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# Structure of the *SAD* Mutation and the Location of Control Sites at Silent Mating Type Genes in *Saccharomyces cerevisiae*

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**The *SAD* mutation, an extra mating type cassette, has been shown to arise from an unequal mitotic crossover between the *MAT* and *HMR* loci, resulting in the formation of a hybrid cassette and a duplication of the *MAT*-*HMR* interval. The *SAD* cassette contains the "a" information and left-hand flanking regions from the parental *HMR*a cassette and the right-hand flanking sequences of the parental *MAT* cassette. This arrangement of flanking sequences causes a leaky but reproducible mating phenotype correlated with a low-level expression of the cassette as measured by RNA blotting. This weak expression is attributed to the loss of one flanking control site normally present at the silent *HM* storage loci.**

The genes controlling mating type in bakers' yeast, *Saccharomyces cerevisiae*, are found in structurally homologous DNA cassettes residing at three genetic loci on chromosome III (8, 18, 24). These cassettes are of two types: **a** cassettes, which contain **a** mating type information, and α cassettes, which contain α mating type information. The mating type of an individual cell is determined by the cassette residing at the *MAT* locus (*MAT***a** or *MAT*α), where the genes are expressed constitutively. At the other two loci, *HML* (left) and *HMR* (right), the cassettes are normally not expressed. However, recessive mutations in four different genes located elsewhere in the genome, known as the *SIR* (silent information regulator) genes, allow expression of the *HML* and *HMR* loci at levels comparable to that of the *MAT* locus (6, 12; J. D. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979). Thus, the normally silent *HML* and *HMR* loci are apparently under negative regulation mediated by the products of the *SIR* genes. The organization of the mating type genes is diagrammed in Fig. 1.

It is reasonable to assume that control of the silent mating type cassettes involves the recognition of one or more regulatory sequences (possibly the site of *SIR* action) present at the *HM* loci and absent at the *MAT* locus. Possible models for the location of such sites and the mechanism of regulation are complicated, however, by the observed arrangement of the transcription units within the cassettes. In both **a** and α cassettes, two transcripts are made divergently from sites within the regions shared among the *MAT* and *HM* loci. Because the sequences of the expressed and unexpressed cassettes have been found to be identical (2, 18), the regulatory sequences must lie outside the limits of cassette homology, at least 750 base pairs (bp) from the actual sites of transcription initiation within the cassettes. These regulatory sequences must therefore be capable of long-range negative effects on transcription.

Recently, a mutation known as *SAD* (suppressor of **a** deficiency) has been described that exhibits some of the properties expected of an additional **a** cassette with a low level of expression (9, 10). *SAD* was identified as a mutation that allows *MAT*α/*MAT*α diploids to sporulate. However, although *MAT*α/*MAT*α *SAD* strains mimic *MAT***a**/*MAT*α diploids with regard to sporulation, they display the α mating phenotype in contrast to the nonmating phenotype characteristic of *MAT***a**/*MAT*α diploids.

Explanation of the Sad phenotype requires some knowledge of the normal roles of the *MAT*α and *MAT***a** gene products inferred from previous genetic studies. Analysis of mutations isolated in vivo (22) and in vitro (25) has led to the hypothesis that the *MAT***a***1* gene product acts cooperatively with one of the two *MAT*α products, *MAT*α*2*, both to turn off α-specific genes (by turning off *MAT*α*1* transcription) and to turn on sporulation-specific genes. *MAT***a***1* function is apparently not required at all to achieve the normal **a** mating phenotype, but it is necessary to achieve the nonmating, sporulation-proficient phenotype characteristic of **a**/α diploids. Thus, *mat***a***1*$^-$ haploids still mate as **a**'s, but *mat***a***1*/*MAT*α diploids exhibit the α mating type and are unable to sporulate.

Intermediate expression of a normally silent **a** cassette has been characterized previously in strains carrying the *sir1-1* mutation (20) along with a normal *HMR***a** allele. Such strains cause enough expression of *HMR* to allow *MAT*α/*MAT*α cells to sporulate but not enough to yield to the nonmating phenotype. This phenotype, which is identical to that observed for *SAD* is also associated with a low-level amount of transcription from *HMR* (19).

