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Identification of *SAS4* and *SAS5*, Two Genes That Regulate Silencing in *Saccharomyces cerevisiae*

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ABSTRACT

In *Saccharomyces cerevisiae*, chromatin-mediated silencing inactivates transcription of the genes at the *HML* and *HMR* cryptic mating-type loci and genes near telomeres. Mutations in the Rap1p and Abf1p binding sites of the *HMR-E* silencer (*HMR***a**-*e***) result in a loss of silencing at *HMR.* We characterized a collection of 15 mutations that restore the α-mating phenotype to *MAT*α *HMR***a**-*e*** strains. These mutations defined three complementation groups, two new groups and one group that corresponded to the previously identified *SAS2* gene. We cloned the genes that complemented members of the new groups and identified two previously uncharacterized genes, which we named *SAS4* and *SAS5.* Neither *SAS4* nor *SAS5* was required for viability. Null alleles of *SAS4* and *SAS5* restored *SIR4*-dependent silencing at *HMR*, establishing that each is a regulator of silencing. Null alleles of *SAS4* and *SAS5* bypassed the role of the Abf1p binding site of the *HMR-E* silencer but not the role of the ACS or Rap1p binding site. Previous analysis indicated that *SAS2* is homologous to a human gene that is a site of recurring translocations involved in acute myeloid leukemia. Similarly, *SAS5* is a member of a gene family that included two human genes that are the sites of recurring translocations involved in acute myeloid leukemia.

TRANSCRIPTION is regulated by factors that act locally at promoters and enhancers, as well as by factors that influence the chromatin structure of genes. There are now five well-described ATP-dependent chromatin remodeling complexes, SWI/SNF, RSC, NURF, CHRAC, and AFC, that use the energy of ATP hydrolysis to alter the relationship between DNA and core histone proteins and activate (Cairns 1998; Varga-Weisz and Becker 1998) or repress (Holstege *et al.* 1998; Schnitzler *et al.* 1998) transcription. Histone acetylation also influences chromatin structure and plays a role in the activation of transcription. Several proteins that possess histone acetylase activity activate transcription of specific genes, whereas others are components of the general transcription machinery (Bannister and Kouzarides 1996; Brownell and Allis 1996; Brownell *et al.* 1996; Mizzen *et al.* 1996; Kuo *et al.* 1998).

Reciprocal translocations that form in-frame gene fusions are common in human leukemias (Cleary 1991; Rabbitts 1994). The translocation partners identified to date encode proteins that are known or suspected to activate transcription, including DNA binding proteins that are transcription activators and histone acetylases (Cleary 1991, 1992; Rabbitts 1994; Rowley *et al.* 1997; Waring and Cleary 1997; Carapeti *et al.* 1998). Thus, altered patterns of transcription, possibly as a result of altered chromatin structures at promoters, have been implicated in the etiology of human leukemia.

At the other end of the spectrum, there are a growing number of proteins that block the expression of genes by causing the formation of an inactive chromatin structure that contains those genes. Well-characterized examples include the proteins that mediate heterochromatin formation and cause the classically defined position effects on gene expression. In Saccharomyces, heterochromatin formation is responsible for silencing the mating-type genes at *HML* and *HMR* and for silencing reporter genes inserted near telomeres (Grunstein 1998; Lustig 1998). A related form of silencing inactivates reporter genes inserted into the rDNA (Bryk *et al.* 1997; Smith and Boeke 1997). Silencing at *HML*, *HMR*, and at the telomeres involves the assembly of a repressive chromatin structure that contains the Sir2, Sir3, and Sir4 proteins that bind to each other and to core histones (Moretti *et al.* 1994; Hecht *et al.* 1995, 1996; Strahl-Bolsinger *et al.* 1997). In addition, silenced chromatin contains hypoacetylated nucleosomes, implicating a role for acetylation and deacetylation in regulating regional effects on gene expression (Braunstein *et al.* 1996; Rundlett *et al.* 1998). An understanding of how particular patterns of histone acetylation are established and how repressive chromatin structures are assembled in particular chromosomal regions is lacking.

The work presented here extends our dissection of silencing in Saccharomyces. Silencing of *HML* and *HMR* is mediated by flanking regulatory sites known as silenc-

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ers (Abraham *et al.* 1984; Feldman *et al.* 1984; Brand *et al.* 1985). The best-characterized silencer, *HMR-E*, consists of binding sites for three proteins: the two transcription factors, Abf1p and Rap1p, and ORC, the replication initiator protein. The combination of these proteins is thought to recruit the *SIR* proteins to the silencer (Chien *et al.* 1993; Moretti *et al.* 1994; Fox *et al.* 1997). Mutations in any one of the *HMR-E* binding sites, in an otherwise wild-type cell, have little effect on silencing (Brand *et al.* 1987; Kimmerly *et al.* 1988). In contrast, *HMR***a**-*e***, a double mutant silencer with lesions in both the Rap1p and Abf1p binding sites, has a substantial defect in *HMR* silencing (Kimmerly *et al.* 1988). Previous work demonstrated that mutations in some genes, such as *SAS2*, can suppress the silencing defect caused by this mutant silencer (Axelrod and Rine 1991; Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997). *SAS2* encodes an acetylase homolog, suggesting that analysis of *SAS2* and functionally related genes may provide a genetic entrée to genes that regulate the modification of histones and the assembly/disassembly of particular chromatin structures (Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997). In addition, *SAS2* is highly similar to *MOZ*, a human gene involved in acute myeloid leukemia (Reifsnyder *et al.* 1996).

In this study, we present the analysis of additional mutations that restore silencing of *HMR* flanked by the *HMR***a**-*e*** silencer. This work identified two new genes, *SAS4* and *SAS5*, and established that these genes were regulators of silencing. *SAS4* lacked any recognizable homolog. *SAS5* had similarity to *ANC1*, a yeast gene implicated in transcriptional activation and chromatin remodeling, and to *AF-9* and *ENL*, two human genes that are the sites of recurring translocations that contribute to leukemia.

MATERIALS AND METHODS

Dominance tests: The original *sas* mutant strains (DRY22-29 and DRY31-DRY42) were of the α-mating type and contained the *HMR***a**-*e*** allele. Two tests were used to determine which mutants contained dominant mutations and which contained recessive mutations. In the first test, the *sas* mutants were mated to a *mat*aΔ*p HMR***a**-*e*** strain (DRY1351) in which the promoter region of the *MAT***a***1* gene is deleted, and the mating phenotype of the diploid was tested. Among the 20 mutants tested, 2 were dominant mutants that could suppress the silencing defect of both *HMR***a**-*e*** alleles and had the α-mating phenotype. The remaining 18 mutants produced diploids that were nonmating, and thus were either recessive, *cis*-dominant, or weakly dominant. In a second test, the remaining 18 mutants were mated to a *mat*aΔ*p* strain (DRY1352) that contained a null allele of *HMR* in which the entire locus was replaced with the *URA3* gene (*mat***a**Δ*p hmr::URA3*). Of the 18 diploids, 15 were unable to mate and hence were judged to be recessive.

