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SAD Mutation of *Saccharomyces cerevisiae* Is an Extra **a** Cassette

YONA KASSIR,[1] JAMES B. HICKS,[2] AND IRA HERSKOWITZ[3]†

Department of Genetics, Hebrew University, Jerusalem, Israel[1]*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*[2]*; and Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403*[3]

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Sporulation of *Saccharomyces cerevisiae* ordinarily requires the **a**1 function of the **a** mating type locus. *SAD* is a dominant mutation that allows strains lacking **a**1 (*MATα/MATα* and *mat***a***1/MATα* diploids) to sporulate. We provide functional and physical evidence that *SAD* is an extra cassette in the yeast genome, distinct from those at *HML*, *MAT*, and *HMR*. The properties of *SAD* strains indicate that the **a** cassette at *SAD* produces a limited amount of **a**1 product, sufficient for promoting sporulation but not for inhibiting mating and other processes. These conclusions come from the following observations. (i) *SAD* did not act by allowing expression of *HMR***a**: *mat***a***1/MATα* diploids carrying *SAD* and only α cassettes at *HML* and *HMR* sporulated efficiently. (ii) *SAD* acted as an **a** cassette donor in *HMLα HMRα* strains and could heal a *mat***a***1* mutation to *MAT***a** as a result of mating type interconversion. (iii) The genome of *SAD* strains contained a single new cassette locus, as determined by Southern hybridization. (iv) Expression of **a** functions from the *SAD* **a** cassette was limited by Sir: *sir*$^-$ *SAD* strains exhibited more extreme phenotypes than *SIR SAD* strains. This observation indicates that *SAD* contains not only cassette information coding for **a**1 (presumably from *HMR***a**) but also sites for Sir action.

The mating type locus (*MAT*) of the yeast *Saccharomyces cerevisiae* determines the yeast cell type. Cells that are homoallelic for *MAT***a** (that is, *MAT***a** haploids or *MAT***a**/*MAT***a** diploids) exhibit **a** mating type, and cells that are homoallelic for *MATα* exhibit α mating type. Mating between **a** and α cells yields the third cell type, **a**/α, which is unable to mate but can be induced to sporulate (20). *MAT***a**/*MAT***a** and *MATα/MATα* diploids do not sporulate. Each mating type locus allele contributes a function (coded by genes *MATα2* and *MAT***a***1*) necessary for sporulation by **a**/α cells (10, 14, 24). For example, mutants defective in *MAT***a** (*mat***a***1*) form diploids (*mat***a***1/MATα*) that mate as α and do not sporulate (10).

The *S. cerevisiae* genome contains silent genetic blocks (cassettes) equivalent to *MAT***a** and *MATα* information (reviewed in reference 4). In standard laboratory strains, a silent **a** cassette is present at *HMR* (*HMR***a**), and a silent α cassette is present at *HML* (*HMLα*). Other strains exist with an **a** cassette at *HML* (*HML***a**) or an α cassette at *HMR* (*HMRα*). The information at *HML* or *HMR* can become expressed by being transposed to the mating type locus in a process catalyzed by the *HO* gene, which results in a substitution for the cassette previously at *MAT*. The cassettes at *HML* and *HMR* are kept silent by the actions of four genes: *SIR1*, *SIR2*, *SIR3*, and *SIR4* (J. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979), two of which correspond to genes *MAR1* (11) and *CMT* (3). A mutation in any of these genes in *ho* strains leads to expression of the cassettes at *HML* and *HMR* without their transposition. Inactivation of a *SIR* gene in a haploid *HMLα HMR***a** strain leads to the phenotype of an **a**/α cell, in particular, a nonmating behavior.

We have previously described a mutation (*SAD*, suppressor of **a** deficiencies) that enables *MATα/MATα* and *mat***a***1/MATα* cells to sporulate efficiently (8, 9). This analysis revealed the following. (i) *SAD* is an unstable single mutation. (ii) *SAD* is dominant to the wild-type *sad*$^+$ allele. (iii) *SAD* is located on chromosome III, 40 centimorgans distal to *MAT* (between *THR4* and *HMR***a**). (iv) Sporulation promoted by *SAD* is independent of *MAT***a** but requires *MATα2*.

In this paper, we provide both functional and physical evidence that *SAD* is an extra **a** cas-

† Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143.

TABLE 1. Strain list[a]