The possibility that *SAD* represents an extra, albeit somewhat leaky, silent cassette was further suggested by its abilities to yield the full **a**/α phenotype in Sir$^-$ strains and to act as an **a** cassette donor during homothallic switches of cell types (11). Genetic mapping places *SAD* between *THR4* and *HMR* on chromosome III, a site not previously associated with mating type cassettes (10).

In this paper we report a physical analysis of the *SAD* locus and its expression. We confirmed the existence of an additional **a** cassette in *SAD* strains and have determined that *SAD* is the fusion of *MAT* and *HMR***a**. The novel phenotypes associated with the *SAD* fusion can be most easily explained by the loss of a minor negative regulatory site involved in control of silent cassette expression (1; J. Abraham, K. A. Nasmyth, J. N. Strathern, A. J. S. Klar, and J. B. Hicks, J. Mol. Biol., in press; J. B. Feldman, J. B. Hicks, J. R. Broach, J. Mol. Biol., in press). Based on a comparison of the phenotypes of the *SAD* fusion and other *MAT-HMR* and *MAT-HML* fusions, we suggest that, consis-

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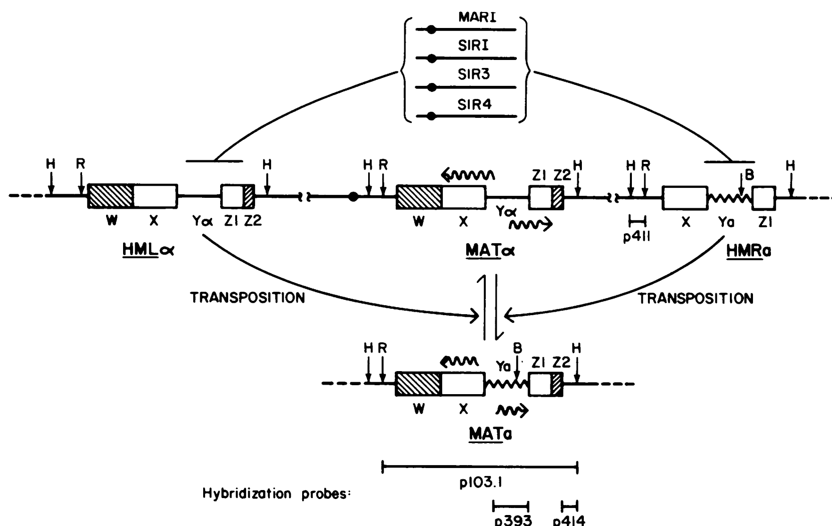


FIG. 1. Schematic diagram of chromosome III and the mating type control system in *S. cerevisiae* (not to physical or genetic scale), showing the arrangement of the mating type cassettes at *MAT*, *HML*, and *HMR*. The DNA segments labeled X (704 bp) and Z1 (239 bp) are homologous at all three sites. The W (723 bp) and Z2 (88 bp) segments are common to *HML* and *MAT* but not to *HMR*. Each cassette also contains one of two alternative core segments designated Yα (747 bp) and Ya (642 bp). The mating type of a cell is determined by which core sequence (Yα or Ya) resides at the *MAT* locus and is thus transcribed (wavy arrows). The *HML* and *HMR* cassettes have the potential to make fully functional α or **a** gene products but are kept silent by the action of the *SIR* genes, which are located elsewhere in the genome. Interconversion of *MAT***a** and *MAT*α alleles can occur by transposition as shown. Restriction sites used in the blotting analysis are designated H (*Hin*dIII), B (*Bgl*II), and R (*Eco*RI).

tent with the work cited above, the major control site for the action of the *SIR* gene products is located in a region to the left of the *HM* loci, whereas a less essential regulatory site lies on the right side of at least the *HMR* locus. Furthermore, we have shown that transcription of the *SAD* cassette is below the level of detection by gel blotting analysis and probably represents less than 5% of normal *MAT***a** expression. Thus, the amount of **a***1* product necessary to support sporulation is a small fraction of that normally produced.