Complementation analysis: As described in the text, complementation analysis was performed by crossing each of the original *sas* mutants to *mat*aΔ*p HMR***a**-*e*** strains harboring a deletion of *SAS2* (DRY1356), *SAS4* (DRY1354), or *SAS5* (DRY1358). The resulting *mat*aΔ*p/MAT*α *HMR***a**-*e***/*HMR***a**-*e*** *sas*Δ/*sas*- diploids were tested for the α-mating phenotype. Diploids with the α-mating phenotype indicated that the original mutation being tested did not complement the *SAS* null allele, whereas diploids with the nonmating phenotype indicated that the original mutation being tested complemented the *SAS* null allele. By this criterion, the original mutant strains that comprised the *SAS2* complementation group were DRY23, DRY26, DRY28, DRY29, DRY30, DRY33, DRY34, DRY35, and DRY41 (Table 1). The mutant strains that comprised the *SAS4* complementation group were DRY25, DRY27, DRY32, and DRY38. The mutant strains that comprised the *SAS5* complementation group were DRY24 and DRY40.

Allelism tests: Allelism between the *sas4*Δ*::kanMX* allele and the original *sas4-1* allele was tested in 29 tetrads from a cross between a *MAT***a** *HMR***a**-*e*** *sas4*Δ*::kanMX* strain (DRY1360) and the original *MAT*α *HMR***a**-*e*** *sas4-1* mutant strain (DRY24). Each of the tetrads from this cross contained two segregants with the α-mating phenotype, indicating that *sas4-1* and the *sas4*Δ*::kanMX* mutations were allelic. Similarly, *MAT***a**/*MAT*α diploids homozygous for *HMR***a**-*e*** and *sas5-1*/*sas5*Δ*::HIS3* (derived from DRY24 crossed to DRY1391) segregated two α-mating competent and two **a**-mating segregants in each of 39 tetrads. Thus *sas5*Δ*::HIS3* and *sas5-1* were allelic.

Cloning of *SAS4* and *SAS5* genes: A yeast genomic library in a LEU2-CEN vector was transformed into DRY601 (*sas4-1*) or DRY342 (*sas5-1*) (Spencer *et al.* 1990). Approximately 3000 colonies were screened for the nonmating phenotype. Plasmids were isolated from nonmating colonies and retransformed into DRY601 or DRY342. Plasmids that conferred the nonmating phenotype upon retransformation were mapped and partially sequenced. The partial sequence was used to identify the complete sequence from GenBank.

Disruption of *SAS4* and *SAS5*: The entire coding regions of the *SAS4* and *SAS5* genes were deleted by PCR-mediated gene disruption (Baudin *et al.* 1993). Disruption of *SAS4* was as follows. The *kanMX4* gene of plasmid pDR760 was amplified by PCR using the 5′-ccgaaaatttctacagcattaaaagcatatgagagttcat cacgttgtaaaacgacggcc-3′ and 5′-atattgaatttcatttacaccatcgcatta tattagttcaaaggctcgtatgttgtgtgg-3′ primers. pDR760 contains an *Eco*RI-*Bam*HI *kanMX4* fragment from pFA6 (Wach *et al.* 1994) inserted into *Eco*RI-*Bam*HI-cleaved pUC18. *sas4*Δ::*kanMX* strains were constructed by transformation of the PCR products and confirmed by DNA blot analysis. Disruption of *SAS5* was as follows: the *HIS3* gene of plasmid pJJ217 (Jones and Prakash 1990) was amplified by PCR using the 5′-tctatgttttcag gcattgtttaatttcatgatggctgtccggcctcctctagtacactc-3′ and 5′-ctttt tttttttttggtgccatatataatagacgctcttttgcgcgcctcgttcagaatg-3′ primers. *sas5*Δ*::HIS3* strains were constructed by transformation of the PCR products and confirmed by DNA blot analysis.

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.

Yeast strain construction: Two isogenic sets of strains were used in this work. The first was derived from JRY2069, the second from W303-1a. *SAS4* and *SAS5* were disrupted in JRY2069 to generate DRY1373 and DRY1374, respectively. The W303 derivatives containing disruptions of *SAS4* or *SAS5* were generated as follows. *SAS4* was disrupted in DRY439 to generate DRY1364, in CAF23 to generate DRY1370, in CAF68 to generate DRY1366, in CAF176 to generate DRY1369, in CAF179 to generate DRY1365, and in CAF396 to generate DRY1368. One copy of *SAS4* was disrupted in the diploid strain DRY1338 to generate DRY1361 (*MAT***a**/*MAT*α *HMR***a**-*e***/*HMR-ssabf1-::ADE2 ade2*Δ*::HIS3*/*ade2*Δ*::LEU2*). DRY1322 and DRY1360 were segregants derived from DRY1361. The *HMR-ssabf1-::ADE2* allele of DRY1361 gives rise to a pink colony