Strain	Relevant genotype	Source or reference
531	*MAT***a** *RME*	9
535	*MATα RME*	9
17-15b	*mat***a***1 SAD*	9
17-16	*mat***a***1 RME*	9
XG99-Y4	*MATα SAD RME*	9
XH8C-27a	*ho MATα HMLα HMRα*	J. Hicks
XJ116-6a	*mat***a***1 cry1-3 sir1-1*	19
XR29-10c	*MATα cry1-3 sir1-1*	19
XR55e-22c	*MATα HO HMLα HMRα*	17
XR160-5d	*mat***a***1 ho HMRα*	J. Rine
YD106-9c	*ho mat***a***1 HMRα SAD*	YD106
YD106-13a	*ho MATα HMLα HMRα SAD*	YD106
YD106	*mat***a***1 SAD HMR***a***/MATα sad*$^+$ *HMRα*	XH8c-27a × 17-15b
YD108	*MAT***a** *sad*$^+$*/MATα SAD SIR1/sir1-1*	XG99-Y4 × XR29-10c
YD115	*mat***a***1 sad*$^+$*/MATα SAD SIR1/sir1-1*	XG99-Y4 × XJ116-6a
YD117	*mat***a***1 sad*$^+$ *HMRα/MATα SAD HMRα*	YD106-13a × XR160-5d
YD118	*mat***a***1* SAD HMRα*/MATα sad*$^+$ *HMRα*	YD106-9c × XH8c-27a
YD127[b]	*ho mat***a***1 SAD HMRα* × *HO MATα sad*$^+$ *HMRα*	YD106-9c × XR55e-22c
YD128[b]	*ho MATα SAD HMRα* × *HO MATα sad*$^+$ *HMRα*	YD106-13a × XR55e-22c
YD129	*mat***a***1 SAD HMRα/MATα sad*$^+$ *HMRα sir1/SIR1*	YD106-9c × XR197B-6d
XJ104-25a	*matα 2 sir1-1*	19
2006	*MATα HMRα sir4-1*	Y. Kassir; J. Rine, Ph.D. thesis
2007	*mat***a***1 SAD HMRα/MATα sad*$^+$ *HMRα sir4/SIR4*	YD106-9c × 2006

[a] Strains are assumed to be *HMLα*, *ho*, and *sad*$^+$ in all cases where not indicated otherwise.
[b] Diploids formed between the indicated strains were unstable and gave rise to stable *MAT***a***/MATα* diploids as described in the text.

sette. The ability of *SAD* to allow *MATα/MATα* and *mat***a***1/MATα* strains to sporulate occurs by expression of this additional **a** cassette.

MATERIALS AND METHODS

Media. YEPD agar (rich medium), SPOR agar (for induction of sporulation), and SD agar (for scoring nutritional markers) are described in reference 5.

Strains. Strains are described in Table 1.

Genetic techniques. Mating, induction of sporulation, tetrad analysis, and mating type assays are described in reference 5.

Scoring *SAD* and *HMR*. The presence of *SAD* was determined by its ability to promote efficient sporulation of *mat***a***1/MATα* diploids. *mat***a***1* or *MATα* segregants to be tested were mated with *MATα sad*$^+$ or *mat***a***1 sad*$^+$ strains, respectively, and the diploids were tested for efficient sporulation; *sad*$^+$*/SAD* diploids sporulate, whereas *sad*$^+$*/sad*$^+$ diploids do not. The presence of *HMR***a** was determined by mating *ho HMLα mat***a***1* or *ho HMLα MATα* segregants to *HO HMLα MATα HMRα* strain XR55e-22c. (Matings between XR55e-22c and *MATα* strains occur readily because XR55e-22c mates as an **a** with an efficiency of approximately 10^{-2} to 10^{-3} [unpublished observations].) Because *HO* is dominant to *ho* (see, for example, reference 6), *ho/HO HMLα mat***a***1 HMR***a***/HMLα MATα HMRα* cells give rise to *MAT***a***/MATα* cells, which are able to sporulate (23). In contrast, *ho/HO HMLα mat***a***1 HMRα/HMLα MATα HMRα* strains cannot switch to *MAT***a***/MATα* and thus do not yield sporulating cells.

Hybridization analysis. Hybridization analysis by the method of Southern (21) was performed as described previously (7). The probe used was plasmid 26.3 (13), which carries the *Eco*RI-*Hin*dIII fragment containing the entire *HMLα* locus.

RESULTS

Independence of *SAD* from *HMR*a. As described above, *HMR***a** is a locus of unexpressed **a** information, and the *SAD* mutation behaves as if it supplies the information of an **a** cassette in that it allows *mat***a***1/MATα* strains to sporulate. Because the original *SAD* strains are *HMR***a** and because both *SAD* and *HMR* are on the right arm of chromosome III, we have examined the possibility that *SAD* acts by allowing expression of *HMR***a**. We have thus determined whether *SAD* can promote sporulation in an *HMRα* strain. Such a strain was constructed as follows. Diploid strain YD106 was formed by mating *ho HMLα mat***a***1 SAD HMR***a** strain 17-15b with *ho HMLα MATα HMRα* strain XH8c-27a and yielded 2*mat***a***1*:2*MATα* segregants in each tetrad upon sporulation. These segregants were then mated to *sad*$^+$ tester strains to score *SAD* and *HMR* alleles (Table 2). The resulting diploids were scored for their ability to sporulate, and genotypes were assigned as follows. (i) The presence of *SAD* was indicated by the ability of *mat***a***1/MATα* cells (formed by matings between *mat***a***1* segregants and tester A or between *MATα* segregants and tester D) to sporulate efficiently. (ii) Because we did not know the

TABLE 2. Scoring of *SAD* and *HMR* alleles in *mata1* and *MATα* segregants from YD106 (*mata1 SAD HMR***a**/*MATα sad⁺ HMR*)[a]

Segregants	Sporulation ability of diploids formed by mating with tester strains[a]				Presumptive genotype of segregant
	A (*ho MATα HMR***a**)	B (HO *MATα HMRα*)	C (ho *MAT***a** *HMR***a**)	D (*ho mata1 HMR***a**)	
mata1	+	+			*mata1 SAD*
	−	+			*mata1 sad⁺ HMR***a**
	−	−			*mata1 sad⁺ HMRα*
MATα		+	+	+	*MATα SAD*
		+	+	−	*MATα sad⁺ HMR***a**
		−	+	−	*MATα sad⁺ HMRα*