## MATERIALS AND METHODS

**Strains.** The genotypes of yeast strains used in this study are provided in Table 1.

**Miscellaneous methods.** Transformation of *Escherichia coli* was performed by the method of Mandel and Higa (15). Yeast transformations were performed as described by Beggs (3). Restriction enzymes were obtained from New England Biolabs, Inc., or Bethesda Research Laboratories,

TABLE 1. Genotypes of yeast strains used in this study

| Strain | Genotype | Reference or source |
|---|---|---|
| XG99-Y4 | *HML*α *MAT*α *SAD HMR***a** *ura3 his4-12 leu2-27 ade2 trp1* | Kassir and Herskowitz, 1980 |
| DC176 | *HML*α/*HML*α *MAT*α/*MAT***a** *HMR***a**/*HMR*α *sad*$^+$/*sad*$^+$ | This laboratory |
| YD106-9C | *HML*α *mat***a**-*1 SAD HMR*α *ade2 trp1* | Kassir and Herskowitz, 1980 |
| YD106-13A | *HML*α *MAT*α *SAD HMR*α *trp1* | Kassir and Herskowitz, 1980 |
| YD106-15A | *HML*α *MAT*α *SAD HMR*α *trp1 ade2* | Kassir and Herskowitz, 1980 |
| IH533 | *HML*α *MAT***a** *sad*$^+$ *HMR***a** *ade2 tyr1-2 lys2-2 his7-1 can1 cyh*$^r$ | Ira Herskowitz |
| SX48-8D | *HML*α *MAT*α *SAD HMR*α *sir2-1 ade2 leu2 trp1* | This laboratory |
| JI360 | *HML*α *MAT***a** *HMR***a** *leu2-3 leu2-112 his3 sir1-2::LEU2* | This laboratory |
| HX834 | JI360 × YD106-15A | This laboratory |
| JH834-7D | *HML*α *MAT*α *SAD HMR*α *leu2 his3 trp1* | This laboratory |
| JH834-10C | *HML*α *MAT*α *SAD HMR*α *leu2 his3 trp1 sir1-2::LEU2* | This laboratory |
| DC40 | *HML*α *mat*α *1-5 HMR***a** *sir1-1 leu2-1 ade6 lys2-1 arg4-17 cry1-3* | This laboratory |

Inc., and digestions were performed as recommended by the supplier. Yeast DNA was purified by the method of Cryer et al. (5). Transfer of agarose gel-fractionated DNA to nitrocellulose was accomplished as described by Southern (21), and hybridizations were performed at 65°C for 16 h in a solution consisting of 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.25 mg of calf thymus DNA per ml, and 0.1% sodium dodecyl sulfate. Yeast RNA isolation, fractionation, transfer to diazobenzyloxymethyl-paper, and hybridization to labeled probes were accomplished as described previously (13).

## RESULTS

**Restriction analysis of *SAD*.** Strains containing the *SAD* mutation (Table 1) were obtained from Y. Kassir and have been described previously (10). DNA was isolated from these strains and subjected to restriction endonuclease digestion and Southern blotting analysis as described above. A radioautograph of one such blot is shown in Fig. 2. The probe used for lanes a to e was a $^{32}$P-labeled restriction fragment containing the complete *MAT***a** gene; because of the homology between the mating type cassettes (Fig. 1), this probe hybridizes to the RI fragments derived from each of the mating type loci. In addition to three expected cassette bands corresponding to *MAT*, *HML*, and *HMR*, however, each *SAD* strain was also found to contain a fourth band intermediate in size between those of *MAT* and *HMR* (lanes a, d, and e). This band was present in seven additional strains examined and is lost on reversion of the *SAD* strain to *sad*$^+$ as described below.

