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

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

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

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

#### ABSTRACT

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

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

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

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

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

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

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

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

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

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

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

we investigated whether the *SAS4* and *SAS5* genes had the same set of unique locus-specific regulatory properties as *SAS2*. Furthermore, we investigated a possible mechanism by which *SAS2* acts as a positive regulator of silencing at *HML* and a negative regulator of silencing at *HMR*.

# MATERIALS AND METHODS

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

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

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

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

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

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

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

#### **TABLE 1**

UCC1001*MATa TEL(VIII) adh4::URA3 ade2:101* his3 $\Delta 200$ leu $2\Delta 1$ hs2 $B01^{ss}$ trp1 $\Delta 1$ ura3-52D. Gottschling D. Gottschling D. Gottschling D. Gottschling R. RothsteinUCC1003MATa add4::URA3D. Gottschling D. Gottschling R. RothsteinD. Gottschling D. Gottschling R. RothsteinDRY125MATe intA.:URA3 [bg2::hisGR. RothsteinDRY126MATe intA.:URA3 [bg2::hisGR. RothsteinDRY127MATe intA.:URA3 [bg2::hisGR. RothsteinDRY128MATe intA.:URA3 [bg2::hisGR. RothsteinDRY1284MATe intA.:eventX4 ade2:HIS3R. RothsteinDRY1385MATe intRe** sac5:kanDK4 ade2:HIS3 /ade2::Leu2DRY1381DRY1384MATe intRe** sac5:kanDK4R. RothsteinDRY1371UCC1001 sac5:::HIS3DRY1382DRY1392MATe intRe Sac5:::HIS3DRY1392DRY1391MATe intRe sac5:::HIS3DRY1393DRY1394MATe intRe Sac5:::HIS3DRY1494DRY1494MATe intRe Sac5:::HIS3DRY1494DRY1494MATe intRe Sac5:::HIS3DRY1494DRY1495MATe intRe Sac5:::HIS3DRY1494DRY1496MATe intRe Sac5:::HIS3DRY1494DRY1497DRY1495MATe intRe Sac5:::HIS3DRY1498MATe intRe Sac5:::HIS3DRY1494DRY1499MATe intRe Sac5:::HIS3DRY1494DRY1494MATe intRe Sac5:::HIS3DRY1494DRY1495MATe intRe Sac5:::HIS3DRY1494DRY1496MATe intRe Sac5:::HIS3DRY1494DRY1496MATe intRe Sac5:::HIS3 ADE2DRY149	Strain		Source
UCC1003         MATa adhd::URA3         D. Gottschling           W303.1a*         MATa adhd::LRA3         R. Rothstein           DRY139         MATa the?L hbs?L1.15 hu2:3.112 tp1-1 ura3-1 can1-100         R. Rothstein           DRY128         MATa str4::URA3 lps2:hbsG         R. Rothstein           DRY128         MATa str4::URA3 lps2:hbsG         R. Rothstein           DRY128         MATa str4::URA3 lps2:hbsG         R. Rothstein           DRY128         MATa thra::str5:HIS3         R. Rothstein           DRY138         MATa::htmRa::str5:HIS3         R. Rothstein           DRY138         Sas5A::kanMAY add2::HIS3         R. Rothstein           DRY1381         DRY138S sas5A::kanMAY add2::HIS3         DRY1392           DRY1391         UCC1001 sas5A::kanMAY         DRY1392         UCC1001 sas5A::kanMAY           DRY1392         UCC1001 sas5A::HIS3         DRY1392         DRY1392         DRY1393           DRY1392         UCC1001 sas5A::HIS3         DRY1392         DRY1494         MATa: HMRa:e** sis5A::HIS3           DRY1492         IRY5273 [pRS426]         DRY1492         DRY1492         DRY1493           DRY1492         IRY5273 [pRS426]         DRY1492         DRY1493         DRY1493           DRY1495         MATa: str4::LEU2 sas5A::HIS3 ADE2	UCC1001 <sup>a</sup>	MATa TEL(VIIL) adh4::URA3 ade2-101°C his3- $\Delta$ 200 leu2- $\Delta$ 1 lys2-801 <sup>am</sup> trp1- $\Delta$ 1 ura3-52	D. Gottschling
W303.1a <sup>2</sup> MATa ade21 his311.15 hu23.112 trpl-1 ura3-1 can1-100       R. Rothstein         DRY1235       MATo: str42::URA3 hy22.::hisG          DRY1236       MATo: str42::URA3 hy22.::hisG          DRY1236       MATo: str42::URA3 hy22.::hisG          DRY1236       MATo: str42::URA3 hy22.::hisG          DRY1237       MATo: HMRa e** str42::inamX4 ade2::HIS3          DRY1388       MATo: HMRa e** str44::inamX4 ade2::HIS3/ade2::Leu2          DRY1384       MATo: HMRa e** str44::inamX4 ade2::HIS3/ade2::Leu2          DRY1385       MATo: HMRa e** str44::XamMX4           DRY1371       UCC1001 sas51::HIS3           DRY1399       MATo: HMRa e** str45::STS3           DRY1499       MATo: HMRa e** str51::STS3           DRY1440       IRYS273 [pRS426]           DRY1452       IRYS273 [pRS426:STR0]           DRY1655       MATa str41::LEV2 sas51::HIS3 ADE2	UCC1003	MATa adh4::URA3	D. Gottschling
DRY1235       MATe. HMRa.SSAI         DRY1235       MATe. ist/a::UKA3 [sp2::.hisG         DRY1236       MATe. ist/a::UKA3 [sp2::.hisG         DRY1237       MATe. ist/a::UKA3 [sp2::.hisG         DRY1238       MATe. ist/a::UKA3 [sp2::.hisG         DRY1234       MATe. ist/mate.*** saisA::IstamXVI add2::HIS3         DRY1332       MATe. ist/mate.** saisA::IstamXVI add2::HIS3         DRY1338       MATe. ist/Mate.*** saisA::IstamXVI add2::HIS3         DRY1336       MATe. ist/Mate.ssi:AstamXVI add2::HIS3         DRY1336       MATe. ist/Mate.ssi:AstamXVI add2::HIS3         DRY1331       UCC1001 saisA::hanXVI         DRY1332       UCC1001 saisA::hINS3         DRY1332       UCC1001 saisA::HIS3         DRY1332       UCC1003 saisA::HIS3         DRY1342       MATe. HMTe.*** saisA::HIS3         DRY1432       UCC1003 saisA::HIS3         DRY1432       IPKS273 [pRS426]         DRY1444       MATe. HMTe.*** saisA: HIS3         DRY1452       IPKS273 [pRS426]         DRY1454       MATe. saisA::HIS3 ADE2         DRY1455       MATa saisA::HIS3 ADE2         DRY1656       MATa saisA::HIS3 ADE2         DRY1650       MATa saisA::HIS3 ADE2         DRY1650       MATa saisA::HIS3 ADE2	W303-1a <sup>b</sup>	MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100	R. Rothstein
DRY1235       MATa sirda::UR43 [ys2a::hisG         DRY1246       MATa sirda::UR43 [ys2a::hisG         DRY1241       MATa sirda::UR43 [ys2a::hisG         DRY1244       MATa sirda::UR43 [ys2a::hisG         DRY1254       MATa (HMRe*** sas5a):ABS3         DRY1354       MATa (HMRe*** sas5a):ABC2 ade2::HIS3/ade2::Leu2         DRY1358       MATa (HMRe***/HMRas5a):ABC2 ade2::HIS3/ade2::Leu2         DRY1358       MATa (HMRe***/HMRAs5a):ABC2 ade2::HIS3/ade2::Leu2         DRY1351       UCC1001 sas42::RiM34         DRY1352       UCC1001 sas42::RiM34         DRY1352       UCC1001 sas42::HIS3         DRY1359       UCC1001 sas45::HIS3         DRY1359       UCC1001 sas45::HIS3         DRY1448       MATa (IMRe*** sas52::HIS3         DRY1448       MATa (IRKe*** sas52:HIS3         DRY1448       MATa (IRKe*** sas52:HIS3         DRY1448       MATa (IRKe*** sas52:HIS3         DRY1448       MATa (IRKe*** sas52): [pRS426]         DRY1448       MATa (IRKe*** Sas52): [pRS426]         DRY1450       IRY5273 [pRS450 (RS426:SIR4]         DRY1454       MATa sas2.:ITRP1 ADE2         DRY1655       MATa sas2.:ITRP1 ADE2         DRY1656       MATa sas2.:ITRP1 ADE2         DRY1657       MATa sas2.:ITRP1 ADE2	DRY439	$MAT\alpha HMRa-SS\Delta I$	
DRV1286       MATa sird>LiCRA3 [ps2:::hbG         DRV1284       MATa sird>LiCRA3 [ps2:::hbG         DRV1314       MATa (ThMRa e** sas5\LHS3         DRV1322       MATa (ThMRa ** sas5\LHS3         DRV1338       Sas4\L:kanMX4 abd2::HIS3/add2::Leu2         DRV1384       MATa (ThMRa **. Sas5\LHS3         DRV1384       MATa (ThMRa **. Sas5\L'HS3         DRV1384       MATa (ThMRa **. Sas5\L'HS3         DRV1394       MATa (ThMRa **. Sas5\L'HS3         DRV1392       UCC1001 sas4\L:kanMX4         DRV1392       UCC1001 sas5\L'HS3         DRV1392       UCC1003 sas5\L'HS3         DRV1494       MATa (ThMRa ** sas5\L'HS3         DRV1492       UCC1003 sas5\L'HS3         DRV1494       MATa (ThMRa ** sas5\L'HS3         DRV1492       UCC1003 sas5\L'HS3         DRV1494       MATa (ThMRa ** sas5\L'HS3         DRV1495       MATa sas5\L'HS3<	DRY1235	$MAT\alpha$ sir4 $\Delta$ ::URA3 lys2 $\Delta$ ::hisG	
