AXELROD and RINE 1991). Unlike *CDC7*, null alleles of *SAS2* were viable and were also capable of restoring silencing at *HMR*. Thus, this suppression reflected the phenotype of a null allele. The restoration of silencing by *sas2Δ* was dependent upon the function of the *SIR* genes, which distinguished *SAS2* from *SUM1-1*, a mutation that causes SIR-independent silencing (KLAR *et al.* 1985; LAURENSON and RINE 1991).

One of the most striking properties of the *sas2Δ* allele was its opposite phenotype at *HML vs. HMR*. Specifically, in combination with *sir1Δ*, which caused partial derepression of *HML*, the *sas2Δ sir1Δ* double mutant was dramatically derepressed at *HML*. *HMR*, however, was no more derepressed in a *sas2Δ sir1Δ* double mutant that it was in a *sir1Δ* mutant strain. The simplest interpretation was that the Sas2 protein must somehow contribute to silencing at *HML* and antagonize silencing at *HMR*. There have been several indications that silencing at *HML* is weaker than silencing of *HMR*, but *SAS2* is the first gene known to have opposing effects at the two silent mating-type loci. These opposite phenotypes may reflect differences in the nature of the chromatin structure or differences in the silencers at the two loci. If Sas2 is an acetyltransferase, it may have two (or more) cellular targets, each of which have separate roles at *HML* and *HMR*. In this regard, it should be noted that *orc* mutations derepress both *HML* and *HMR*. Thus ORC is unlikely to mediate the opposing effects of *sas2Δ* at *HML* and *HMR*.

Double mutant analysis indicated that the ARS consensus sequence of the silencer, the SIR proteins, and the ORC proteins were required to restore silencing at the mutant silencer, whereas Rap1, Sir1 and Abf1 were not required. Because *sas2Δ* increased silencer function in an ORC-dependent manner, it is possible to think of Sas2 as a protein that inhibits ORC function, perhaps directly by acetylation of ORC or an accessory protein. Some support for this view was provided by the ability of *sas2Δ* to partially suppress the temperature sensitivity of *orc2-1*; this presumably reflects an impaired ability to initiate replication at origins of replication. However, a decrease in replication initiation could, in principle, be compensated for by facilitating other related processes, such as the processivity of DNA replication or the assembly of chromatin at the time of DNA replication. Thus, *SAS2* may inhibit the function of processivity factors, such as PCNA (AYYAGARI *et al.* 1995) and RF-C (CULLMANN *et al.* 1995). By this model, replication elongation would be more processive in *sas2* mutants, placing a decreased demand on initiation to replicate the genome.

Histone acetylation has been implicated in silencing in yeast. Thus, enzymes that are capable of acetylating histones and other proteins are potentially of great relevance. The inferred protein sequence of Sas2 implied that Sas2 may be a member of the protein acetyltransferase family. Over 33 consecutive amino acids, Sas2 is similar to *S. cerevisiae* Hat1, an acetyltransferase for histone H4 (KLEFF *et al.* 1995), and to the *E. coli* RimI protein, which acetylates the N-terminal alanine of ribosomal protein S18 (YOSHIKAWA *et al.* 1987) (Figure 8). Gcn5, a bona fide yeast acetyltransferase (BROWNELL *et al.* 1996), is even less homologous to Hat1 and RimI than is Sas2, providing support for the notion that *SAS2* encodes a protein acetyltransferase. Although such comparisons cannot prove that Sas2 is an acetyltransferase, Sas2 is likely to be either an acetyltransferase or a protein that shares some property with acetyltransferases. Some relatives of Sas2 have a role in silencing, and some do not. A null allele of *HAT1* did not restore silencing at *HMR* flanked by a mutant silencer (A. EHRENHOFER-MURRAY and J. RINE, unpublished data). In contrast, a null allele of *SAS3 (YBF2)*, a close homologue of *SAS2*, does restore silencing at *HMR* (REIFSNYDER *et al.* 1996). Thus, even if the biochemical activity of Sas2 and its relatives can be predicted from their sequence, the phenotype of the mutant alleles cannot. This point bears on the possible function of other Sas2 homologues. Specifically, the Tat-interacting protein Tip (KAMINE *et al.* 1996) and the human proteinase C inhibitor (MEIJERS and CHUNG 1991) are homologues of Sas2. Even if both proteins are acetyltransferases, the consequences of acetylation in these two contexts are likely to be rather different.