TABLE 1

Yeast strains

Strain	Genotype
JRY2069[a]	*MAT*α *HMR***a***-e*** ade2-101oc his3 lys2 tyr1 ura3-52*
DRY23	*sas2-1*
DRY24	*sas5-1*
DRY25	*sas4-1*
DRY26	*sas2-2*
DRY27	*sas4-2*
DRY28	*sas2-3*
DRY29	*sas2-4*
DRY31	*sas2-5*
DRY32	*sas4-3*
DRY33	*sas2-6*
DRY34	*sas2-7*
DRY35	*sas2-8*
DRY38	*sas4-4*
DRY40	*sas5-2*
DRY41	*sas2-9*
DRY1373	*sas4Δ::kanMX4*
DRY1374	*sas5Δ::HIS3*
W303-1a[b]	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*
JRY3009	*MAT*α
JRY5273	*MAT*α *HMR***a***-e***
DRY439	*MAT*α *HMR-SS ΔI*
DRY1314	*MAT*α *HMR***a***-e*** sas5Δ::HIS3*
DRY1322	*MAT*α *HMR***a***-e*** sas4Δ::kanMX4 ade2Δ::HIS3*
DRY1351	*mat***a***Δp HMR***a***-e*** ade2Δ::HIS3*
FRY1352	*mat***a***Δp HMR***a***-e*** hmrΔ::URA3 ade2Δ::HIS3*
DRY1354	*matΔp HMR***a***-e*** sas4Δ::kanMX4 ade2Δ::HIS3*
DRY1356	*mat***a***Δp HMR***a***-e*** sas2-Δ1::TRP1*
DRY1358	*matΔp HMR***a***-e*** sas5Δ::HIS3*
DRY1360	*MAT***a** *HMR***a***-e*** sas4Δ::kanMX4 ade2Δ::HIS3*
DRY1364	*MAT*α *HMR-SS ΔI sas4Δ::kanMX4*
DRY1365	*MAT*α *HMR-SS* (ACS $Rap1p_{bs}$ $abf1^{-}_{bs}$)[c] *ΔI sas4Δ::kanMX4*
DRY1366	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$) *sas4Δ::kanMX4*
DRY1368	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$) *sas4Δ::kanMX4*
DRY1369	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$)*ΔI sas4Δ::kanMX4*
DRY1370	*MAT*α *HMR-SS* (ACS $Gal4p_{bs}$ $Abf1p_{bs}$)*ΔI sas4Δ::kanMX4*
DRY1391	*MAT***a** *HMR***a***-e*** sas5Δ::HIS3*
DRY1397	*MAT*α *HMR***a***-e*** sir4Δ::LEU2 sas4Δ::kanMX4*
DRY1398	*MAT*α *HMR***a***-e*** sir4Δ::LEU2 sas5Δ::HIS3*
DRY1426	*MAT***a***/MAT*α *sas4Δ::kanMX4/sas4Δ::kanMX4 ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*
DRY1428	*MAT***a***/MAT*α *sas5Δ::HIS3/sas5Δ::HIS3 ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*
DRY1568	*MAT***a***/MAT*α *ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*
DRY2109	*MAT*α *HMR-SS ΔI sas5Δ::HIS3*
DRY2110	*MAT*α *HMR-SS* (ACS $Rap1p_{bs}$ $abf1^{-}_{bs}$) *ΔI sas5Δ::HIS3*
DRY2111	*MAT*α *HMR-SS* (ACS $Gal4p_{bs}$ $Abf1p_{bs}$) *sas5Δ::HIS3*
DRY2112	*MAT*α *HMR-SS* (ACS $Gal4p_{bs}$ $Abf1p_{bs}$) *ΔI sas5Δ::HIS3*
DRY2113	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$) *sas5Δ::HIS3*
DRY2114	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$) *ΔI sas5Δ::HIS3*
CAF23	*MAT*α *HMR-SS* (ACS $Gal4p_{bs}$ $Abf1p_{bs}$) *ΔI*
CAF68	*MAT*α *HMR-SS* (ACS $Gal4p_{bs}$ $Abf1p_{bs}$)
CAF176	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$) *ΔI*
CAF179	*MAT*α *HMR-SS* (ACS $Rap1p_{bs}$ $abf1^{-}_{bs}$) *ΔI*
CAF396	*MAT*α *HMR-SS* ($Gal4p_{bs}$ $Rap1p_{bs}$ $Abf1p_{bs}$)
FRY342[d]	*MAT*α *HMR***a***-e*** sas5-1 ade2-101oc leu2-3,112 ura3-52*
DRY601	*MAT*α *HMR***a***-e*** sas4-1 ade2-101oc leu2-3,112 ura3-52*

[a] Strains below are isogenic to JRY2069 except as noted.

[b] Strains below are isogenic to W303-1a except as noted.

[c] DNA sequence elements of the synthetic silencer are noted within the parentheses; a bs subscript indicates that the element is a binding site for the specified protein.

[d] DRY342 and DRY601 are congenic with JRY2069, as described in materials and methods.

color in an otherwise *ade2-* background, allowing the alleles of *HMR* to be unambiguously assigned in segregants of DRY1361. *SAS5* was disrupted in JRY5273 to generate DRY1314, in DRY439 to generate DRY2109, in CAF23 to generate DRY2112, in CAF68 to generate DRY2111, in CAF176 to generate DRY2114, in CAF179 to generate DRY2110, and in CAF396 to generate DRY2113.

All other strains isogenic with W303 were derived by cross. DRY1351, DRY1352, and DRY1354 were segregants from a cross between DRY1322 and JRY4186, a *mata*Δ*p hmr::URA3* derivative of W303-1a described previously (Loo *et al.* 1995). DRY1358 was a segregant from a cross between JRY4186 and DRY1314. DRY1356 was a segregant from a cross between JRY4186 and JRY5274, a *MAT*α *HMR***a**-*e*** *sas2-*Δ*1* derivative of W303-1a described previously (Ehrenhofer-Murray *et al.* 1997). DRY1391 is a *MAT***a** *HMR***a**-*e*** *sas5*Δ*::HIS3* strain derived from a cross between DRY1314 and DRY1803 (*MAT***a** *HMR-ssabf1-::ADE2 ade2*Δ*::LEU2*). As described above, *HMR-ssabf1::ADE2* allows assignment of the alleles of *HMR* in the segregants.

*sir4*Δ*::LEU2 sas* strains were generated by cross to a *MAT*α *sir4*Δ*::LEU2 HMR-SS::ADE2* strain. Since this strain lacks the *HMR***a** genes it has the α-mating phenotype. In addition, the presence of the *ADE2* gene at *HMR* allows unambiguous assignment of *HMR* alleles in segregants. DRY1397 was a segregant from a cross between DRY1360 and DRY1804 (W303-1a; *MAT*α *sir4*Δ*::LEU2 HMR-SS::ADE2 lys2*Δ). Similarly, DRY1398 was a segregant from a cross between DRY1391 and DRY1804. The CAF strains were provided by C. Fox.

DRY601 and DRY342, the strains used to clone *SAS4* and *SAS5*, were segregants derived from crosses between YAA87 (*mata1 HMR***a**-*e*** *ade2-101*^{oc} *leu2-3,112 ura3-52*; Axelrod and Rine 1991) and DRY25 (*sas4-1*) or DRY24 (*sas5-1*), respectively.

Quantitative and patch mating assays: Quantitative matings were performed essentially as described previously (Ehrenhofer-Murray *et al.* 1997). Cells were grown to an OD_{600} of 0.5–1.0 in rich medium supplemented with adenine. Serial dilutions of test strains were mixed with 1.2×10^7 cells of a *MAT***a** lawn (JRY2726) or a *MAT*α lawn (JRY2728) and plated onto YM medium supplemented with adenine. Equivalent dilutions of test strains were plated onto solid rich medium to determine the number of viable cells/dilution. Mating efficiencies were calculated as the number of diploids formed per viable cell plated and were normalized to the efficiency of an isogenic wild-type strain. Values reported are the average of two to eight independent trials performed with at least two independent isolates of each strain tested.

Sequence comparison: Proteins with similarity to Sas5p were identified using the tblastn program against the nonredundant sequences in GenBank. Alignment of the proteins with similarity to Sas5p was carried out using Blockmaker, ClustalW, and Multishade. Alignment and comparison were carried out using the resources provided at the NCSA Biology Workbench (http://biology.ncsa.uiuc.edu) using default parameters.