DRY1264 MATk sird2.::LEU2 ' PNY1314 MATk HMRa *** sas5AHS2 DRY1322 MATk HMRa *** sas5AHS3 DRY1332 MATk i HMRa *** sas5AHS3 DRY1334 MATa \circ HMRa *** sas5AHS3 DRY1334 MATa \circ HMRa *** sas5AHS3 DRY1334 DRY1338 Sas4A::kanMX4/SAS4 DRY1372 UCC1001 sas42.:kanMX4 DRY1372 UCC1001 sas52::HIS3 DRY1380 MATk HMRa *** sas53::HIS3 DRY1391 MATk HMRa *** sas53::HIS3 DRY1392 UCC1003 sas52::HIS3 DRY1492 UCC1003 sas52::HIS3 DRY1492 UCC1003 sas52::HIS3 DRY1493 MATk HMRa *** sas53::HIS3 DRY1494 MATk HMRa *** sas52::HIS3 DRY1495 MATk HMRa *** sas52::HIS3 DRY1495 MATk HMRa *** sas52::HIS3 DRY1494 MATk HMRa *** sas52::HIS3 DRY1495 MATk HMRa *** sas52::HIS3 DRY1495 MATk HMRa *** sas52::HIS3 DRY1495 MATk IRMS ** sas52::HIS3 DRY1495 MATk IRMS ** sas52::HIS3 DRY1495 MATk iRMS ** sas52::HIS3 DRY1495 MATk iRMS *** sas52::HIS3 DRY1495 MATk iRMS *** sas52::HIS3 DRY1495 MATk iRMS *** sas52::HIS3 DRY1495 MATk is sas52::HIS3 DRY1495 DRY1695 MATk is sas52::HIS3 DRY1495 MATk is sas52::HIS3 DRY2 DRY1695 MATk is ADE2 DRY1695 MATk is ADE2 DRY1695 MATk is ADE2 DRY1695 MATk is ADE	DRY1236	MATa sir4 $\Delta$ ::URA3 lys2 $\Delta$ ::hisG	
DRY1314       MATG. HMRa.e** sas5AHIS3         DRY1328       MATa.fmRa.e** sas5A::kanMX4 ade2::HIS3         DRY1338       MATa.fmRa.eS3: sas6.::kanMX4         DRY1341       DRY1338         DRY1351       DCC1001 sas5.::HIS3         DRY1352       UCC1001 sas5.::HIS3         DRY1361       DRY1372         UCC1001 sas5.::HIS3         DRY1392       UCC1001 sas5.::HIS3         DRY1392       UCC1001 sas5.::HIS3         DRY1392       UCC1003 sas5.::HIS3         DRY1484       MATG. HMTGa.e** sas53.::HIS3         DRY1482       IRY5273 [pRS426]         DRY1484       MATG. HMTGa.e** sas53.::HIS3         DRY1452       IRY5273 [pRS426]         DRY1454       MATG. HMTGa.e** sas53.::HIS3         DRY1455       IRY5273 [pRS426]         DRY1456       MATG. sas53.::HIS3 ADE2         DRY1656       MATa sas42.:::TRP1 ADE2         DRY1656       MATa sas53.::HIS3 ADE2         DRY1657       MATa sas53.::HIS3 ADE2         DRY1658       MATa sit13.::LEU2 sas53.::HIS3 ADE2         DRY1660       MATa sit13.::LEU2 sas53.::HIS3 ADE2         DRY1661       MATa sas54.::TRP1 ADE2         DRY1662       MATa sit13.::LEU2 sas53.::HIS3 ADE2         DRY1664 <t< td=""><td>DRY1264</td><td><math>MAT\alpha</math> sir3<math>\Delta</math>::LEU2</td><td></td></t<>	DRY1264	$MAT\alpha$ sir3 $\Delta$ ::LEU2	
DRY1322       MATe. HMRe.*** sas4:::kanMX4 ade2::HIS3         DRY1338       MATe./a. HMRe.***.HMRe.ssb:::ADE2 ade2::HIS3/ade2::Leu2         DRY1361       DRY1372         DRY1371       DRY1372         UCC1001       sas4::kanMX4         DRY1381       MATe./HMRe.SSL:sas4::kanMX4         DRY1372       UCC1001         DRY1373       UCC1001         DRY1389       MATe. HMRe.** sit13::LEU2 hg2:thisG         DRY1424       MATe. HMRe.** sit13::LEU2 hg2:thisG         DRY1424       MATe. HMRe.** sit13::LEU2 hg2:thisG         DRY1424       MATe. HMRe.** sit13::LEU2 hg2:thisG         DRY1448       MATe. [pRS426]         DRY1452       JRY5273 [pDR580 (pRS426-SJR4)]         DRY1454       JRY5273 [pDR580 (pRS426-SJR4)]         DRY1455       MATe. sas51::HIS3 ADE2         DRY1460       JRY5273 [pDR580 (pRS426-SJR4)]         DRY1656       MATa sas51::HIS3 ADE2         DRY1656       MATa sas51::HIS3 ADE2         DRY1658       MATa sit11::LEU2 sas51::HIS3 ADE2         DRY1659       MATa sit11::LEU2 sas51::HIS3 ADE2         DRY1660       MATa sit11::LEU2 sas51::HIS3 ADE2         DRY1661       MATa sas21::TRP1 ADE2         DRY1663       MATa sas21::TRP1 sas51::HIS3 ADE2         D	DRY1314	$MAT\alpha$ $HMRa-e^{**}$ sas5 $\Delta$ HIS3	
DRY138MATa'o: HINRa **':/HNRa sub::ADE2 ade2::HIS3/ade2::Leu2DRY1381DRY1383 sab::A::RAIMMA'SASADRY1384MATo: HINRa SSA1 sas4 $\Delta$ ::kaIMMA'DRY1371UCC1001 sas4 $\Delta$ ::kaIMMA'DRY1372UCC1001 sas4 $\Delta$ ::HIS3DRY1373UCC1001 sas5 $\Delta$ ::HIS3DRY1394MATa HINRa *** sas5 $\Delta$ ::HIS3DRY1395UCC1003 sas5 $\Delta$ ::HIS3DRY1399MATo: HINRa *** sas5 $\Delta$ :HIS3DRY1444MATo: HINRa *** sas5 $\Delta$ :HIS3DRY1445IRY5273 [pRS426]DRY1452IRY5273 [pDR580 (pRS426-SIR4)]DRY1454MATo: ALMRa ** sas5 $\Delta$ HIS3 [pRS426]DRY1455MATa sas4 $\Delta$ :TIRP1 ADE2DRY1656MATa sas4 $\Delta$ :TIRP1 ADE2DRY1657MATa sas4 $\Delta$ :TIRP1 ADE2DRY1658MATa sas4 $\Delta$ :TIRP1 ADE2DRY1659MATa sas4 $\Delta$ :TIRP1 ADE2DRY1650MATa sas4 $\Delta$ :TIRP1 ADE2DRY1651MATa sas4 $\Delta$ :TIRP1 ADE2DRY1652MATa sas4 $\Delta$ :TIRP1 ADE2DRY1654MATa sas4 $\Delta$ :TIRP1 ADE2DRY1655MATa sas4 $\Delta$ :TIRP1 ADE2DRY1656MATa sas4 $\Delta$ :TIRP1 ADE2DRY1657MATa sas4 $\Delta$ :TIRP1 Sas5 $\Delta$ :HIS3 ADE2DRY1661MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1662MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1663MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1664MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1679MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1680MATa sas4 $\Delta$ :TIRP1 sas4 $\Delta$ :TIRP1 ADE2DRY1797MATa sas4 $\Delta$ :TIRP1 Sas5 $\Delta$ :HIS3 ADE2DRY1798MATa sas4 $\Delta$	DRY1322	MATα HMR <b>a</b> -e** sas4Δ::kanMX4 ade2::HIS3	
DRY1361 DRY1338 sas4 $\Delta$ :kanMX4/SA54 DRY1364 MATc. HMRa.SSD sas4 $\Delta$ :kanMX4 DRY1371 UCC1001 sas4 $\Delta$ :kanMX4 DRY1372 UCC1001 sas5 $\Delta$ :HIS3 DRY1392 UCC1003 sas5 $\Delta$ :HIS3 DRY1392 UCC1003 sas5 $\Delta$ :HIS3 DRY1399 MATc. HMRa.e** sas5 $\Delta$ :HIS3 DRY1499 MATc. HMRa.e** sas5 $\Delta$ :HIS3 DRY1499 MATc. HMRa.e** sas5 $\Delta$ :HIS3 DRY1452 IRY5273 [pRS426] DRY1452 IRY5273 [pRS426] DRY1454 MATc. HMRa.e** sas5 $\Delta$ :HIS3 [pRS426] DRY1455 MATa sas5 $\Delta$ :HIS3 (pRS426] DRY1456 MATa sas5 $\Delta$ :HIS3 (pRS426] DRY1457 [pRS4273 [pDRS43 (pRS426-SIR4)] DRY1456 MATa sas5 $\Delta$ :HIS3 ADE2 DRY1655 MATa sas5 $\Delta$ :HIS3 ADE2 DRY1656 MATa sas5 $\Delta$ :HIS3 ADE2 DRY1656 MATa sas5 $\Delta$ :HIS3 ADE2 DRY1658 MATa sirl $\Delta$ :LEU2 sas5 $\Delta$ :HIS3 ADE2 DRY1660 MATa sas5 $\Delta$ :HIS3 ADE2 DRY1660 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1660 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1660 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1661 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1662 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1663 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1664 MATa sas2 $\Delta$ :T:TRP1 SADE2 DRY1664 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1664 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1665 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1666 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1667 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1668 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1668 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1669 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1669 MATa sas2 $\Delta$ :T:TRP1 ADE2 DRY1669 MATa s	DRY1338	MATa/\a HMRa-e**/HMRa-ssb::ADE2 ade2::HIS3/ade2::Leu2	
DRY1364       MATe, HMRa SSJ sar4a::kanMX4         DRY1371       UCC1001 sar5A:::HIS3         DRY1372       UCC1001 sar5A:::HIS3         DRY1391       MATa HMRa e** sar5A::HIS3         DRY1392       UCC1003 sar5A::HIS3         DRY1393       MATe, HMRa e** sar5A::HIS3         DRY144       MATe, HMRa e** sir1A::LEU2 lys2::hisG         DRY1452       JRY5273 [pRS426]         DRY1454       MATe, HMRa e** sar5A::HIS3         DRY1455       JRY5273 [pRS426]         DRY1464       MATe, HMRa e** sar5A:HIS3 [pRS426]         DRY1455       MATa sar5A::HIS3 [pRS426]         DRY1464       JRY5273 [pDR550 (pRS426-SIR4)]         DRY1655       MATa sar5A::HIS3 ADE2         DRY1656       MATa sar5A::HIS3 ADE2         DRY1657       MATa sar5A::HIS3 ADE2         DRY1658       MATa sar5A::HIS3 ADE2         DRY1660       MATa sar5A::HIS3 ADE2         DRY1661       MATa sar5A::HIS3 ADE2         DRY1662       MATa sar5A::HIS3 ADE2         DRY1663       MATa sar5A::HIS3 ADE2         DRY1664       MATa sar5A::HIS3 ADE2         DRY1665       MATa sar5A::HIS3 ADE2         DRY1666       MATa sar5A::HIS3 ADE2         DRY1667       MATa sar5A::HIS3 ADE2	DRY1361	DRY1338 sas4∆::kanMX4/SAS4	