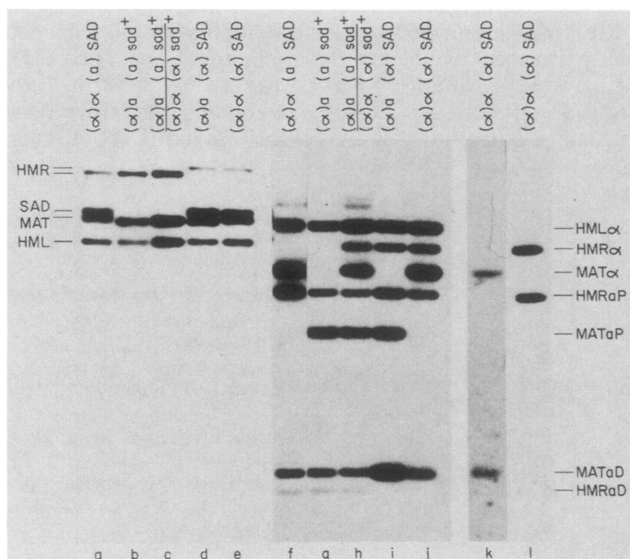


FIG. 2. Radioautographs of Southern blots of DNA from representative *SAD* strains cut with *Eco*RI (lanes a to e) and a combination of *Hin*dIII and *Bgl*II (lanes f to l). The cassette (**a** or α) residing at each of the mating type loci (*HML*, *MAT*, and *HMR*) in each strain (as determined by genetic methods) is shown above each lane. Parenthesis denote silent *HML* and *HMR* cassettes. Lanes a to j were probed with plasmid p103.1, lane k was probed with p414, and lane l was probed with p411. Locations of the probes are shown in Fig. 1. Strains used in this analysis were XG99-Y4 (lanes a and f), IH-533 (lanes b and g), DC176 (lanes c and h), YD106-9C (lanes d and i), and YD106-13A (lanes e, j, k, and l). Genotypes are given in Table 1.

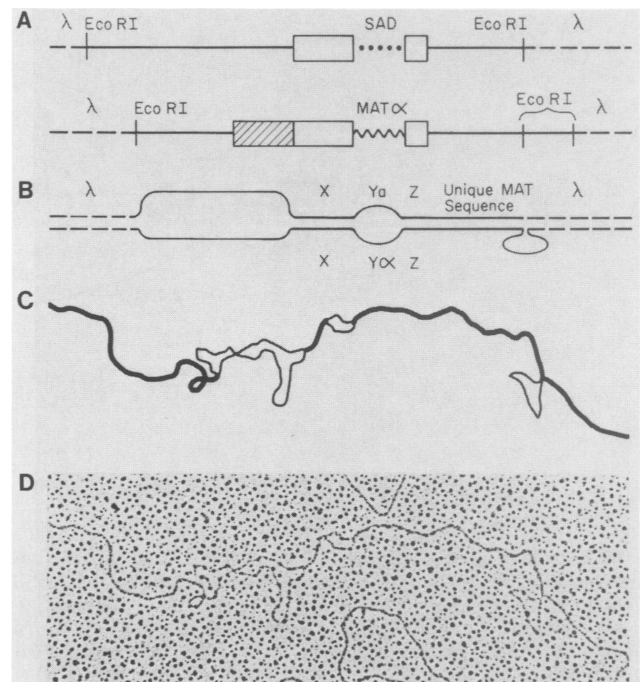


FIG. 3. Heteroduplexes between lambda transducing phage, one containing the *Eco*RI fragment corresponding to the *MAT*α locus plus an adjacent fragment, and the other containing the *Eco*RI fragment corresponding to the *SAD* cassette. (A) Diagram of the structure of each insert; (B) schematic of the hybrid molecule; (C) tracing of the hybrid shown in (D). The ends of the arms of the phage could be traced completely but are not shown in this enlargement.

*SAD* strains thus exhibit a fourth cassette not previously observed in haploid strains. We therefore investigated whether the *SAD* restriction fragment represented the insertion of a mating type cassette at a new site or was the result of a rearrangement of previously existing sequences. Figure 2, lanes f to l, show combined *Hin*dIII-*Bgl*II digestion of the same strains shown in lanes a to e. *Bgl*II cuts only in **a** cassettes and nowhere else in the *Hin*dIII fragments from the *HML*, *HMR*, or *MAT* cassette. This site provides a convenient assay for **a** cassettes at any locus. Examination of the results shown in lanes f to l demonstrates that although the *SAD* cassette contributes a new *Eco*RI restriction fragment, it does not contribute any new bands to the *Hin*dIII-*Bgl*II double digestion pattern. In lane j, the only **a** sequences present should reside in the *SAD* cassette, and the digestion yields fragments identical in size to *HMR***a** (proximal) and *MAT***a** (distal). Using subcloned restriction fragments containing no cassette homology as specific probes for regions flanking *MAT* and *HMR*, we have further shown that the *Hin*dIII-*Bgl*II fragments characteristic of *SAD* contain sequences unique to the proximal side of *HMR* and to the distal side of *MAT* (Fig. 2, lanes k and l). The locations of these restriction sites are shown in Fig. 1. The restriction patterns for enzymes *Pst*I, *Xho*I, and *Bam*HI (data not shown) are likewise consistent with this interpretation. We therefore interpret *SAD* to be a hybrid cassette generated by recombination between the *HMR***a** and *MAT*α cassettes.