RESULTS

sas **mutations define three complementation groups:** To identify genes that regulate position effect silencing in yeast, we analyzed mutations that potentially restored silencing at an *HMR* locus flanked by an *HMR-E* silencer containing mutations in two domains. This mutant silencer is known as *HMR***a**-*e***, with the lowercase *e* designating a loss of function and the two asterisks indicating the mutations in the Rap1p and Abf1p binding sites. *MAT*α strains containing the *HMR***a**-*e*** allele display the nonmating phenotype characteristic of **a**/α diploids due to the simultaneous expression of both the α-genes at *MAT* and the **a**-genes at *HMR* (Kimmerly *et al.* 1988). A previous report described a collection of mutations that restore the α-mating phenotype to *HMR***a**-*e*** strains and thus potentially alter the function of genes that regulate silencing (Axelrod and Rine 1991). Two of these mutations, in fact, restore the α-mating phenotype by suppressing the silencing defects of the *HMR***a**-*e*** silencer. Analysis of these mutations led to the identification of two genes not previously known to play a role in silencing. One of these genes, *CDC7*, encodes a protein kinase required for cell-cycle progression, and the other, *SAS2*, encodes a homolog of a human gene involved in leukemia, as described above (Axelrod and Rine 1991; Ehrenhofer-Murray *et al.* 1997). In an effort to identify novel regulators of silencing and stimulated by the connection between *SAS2* and human leukemia, we performed a systematic analysis of the remaining mutations. We found that 15 mutant strains from 10 independently mutagenized cultures contained recessive mutations responsible for restoring the α-mating phenotype to *MAT*α *HMR***a**-*e*** strains (see materials and methods). The genes responsible for the α-mating phenotype of these mutants were referred to generically as *SAS* genes, as before, to reflect that they had *S*omething to do *A*bout *S*ilencing.

To determine whether the *sas* phenotype of the mutants was due to a mutation in a single nuclear gene, three mutants were chosen for initial characterization (DRY23, DRY24, and DRY25). Each mutant was mated to a *mat***a**1 HMR**a**-*e*** strain, forming a *MAT*α/*mat***a**Δ*p* diploid homozygous for *HMR***a**-*e*** and heterozygous for the mutation of interest. Tetrad analysis showed that in each case the suppressor of the *HMR***a**-*e*** mutation segregated as a single nuclear mutation (see materials and methods). To determine the number of mutant genes represented among the *sas* mutants, a complementation analysis was performed. Each of the 15 mutants contained mutations that fell into one of three complementation groups. One group corresponded to the *SAS2* gene. The other mutations fell into two new complementation groups that corresponded to the newly identified genes *SAS4* and *SAS5.* The complementation analysis was confirmed with null alleles of *SAS4* and *SAS5*, as discussed below.

Identification of the ***SAS4*** **and** ***SAS5*** **genes:** To clone wild-type copies of the *SAS4* and *SAS5* genes, a *MAT*α *HMR***a**-*e*** *sas4-1* strain (DRY601) and a *MAT*α *HMR***a**-*e*** *sas5-1* strain (DRY342) were transformed with a yeast genomic library in a centromere-containing vector. Transformants were screened for clones that could complement the *sas* phenotype. Complementation restored the α-mating phenotype of the *sas4* and *sas5* mutants to the nonmating phenotype of *SAS* strains. In the case of the *sas4-1* mutant, two overlapping and complement-

ing clones each contained a 2.0-kb *Sal*I-*Hin*dIII fragment of genomic DNA that, when subcloned into a Cen vector, could complement *sas4-1.* This fragment contained only a single open reading frame from chromosome IV previously known only by the systematic name of YDR181c. In the case of *sas5-1*, a single complementing plasmid clone was recovered. Subcloning analysis of the insert in this plasmid established that a 1.5-kb *Xba*I-*Sma*I fragment could complement the *sas5-1* mutation. This fragment contained only a single open reading frame from chromosome XV previously known by two names, YOR213c and SC33KB_3. Allelism tests confirmed that the genes that complemented *SAS4* and *SAS5* were indeed the *SAS4* and *SAS5* structural genes, respectively (see materials and methods).

***SAS4* and *SAS5* are nonessential genes:** Silencing in Saccharomyces is not an essential function and cells completely defective in silencing have normal growth rates and survival qualities. Silencing, however, is mediated by a combination of proteins some of which are essential for life, such as ORC, Rap1p, and Abf1p, and others that are nonessential, such as the *SIR* proteins (Shore and Nasmyth 1987; Foss *et al.* 1993; Loo *et al.* 1995). To determine whether *SAS4* was essential for life, the entire coding region of *SAS4* was replaced with the *kanMX* coding region, which confers G418 resistance, on one chromosome of an **a**/α diploid strain heterozygous for the HMRa-e** allele. Analysis of 28 tetrads from this diploid, upon sporulation, revealed that each tetrad contained four viable spores and the *sas4Δ::kanMX*-containing spores showed no obvious growth defect. Thus *SAS4*, like the *SIR* genes, encoded a protein dispensable for growth. Among the segregants from this diploid, each of the MATα HMRa-e** sas4Δ::kanMX segregants had the α-mating phenotype. Thus, suppression of the *HMR-E* silencer defect reflected the null phenotype of *SAS4* (Figure 1).

The same strategy was used to test whether *SAS5* was an essential gene. As with *sas4* mutants, *sas5* mutants were viable and had a normal growth rate. Moreover, MATα HMRa-e** sas5Δ::HIS3 segregants were mating proficient. Thus, *SAS5* was not essential for viability, and suppression of the HMRa-e** silencer defect reflected the null phenotype of *SAS5* (Figure 1).

Complementation analysis with some of the original *sas* mutants indicated that *SAS4* and *SAS5* were newly characterized genes. To test more rigorously the assignment of mutants to complementation groups, complementation analysis was repeated using null alleles of *SAS2*, *SAS4*, and *SAS5.* A mataΔp HMRa-e** sas4Δ::kanMX strain (*mat***a***Δp* indicates a deletion of the *MAT***a**1 promoter; Loo and Rine 1994) (DRY1354) was mated to each of the original 15 recessive *sas* mutants to determine which contained lesions in the *SAS4* gene. Similar experiments were performed with a strain containing a *sas2Δ::TRP1* allele (DRY1356) and with a strain containing the *sas5Δ::HIS3* allele (DRY1358). The results