DRY1371       UCC1001 sas43::kanMX4         DRY1372       UCC1001 sas43::HIS3         DRY1391       MATa HMRa e** sir13::LEU2 lys2::hisG         DRY1392       UCC1003 sas53::HIS3         DRY1399       MATa HMRa e** sir13::LEU2 lys2::hisG         DRY1484       MATa (mRs428)         DRY1485       JRY5273 [pRS426]         DRY1484       MATa (mRs428)         DRY1485       JRY5273 [pRS426]         DRY1486       MATa (mRs428)         DRY1485       JRY5273 [pRS426]         DRY1486       MATa (mRs428)         DRY1486       MATa (mRs428)         DRY1486       MATa (mRs428)         DRY1486       MATa (mRs428)         DRY1685       MATa sas2 \L:TRP1 ADE2         DRY1686       MATa sas42.:kan ADE2         DRY1685       MATa sir13.:LEU2 sas2 \L:TRP1 ADE2         DRY1686       MATa sir13.:LEU2 sas2 \L:TRP1 ADE2         DRY1686       MATa sir13.:LEU2 sas2 \L:TRP1 ADE2         DRY1687       MATa sas2 \L:TRP1 ADE2         DRY1688       MATa sir13.:LEU2 sas2 \L:TRP1 ADE2         DRY1680       MATa sas2 \L:TRP1 sas4.::kanMX4 ADE2         DRY1681       MATa sas2 \L:TRP1 sas4.::kanMX4 aDE2         DRY1682       MATa sas2 \L:TRP1 sas4.::kanMX4 sas5 <t< td=""><td>DRY1364</td><td><math>MAT\alpha</math> <math>HMRa</math>-SS<math>\Delta I</math> sas4<math>\Delta</math>::kanMX4</td><td></td></t<>	DRY1364	$MAT\alpha$ $HMRa$ -SS $\Delta I$ sas4 $\Delta$ ::kanMX4	
DRY1372       UCC1001 sas51::HIS3         DRY1391       MATa HMRa e** sas51::HIS3         DRY1392       UCC1003 sas51::HIS3         DRY1393       MATa HMRa e** sis12.:LEU2 lys2::hisG         DRY1444       MATa (HMRa e** sis12.:LEU2 lys2::hisG         DRY1445       MATa (HMRa e** sas51.:HIS3         DRY1446       MATa (PRS426]         DRY1452       IRY5273 [pRS426]         DRY1454       MATa (MRa e** sas51.HIS3 [pRS426]         DRY1455       MATa sas21:TRP1 ADE2         DRY1656       MATa sas24.:TRP1 ADE2         DRY1656       MATa sas24.:TRP1 ADE2         DRY1656       MATa sas24.:TRP1 ADE2         DRY1657       MATa sas24.:TRP1 ADE2         DRY1658       MATa sin12.:LEU2 sas24.:TRP1 ADE2         DRY1659       MATa sin12.:LEU2 sas24.::TRP1 ADE2         DRY1660       MATa sin12.:LEU2 sas24.::RanMX4 ADE2         DRY1661       MATa sas24.::TRP1 sas52.:HIS3 ADE2         DRY1662       MATa sas24.::TRP1 sas52.:HIS3 ADE2         DRY1663       MATa sas24.::TRP1 sas52.:HIS3 ADE2         DRY1664       MATa sas24.::TRP1 sas52.:HIS3 ADE2         DRY1665       MATa sas24.::TRP1 sas52.:HIS3 ADE2         DRY1664       MATa sas52.:HIS3 ADE2         DRY1665       MATa sas52.:HIS3 ADE2 <td>DRY1371</td> <td>UCC1001 sas4∆::kanMX4</td> <td></td>	DRY1371	UCC1001 sas4∆::kanMX4	
DRY1391       MATa HMRa** sas5.::HIS3         DRY1392       UCC1003 sas5.::HIS3         DRY1399       MATa (HMRa** sin12:::LU2/ sp2:::hisG         DRY1444       MATa (IMRa*** sin12:::LU2/ sp2:::hisG         DRY1445       MATa (IMRa*** sin12::LU2/ sp2:::hisG         DRY1446       MATa (IMRa*** sin12::LU2/ sp2:::hisG         DRY1447       IRY5273 [pRS426]         DRY1460       IRY5273 [pRS426]         DRY1461       IRY5273 [pRS580 (pRS426-SIR3)]         DRY1655       MATa sas42::I:TRP1 ADE2         DRY1656       MATa sas52::HIS3 ADE2         DRY1657       MATa sas52::HIS3 ADE2         DRY1658       MATa sirl2::LEU2 sas42::I:TRP1 ADE2         DRY1659       MATa sirl2::LEU2 sas42::HIS3 ADE2         DRY1661       MATa sas52::HIS3 ADE2         DRY1662       MATa sas52::HIS3 ADE2         DRY1663       MATa sas42::kan MX4 ADE2         DRY1664       MATa sas52::HIS3 ADE2         DRY1665       MATa sas52::HIS3 ADE2         DRY1666       MATa sas52::HIS3 ADE2         DRY1661       MATa sas52::HIS3 ADE2         DRY1662       MATa sas52::HIS3 ADE2         DRY1663       MATa sas52::HIS3 ADE2         DRY1664       MATa sas52::HIS3 ADE2         DRY1797       MATo	DRY1372	UCC1001 <i>sas5</i> ∆::HIS3	
DRY1392       UCC1003 sas5Δ::HIS3         DRY1399       MATα HMRa e** sirl∆::LEU2 lys2::hisG         DRY144       MATα HMRa eSSAT sas5Δ::HIS3         DRY1448       MATα (pRS426)         DRY1448       MATα (pRS426)         DRY1448       MATα (pRS426)         DRY1452       JRY5273 (pRS426)         DRY1460       JRY5273 (pDR583 (pRS426-SIR4))         DRY1655       MATa sas2-Δ::TRP1 ADE2         DRY1656       MATa sas2-Δ::TRP1 ADE2         DRY1656       MATa sas4-::kan ADE2         DRY1656       MATa sas2-Δ::TRP1 ADE2         DRY1657       MATa sas2-Δ::TRP1 ADE2         DRY1658       MATa sirl∆::LEU2 sas2-Δ::KanNAX ADE2         DRY1660       MATa sirl∆::LEU2 sas4-Δ::kanNX4 ADE2         DRY1661       MATa sas2-Δ::TRP1 sas4-MARA         DRY1662       MATa sas2-Δ::TRP1 sas4-::kanNX4 ADE2         DRY1663       MATa sas2-Δ::TRP1 sas4-::kanNX4 ADE2         DRY1664       MATa sas2-Δ::TRP1 sas4-::kanNX4 sas5-Δ::HIS3 ADE2         DRY1664       MATa sas2-Δ::TRP1 sas4-::kan MX4 sas5-Δ::HIS3 ADE2         DRY1679       MATc sas2-Δ::TRP1 sas4-::kan X4 sas5-Δ::HIS3 ADE2         DRY1680       MATc sas2-Δ::TRP1 sas4-::kan X4 sas5-Δ::HIS3 ADE2         DRY1798       MATc sas4-::kanMX4 ADE2         DRY1799	DRY1391	MATa HMRa-e** sas5:::HIS3	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	DRY1392	UCC1003 <i>sas5</i> ∆::HIS3	
DRY1424MATα HMRa-SSΔ1 sas5Δ::HIS3DRY1424MATα [pRS426]DRY1455MATα HMRa-e** sas5Δ HIS3 [pRS426]DRY1456MATα HMRa-e** sas5Δ HIS3 [pRS426]DRY1456MATα HMRa-e** sas5Δ HIS3 [pRS426]DRY1456MATα HMRa-e** sas5Δ HIS3 [pRS426]DRY1460IRY5273 [pDR583 (pRS426-SIR3)]DRY1655MATa sas2Δ1::TRP1 ADE2DRY1656MATa sas4Δ::kan ADE2DRY1657MATa sas5Δ::HIS3 ADE2DRY1658MATa sir1Δ::LEU2 sas4Δ::kanNAVA ADE2DRY1659MATa sir1Δ::LEU2 sas4Δ::kanNAVA ADE2DRY1660MATa sir1Δ::LEU2 sas4Δ::kanNAVA ADE2DRY1661MATa sir1Δ::LEU2 sas4Δ::kanNAVA ADE2DRY1662MATa sir1Δ::LEU2 sas4Δ::kanNAVA ADE2DRY1663MATa sas2Δ1::TRP1 sas4Δ::kanNAVA ADE2DRY1664MATa sas2Δ1::TRP1 sas4Δ::kan NAVA sas5Δ::HIS3 ADE2DRY1679MATα sas2Δ1::TRP1 sas4Δ::kan NAVA sas5Δ::HIS3 ADE2DRY1797MATα sas2Δ1::TRP1 ADE2DRY1799MATα sas2Δ1::TRP1 ADE2DRY1799MATα sas2Δ1::TRP1 ADE2DRY1799MATα sas2Δ1::TRP1 ADE2DRY1799MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2 lss2Δ::hisGDRY1790MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1790MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1790MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1791MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1790MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1791MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1792MATα sir1Δ::LEU2 sas2Δ1::TRP1 ADE2DRY1793MATα sir1Δ::LEU2 sas2Δ1::HIS3 ADE2	DRY1399	MATα HMR <b>a</b> -e** sir1Δ::LEU2 lys2::hisG	
DRY1448 $MAT_{\alpha}$ [pRS426]DRY1452JRY5273 [pRS426]DRY1454 $MAT_{\alpha}$ HMRa-e**DRY1450JRY5273 [pDR583 (pRS426-SJR4)]DRY1464JRY5273 [pDR580 (pRS426-SJR4)]DRY1655 $MAT_{\alpha}$ sas2- $\Delta 1::TRP1 ADE2$ DRY1656MAT_{\alpha} sas2- $\Delta 1::TRP1 ADE2$ DRY1657MAT_{\alpha} sas2- $\Delta 1::TRP1 ADE2$ DRY1658MAT_{\alpha} sas2- $\Delta 1::TRP1 ADE2$ DRY1659MAT_{\alpha} sas2- $\Delta 1::TRP1 ADE2$ DRY1651MAT_{\alpha} sas2- $\Delta 1::TRP1 ADE2$ DRY1652MAT_{\alpha} sir1 $\Delta ::LEU2$ sas2- $\Delta 1::TRP1 ADE2$ DRY1654MAT_{\alpha} sir1 $\Delta ::LEU2$ sas2- $\Delta 1::TRP1 ADE2$ DRY1655MAT_{\alpha} sir1 $\Delta ::LEU2$ sas2- $\Delta 1::TRP1$ ADE2DRY1661MAT_{\alpha} sir2- $\Delta 1::TRP1$ sas2- $\Delta 1::T$	DRY1424	$MAT\alpha HMRa-SS\Delta I sas5\Delta::HIS3$	