[a] +, Diploids gave rise to sporulating cells; −, diploids did not give rise to sporulating cells. All tester strains are *sad⁺* and *HMLα*. A, *ho MATα HMR***a** (strain 535); B, *HO MATα HMRα* (strain XR55e-22c); C, *ho MAT***a** *HMR***a** (strain 531); D, *ho mata1 HMR***a** (strain 17–16). Other details are described in the text.

phenotype of a *SAD HMRα* recombinant, *HMR* was scored only in *sad⁺* segregants. The presence of the *HMR***a** allele was indicated by the ability of diploids formed between *mata1* or *MATα* segregants and tester B to give rise to sporulating cells; segregants with the *HMRα* allele do not form diploids capable of sporulation (see above). (iii) The final step in this analysis was to assign the *HMR* allele to *SAD* segregants by assuming 2*HMR***a**:2*HMRα* segregation. The relationship between the types of segregants and their behavior when crossed to the tester strains is summarized in Table 2. Results are given in Table 3.

Strain YD106 yielded 43 2*SAD*:2*sad⁺* tetrads, 28 1*SAD*:3*sad⁺* tetrads, and 3 0*SAD*:4*sad⁺* tetrads. The high frequency of loss of *SAD* is comparable to that reported previously (8, 9). Of the 114 *SAD* segregants, 36 were *HMRα*. *HMLα mata1 SAD HMRα* segregant YD106-9c was then mated to *HMLα MATα HMRα* strain XH8C-27a to form diploids of genotype:

$$\text{YD118} \quad \frac{HML\alpha \ \ mata1 \ \ SAD \ \ HMR\alpha}{HML\alpha \ \ MAT\alpha \ \ sad^{+} \ \ HMR\alpha}$$

Likewise, *HMLα MATα SAD HMRα* segregant YD106-13a was mated to *HMLα mata1 HMRα* strain XR160-5d to form diploids of genotype:

$$\text{YD117} \quad \frac{HML\alpha \ \ mata1 \ \ sad^{+} \ \ HMR\alpha}{HML\alpha \ \ MAT\alpha \ \ SAD \ \ HMR\alpha}$$

YD118 and YD117 sporulated efficiently, and *sad⁺* segregants were analyzed for *HMR*: only *HMRα* segregants were observed in 23 tetrads from YD118 and in 27 tetrads from YD117. We conclude that the ability of *SAD* to support sporulation of *mata1*/*MATα* diploids is independent of *HMR***a**.

An a cassette for mating type interconversion supplied by *SAD*. As noted above, the ability of *SAD* to bypass the requirement of the **a** mating type locus can be explained if *SAD* is itself an expressed **a** cassette. To determine whether *SAD* can supply an **a** cassette for mating type interconversion, we have constructed strains carrying *SAD* but lacking the ordinary sources of **a** cassettes at *HMR* (or *HML*). Two such crosses have been performed, between *ho SAD HMRα* segregants from YD106 (*mata1* strain YD106-9c and *MATα* strain YD106-13a) and *HO MATα sad⁺ HMRα* strain XR55e-22c:

$$\text{YD127} \quad \frac{ho}{HO}\ \frac{HML\alpha \ \ mata1 \ \ SAD \ \ HMR\alpha}{HML\alpha \ \ MAT\alpha \ \ sad^{+} \ \ HMR\alpha}$$

$$\text{YD128} \quad \frac{ho}{HO}\ \frac{HML\alpha \ \ MAT\alpha \ \ SAD \ \ HMR\alpha}{HML\alpha \ \ MAT\alpha \ \ sad^{+} \ \ HMR\alpha}$$

If *SAD* provides a source of **a** cassettes for mating type interconversion, then diploids formed by these matings should be able to switch to *MAT***a**/*MATα* and thus form colonies containing sporulating cells incapable of mating. With some exceptions (discussed below), such colonies were indeed observed. All 39 YD127 colonies were nonmating; 36 sporulated. Forty-three of 46 YD128 colonies were nonmating and sporulation proficient; 3 mated as α and did not sporulate. To determine whether these diploid colonies were indeed *MAT***a**/*MATα*, individual diploids from matings YD127 and YD128 (YD127-1 and YD128-1) were sporulated, and segregants were analyzed for *MAT*, *HO*, and *SAD*. Results are given in Table 4. The most important finding was that all of the segregants that mate as **a** supported sporulation after mating with *MATα* strains. This result indicates that the original diploids are *MAT***a**/*MATα* (and not, for example, *mata1*/*MATα SAD*/*sad⁺*). These results therefore show that *SAD* can provide an **a** cassette for mating type interconversion. In addition, the behavior of YD127-1 shows that *SAD* is able to heal the mating type locus defect

TABLE 3. Production of *SAD HMR*α segregants from YD106[a]

Tetrad type[b]	Segregant[c]	No. of tetrads
PD	*sad*$^+$ *HMR*α	17
	sad$^+$ *HMR*α	
	*SAD HMR***a**	
	*SAD HMR***a**	
T	*sad*$^+$ *HMR*α	25
	sad$^+$ *HMR***a**	
	*SAD HMR***a**	
	*SAD HMR*α	
NPD	*sad*$^+$ *HMR***a**	1
	sad$^+$ *HMR***a**	
	*SAD HMR*α	
	*SAD HMR*α	
Others	*sad*$^+$ *HMR*α	19
	sad$^+$ *HMR*α	
	sad$^+$ *HMR***a**	
	*SAD HMR***a**	
	sad$^+$ *HMR*α	9
	sad$^+$ *HMR***a**	
	sad$^+$ *HMR***a**	
	SAD$^+$ *HMR*α	
	sad$^+$ *HMR*α	3
	sad$^+$ *HMR*α	
	sad$^+$ *HMR***a**	
	sad$^+$ *HMR***a**	

[a] YD106 is *mata1 SAD HMR***a**/*MAT*α *sad*$^+$ *HMR*α.