Several features of *sas2Δ* are reminiscent of Su(var) mutations. These mutations suppress the variegated phenotype of specific chromosome rearrangements in Drosophila (WEILER and WAKIMOTO 1995). Some Su(var) mutations have identified potential heterochromatin components, whereas others appear to be involved in chromatin assembly or modification. Interestingly, some Su(var) mutations have opposite effects on the variegation of euchromatic and heterochromatic genes (HEARN *et al.* 1991), which bears similarities to the opposing effects of *sas2Δ* on *HML* and *HMR*. Inhibitors of histone deacetylases have been long known to affect some position effects on gene expression in Drosophila (MOTTUS *et al.* 1980; REUTER *et al.* 1982), but the interpretation of inhibitor studies is always limited by the possibility of an inhibitor having a secondary effect. The discovery of mutations affecting protein acetyltransferases and acetyltransferase homologues by their effects on gene expression imply that dynamic acetylation of chromatin components may play an important role in aspects of gene regulation, much as phosphorylation plays a regulatory role in metabolism.

We thank LORRAINE PILLUS for sharing information befeore publication and for stimulating discussions. We thank DAVID SHORE for supplying the *rap1-12* mutation; M. GRUNSTEIN for providing histone mutants; CATHERINE FOX, STEPHEN LOO and ROHINTON KAMAKAKA for strains and plasmids; PATRICK ERRADA for expert help in sequencing *SAS2*; and PAUL LASKI for help constructing the *sas2Δ* allele. We thank the members of our laboratories for many stimulating discussions. This work was supported by the Swiss National Foundation and the Janggen-Pöhn Foundation (A.E.), an American Cancer Society postdoctoral fellowship (D. R.) and by a grant from the National Institutes of Health (GM-31105).

LITERATURE CITED

ALTSCHUL, S. F., W. GISH, W. MILLER, E. W. MYERS and D. J. LIPMAN, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

APARICIO, O. M., and D. E. GOTTSCHLING, 1994 Overcoming telomeric silencing: a trans-activator competes to establish gene expression in a cell cycle-dependent way. Genes Dev **8:** 1133–1146.

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.

AYYAGARI, R., K. J. IMPELLIZZERI, B. L. YODER, S. L. GARY and P. M. BURGERS, 1995 A mutational analysis of the yeast proliferating cell nuclear antigen indicates distinct roles in DNA replication and DNA repair. Mol. Cell. Biol. **15:** 4420–4429.

BELL, S. P., R. KOBAYASHI and B. STILLMAN, 1993 Yeast origin recognition complex functions in transcription silencing and DNA replication. Science **262:** 1844–1849.

BLACKBURN, E. H., 1994 Telomeres: no end in sight. Cell **77:** 621–623.

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.

BRAUNSTEIN, M., A. B. ROSE, S. G. HOLMES, C. D. ALLIS and J. R. BROACH, 1993 Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev. **7:** 592–604.

BROWNELL, J. E., J. ZHOU, T. RANALLI, R. KOBAYASHI, D. G. EDMONDSON *et al.*, 1996 Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84:** 843–851.

CULLMANN, G., K. FIEN, R. KOBAYASHI and B. STILLMAN, 1995 Characterization of the five replication factor C genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:** 4661–4671.

FOSS, M., F. J. MCNALLY, P. LAURENSON and J. RINE, 1993 A role of the origin recognition complex (ORC) in transcriptional silencing and DNA replication in *Saccharomyces cerevisiae*. Science **262:** 1838–1844.

FOX, C. A., A. E. EHRENHOFER-MURRAY, S. LOO and J. RINE, 1996 The role of ORC and Sir1 in the S-phase requirement for silencing. Science (in press).

HEARN, M. G., A. HEDRICK, T. A. GRIGLIATTI and B. T. WAKIMOTO, 1991 The effect of modifiers of position-effect variegation on the variegation of heterochromatic genes of *Drosophila melanogaster*. Genetics **128:** 785–797.

JEPPESEN, P., and B. M. TURNER, 1993 The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell **74:** 281–289.

JOHNSON, L. M., A. G. FISHER 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.

KAMINE, J., B. ELANGOVAN, T. SUBRAMANIAN, D. COLEMAN and G. CHINNADURAI, 1996 Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. Virology **216:** 357–366.

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.

KLAR, A. J., S. N. KAKAR, J. M. IVY, J. B. HICKS, G. P. LIVI *et al.*, 1985 *SUM1*, an apparent positive regulator of the cryptic mating-type loci in *Saccharomyces cerevisiae*. Genetics **111:** 745–758.

KLEBE, R. J., J. V. HARRISS, Z. D. SHARP and M. G. DOUGLAS, 1983 A general method for polyethylene-glycol-induced genetic transformation of bacteria and yeast. Gene **25:** 333–341.