DRY1452       JRY5273 [pRS426]         DRY1456       MATα HMRa*** sa5Δ HIS3 [pRS426]         DRY1460       JRY5273 [pDR583 (pRS426-SIR4)]         DRY1464       JRY5273 [pDR590 (pRS426-SIR4)]         DRY1464       JRY5273 [pDR590 (pRS426-SIR4)]         DRY1655       MATa sas2-A1::TRP1 ADE2         DRY1656       MATa sas2-A1::TRP1 ADE2         DRY1657       MATa sas2-A1::TRP1 ADE2         DRY1658       MATa sir1-:LEU2 sas2-A1::TRP1 ADE2         DRY1659       MATa sir1-:LEU2 sas2-A1::TRP1 ADE2         DRY1661       MATa sir1-:LEU2 sas2-A1::TRP1 SADE2         DRY1662       MATa sir2-A1::TRP1 sas4-::HIS3 ADE2         DRY1663       MATa sas2-A1::TRP1 sas4-::HIS3 ADE2         DRY1664       MATa sas2-A1::TRP1 sas4-::kan MX4 ADE2         DRY1665       MATa sas2-A1::TRP1 sas4-::kan MX4 sas5::HIS3 ADE2         DRY1666       MATa sas2-A1::TRP1 sas4-::kan MX4 sas5::HIS3 ADE2         DRY1679       MATα sas2-A1::TRP1 ADE2         DRY1797       MATα sas2-A1::TRP1 ADE2         DRY1797       MATα sas2-A1::TRP1 ADE2         DRY1798       MATα sas2-A1::TRP1 ADE2         DRY1799       MATα sas2-A1::TRP1 ADE2         DRY1790       MATα sir1-::LEU2 sas2-A1::TRP1 ADE2         DRY1790       MATα sir1-::LEU2 sas2-A1::TRP1	DRY1448	$MAT\alpha$ [pRS426]	
DRY1456MATα HMRa-e** sas5Δ HIS3 [pRS426]DRY1460JRY5273 [pDR583 (pRS426.SIR4)]DRY1464JRY5273 [pDR580 (pRS426.SIR4)]DRY1655MATa sas2-Δ1::TRP1 ADE2DRY1656MATa sas5Δ::HIS3 ADE2DRY1657MATa sas5Δ::HIS3 ADE2DRY1658MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1659MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1659MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1659MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1660MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1661MATa sas2-Δ1::TRP1 sas4Δ::kanMX4 ADE2DRY1662MATa sas2-Δ1::TRP1 sas4Δ::kanMX4 ADE2DRY1663MATa sas2-Δ1::TRP1 sas4Δ::kanMX4 ADE2DRY1664MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2DRY1664MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 ADE2DRY1797MATα sas2-Δ1::TRP1 ADE2DRY1798MATα sas2-Δ1::TRP1 ADE2DRY1799MATα sas2-Δ1::TRP1 ADE2DRY1799MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1790MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1790MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1800MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2DRY1801MATα sir1Δ::LEU2 asa5Δ::HIS3 ADE2IRY3009MATαIRY4621MATα sir1Δ::LEU2 ADE2 lys2Δ::hisGIRY4622MATa sir1Δ::LEU2 asa5Δ::HIS3 ADE2IRY4624MATα sir1Δ::LEU2 asa5Δ::HIS3 ADE2IRY4624MATα sir1Δ::LEU2 asa5Δ::HIS3 ADE2IRY4624MATα sir1Δ::LEU2 asa5Δ::HIS3 ADE2IRY4624MATα sir1Δ::LEU2 asa5Δ::HIS3 ADE2 <t< td=""><td>DRY1452</td><td>JRY5273 [pRS426]</td><td></td></t<>	DRY1452	JRY5273 [pRS426]	
DRY1460       JRY5273 [pDR583 (pRS426-SJR3)]         DRY1464       JRY5273 [pDR590 (pRS426-SJR3)]         DRY1655       MATa sas2-Δ1::TRP1 ADE2         DRY1656       MATa sas2-Δ1::TRP1 ADE2         DRY1657       MATa sas2-Δ1::TRP1 ADE2         DRY1658       MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2         DRY1658       MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2         DRY1659       MATa sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2         DRY1660       MATa sir1Δ::LEU2 sas4-::HIS3 ADE2         DRY1661       MATa sas2-Δ1::TRP1 sas5Δ::HIS3 ADE2         DRY1662       MATa sas2-Δ1::TRP1 sas4Δ::kanMX4 ADE2         DRY1663       MATa sas2-Δ1::TRP1 sas5Δ::HIS3 ADE2         DRY1664       MATa sas2-Δ1::TRP1 ADE2         DRY1665       MATa sas2-Δ1::TRP1 ADE2         DRY1666       MATa sas2-Δ1::TRP1 ADE2         DRY1797       MATα sas2-Δ1::TRP1 ADE2         DRY1798       MATα sas2-Δ1::TRP1 ADE2         DRY1799       MATα sas2-Δ1::TRP1 ADE2         DRY1799       MATα sas2-Δ1::TRP1 ADE2         DRY1798       MATα sas2-Δ1::TRP1 ADE2         DRY1799       MATα sas2-Δ1::TRP1 ADE2         DRY1800       MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2         DRY1802       MATα sir1Δ::LEU2 sas2-Δ1::TRP1         DRY1803       J. Rine <td>DRY1456</td> <td><math>MAT\alpha HMR</math>a-<math>e^{**}</math> sas5<math>\Delta</math> HIS3 [pRS426]</td> <td></td>	DRY1456	$MAT\alpha HMR$ a- $e^{**}$ sas5 $\Delta$ HIS3 [pRS426]	
DRY1464       JRY5273 [pDR590 (pRS426-SIR3)]         DRY1655       MATa sas2-A1::TRP1 ADE2         DRY1656       MATa sas42::kan ADE2         DRY1657       MATa sas42::kan ADE2         DRY1658       MATa sir1A::LEU2 sas2-A1::TRP1 ADE2         DRY1659       MATa sir1A::LEU2 sas2-A1::TRP1 ADE2         DRY1659       MATa sir1A::LEU2 sas2-A1::TRP1 ADE2         DRY1660       MATa sir1A::LEU2 sas2-A1::TRP1 ADE2         DRY1661       MATa sas2-A1::TRP1 sas5A::HIS3 ADE2         DRY1662       MATa sas2-A1::TRP1 sas5A::HIS3 ADE2         DRY1663       MATa sas2-A1::TRP1 sas5A::HIS3 ADE2         DRY1664       MATa sas2-A1::TRP1 sas5A::HIS3 ADE2         DRY1665       MATa sas2-A1::TRP1 sas5A::HIS3 ADE2         DRY1664       MATa sas2-A1::TRP1 ADE2         DRY1797       MATa sas2-A1::TRP1 ADE2         DRY1798       MATa sas2-A1::TRP1 ADE2         DRY1799       MATa sas5-A1::TRP1 ADE2         DRY1799       MATa sas5-A1::TRP1 ADE2         DRY1800       MATa sas5-A1::TRP1 ADE2         DRY1800       MATa sas5-A1::TRP1 ADE2         DRY1800       MATa sas5-A1::TRP1 ADE2         DRY1801       MATa sas5-A1::TRP1 ADE2         DRY1802       MATa sinf-A::LEU2 sas5-A::HIS3 ADE2         DRY1804       MATa si	DRY1460	JRY5273 [pDR583 (pRS426- <i>SIR4</i> )]	
DRY1655 $MATa sas2 \Delta 1::TRP1 ADE2$ DRY1656 $MATa sas4 \Delta::kan ADE2$ DRY1657 $MATa sas4 \Delta::kan ADE2$ DRY1658 $MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2$ DRY1659 $MATa sir1\Delta::LEU2 sas2 \Delta ::KanMX4 ADE2$ DRY1650 $MATa sir1\Delta::LEU2 sas2 \Delta ::KanMX4 ADE2$ DRY1661 $MATa sir1\Delta::LEU2 sas2 \Delta ::HIS3 ADE2$ DRY1661 $MATa sas2 \Delta 1::TRP1 sas4 \Delta::kanMX4 ADE2$ DRY1661 $MATa sas2 \Delta 1::TRP1 sas4 \Delta::kanMX4 ADE2$ DRY1663 $MATa sas2 \Delta 1::TRP1 sas4 \Delta::kan MX4 sas5 \Delta::HIS3 ADE2$ DRY1664 $MATa sas2 \Delta 1::TRP1 sas4 \Delta::kan MX4 sas5 \Delta::HIS3 ADE2$ DRY167 $MATa sas2 \Delta 1::TRP1 sas4 \Delta::kan MX4 sas5 \Delta::HIS3 ADE2$ DRY1684 $MATa sas2 \Delta 1::TRP1 ADE2$ DRY1695 $MATa sas2 \Delta 1::TRP1 ADE2$ DRY1797 $MATa sas2 \Delta 1::TRP1 ADE2$ DRY1797 $MATa sas5 \Delta::HIS3 ADE2$ DRY1798 $MATa sas5 \Delta::HIS3 ADE2$ DRY1799 $MATa sas5 \Delta::HIS3 ADE2$ DRY1800 $MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1802MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1804MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1805MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1806MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1802MATa sir1\Delta::LEU2 sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1804MATa sir1\Delta::LEU2 lys2::hisGDRY1805MATa sir1\Delta::LEU2 lys2::hisGDRY1804MATa sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1805MATa sas2 \Delta 1::TRP1 ADE2 lys2 \Delta::hisGDRY1806MATa sa$	DRY1464	JRY5273 [pDR590 (pRS426- <i>SIR3</i> )]	
DRY1656MATa sas4 $\Delta$ ::kan ADE2DRY1657MATa sas5 $\Delta$ ::HIS3 ADE2DRY1657MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1658MATa sir1 $\Delta$ ::LEU2 sas4 $\Delta$ ::kanMX4 ADE2DRY1659MATa sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2DRY1660MATa sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2DRY1661MATa sas2 $\Delta$ 1::TRP1 sas5 $\Delta$ ::HIS3 ADE2DRY1662MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1663MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kan MX4 ADE2DRY1664MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kan MX4 sas5 $\Delta$ ::HIS3 ADE2DRY1797MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1798MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1799MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1800MAT $\alpha$ sas4 $\Delta$ ::kanMX4 ADE2DRY1801MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1804MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1805MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1800MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1801MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1804MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1805MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1804MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2DRY1805MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2DRY1806MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2DRY1807MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2DRY1808J. RineJRY2099MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2DRY1809MAT $\alpha$ sir1 $\Delta$ ::LEU2 aDE2JRY1809J. Rine <td>DRY1655</td> <td>MATa sas2-<math>\Delta</math>1::TRP1 ADE2</td> <td></td>	DRY1655	MATa sas2- $\Delta$ 1::TRP1 ADE2	