[b] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

[c] Genotypes were assigned as described in the text and in Table 2. The *HMR* genotype of *SAD* segregants was inferred from the *HMR* genotype of *sad*$^+$ segregants, assuming 2 *HMR***a**:2 *HMR*α segregation.

of *mat***a***1* strains (just as *HMR***a** is able to heal the mutation in *mat***a***1* strains; 12, 17, 23). It is clear from the data of Table 4 that both YD127-1 and YD128-1 contain both *sad*$^+$ and *SAD* alleles of their parents. The transposition of information from *SAD* to the mating type locus thus appears to be a nonreciprocal transfer of information as in the normal interconversion process (Fig. 1).

Analysis of tetrads obtained from YD127-1 and YD128-1 provides further evidence that *SAD* is a source of **a** cassettes. Given that YD127-1 and YD128-1 are *ho*/*HO MAT***a**/*MAT*α *sad*$^+$/*SAD*, the occurrence of certain distinctive tetrads indicates that *HO MAT*α *SAD HMR*α strains can switch to *MAT***a** (Table 5).

As in previous segregations by *SAD*/*sad*$^+$ strains, *SAD* appears to be lost in a large fraction of segregants: only 23 of 108 *MAT*α segregants from YD127-1 are *SAD*, and only 20 of 53 *MAT*α segregants from YD128-1 are *SAD*. Although this loss of *SAD* may occur as in previous diploids, it is also possible that *HO* promotes loss of *SAD*. An interesting possibility is that *SAD* is not only a donor of **a** cassettes but can also be a recipient of cassettes. In other words, some cases in which *SAD* appears to be lost may be situations in which the **a** cassette at *SAD* has been replaced by an α cassette from *HML*α or *HMR*α. Such an event can also account for the formation of diploids (YD128), produced by mating an *ho MAT*α *SAD HMR*α strain with an *HO MAT*α *sad*$^+$ *HMR*α strain, which gives rise to diploids that are able to mate but are incapable of sporulating.

Physical evidence that *SAD* strains contain an extra cassette. The analyses described above show that *SAD* behaves functionally in two respects as if it is an **a** cassette: it provides **a**1 function to allow *mat***a***1*/*MAT*α and *MAT*α/*MAT*α diploids to sporulate, and it acts as a source of **a** cassettes in mating type interconversion. To determine physically whether the *SAD* mutation is an additional cassette in the yeast genome, we analyzed DNA from *SAD* strains by Southern hybridization, using a probe that is homologous to the three standard cassette loci. In this case, we used a probe containing the entire *HML*α locus, which hybridizes to *HML*, *HMR*, and *MAT* because the unique segments of **a** and α cassettes are flanked by homologous sequences (7, 15, 26). We likewise found that the cassette probe hybridized to three bands in *sad*$^+$ strain 17-15 (Fig. 2, lane c). A striking result was found for three different *SAD* strains: all contained not only the three bands corresponding to cassettes at *HML*, *HMR*, and *MAT*, but an additional band as well. *SAD* strains thus contain an additional cassette locus that is not present in wild-type strains.

Expression of a more severe phenotype by *SAD sir1-1* double mutants than by either single mutant. Previous work has shown that *SAD* supplies **a** information which restores sporulation to *mat***a***1*/*MAT*α cells to normal levels but does not affect mating ability, for example, in *MAT*α cells (8, 9). These observations can be explained if *SAD* is expressed only under meiotic conditions or if it allows only a limited amount of expression of an **a** cassette that is sufficient for supporting sporulation but not for inhibiting mating. The mutation *sir1-1* behaves in a similar manner: *sir1-1* allows *mat***a***1*/*MAT*α strains to sporulate but does not affect mating ability of *MAT*α strains (19). Because both *sir1-1* and *SAD* appear to allow only limited expression of **a** information, we wished to determine whether a *SAD sir1-1* double mutant would exhibit more extensive expression of **a** cassette functions. In particular, we have determined whether *MAT*α *SAD sir1-1* strains have a nonmating phenotype.

To construct a *MAT*α *SAD sir1-1* strain, two

TABLE 4. Segregation from diploids YD127-1 and YD128-1[a]

Inferred genotype	Mates with:	Sporulation[b]	Sporulation of diploids formed with *ho* strain:[c]		No. observed	
			*mat*a*1 HMR*a	*mat*a*1 HMR*α	127-1	128-1
*ho MAT***a**	α	−			88	48
*HO MAT***a**	—[d]	+			22	17
*ho MAT*α *sad*$^+$	**a**	−	−	−	52	19
*ho MAT*α *SAD*	**a**	−	+	+	13	10
*HO MAT*α *sad*$^+$	**a**	−	+	−	33	14
*HO MAT*α *SAD*	—[d]	+			10	10

[a] YD127-1 is a diploid produced by mating between *ho mat***a***1 SAD HMR*α strain YD106-9c and *HO MAT*α *sad*$^+$ *HMR*α strain XR55e-22c. YD128-1 is a diploid produced by mating between *ho MAT*α *SAD HMR*α strain YD106-13a and XR55e-22c. Genotypes were deduced from mating and sporulation ability of spore clones and by sporulation ability of diploids as described here and in the text.