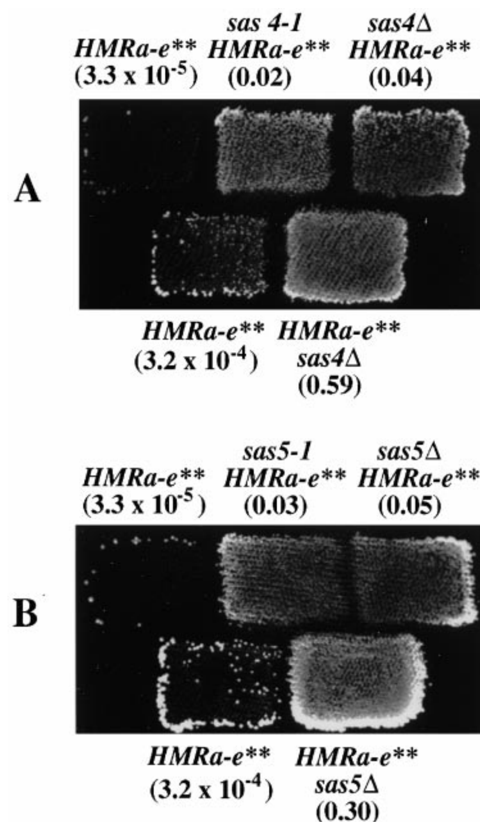

Figure 1.—Mating phenotypes of strains with *sas4-1*, *sas5-1*, *sas4Δ*, and *sas5Δ* alleles in two strain backgrounds. (A) Comparison of α-mating phenotype in MATα HMRa-e** strains with *SAS4* or *sas4* mutant alleles in JRY2069 (top) and in W303 (bottom). Results of quantitative mating analysis are presented in parentheses. (B) Comparison of α-mating phenotype in MATα HMRa-e** strains with *SAS5* or *sas5* mutant alleles in JRY2069 (top) and in W303-1a (bottom). Results of quantitative mating analysis are presented in parentheses. Isogenic strains shown are HMRa-e** (JRY2069), *sas4-1* (DRY24), *sas4Δ* (DRY1374), *sas5-1* (DRY25), and *sas5Δ* (DRY1373). Strains isogenic with JRY3009 (*MATα* W303-1a) are HMRa-e** (JRY5273), *sas4Δ* (DRY1322), and *sas5Δ* (DRY1314).

from these complementation tests clearly revealed that 9 mutants contained a *sas2* mutant allele, 4 contained a *sas4* mutant allele, and 2 contained a *sas5* mutant allele (see materials and methods). Transformation experiments revealed that each mutant could be complemented only by plasmids containing a wild-type copy of the corresponding *SAS* gene (data not shown). Based upon these multiple lines of evidence, we have renamed YDR181c as *SAS4* and YOR213c as *SAS5.*

***SAS4* and *SAS5* are regulators of silencing:** The experiments described above established that mutations in *SAS4* and *SAS5* restored the α-mating phenotype in *MATα* cells containing the HMRa-e** mutation. There are two ways of restoring the α-mating phenotype: the *SAS4* and *SAS5* mutations could block **a**1 function in some way such that the **a**1/α2 repressor fails to repress expression of α1; alternatively, the *SAS4* and *SAS5* mutations could restore silencing of the mutant *HMR* locus.

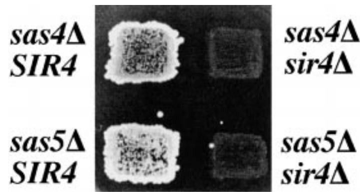

Figure 2.—*SAS4* and *SAS5* mutants restore silencing of *HMR***a**-*e***. The α-mating phenotype of *HMR***a**-*e*** *sas4*Δ strains was abolished by deletion of *SIR4* (top). Similarly, the α-mating phenotype of *HMR***a**-*e*** *sas5*Δ strains is abolished by deletion of *SIR4* (bottom). Hence, *SAS4* and *SAS5* mutations restore *SIR*-dependent silencing. The strains shown were *sas4*Δ *SIR4* (DRY1322), *sas4*Δ *sir4*Δ (DRY1397), *sas5*Δ *SIR4* (DRY1314), and *sas5*Δ *sir4*Δ (DRY1398).

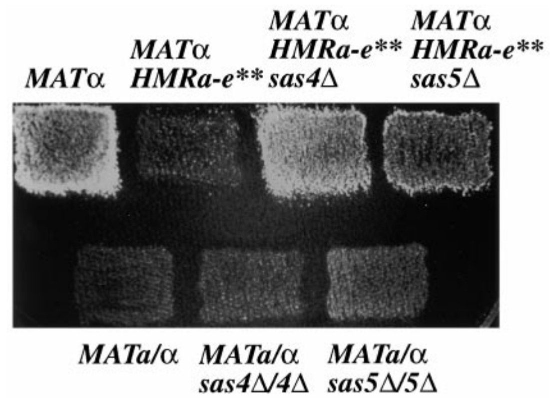

Figure 3.—*SAS4* and *SAS5* are not required for *MAT***a**-gene expression. Deletion of *SAS4* or *SAS5* results in the α-mating phenotype in haploid *MAT*α *HMR***a**-*e*** strains and does not result in the α-mating phenotype in *MAT***a**/*MAT*α diploids. Strains shown are *MAT*α (JRY3009), *HMR***a**-*e*** (JRY5273), *sas4*Δ (DRY1322), *sas5*Δ (DRY1314), *MAT***a**/*MAT*α (DRY 1568), *sas4*Δ/*sas4*Δ (DRY1426), and *sas5*Δ/*sas5*Δ (DRY1428).

We distinguished between these models by determining whether the α-mating phenotype in the *sas* mutants depended upon the function of *SIR4*, which is required for silencing.

The *SIR4* dependence of the *sas4* and *sas5* phenotypes was tested by crossing both a *MAT***a** *HMR***a**-*e*** *sas4*Δ*::kanMX* strain (DRY1360) and an isogenic *MAT***a** *HMR***a**-*e*** *sas5*Δ*::HIS3* strain (DRY1391) to an isogenic *MAT*α *HMR-SS::ADE2 sir4*Δ*::LEU2* strain (DRY1376) in which the natural *HMR-E* silencer was replaced by a synthetic silencer and the *MAT***a** genes normally found at *HMR* were replaced by *ADE2* (McNally and Rine 1991; Rivier *et al.* 1999). Thus, the *HMR* allele present in all segregants from these crosses could be unambiguously identified. Nine *MAT*α *HMR***a**-*e*** *sas4*Δ*::kanMX sir4*Δ*::LEU2* segregants were identified from the first cross and 12 *MAT*α *HMR***a**-*e*** *sas5*Δ*::HIS3 sir4*Δ*::LEU2* segregants were identified from the second cross. All of these segregants were unable to mate, whereas all the *MAT*α *HMR***a**-*e*** *sas4*Δ*::kanMX SIR4* segregants and all the *MAT*α *HMR***a**-*e*** *sas5*Δ*::HIS3 SIR4* segregants were able to mate (Figure 2). The *SIR4* dependence of the *sas* mutant phenotypes established that *sas* mutants restored silencing *per se.*

The previous experiments established that the *sas4* and *sas5* phenotypes were dependent on silencing functions. Nevertheless, these experiments did not eliminate the formal possibility that *sas4* or *sas5* mutations might also affect *MAT***a**1 function. Therefore, two **a**/α diploids were constructed, one homozygous for *sas4*Δ (DRY 1426) and one for *sas5*Δ (DRY1428). Both diploids had the nonmating phenotype of a wild-type **a**/α diploid. Thus, the effect of *sas4* and *sas5* on mating phenotype was exclusively through a silencing mechanism (Figure 3).