DRY1657MATa sas5 $\Delta$ ::HIS3 ADE2DRY1658MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1659MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1660MATa sir1 $\Delta$ ::LEU2 sas4 $\Delta$ ::HIS3 ADE2DRY1661MATa sas2 $\Delta$ 1::TRP1 sas5 $\Delta$ ::HIS3 ADE2DRY1662MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1663MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1664MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1664MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kan MX4 sas5 $\Delta$ ::HIS3 ADE2DRY1664MATa sas2 $\Delta$ 1::TRP1 ADE2DRY1797MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1798MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1799MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1790MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1791MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1792MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1793MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1794MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1795MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1800MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1801MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisGJ. RineJRY4621MAT $\alpha$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisGJ. RineJRY4624MAT $\alpha$ hMR $a$ -SSA1 sir1 $\Delta$ ::HIS3JRY4624MAT $\alpha$ hMR $a$ -SSA1 sir1 $\Delta$ ::HIS3JRY573MAT $\alpha$ hMR $a$ -e**JRY573MAT $\alpha$ his4JRY2728'MAT $\alpha$ his4J. RineJRY2728'MAT $\alpha$ his4J. Rine <td>DRY1656</td> <td>MATa sas4∆::kan ADE2</td> <td></td>	DRY1656	MATa sas4∆::kan ADE2	
DRY1658MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1659MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ ::KanMX4 ADE2DRY1660MATa sir1 $\Delta$ ::LEU2 sas2 $\Delta$ ::HIS3 ADE2DRY1661MATa sas2 $\Delta$ 1::RP1 sas2 $\Delta$ ::HIS3 ADE2DRY1662MATa sas2 $\Delta$ 1::RP1 sas2 $\Delta$ ::Kan SADE2DRY1663MATa sas2 $\Delta$ 1::RP1 sas2 $\Delta$ ::Kan Sas2 $\Delta$ DRY1664MATa sas2 $\Delta$ 1::RP1 sas2 $\Delta$ ::Kan MX4 ADE2DRY1679MATa sas2 $\Delta$ 1::RP1 ADE2DRY1684MATa sas2 $\Delta$ 1::RP1 ADE2DRY1797MAT $\alpha$ sas2 $\Delta$ 1::RP1 ADE2DRY1798MAT $\alpha$ sas2 $\Delta$ 1::RP1 ADE2DRY1799MAT $\alpha$ sas2 $\Delta$ 1::RP1 ADE2DRY1800MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1801MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1804MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1805MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1806MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1807MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1808MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2DRY1809MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::RP1 ADE2JRY4021MAT $\alpha$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisGJ. RineJRY4624MAT $\alpha$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisGJRY4624MAT $\alpha$ sir1 $\Delta$ ::LEU2 lys2::hisGJRY5071MAT $\alpha$ sas2 $\Delta$ 1::TRP1JRY5273MAT $\alpha$ HMR $a$ e**JRY2728'MAT $\alpha$ his4J. RineJRY2728'MAT $\alpha$ his4J. RineJRY2728'MAT $\alpha$ his4J. RineJRY2728'MAT $\alpha$	DRY1657	MATa sas5\Delta::HIS3 ADE2	
DRY1659MATa sir1Δ:::LEU2 sas4Δ::kanMX4 ADE2DRY1660MATa sir1Δ:::LEU2 sas5Δ::HIS3 ADE2DRY1661MATa sas2Δ1::TRP1 sas5Δ::HIS3 ADE2DRY1662MATa sas2Δ1::TRP1 sas5Δ::HIS3 ADE2DRY1663MATa sas4Δ::kan sas5Δ::HIS3 ADE2DRY1664MATa sas4Δ::kan sas5Δ::HIS3 ADE2DRY1674MATa sas4Δ::kan Sas5Δ::HIS3 ADE2DRY1685MATa sas4Δ::kan Sas5Δ::HIS3 ADE2DRY1694MATa sas4Δ::kan MX4 sas5Δ::HIS3 ADE2DRY1797MATα sas4Δ::kan MX4 ADE2DRY1798MATα sas4Δ::kanMX4 ADE2DRY1799MATα sas5Δ::HIS3 ADE2DRY1800MATα sin1Δ::LEU2 sas4Δ::kanMX4 ADE2DRY1801MATα sin1Δ::LEU2 sas4Δ::kanMX4 ADE2DRY1802MATα sin1Δ::LEU2 sas4Δ::kanMX4 ADE2JRY3009MATα sin1Δ::LEU2 sas4Δ::kanMX4 ADE2JRY4621MATα sin1Δ::LEU2 sas5Δ::HIS3 ADE2JRY4622MATa sin1Δ::LEU2 sas5Δ::HIS3 ADE2JRY4624MATα sin1Δ::LEU2 sas5Δ::HIS3 ADE2JRY4625MATα sin1Δ::LEU2 sas5Δ::HIS3 ADE2JRY4626J. RineJRY4627MATα sin1Δ::LEU2 lys2::hisGJ. RineJ. RineJRY4624MATα hMRa-sSMI sin1Δ::HIS3JRY5071MATα sas2-Δ1::TRP1JRY5273MATα hMRa-e**JRY2726'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'MATα his4JRY2728'<	DRY1658	MATa sir1 $\Delta$ ::LEU2 sas2- $\Delta$ 1::TRP1 ADE2	
DRY1660       MATa sir1Δ:::LEU2 sas5Δ:::HIS3 ADE2         DRY1661       MATa sas2-Δ1:::TRP1 sas5Δ:::HIS3 ADE2         DRY1662       MATa sas2Δ1::TRP1 sas4Δ::kanMX4 ADE2         DRY1663       MATa sas2Δ1::TRP1 sas4Δ::kanMX4 ADE2         DRY1664       MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2         DRY1664       MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2         DRY1797       MATα sas2-Δ1::TRP1 ADE2         DRY1798       MATα sas2-Δ1::TRP1 ADE2         DRY1799       MATα sas2-Δ1::TRP1 ADE2         DRY1790       MATα sas2-Δ1::TRP1 ADE2         DRY1791       MATα sas2-Δ1::TRP1 ADE2         DRY1792       MATα sas2-Δ1::TRP1 ADE2         DRY1800       MATα sas2-Δ1::TRP1 ADE2 lys2Δ::hisG         DRY1801       MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2 lys2Δ::hisG         DRY1802       MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2         DRY1802       MATα sir1Δ::LEU2 sas2-Δ1::TRP1         DRY1802       MATα sir1Δ::LEU2 sas2-Δ1::TRP1         DRY1802       MATα sir1Δ::LEU2 sas2-Δ1::TRP1         JRY4621       MATα sir1Δ::LEU2 ADE2 lys2Δ::hisG       J. Rine         JRY4622       MATα sir1Δ::LEU2 sir15:G       J. Rine         JRY4624       MATα HMRa-SSAI sir1Δ::HIS3       J. Rine         JRY5071       MATα sas2-Δ1::TRP1 <t< td=""><td>DRY1659</td><td>MATa sir1\\::LEU2 sas4\\::kanMX4 ADE2</td><td></td></t<>	DRY1659	MATa sir1\\::LEU2 sas4\\::kanMX4 ADE2	
DRY1661MATa sas2 $\Delta$ 1::TRP1 sas5 $\Delta$ ::HIS3 ADE2DRY1662MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1663MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kanMX4 ADE2DRY1664MATa sas2 $\Delta$ 1::TRP1 sas4 $\Delta$ ::kan MX4 sas5 $\Delta$ ::HIS3 ADE2DRY1797MAT $\alpha$ sas2 $\Delta$ 1::TRP1 ADE2DRY1798MAT $\alpha$ sas4 $\Delta$ ::kanMX4 ADE2DRY1799MAT $\alpha$ sas4 $\Delta$ ::kanMX4 ADE2DRY1800MAT $\alpha$ sis4 $\Delta$ ::kanMX4 ADE2DRY1801MAT $\alpha$ sis4 $\Delta$ ::kanMX4 ADE2DRY1802MAT $\alpha$ sis4 $\Delta$ ::kanMX4 ADE2DRY1804MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas2 $\Delta$ 1::TRP1 ADE2 lys2 $\Delta$ ::hisGDRY1805MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas4 $\Delta$ ::kanMX4 ADE2DRY1806MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1807MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1808MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1809MAT $\alpha$ sis1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1802MAT $\alpha$ sis1 $\Delta$ ::LEU2 sis5 $\Delta$ ::HIS3 ADE2JRY4621MAT $\alpha$ sis1 $\Delta$ ::LEU2 sis5 $\Delta$ ::HIS3JRY4622MAT $\alpha$ sis1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY4624MAT $\alpha$ sis1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY4625MAT $\alpha$ sis1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY6071MAT $\alpha$ sas2 $\Delta$ 1::TRP1JRY5071MAT $\alpha$ sas2 $\Delta$ 1::TRP1JRY2726'MAT $\alpha$ his4JRY2728'MAT $\alpha$ his4	DRY1660	MATa sir1\\::LEU2 sas5\\::HIS3 ADE2	
DRY1662       MATa sas2Δ1::TRP1 sas4Δ::kanMX4 ADE2         DRY1663       MATa sas2Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2         DRY1664       MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2         DRY1797       MATα sas2-Δ1::TRP1 ADE2         DRY1798       MATα sas4Δ::kanMX4 ADE2         DRY1799       MATα sas4Δ::kanMX4 ADE2         DRY1800       MATα sas5Δ::HIS3 ADE2         DRY1801       MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2 lys2Δ::hisG         DRY1802       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1802       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1802       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1802       MATα sir1Δ::LEU2 sas5Δ::HIS3 ADE2         JRY3009       MATα sir1Δ::LEU2 sas5Δ::HIS3 ADE2         JRY4621       MATα sir1Δ::LEU2 ADE2 lys2Δ::hisG       J. Rine         JRY4622       MATa sir1Δ::LEU2 ADE2 lys2Δ::hisG       J. Rine         JRY4624       MATα sir1Δ::LEU2 lys2::hisG       J. Rine         JRY4624       MATα sir1Δ::HIS3       J. Rine         JRY5071       MATα sas2-Δ1::TRP1       J. Rine         JRY5273       MATα HMRa-e**       J. Rine         JRY2726'       MATα his4       J. Rine         JRY2728'       MATα his4       J. Rine	DRY1661	MATa sas2-\[]::TRP1 sas5\]::HIS3 ADE2	