[b] Sporulation of unmated spore clones.

[c] *mat***a***1 HMR***a** was strain 17-16; *mat***a***1 HMR*α was strain XR160-5d. Strains of postulated genotype *ho MAT***a** sporulated when mated with an *ho MAT*α strain; those of genotype *ho MAT*α and *HO MAT*α *sad*$^+$ sporulated when mated with an *ho MAT***a** strain.

[d] Some *HO MAT***a** strains mated weakly with the *MAT*α tester; some *HO MAT*α strains mated weakly with the *MAT***a** tester.

diploids (YD108 and YD115) were constructed as follows:

YD108 *MAT*α *SAD* (XG99-Y4)
× *MAT***a** *sir1-1* (XR29-10c)

YD115 *MAT*α *SAD* (XG99-Y4)
× *mat***a**1 *sir1-1* (XJ116-6a)

The diploids were sporulated, and segregants were examined for mating ability and for the presence of *SAD* and *sir1-1*. *SAD* was scored in *MAT*α segregants by its ability to promote sporulation of *mat***a***1/MAT*α diploids. (Because YD108 is an **a**/α diploid, *SAD* was scored only among *MAT*α segregants.) The presence of *sir1-1* was determined by scoring sporulation in different tester strains (Table 6). Segregation data for YD108 and YD115 are given in Table 7.

In contrast to a standard **a**/α diploid, which yields 2**a**:2α segregants in each tetrad, YD108 yielded 15 2**a**:2α tetrads and 13 2**a**:1α:1nm (nonmating) tetrads; YD115 yielded 22 2**a**:2α tetrads, 18 2**a**:1α:1nm, and 2 2**a**:2nm tetrads. Thus, we observed a deficiency in α segregants correlated with the appearance of nonmating segregants. Assuming Mendelian segregation of *SAD* and *sir1-1*, the nonmating segregants are genotypically *MAT*α *SAD sir1-1*. To confirm this deduction, the nonmating segregants were crossed to a *mat*α*2 sir1-1* tester (strain XJ104-25a to score the *sir1-1* allele; Table 6). All of the nonmating segregants were *sir1-1*. We attempted to determine the presence of *SAD* by crossing the nonmating segregants with *mat***a***1 SIR1* strain 17-16: sporulation indicated the presence of *SAD*; inability to sporulate indicated the presence of *sad*$^+$. In most cases, the diploids formed between the nonmating segregants and strain 17-16 were not capable of sporulating. We assume that the nonmating segregants originally did contain *SAD*, but that this mutation (which is unstable) was lost as a result of selection for mating with strain 17-16. In agreement with this view, we observed that the nonmating segregants upon subculturing readily gave rise to cells that mated as α. These results indicate that *SAD sir1-1*

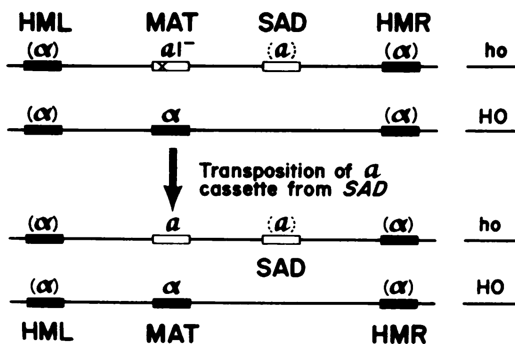

FIG. 1. *SAD* supplies an **a** cassette for mating type interconversion and for healing a *MAT***a** mutation. The structures of chromosome III homologs of diploid strain YD127 are drawn in the top two lines and show the relative positions of *HML*, *MAT*, *SAD*, and *HMR* (not drawn to physical or genetic scale). The lower two lines indicate the genotype resulting from a mating type interconversion event. Solid rectangles indicate α cassettes (expressed at *MAT* and silent at *HML* and *HMR*); open rectangles indicate **a** cassettes. The X within the **a** cassette on the top line indicates the presence of the *mat***a***1* mutation. Broken parentheses around the *SAD* **a** cassette indicate that this cassette is partially expressed and partially repressed (see text).

TABLE 5. Inferred genotypes of selected tetrads from YD127-1 and YD128-1[a]

Tetrad type[b]	No. observed		Inferred genotype[c]	
	YD127-1	YD128-1		
NM	1	3	*HO MAT***a** *sad*$^+$	*HO MAT***a** *sad*$^+$
NM			*HO MAT*α *SAD*	*HO MAT*α *SAD*
a			*ho MAT***a** *sad*$^+$	*ho MAT***a** *SAD*
α			*ho MAT*α *SAD*	*ho MAT*α *sad*$^+$
NM	3	2	*HO MAT*α *SAD*	*HO MAT*α *SAD*
α			*HO MAT*α *sad*$^+$	*HO MAT*α *sad*$^+$
a			*ho MAT***a** *SAD*	*ho MAT***a** *sad*$^+$
a			*ho MAT***a** *sad*$^+$	*ho MAT***a** *sad*$^+$
NM	0	1	*HO MAT*α *SAD*	
NM			*HO MAT*α *SAD*	
a			*ho MAT***a** *sad*$^+$	
a			*ho MAT***a** *sad*$^+$	

[a] YD127-1 and YD128-1 are *HO/ho MAT***a***/MAT*α *SAD/sad*$^+$.