Efficient silencing by null alleles of *SAS4* and *SAS5* depends on the ACS and Rap1p binding site of a synthetic *HMR-E* silencer: In the context of the wild-type *HMR-E* silencer the Rap1p and Abf1p binding sites and the ARS consensus sequence element (ACS) appear to have redundant functions; mutation of any individual element does not disrupt silencing, whereas mutation of any pairwise combination of elements does (Brand *et al.* 1987; Kimmerly *et al.* 1988). In principle, null alleles of *SAS4* or *SAS5* could restore silencing to the *HMR***a**-*e*** silencer by bypassing the role of the Rap1p binding site, the Abf1p binding site, or both. Alternatively, null alleles of *SAS4* or *SAS5* could restore silencing by increasing the activity of the silencer elements that remain in *HMR***a**-*e*** strains, namely, the ACS of *HMR-E* or the *HMR-I* silencer. To explore these possibilities we systematically tested which silencer elements were required for silencing in *sas4*Δ and *sas5*Δ strains. To make these experiments simpler to interpret, we used mutant forms of a synthetic silencer (*HMR-SS*) that lack some of the apparent functional redundancy that complicates analysis of mutant forms of the natural *HMR-E* silencer (McNally and Rine 1991; Rivier *et al.* 1999). Previous analysis indicates that restoration of silencing by null alleles of *SAS2* does not depend on either the Abf1p binding site of the synthetic silencer or the *HMR-I* silencer. In contrast, null alleles of *SAS2* do not suppress mutations in the ACS of the synthetic silencer and only partially suppress defects in the Rap1p binding site. Thus, the silencing that results from null alleles of *SAS2* depends on the ACS and Rap1p binding sites of the synthetic silencer.

Deletion of the *HMR-I* silencer from a strain containing the synthetic silencer (*HMR-SS* Δ*I*) (DRY439) resulted in a 10-fold loss of silencing as judged by decreased mating efficiency relative to a strain that contained the synthetic silencer and *HMR-I* (*HMR-SS*) (DRY874; Figures 4 and 5). Deletion of either *SAS4* (DRY1364; Figure 4) or *SAS5* (DRY2109; Figure 5) restored silencing in an *HMR-SS* Δ*I* strain to wild-type levels. Therefore, silencing did not depend on *HMR-I* in either *sas4*Δ or *sas5*Δ strains. We next investigated the role of the Abf1p binding site in strains lacking *SAS4* or *SAS5.* Mutation of the Abf1p binding site of the

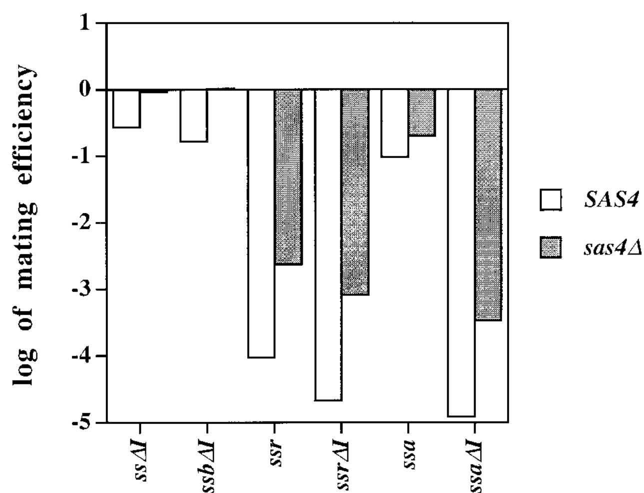

Figure 4.—The ACS and Rap1p binding sites of the synthetic *HMR-E* silencer (*HMR-SS*) contribute to silencing in *sas4*Δ strains. The logarithmic values of quantitative mating assays for strains containing the indicated alleles of *HMR-E* are shown. Open bars indicate the values of *SAS4* strains, solid bars the value of *sas4*Δ strains. The strains shown are DRY439 and DRY1364 [*HMR-SS* Δ*I* (*ss*Δ*I*)], DRY879 and DRY1365 [*HMR-SS abf1-* Δ*I* (*ssb*Δ*I*)], DRY875 and DRY1366 [*HMR-SS rap1-* (*ssr*)], DRY882 and DRY1370 [*HMR-SS rap1-* Δ*I* (*ssr*Δ*I*)], DRY881 and DRY1368 [*HMR-SS acs-* (*ssa*)], DRY878 and DRY1369 [*HMR-SS acs-* Δ*I* (*ssa*Δ*I*)].

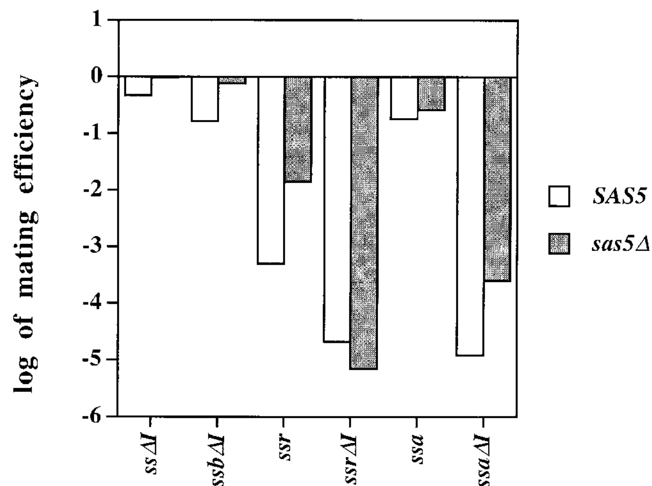

Figure 5.—The ACS and Rap1p binding sites of the synthetic *HMR-E* silencer (*HMR-SS*) contribute to silencing in *sas5*Δ strains. The logarithmic values of quantitative mating assays for strains containing the indicated alleles of *HMR-E* are shown. Open bars indicate the values of *SAS5* strains, solid bars the value of *sas5*Δ strains. The strains shown are DRY439 and (DRY2109) [*HMR-SS* Δ*I* (*ss*Δ*I*)], DRY879 and (DRY2110) [*HMR-SS abf1-* Δ*I* (*ssb*Δ*I*)], DRY875 and (DRY2111) [*HMR-SS rap1-* (*ssr*)], DRY882 and (DRY2112) [*HMR-SS rap1-* Δ*I* (*ssr*Δ*I*)], DRY881 and (DRY2113) [*HMR-SS acs-* (*ssa*)], DRY878 and (DRY2114) [*HMR-SS acs-* Δ*I* (*ssa*Δ*I*)].

synthetic silencer in a strain lacking *HMR-I* (JRY4889) (*HMR-SS abf1-* Δ*I*) resulted in a 2- to 3-fold decrease in mating efficiency. Deletion of either *SAS4* (DRY1365; Figure 4) or *SAS5* (DRY2110; Figure 5) in an *HMR-SS abf1-* Δ*I* strain restored silencing to wild-type levels. Therefore silencing did not depend on the Abf1p binding site in *sas4*Δ or *sas5*Δ strains. Collectively, these and previous data revealed that neither the Abf1p binding site of the synthetic silencer or *HMR-I* is required for silencing in *sas2*Δ, *sas4*Δ, or *sas5*Δ strains.