DRY1663MATa sas4 $\Delta$ ::kan sas5 $\Delta$ ::HIS3 ADE2DRY1664MATa sas2- $\Delta$ 1::TRP1 sas4 $\Delta$ ::kan MX4 sas5 $\Delta$ ::HIS3 ADE2DRY1797MAT $\alpha$ sas2- $\Delta$ 1::TRP1 ADE2DRY1798MAT $\alpha$ sas4 $\Delta$ ::kanMX4 ADE2DRY1799MAT $\alpha$ sas5 $\Delta$ ::HIS3 ADE2DRY1800MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas2- $\Delta$ 1::TRP1 ADE2 lys2 $\Delta$ ::hisGDRY1801MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas4 $\Delta$ ::kanMX4 ADE2DRY1802MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas4 $\Delta$ ::kanMX4 ADE2DRY1803MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1804MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY1805MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY3009MAT $\alpha$ sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2JRY4621MAT $\alpha$ sir1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY4622MAT $\alpha$ sir1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY4624MAT $\alpha$ sir1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY4624MAT $\alpha$ sir1 $\Delta$ ::LEU2 lys2 $\Delta$ ::hisGJRY5071MAT $\alpha$ sas2- $\Delta$ 1::TRP1JRY5273MAT $\alpha$ HMR $\mathbf{a}$ -e**JRY2726'MAT $\alpha$ his4JRY2728'MAT $\alpha$ his4	DRY1662	MATa sas2\1::TRP1 sas4\::kanMX4 ADE2	
DRY1664       MATa sas2-Δ1::TRP1 sas4Δ::kan MX4 sas5Δ::HIS3 ADE2         DRY1797       MATα sas2-Δ1::TRP1 ADE2         DRY1798       MATα sas2-Δ1::TRP1 ADE2         DRY1799       MATα sas5Δ::HIS3 ADE2         DRY1800       MATα sir1Δ::LEU2 sas2-Δ1::TRP1 ADE2 lys2Δ::hisG         DRY1801       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1802       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1804       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1805       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1806       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         DRY1807       MATα sir1Δ::LEU2 sas4Δ::kanMX4 ADE2         JRY3009       MATα       J. Rine         JRY4621       MATα sir1Δ::LEU2 abs5Δ::HIS3 ADE2       J. Rine         JRY4622       MATα sir1Δ::LEU2 lys2Δ::hisG       J. Rine         JRY4624       MATα sir1Δ::LEU2 lys2::hisG       J. Rine         JRY5071       MATα sas2-Δ1::TRP1       J. Rine         JRY5273       MATα HMRa-e**       J. Rine         JRY2726'       MATα his4       J. Rine         JRY2728'       MATα his4       J. Rine	DRY1663	MATa sas4 $\Delta$ ::kan sas5 $\Delta$ ::HIS3 ADE2	
DRY1797 $MAT\alpha sas2-\Delta1::TRP1 ADE2$ DRY1798 $MAT\alpha sas4\Delta::kanMX4 ADE2$ DRY1799 $MAT\alpha sas5\Delta::HIS3 ADE2$ DRY1800 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1801 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ JRY4621 $MAT\alpha sir1\Delta::LEU2 lys2\Delta::hisG$ JRY4622 $MAT\alpha sir1\Delta::LEU2 lys2\Delta::hisG$ JRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ JRY5071 $MAT\alpha sas2-\Delta1::TRP1$ JRY5273 $MAT\alpha HMRa-e^{**}$ JRY2726' $MAT\alpha his4$ JRY2728' $MAT\alpha his4$	DRY1664	MATa sas2-\1::TRP1 sas4\::kan MX4 sas5\::HIS3 ADE2	
DRY1798 $MAT\alpha sas4\Delta::kanMX4 ADE2$ DRY1799 $MAT\alpha sas5\Delta::HIS3 ADE2$ DRY1800 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1801 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ JRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ JRY4622 $MAT\alpha sir1\Delta::LEU2 lys2\Delta::hisG$ JRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ JRY5071 $MAT\alpha sas2-\Delta1::TRP1$ JRY5273 $MAT\alpha HMRa-e^{**}$ JRY2726' $MAT\alpha his4$ JRY2728' $MAT\alpha his4$	DRY1797	MATα sas2-Δ1::TRP1 ADE2	
DRY1799 $MAT\alpha sas5\Delta::HIS3 ADE2$ DRY1800 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1801 $MAT\alpha sir1\Delta::LEU2 sas2-\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ JRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ JRY4622 $MATa sir1\Delta::LEU2 lys2::hisG$ JRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ JRY5071 $MAT\alpha sas2-\Delta1::TRP1$ JRY5273 $MAT\alpha HMRa-e^{**}$ JRY2726' $MATa his4$ JRY2728' $MAT\alpha his4$	DRY1798	$MAT\alpha$ sas4 $\Delta$ ::kanMX4 ADE2	
DRY1800 $MAT\alpha sir1\Delta::LEU2 sas2:\Delta1::TRP1 ADE2 lys2\Delta::hisG$ DRY1801 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ JRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ JRY4622 $MATa sir1\Delta::LEU2 lys2::hisG$ JRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ JRY5071 $MAT\alpha sas2:\Delta1::TRP1$ JRY5273 $MAT\alpha HMRa-e^{**}$ JRY2726' $MATa his4$ JRY2728' $MAT\alpha his4$	DRY1799	MATα sas5Δ::HIS3 ADE2	
DRY1801 $MAT\alpha sir1\Delta::LEU2 sas4\Delta::kanMX4 ADE2$ DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ JRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ JRY4622 $MATa sir1\Delta::LEU2 lys2::hisG$ JRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ JRY5071 $MAT\alpha sas2-\Delta 1::TRP1$ JRY5273 $MAT\alpha HMRa-e^{**}$ JRY2726' $MATa his4$ JRY2728' $MAT\alpha his4$	DRY1800	$MAT\alpha$ sir1 $\Delta$ ::LEU2 sas2- $\Delta$ 1::TRP1 ADE2 lys2 $\Delta$ ::hisG	
DRY1802 $MAT\alpha sir1\Delta::LEU2 sas5\Delta::HIS3 ADE2$ JRY3009 $MAT\alpha$ J. RineJRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ J. RineJRY4622 $MATa sir1\Delta::LEU2 lys2::hisG$ J. RineJRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ J. RineJRY5071 $MAT\alpha sas2-\Delta 1::TRP1$ J. RineJRY5273 $MAT\alpha HMRa-e^{**}$ J. RineJRY2726' $MATa his4$ J. RineJRY2728' $MAT\alpha his4$ J. Rine	DRY1801	$MAT\alpha$ sir1 $\Delta$ ::LEU2 sas4 $\Delta$ ::kanMX4 ADE2	
JRY3009 $MAT_{\alpha}$ J. RineJRY4621 $MAT_{\alpha}$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisGJ. RineJRY4622 $MAT_{\alpha}$ sir1 $\Delta$ ::LEU2 lys2::hisGJ. RineJRY4624 $MAT_{\alpha}$ HMRa-SS $\Delta$ I sir1 $\Delta$ ::HIS3J. RineJRY5071 $MAT_{\alpha}$ sas2- $\Delta$ 1::TRP1J. RineJRY5273 $MAT_{\alpha}$ HMRa-e**J. RineJRY2726'MAT_{\alpha} his4J. RineJRY2728'MAT_{\alpha} his4J. Rine	DRY1802	$MAT\alpha$ sir1 $\Delta$ ::LEU2 sas5 $\Delta$ ::HIS3 ADE2	
JRY4621 $MAT\alpha sir1\Delta::LEU2 ADE2 lys2\Delta::hisG$ J. RineJRY4622 $MATa sir1\Delta::LEU2 lys2::hisG$ J. RineJRY4624 $MAT\alpha HMRa-SS\Delta I sir1\Delta::HIS3$ J. RineJRY5071 $MAT\alpha sas2-\Delta 1::TRP1$ J. RineJRY5273 $MAT\alpha HMRa-e^{**}$ J. RineJRY2726' $MATa his4$ J. RineJRY2728' $MAT\alpha his4$ J. Rine	JRY3009	ΜΑΤα	J. Rine
JRY4622MATa sir1 $\Delta$ ::LEU2 lys2::hisGJ. RineJRY4624MAT $\alpha$ HMRa-SS $\Delta$ I sir1 $\Delta$ ::HIS3J. RineJRY5071MAT $\alpha$ sas2- $\Delta$ 1::TRP1J. RineJRY5273MAT $\alpha$ HMRa-e**J. RineJRY2726'MATa his4J. RineJRY2728'MAT $\alpha$ his4J. Rine	JRY4621	$MAT\alpha$ sir1 $\Delta$ ::LEU2 ADE2 lys2 $\Delta$ ::hisG	J. Rine
JRY4624 $MAT \alpha$ $HMRa$ - $SS\Delta I sir1\Delta::HIS3$ J. RineJRY5071 $MAT \alpha$ $sas2-\Delta 1::TRP1$ J. RineJRY5273 $MAT \alpha$ $HMRa$ - $e^{**}$ J. RineJRY2726 <sup>c</sup> $MAT \alpha$ his4J. RineJRY2728 <sup>c</sup> $MAT \alpha$ his4J. Rine	JRY4622	MATa sir1 $\Delta$ ::LEU2 lys2::hisG	J. Rine
JRY5071 $MAT\alpha sas2-\Delta1::TRP1$ J. RineJRY5273 $MAT\alpha HMRa-e^{**}$ J. RineJRY2726c $MATa his4$ J. RineJRY2728c $MAT\alpha his4$ J. RineJRY2728c $MAT\alpha his4$ J. Rine	JRY4624	$MAT\alpha$ $HMRa$ -SS $\Delta I$ sir1 $\Delta$ ::HIS3	J. Rine
JRY5273       MATα HMRa-e**       J. Rine         JRY2726°       MATa his4       J. Rine         JRY2728°       MATα his4       J. Rine	JRY5071	$MAT\alpha sas2-\Delta1::TRP1$	J. Rine
JRY2726 <sup>c</sup> MATa his4 J. Rine JRY2728 <sup>c</sup> MATa his4 J. Rine	JRY5273	MATa HMR <b>a</b> -e**	J. Rine
JRY2728 <sup>c</sup> MATa his4 J. Rine	JRY2726 <sup>c</sup>	MATa his4	J. Rine
	JRY2728 <sup>c</sup>	MATa his4	J. Rine