[b] NM, Nonmating, sporulation-proficient spore clone; **a**, mating as **a**; α, mating as α. A total of 28 tetrads were analyzed from YD127-1 and YD128-1.

[c] Listed are the genotypes that can produce the tetrad types observed, assuming 2:2 segregation for *HO*, *MAT*, and *SAD*. Although the assumption of 2:2 segregation for *SAD* is not valid in general (because *SAD* is lost with high efficiency), certain of these tetrad types can occur only if *SAD* is not lost. Loss of *SAD* was apparent in other tetrads analyzed (data not shown; see Table 4).

strains have a more extreme phenotype than either single mutation alone in inhibiting mating by *MAT*α cells.

A further indication that *SAD* and *sir1-1* lead to a more severe phenotype comes from observations on *mat***a***1* segregants from YD115. *mat***a***1 sir1-1* (*HML*α *HMR***a** *sad*$^+$) strains give a "bimating" phenotype: colonies showed a mating reaction with both **a** and α tester strains. In contrast, *mat***a***1 SAD sir1-1* segregants obtained from YD115 mated only as **a**. As described previously (19), we interpret the bimating behavior of colonies grown from *mat***a***1 sir1-1* cells to result from a mixed population of cells, some that mate as **a**, others as α. We presume that the *sir1-1* mutation allows only a very low level expression of the *HM* loci: in some cell division cycles, *HML*α is expressed and *HMR***a** is not. Such a cell will have an α phenotype. In other cell cycles, *HML*α is not expressed at an adequate level, and thus the cell has an **a** cell phenotype. We imagine that *mat***a***1 SAD sir1-1* cells (which have an overall higher level of **a**1 function than *mat***a***1 sad*$^+$ *sir1-1* cells) also give rise to colonies containing two types of cells. In this case, low level expression of α functions from *HML*α leads to a cell with an **a**/α (nonmating) phenotype due to the increased **a**1 product from *SAD*. Inadequate expression of *HML*α again allows the cell to exhibit an **a** phenotype.

Limitation of *SAD* expression by Sir. The above analysis showed that *sir1-1 SAD* strains

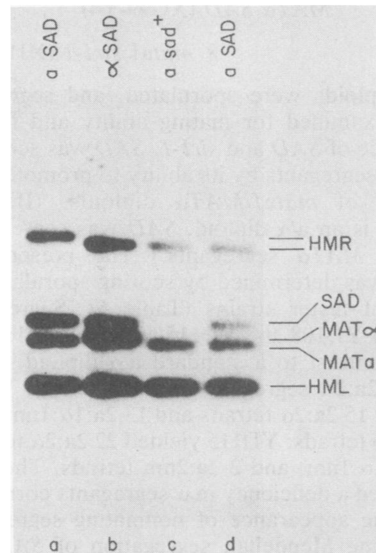

FIG. 2. Hybridization analysis of *SAD* and *sad*$^+$ strains to assay for the presence of additional cassettes. DNA extracted from the indicated strains was digested with *Eco*RI, fractionated by electrophoresis, and probed with an *HML*α probe as described in the text. The analyzed strains were: lane a, Y101 (*MAT***a** *SAD*); lane b, XG99-Y1 (*MAT*α *SAD*); lane c, 17-15 (*mat***a***1 sad*$^+$); lane d, 17-18 (*mat***a***1 SAD*). Full genotypes and origins of these strains are given in reference 9. Positions of bands corresponding to *HML*, *HMR*, *MAT*, and *SAD* are indicated.

that are *HMR***a** exhibit a higher level of **a**1 function than either *sir1-1 HMR***a** or *SAD HMR***a** strains. Because the *sir1-1 SAD HMR***a** strains contain **a** cassettes at both *SAD* and *HMR***a**, it was not possible to determine whether the increased level of **a**1 function resulted from increased production of **a**1 from *SAD* due to the *sir1* mutation or simply from **a**1 produced independently from *SAD* and from *HMR***a**. To determine whether *SAD* expression is still sensitive to Sir, we examined the behavior of strains whose only **a** cassette is at *SAD*. We thus constructed strains that were *HML*α *MAT*α *SAD HMR*α and that were either *SIR*⁺ or carried a mutation in the *SIR1* or *SIR4* genes. If *SAD* expression is limited by Sir action, then *HML*α *MAT*α *SAD HMR*α strains that are *sir*⁻ should express a higher level of **a**1 function from *SAD* and thus exhibit a nonmating phenotype.

SAD sir recombinants were constructed by crossing two strains that are both *HML*α *HMR*α to form the following diploids:

YD129 *mat***a***1 SAD* × *MAT*α *sir1-1*

YD2007 *mat***a***1 SAD* × *MAT*α *sir4-1*

For simplicity, we present the results only for *MAT*α segregants (Table 8). The striking result was that a large fraction of these *MAT*α segregants were now defective in mating: 48% from YD129 and 17% from YD2007. Because *SAD* and *sir1* or *sir4* are segregating in these crosses, we presume that a *MAT*α strain carrying both *SAD* and *sir* has a nonmating phenotype. Analysis of segregants confirms this point (data not shown). Why the fraction of nonmating segregants from YD129 was so high is unexplained. These results clearly suggest that the level of expression of the *SAD* **a** cassette is enhanced in the absence of Sir and thus that the *SAD* **a** cassette is still negatively regulated by Sir.