We next investigated the role of the ACS of the synthetic silencer in silencing in *sas4*Δ and *sas5*Δ strains. Deletion of the ACS of the synthetic silencer (DRY881) (*HMR-SS acs-*) in an otherwise wild-type strain resulted in an approximately 10-fold decrease in silencing as judged by mating efficiency. Deletion of *SAS4* (DRY1368; Figure 4) or *SAS5* (DRY2113; Figure 5) from an *HMR-SS acs-* strain did not increase mating efficiency more than 2-fold and did not restore mating to wild-type levels. Thus, the ACS of the synthetic silencer was required for the efficient restoration of silencing by null alleles of *SAS4* and *SAS5.* Strains lacking the ACS of the synthetic silencer and *HMR-I* (*HMR-SS acs-* Δ*I*) (DRY878) have a mating efficiency that is approximately five orders of magnitude less than strains with a wild-type *HMR* allele. Deletion of *SAS4* (DRY1369) or *SAS5* (DRY2114) from an *HMR-SS acs-* Δ*I* strain resulted in an increase in silencing, but only to a level that was approximately four orders of magnitude less than wild type. Thus, the ACS was required for efficient restoration of silencing by null alleles of *SAS4* and *SAS5*, both in the presence and absence of *HMR-I.* Previous analysis indicated that null alleles of *SAS2* were not capable of even slight suppression of silencing in either *HMR-SS acs-* or *HMR-SS acs-* Δ*I* strains as seen here for null alleles of *SAS4* and *SAS5.* To determine whether null alleles of *SAS2* were phenotypically distinct from null alleles of *SAS4* or *SAS5*, we compared the mating efficiency of *HMR-SS acs-* Δ*I* strains lacking *SAS2*, *SAS4*, or *SAS5.* By our assays, deletion of *SAS2* resulted in the same slight suppression of the silencing defect of the *HMR-SS acs-* Δ*I* allele as did deletion of *SAS4* or *SAS5* (data not shown). Thus, the dependence of silencing on the ACS by null alleles of *SAS2*, *SAS4*, or *SAS5* was indistinguishable by the assays used here.

Finally, we investigated the contribution of the Rap1p binding site to silencing in *sas4*Δ and *sas5*Δ strains. Deletion of the Rap1p binding site of the synthetic silencer results in a reduction of mating efficiency by three to four orders of magnitude in the presence of *HMR-I* (*HMR-SS rap1-*) (DRY875). Deletion of either *SAS4* (DRY1366) or *SAS5* (DRY2111) from an *HMR-SS rap1-* strain resulted in an increase in mating efficiency, but only to a level that was two to three orders of magnitude less than wild type. Therefore, the Rap1p binding site of the synthetic silencer was required for efficient restoration of silencing by null alleles of *SAS4* and *SAS5.* These results were similar to previous results that deletion of *SAS2* partially restores silencing to the *HMR-SS rap1-* allele, indicating that null alleles of *SAS2*, *SAS4*,

and *SAS5* have similar phenotypes in this context. To further explore the role of the Rap1p binding site in silencing in *sas4*Δ and *sas5*Δ strains, we analyzed the *HMR-SS rap1-* allele in the absence of *HMR-I* (*HMR-SS rap1-* Δ*I*). A strain containing this *HMR-SS rap1-* Δ*I* allele (DRY882) mated approximately five orders of magnitude less well than a strain containing the wild-type allele of *HMR* (Figures 4 and 5). Deletion of *SAS4* from this *HMR-SS rap1-* Δ*I* strain (DRY1370) resulted in an increase in mating efficiency, but only to a level that was three to four orders of magnitude less than wild type (Figure 4). Comparable levels of silencing were previously reported for a *sas2*Δ *HMR-SS rap1-* Δ*I* strain (Ehrenhofer-Murray *et al.* 1997). In contrast, deletion of *SAS5* from an *HMR-SS rap1-* Δ*I* strain (DRY2112) did not result in an increase in mating efficiency (Figure 5). Thus, by these criteria, the Rap1p binding site was required for efficient restoration of silencing by null alleles of *SAS2*, *SAS4*, and *SAS5*, and furthermore, the Rap1p binding site made a more significant contribution to restoration of silencing by null alleles of *SAS5* than by null alleles of *SAS2* or *SAS4.* Collectively, the results presented here and previously indicated that the suppression of silencing defects at *HMR* in *sas2*Δ, *sas4*Δ, and *sas5*Δ strains depends on the ACS and Rap1p binding sites of the synthetic silencer, and not on the Abf1p binding site or *HMR-I.*

Sas5p was a family member of a protein implicated in human leukemia: Comparison of the predicted protein sequence of *SAS5* with other proteins encoded by the yeast genome revealed one strong paralog, *ANC1.* The Blastp comparison of Sas5p and Anc1p resulted in a score of 10^{-26}, with two regions of similarity that together span the majority of the length of both proteins. *ANC1* was originally identified as a potential regulator of the actin cytoskeleton, but more recent evidence indicates that *ANC1* encodes a protein intimately connected with transcription (Henry *et al.* 1994; Kim *et al.* 1994; Welch and Drubin 1994; Cairns *et al.* 1996). Previous analysis of the predicted protein encoded by *ANC1* revealed that it has significant sequence similarity to proteins encoded by two human genes, *AF-9* and *ENL*, that are the sites of reciprocal translocations that contribute to acute myeloid leukemia and that it also has significant sequence similarity to a putative protein encoded by the uncharacterized yeast open reading frame *SC33KB_3* (Welch and Drubin 1994; Cairns *et al.* 1996). As revealed here, *SC33KB_3* is identical to *SAS5.* As described previously, the region of highest similarity among these four proteins is a 42-amino-acid region corresponding to amino acids 52–93 of Sas5p. In addition to *ANC1*, our analysis identified a second yeast paralog of *SAS5*, the *YNL107w* gene, whose function was unknown. Comparisons of the *SAS5* sequence with the non-Saccharomyces entries of GenBank revealed similarities to the *YD67* gene of *Schizosaccharomyces pombe* and the *M04B2.3* gene of *Caenorhabditis elegans*, in addition to *AF-9* and *ENL.*