<sup>a</sup> Strains below are isogenic with UCC1001 except as noted.

<sup>b</sup> Strains below are isogenic with W303-1a except as noted.

<sup>c</sup> JRY2726 and JRY2728 are lawn strains for mating assays.

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

Assay for TPE: Silencing of the *TEL(VIIL)* adh4::URA3 gene (Gottschling *et al.* 1990) was measured as a function of growth on medium containing 5-fluoroorotic acid (5-FOA; Guthrie and Fink 1991). Aliquots (5  $\mu$ l) of 10-fold serial dilutions containing from 10<sup>6</sup> to 10<sup>2</sup> cells per aliquot were spotted onto solid minimal medium containing 5-FOA and

incubated for 2–3 days at 30°. As a control for cell viability, 5- $\mu$ l aliquots of the serial dilutions were also spotted onto solid rich medium and onto minimal medium supplemented with uracil.

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

## RESULTS

SAS4 and SAS5 are required for TPE: The HMR-E silencer is composed of an ARS consensus sequence (ACS) element, which is the binding site for ORC, and one binding site each for Rap1p and Abf1p (Brand *et al.* 1987; Kimmerly *et al.* 1988; McNal1y and Rine 1991). SAS2, SAS4, and SAS5 were identified by recessive mutations that restored silencing to an allele of HMR that contained the defective HMRa-e\*\* silencer (Axel-rod and Rine 1991; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1999). This silencer contains a point mutation in the Rap1 binding site and a 1-bp insertion in the Abf1 binding site and is almost completely defective in silencing. Null mutations in SAS2, SAS4, or SAS5 restore silencing to HMRa-e\*\* (Reifsnyder *et al.* 1996; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1996; Ehrenhofer-Murray *et al.* 1997; Xu *et al.* 1999).