TABLE 6. Mating procedure for determining the presence of *sir1-1*[a]

Genotype of segregant	Tester strains		
	*mat***a***1 sir1*	*mat*α*2 sir1*	*MAT*α *sir1*
*MAT*α *sad*⁺	X		
*MAT***a**	X	X	
*mat***a***1 sad*⁺			X
*mat***a***1 SAD*		X	

[a] Diploids were formed between segregants and tester strains, as indicated by X, and assayed for sporulation. Sporulation of resultant diploids indicates that the segregant is *sir1-1*; failure to sporulate indicates that the segregant is *SIR1*. *sir1-1* tester strains are: *mat***a***1*, XJ116-6a; *mat*α*2*, XJ104-25a; *MAT*α, XR29-10c.

TABLE 7. Segregation of *MAT*, *SAD*, and *SIR1* among progeny of *SAD*/*sad*⁺ *sir1*/*SIR1* diploids

Strain[a]	Diploid genotype	Phenotype[b]	No. of segregants		
			sad⁺	*SAD*	Total
YD108	*MAT***a**/*MAT*α	**a** Sir⁺			44
		a Sir⁻			48
		α Sir⁺	15	30	45
		α Sir⁻	27		27
		nm Sir⁻	26	1	27
YD115	*mat***a***1*/*MAT*α	**a** Sir⁺	34	15	49
		a Sir⁻		9	9
		bi Sir⁻	26		26
		α Sir⁺	20	18	38
		α Sir⁻	23		23
		nm Sir⁻	19	4	23

[a] YD108 is *MAT***a** *sad*⁺/*MAT*α *SAD*; YD115 is *mat***a***1 sad*⁺/*MAT*α *SAD*.

[b] **a**, Mating as **a**; α, mating as α; nm, nonmating; bi, bimating (mates with both **a** and α tester strains).

DISCUSSION

Sporulation of *S. cerevisiae* ordinarily requires the **a**1 function of *MAT***a** and the α2 function of *MAT*α. The *SAD* mutation has been identified as a mutation that allows *MAT*α/*MAT*α (8) as well as *mat***a***1*/*MAT*α diploids to sporulate efficiently (9). *SAD*-promoted sporulation still requires α2 function (9). Thus, *SAD* can be viewed either as supplying **a**1 function in some way or as bypassing the need for **a**1 function. We have presented functional and physical evidence that shows that the *SAD* mutation is a new **a** cassette present in the *S. cerevisiae* genome that provides **a**1 function. First, we showed that *SAD* does not act by allowing expression of the **a** cassette at *HMR***a**. Second, we showed that *SAD* can act as a donor of **a** cassettes for mating type interconversion. *MAT*α *SAD* strains carrying only α cassettes at the wild-type library loci, *HML* and *HMR*, are able to switch to *MAT***a**. The **a** cassette at the mating type locus contributed by *SAD* behaves in all respects like a wild-type **a** cassette. If *SAD* is a new **a** cassette, *SAD* strains should contain an extra restriction fragment with homology to a cassette probe. This prediction was confirmed by Southern hybridization. Because *SAD* behaved physiologically in all respects as an **a** cassette, *SAD* must contain at least the 642-base pair Ya region that is unique to **a** cassettes and absent from α cassettes (15, 26). Without further analysis, we cannot be more specific about the precise homology between *SAD* and other loci that harbor cassettes. We discuss physiological evidence below indicating that *SAD* must also contain some of the regions that flank cassettes at *HML* and *HMR*, namely, sites for regulation by Sir.

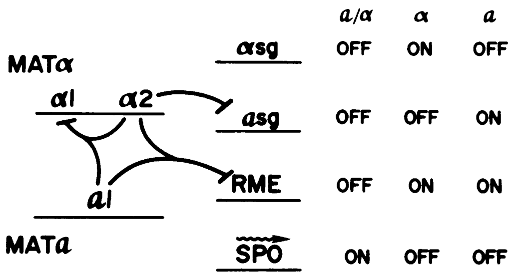

FIG. 3. Control of sporulation and mating in a/α diploids. The mating type loci of an a/α diploid are drawn to the left. αsg, α-specific genes; asg, a-specific genes; *RME*, negative regulator of *SPO* genes (and other genes that are expressed in haploids but not in a/α diploids); *SPO*, genes required for sporulation. Wavy line indicates expression or activity of the *SPO* genes. Line with a blunt end indicates inhibition of synthesis or activity: a1 and α2 inhibit *MATα1* and *RME*; α2 inhibits asg. Expression of αsg, asg, *RME*, and *SPO* genes is indicated to the right for a/α, α, and a cells. In haploid *MATα* cells, αsg are activated by α1; in haploid *MAT*a and *MATα* cells, *SPO* genes are negatively regulated by the *RME* gene product.