KLEFF, S., E. D. ANDRULIS, C. W. ANDERSON and R. STERNGLANZ, 1995 Identification of a gene encoding a yeast histone H4 acetyltransferase. J. Biol. Chem. **270:** 24674–24677.

LAURENSON, P., and J. RINE, 1991 *SUM1-1*: a suppressor of silencing defects in *Saccharomyces cerevisiae*. Genetics **129:** 685–696.

LE, M. H., D. DURICKA and G. H. KARPEN, 1995 Islands of complex DNA are widespread in Drosophila centric heterochromatin. Genetics **141:** 283–303.

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

LOO, S., C. A. FOX, J. RINE, R. KOBAYASHI, B. STILLMAN *et al.*, 1995a The origin recognition complex in silencing, cell-cycle progression, and DNA replication. Mol. Biol. Cell **6:** 741–756.

LOO, S., P. LAURENSON, M. FOSS, A. DILLIN and J. RINE, 1995b Roles of *ABF1*, *NPL3*, and *YCL54* in silencing in *Saccharomyces cerevisiae*. Genetics **141:** 889–902.

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

MEGEE, P. C., B. A. MORGAN, B. A. MITTMAN and M. M. SMITH, 1990 Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science **247:** 841–845.

MEIJERS, J. C., and D. W. CHUNG, 1991 Organization of the gene coding for human protein C inhibitor (plasminogen activator inhibitor-3). Assignment of the gene to chromosome 14. J. Biol. Chem. **266:** 15028–15034.

MICKLEM, G., A. ROWLEY, J. HARWOOD, K. NASMYTH and J. F. DIFFLEY, 1993 Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. Nature **366:** 87–89.

MOTTUS, R., R. REEVES and T. A. GRIGLIATTI, 1980 Butyrate suppression of position-effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. **178:** 465–469.

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.

MULLER, J., 1995 Transcriptional silencing by the Polycomb protein in *Drosophila* embryos. EMBO J. **14:** 1209–1220.

PARK, E. C. and J. W. SZOSTAK, 1990 Point mutations in the yeast

histone H4 gene prevent silencing of the silent mating type locus *HML*. Mol. Cell. Biol. **10:** 4932–4934.

PATTERSON, M., R. A. SCLAFANI, W. L. FANGMAN and J. ROSAMOND, 1986 Molecular characterization of cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 1590–1598.

RASTAN, S., 1994 X chromosome inactivation and the Xist gene. Curr. Opin. Genet. Dev. **4:** 292–297.

REIFSNYDER, C., J. LOWELL, A. CLARKE and L. PILLUS, 1996 Yeast silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nature Genetics **14:** 42–49.

REUTER, G., R. DORN and H. J. HOFFMANN, 1982 Butyrate sensitive suppressor of position-effect variegation mutations in *Drosophila melanogaster*. Mol. Gen. Genet. **188:** 480–485.

ROSE, M. D., P. NOVICK, J. H. THOMAS, D. BOTSTEIN and G. R. FINK, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SHAW, W. V. and A. G. LESLIE, 1991 Chloramphenicol acetyltransferase. Annu. Rev. Biophys. Biophys. Chem. **20:** 363–386.

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

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.

SPENCER, F., S. L. GERRING, C. CONNELLY and P. HIETER, 1990 Mitotic chromosome transmission fidelity mutants in *Saccharomyces cerevisiae*. Genetics **124:** 237–249.

SUSSEL, L., D. VANNIER and D. SHORE, 1993 Epigenetic switching of transcriptional states: *cis*- and *trans*-acting factors affecting establishment of silencing at the *HMR* locus in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 3919–3928.

TAUNTON, J., C. A. HASSIG and S. L. SCHREIBER, 1996 A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272:** 408–411.

TERCERO, J. C., L. E. RILES and R. B. WICKNER, 1992 Localized mutagenesis and evidence for post-transcriptional regulation of *MAK3*. A putative N-acetyltransferase required for double-stranded RNA virus propagation in *Saccharomyces cerevisiae*. J. Biol. Chem. **267:** 20270–20276.

THOMAS, B. J., and R. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

THOMPSON, J. S., X. LING and M. GRUNSTEIN, 1994 Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. Nature **369:** 245–247.

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

WEILER, K. S., and B. T. WAKIMOTO, 1995 Heterochromatin and gene expression in *Drosophila*. Annu. Rev. Genet. **29:** 577–605.

YOSHIKAWA, A., S. ISONO, A. SHEBACK and K. ISONO, 1987 Cloning and nucleotide sequencing of the genes *rimI* and *rimJ* which encode enzymes acetylating ribosomal proteins S18 and S5 of *Escherichia coli* K12. Mol. Gen. Genet. **209:** 481–488.

Communicating editor: F. WINSTON