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

SAS4 and SAS5 are positive regulators of silencing at *HML*: To determine whether SAS4 or SAS5 is required for silencing at *HML*, we used a quantitative mating-type assay to monitor expression of the *HML* $\alpha$  genes. Wild-type *MATa* strains display the **a**-mating phenotype, whereas *MATa* strains in which silencing at *HML* is disrupted display the nonmating phenotype. Similar to deletion of *SIR1* (JRY4622), deletion of either SAS4 (DRY1656) or SAS5 (DRY1657) results in a modest reduction in silencing at *HML* as indicated by quantitative



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

mating analysis (Figure 2A). Therefore, both *SAS4* and *SAS5* contribute to the efficient silencing of *HML* but neither is required for silencing of *HML*.

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

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

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



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

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

Null alleles of SAS4 and SAS5 have phenotypes at HMR opposite to that of a null allele of SIR1: The



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

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



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

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

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

To test whether the HMRa-e\*\* silencing defects could



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

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

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

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

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

## DISCUSSION

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

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

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

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

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

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

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

# LITERATURE CITED

- Abraham, J., J. Feldman, K. A. Nasmyth, J. N. Strathern, A. J. Klar *et al.*, 1983 Sites required for position-effect regulation of mating-type information in yeast. Cold Spring Harbor Symp. Quant. Biol. **47**: 989–998.
- Aparicio, O. M., B. L. Billington and D. E. Gottschling, 1991 Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell **66**: 1279–1287.
  Axelrod, A., and J. Rine, 1991 A role for *CDC7* in repression of
- Axel rod, A., and J. Rine, 1991 A role for *CDC7* in repression of transcription at the silent mating-type locus *HMR* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**: 1080–1091.
- Baudin, A., O. Ozier-Kal ogeropoul os, A. Denouel, F. Lacroute and C. Cullin, 1993 A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. 21: 3329–3330.
- Borrow, J., V. P. Stanton, Jr., J. M. Andresen, R. Becher, F. G. Behm *et al.*, 1996 The translocation t(8;16) (p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREBbinding protein. Nat. Genet. **14**: 33–41.
- Brand, A. H., L. Breeden, J. Abraham, R. Sternglanz and K. Nasmyth, 1985 Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41**: 41–48.
- Brand, A. H., G. Micklem and K. Nasmyth, 1987 A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell **51**: 709–719.
- Bryk, M., M. Banerjee, M. Murphy, K. E. Knudsen, D. J. Garfinkel et al., 1997 Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev. 11: 255–269.
- Chien, C. T., S. Buck, R. Sterngl anz and D. Shore, 1993 Targeting of *SIR1* protein establishes transcriptional silencing at HM loci and telomeres in yeast. Cell **75:** 531–541.
- Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene 110: 119–122.
- Cockell, M., F. Palladino, T. Laroche, G. Kyrion, C. Liu *et al.*, 1995 The carboxy termini of Sir4 and Rap1 affect Sir3 localization: evidence for a multicomponent complex required for yeast telomeric silencing. J. Cell Biol. **129**: 909–924.
- Dubey, D. D., L. R. Davis, S. A. Greenfeder, L. Y. Ong, J. G. Zhu *et al.*, 1991 Evidence suggesting that the ARS elements associated with silencers of the yeast mating-type locus *HML* do not function as chromosomal DNA replication origins. Mol. Cell. Biol. 11: 5346–5355.
- Ehrenhofer-Murray, A. E., D. H. Rivier and J. Rine, 1997 The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. Genetics **145**: 923–934.
- Feldman, J. B., J. B. Hicks and J. R. Broach, 1984 Identification of sites required for repression of a silent mating type locus in yeast. J. Mol. Biol. 178: 815–834.
- Gardner, K. A., J. Rine and C. A. Fox, 1999 A region of the Sir1 protein dedicated to recognition of a silencer and required for interaction with the orc1 protein in *Saccharomyces cerevisiae*. Genetics 151: 31–44.

Gietz, R. D., and R. H. Schiestl, 1991 Applications of high effi-

ciency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. Yeast **7:** 253–263.

- Gotta, M., and S. M. Gasser, 1996 Nuclear organization and transcriptional silencing in yeast. Experientia **52**: 1136–1147.
- Gotta, M., T. Laroche, A. Formenton, L. Maillet, H. Scherthan et al., 1996 The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type Saccharomyces cerevisiae. J. Cell Biol. 134: 1349–1363.
- Gotta, M., S. Strahl-Bolsinger, H. Renauld, T. Laroche, B. K. Kennedy *et al.*, 1997 Localization of Sir2p: the nucleolus as a compartment for silent information regulators. EMBO J. 16: 3243-3255.
- Gottschling, D. E., O. M. Aparicio, B. L. Billington and V. A. Zakian, 1990 Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell **63**: 751–762.
- Grunstein, M., 1997 Molecular model for telomeric heterochromatin in yeast. Curr. Opin. Cell Biol. **9**: 383–387.
- Grunstein, M., 1998 Yeast heterochromatin: regulation of its assembly and inheritance by histones. Cell **93**: 325–328.
- Guthrie, C., and G. R. Fink, 1991 Guide to Yeast Genetics and Molecular Biology. Academic Press, San Diego.
- Hurst, S. T., and D. H. Rivier, 1999 Identification of a compound origin of replication at the *HMR* E locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **274**: 4155–4159.
- Kennedy, B. K., M. Gotta, D. A. Sinclair, K. Mills, D. S. McNabb et al., 1997 Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in S. cerevisiae. Cell 89: 381–391.
- Kimmerly, W., A. Buchman, R. Kornberg and J. Rine, 1988 Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J. 7: 2241–2253.
- Laurenson, P., and J. Rine, 1992 Silencers, silencing, and heritable transcriptional states. Microbiol. Rev. 56: 543–560.
- Loo, S., and J. Rine, 1995 Silencing and heritable domains of gene expression. Annu. Rev. Cell Dev. Biol. 11: 519–548.
- Lustig, A. J., 1998 Mechanisms of silencing in Saccharomyces cerevisiae. Curr. Opin. Genet. Dev. 8: 233–239.
- Lustig, A. J., C. Liu, C. Zhang and J. P. Hanish, 1996 Tethered Sir3p nucleates silencing at telomeres and internal loci in Saccharomyces cerevisiae. Mol. Cell. Biol. 16: 2483–2495.
- Marcand, S., S. W. Buck, P. Moretti, E. Gilson and D. Shore, 1996 Silencing of genes at nontelomeric sites in yeast is controlled by sequestration of silencing factors at telomeres by Rap 1 protein. Genes Dev. 10: 1297–1309.
- McNally, F. J., and J. Rine, 1991 A synthetic silencer mediates SIRdependent functions in Saccharomyces cerevisiae. Mol. Cell. Biol. 11: 5648–5659.
- Moretti, P., K. Freeman, L. Coodly and D. Shore, 1994 Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. Genes Dev. 8: 2257–2269.
- Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsunasawa, M. Gribskov *et al.*, 1989 Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. 8: 2067–2075.
- Park, E. C., and J. W. Szostak, 1992 ARD1 and NAT1 proteins form a complex that has N-terminal acetyltransferase activity. EMBO J. 11: 2087–2093.
- Pillus, L., and J. Rine, 1989 Epigenetic inheritance of transcriptional states in *S. cerevisiae*. Cell 59: 637–647.
- Reifsnyder, C., J. Lowell, A. Clarke and L. Pillus, 1996 Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. 14: 42–49.
- Rivier, D. H., and J. Rine, 1992 An origin of DNA replication and a transcription silencer require a common element. Science 256: 659–663.
- Rivier, D. H., J. L. Ekena and J. Rine, 1999 HMR-I is an origin of replication and a silencer in Saccharomyces cerevisiae. Genetics 151: 521–529.
- Sherman, F., 1991 Getting started with yeast. Methods Enzymol. 194: 3–21.
- Smith, E. R., A. Eisen, W. Gu, M. Sattah, A. Pannuti *et al.*, 1998 ESA1 is a histone acetyltransferase that is essential for growth in yeast. Proc. Natl. Acad. Sci. USA 95: 3561–3565.

- Smith, J. S., and J. D. Boeke, 1997 An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. **11**: 241–254.
- Smith, J. S., C. B. Brachmann, L. Pillus and J. D. Boeke, 1998 Distribution of a limited Sir2 protein pool regulates the strength of yeast rDNA silencing and is modulated by Sir4p. Genetics 149: 1205–1219.
- Stone, E. M., M. J. Swanson, A. M. Romeo, J. B. Hicks and R. Sternglanz, 1991 The *SIR1* gene of *Saccharomyces cerevisiae* and its role as an extragenic suppressor of several mating-defective mutants. Mol. Cell. Biol. **11**: 2253–2262.
- Triolo, T., and R. Sternglanz, 1996 Role of interactions between the origin recognition complex and *SIR1* in transcriptional silencing. Nature 381: 251–253.
- Whiteway, M., R. Freedman, S. Van Arsdell, J. W. Szostak and J. Thorner, 1987 The yeast ARD1 gene product is required for repression of cryptic mating-type information at the HML locus. Mol. Cell. Biol. 7: 3713–3722.
- Xu, E., S. Kim, K. Replogle, J. Rine and D. H. Rivier, 1999 Identification of *SAS4* and *SAS5*, two genes that regulate silencing in *Saccharomyces cerevisiae*. Genetics **153**: 13–23.
- Yamamoto, T., and M. Horikoshi, 1997 Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. J. Biol. Chem. **272:** 30595–30598.

Communicating editor: F. Winston