The a1 function of *MAT*a is necessary for two behaviors of a/α diploids, first, for inhibiting mating, and second, for stimulating sporulation (Fig. 3). Inhibition of mating occurs because a1 and α2 functions inhibit synthesis of the α1 product (13, 16), which activates expression of genes unlinked to the mating type locus that are necessary for mating by α cells (22, 24). The requirement of a1 in promoting sporulation is less clear, but it has been proposed that a1 and α2 again act as negative regulators. In this case, a1 and α2 inhibit expression of a gene (*RME*; 10) that is proposed to inhibit sporulation (18). The behavior of the *SAD* mutation appears to distinguish between these two roles of a1 in that *SAD* stimulates sporulation of *MATα/MATα* and *mata1/MATα* strains but does not inhibit mating in *MATα* strains. *MATα SAD* strains differ from a/α strains also in that they do not inhibit mating type interconversion (see above) or expression of certain genes that have become negatively regulated by a1 and α2 (1). These properties of *SAD* can be explained by proposing that *SAD* does not produce a full level of a1 function, in other words, that the production of a1 from *SAD* is lower than from a fully active a cassette (such as an a cassette at *MAT* in wild-type strains or an a cassette at *HMR* in *sir* mutants). Furthermore, we must argue that a reduced level of a1 is sufficient to promote sporulation but not to inhibit mating. A very different physiological situation, the behavior of *sir1-1* mutants, has been viewed in exactly the same light: *sir1-1* strains express enough a1 to allow *MATα/MATα* strains to sporulate but not enough a1 to inhibit mating by *MATα* strains (19). As discussed more fully below, we derived conditions in which *SAD* does inhibit mating by *MATα* strains. These are conditions that apparently lead to higher level expression of the a cassette at the *SAD* locus. Although our level of understanding of a1-α2 action at the molecular level is incomplete, we offer one specific view as to how the level of a1 might differentially control sporulation and mating. For this argument, we assume first that a1 and α2 (perhaps as a complex) act at sites near the *MATα1* and *RME* genes. We then argue that the affinity of a1 and α2 is higher for the site at *RME* than for the site at *MATα1*. Alternatively, the affinities of a1 and α2 for these two loci are similar. In this case, incomplete inhibition of *MATα1* expression does not reduce α1 levels below a threshold for activation of unlinked genes. In contrast, incomplete inhibition of the *RME* gene allows cells to express enough sporulation functions to proceed through sporulation.

Wild-type *S. cerevisiae* harbors mating type cassettes at three loci, *HML*, *HMR*, and *MAT*. The cassettes at *HML* and *HMR* are silent because of action of the four *SIR* gene products (J. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979), which are thought to act at sites to the left of *HML* and *HMR* loci (13, 16, 25). These cassettes become activated by moving them to *MAT* and thus away from sites of Sir action. The *SAD* mutation behaves as an a cassette that has a level of expression between that of an a cassette at *MAT* (fully expressed) and one at *HMR* or *HML* (fully repressed) (Table 9). What type of rearrangement gave rise to *SAD*? Why does *SAD* expression occur at an intermediate level? First, we consider the possibility that *SAD* resulted from an error in the normal transposition process, such that a cassette from *HMR*a has been transposed to a location other than the normal target, the mating

TABLE 8. Segregation of mating phenotype from *mata1 SAD/MATα sad⁺ sir/SIR* diploids that are homozygous for *HMLα HMRα*

Mating phenotype	Inferred genotype[a]	No. of segregants observed from:	
		YD129 (*sir1/SIR1*)	YD2007 (*sir4/SIR4*)
α	*MATα sad⁺ SIR*, *MATα SAD SIR*, *MATα sad⁺ sir*	35	79
Nonmating	*MATα SAD sir*	32	16

[a] Genotypes were assigned by assuming 2a:2α segregation in tetrads and by sporulation ability of diploids formed between segregants and tester strains as described in Table 6 and in the text. Data are shown only for *MATα* segregants.

TABLE 9. Behavior of a cassettes at *MAT*, *HMR*, and *SAD*

Location of a cassette	Expression of a cassette	Negative regulation by Sir
MAT	On	No
HMR	Off	Yes
SAD	Partially on	Yes[a]

[a] *SAD* is partially sensitive to Sir (see text).

type locus. According to this view, we expect that the new a cassette has been removed from sites of Sir action, in which case its limited expression may result from insertion into a chromatin domain that prevents full expression. Such a chromatin domain override control is seen for integrated mouse mammary tumor virus (2). According to this hypothesis, the level of expression of *SAD* should be independent of Sir. However, we have found that *SAD* is still under Sir control. In particular, we find that whereas *HML*α *MAT*α *HMR*α *SAD* strains mate as α, *HML*α *MAT*α *HMR*α *SAD* strains that are defective in either *SIR1* or *SIR4* have the nonmating phenotype of a/α cells. Thus, these sir^- strains apparently overproduce the a1 function of the a cassette at *SAD*. This observation indicates that the *SAD* rearrangement did not simply result from an improper mating type interconversion event. The observation that *SAD* remains partially sensitive to Sir provides some information on its origin and structure. In particular, we consider it likely that *SAD* arose from a rearrangement involving the standard locus harboring an a cassette and sites of Sir action, namely, *HMR*a. One plausible explanation for the origin of *SAD* is thus that *SAD* is an (insertional) translocation that has moved not only the a cassette from *HMR*a but also some flanking regions that contain sites of Sir action. Further physical characterization (J. B. Hicks, J. N. Strathern, S. Ismail, A. J. S. Klar, and J. R. Broach, manuscript in preparation) indicates that *SAD* is an a cassette flanked on its left by sequences from *HMR* and on its right by sequences from *MAT*.

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