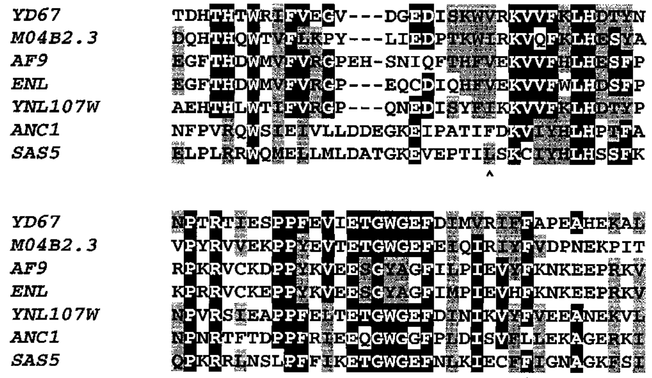

Figure 6.—Sequence similarity among Sas5p-related proteins. Shown is a 76-amino-acid region that spans the 42-amino-acid region of highest similarity among family members. Black shading around letters indicates that at least half of the aligned proteins had identical amino acids. Gray shading around letters indicates that at least half of the family members had similar amino acids. The 42-amino-acid region of highest similarity among the proteins shown is bracketed by the carets. Sequences were aligned and similarity was scored as indicated in materials and methods. Gene names are indicated to the left of the aligned sequence: *YD67* (*S. pombe*), *M04B2.3* (*C. elegans*), *AF-9* and *ENL* (human), *YNL107w*, *ANC1*, and *SAS5* (*S. cerevisiae*).

The alignment of *SAS5* to its related genes indicated that the region of 42 amino acids that is similar among *SAS5*, *ANC1*, *AF-9*, and *ENL* also corresponds to the region of highest similarity with *YNL107w*, *YD67*, and *MO4B23* (Figure 6). Within this region, *SAS5* was 38–51% identical and 56–65% similar to each of the related proteins, suggesting that these proteins are members of a family of proteins that contain a region of conserved function. Although the similarity among the Sas5p-related proteins implies that each has related functions, the region of similarity does not extend over the entire length of these proteins; therefore the family members may not carry out the exact same function. In contrast to Sas5p, the sequence of Sas4p was not highly similar to other known proteins and thus defined a pioneer protein.

DISCUSSION

We characterized 15 recessive mutations that suppressed the silencing defect associated with a mutant *HMR-E* silencer. Nine of the mutations were in the previously characterized *SAS2* gene, which encodes an acetylase homolog. Of the remaining mutations, 4 were in *SAS4* and 2 were in *SAS5.* Cells bearing null alleles of either *SAS4* or *SAS5* were viable, and the phenotypes of the null alleles were indistinguishable from those of the original mutants.

The *sas4* and *sas5* mutations restored the α-mating

phenotype by restoring silencing of *HMR***a**-*e*** rather than by interfering with the function of the *MAT***a**1-encoded protein. This conclusion was based upon the requirement of *SIR4* function for the suppression caused by null alleles of *SAS4* or *SAS5* and by the requirement for the ACS and a Rap1p binding site of the synthetic *HMR-E* silencer for efficient suppression of the silencing defect by a null allele of *SAS4* and *SAS5.* Furthermore, *MAT***a**/*MAT*α diploids homozygous for null alleles of *SAS4* or *SAS5* had the nonmating phenotype, ruling out the possibility that *SAS4* or *SAS5* was required for **a**1 function.

The function of *SAS4* and *SAS5* in silencing: There are at least two ways of thinking about how *sas4* and *sas5* mutations increased silencing mediated by the mutant *HMR***a**-*e*** silencer. One view is that the proteins encoded by these genes directly inhibited the function of ORC or Rap1p at *HMR-E.* Mutation of either gene would then relieve the inhibitory effect, allowing Rap1p or ORC to have increased function at the *HMR-E* silencer. An alternative model is that neither *SAS4* nor *SAS5* had a direct effect at *HMR-E.* Rather, these proteins might have a direct effect on the assembly of silenced chromatin at telomeres. Previous studies have revealed a competition between telomeric silencing and silencing of *HMR* (Buck and Shore 1995). In particular, some mutations that increase silencing at the telomeres result in a decrease in silencing at *HMR* (Buck and Shore 1995; Wotton and Shore 1997). By relieving silencing at telomeres, the *sas4* and *sas5* mutations could favor restoration of silencing at *HMR*, despite the presence of the mutant silencer. Our recent analysis demonstrates that both *SAS4* and *SAS5* are required for the telomeric position effect and, hence, play a positive role in the formation of repressive chromatin (Xu *et al.* 1999). Certainly, the requirement for *SAS4* and *SAS5* in telomeric silencing lends favor to the latter model.

A link between *SAS* genes and human leukemia: Many of the mutations that are known to contribute to human leukemia are reciprocal chromosomal translocations that result in the formation of chimeric genes. Previous work established that *SAS2* is highly similar to the human *MOZ* gene, which is a site of recurring reciprocal translocations that form a chimeric gene with *CBP* in one subtype of acute myeloid leukemia (Borrow *et al.* 1996; Reifsnyder *et al.* 1996). *SAS2* and *MOZ* are members of the MYST gene family whose members have similarity to acetylases (Reifsnyder *et al.* 1996). Furthermore, two MYST family members, *ESA1* and Tip60, are known histone acetylases (Yamamoto and Horikoshi 1997; Smith *et al.* 1998). As described here, mutations in *SAS4* and *SAS5* restore silencing to defective alleles of *HMR-E* as do mutations in *SAS2*, raising the possibility that the functions of these three proteins are related. Remarkably, *SAS5* family members in humans are also found as chimeric genes created by other chromosomal rearrangements in different subtypes of acute myeloid leukemias (Tkachuk *et al.* 1992; Nakamura *et al.* 1993). These data extend the connections between the *SAS* genes and genes that contribute to acute myeloid leukemias.

The relationship between yeast silencing genes and human leukemia genes is further extended by studies of a family of proteins that share a SET domain. Set1p contains a block of ∼130–140 amino acids, known as a SET domain, which is shared among a variety of proteins throughout eukaryotes (Nislow *et al.* 1997). The SET-domain family members have disparate effects on transcription. For instance, *SET1* activates transcription of some genes in yeast and represses transcription of others, including silencing of genes near telomeres (Nislow *et al.* 1997). Similarly, Drosophila proteins with a SET domain include *trithorax* and *enhancer of zeste*, which are responsible for establishing stable activated and repressed states of gene expression in development (Jones and Gelbart 1993). The human trithorax homolog, known variously as *HRX*, *MLL*, and *ALL-1*, is the site of recurring chromosomal translocations that contribute to a variety of human leukemias, including acute myeloid leukemia. Strikingly, the *SAS5*-related genes *AF9* and *ENL* are fused to the *SET1*-related human trithorax homolog in specific types of acute myeloid leukemia (Tenen *et al.* 1997; Waring and Cleary 1997). Hence, both partners in *HRX-AF9* and *HRX-ENL* fusions are related to yeast genes involved in silencing. Based on the parallels described for *SAS2* and *SAS5*, any *SAS4* homolog discovered in humans would be a logical candidate to evaluate for association with chromosomal breakpoints in human leukemias.

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