Using standard procedures for cloning genomic restriction fragments (4) into the bacteriophage vector λgt WES-B (14) we cloned the *Eco*RI fragment containing the *SAD* cassette from strain XG99-Y4 (Table 1). Electron microscopy of

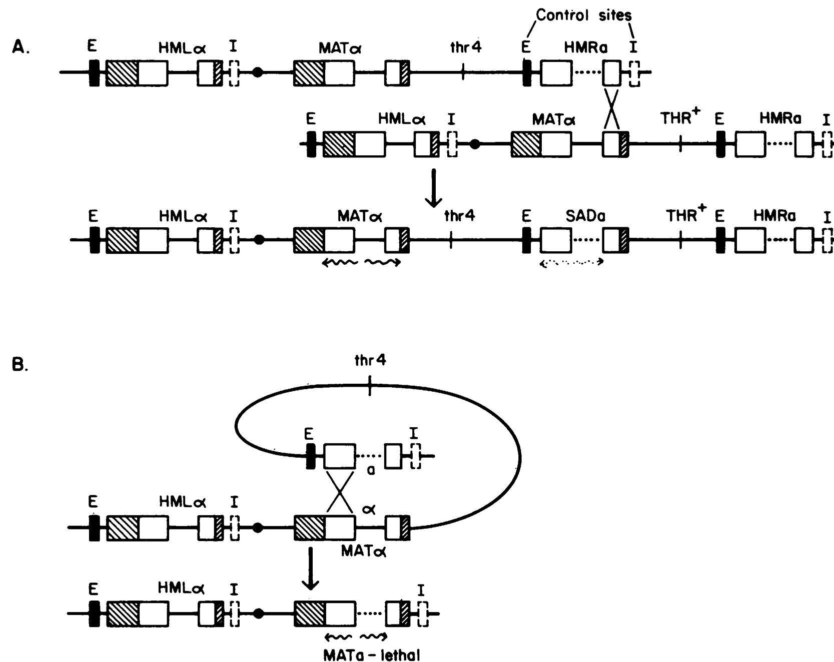


FIG. 4. Proposed recombination events leading to the *SAD* mutation (A) and the **a**-lethal Hawthorne deletion (B). In (A) an unequal crossing over is proposed to occur in the Z1 regions (see Fig. 1) of *HMR* and *MAT* on two different chromosomes, leading to a duplication of the *MAT-HMR* interval and creation of the hybrid *SAD* cassette. The low-level expression exhibited by the *SAD* cassette may result from loss of a control site at the right side (dashed box) (1). In (B) an internal event is proposed to occur in the homologous X regions fusing Y**a** to the WX region of *MAT* and eliminating the *MAT-HMR* interval. The proposed negative control site to the left of *HMR* (and, by analogy, *HML*) is preserved in the *SAD* fusion, leaving the *SAD* fusion under *SIR* gene control, but is eliminated in the deletion event, causing constitutive expression of the Y**a** region originally present at *HMR*. A second control site designated by the dashed box may be present to the right of *HMR* (and likewise *HML*), deletion of which leads to partial expression of the cassette.

heteroduplexes formed between this recombinant phage molecule and a phage containing the *MAT*α cassette (24) were completely consistent with *SAD* being a simple fusion of the *HMR***a** and *MAT*α cassettes (Fig. 3).

The recombination event proposed to generate the *SAD* is shown in Fig. 4A. The *SAD* strain was originally isolated as a *MAT*α/*MAT*α diploid that sporulated at high efficiency but retained the α mating phenotype (9). No mutagenesis was applied in these experiments. We propose that an unequal reciprocal crossover occurred between the Z1 region of *HMR***a** on one homolog and the Z1 region of *MAT*α on the other homolog, thus generating a tamdem duplication of the region between *MAT* and *HMR* (including the *THR4* locus). Although our results do not constitute proof that the complete *MAT-HMR* interval is duplicated, the proposed event provides the simplest explanation for the existing data. We presume that the chromosome carrying the duplication was originally heterozygous for the recessive *thr4* allele because in some crosses between *SAD THR4* and *sad*$^+$ *thr4* strains, only one copy of the *THR4* allele appears to be present (10). Although the parents of the ultimate *SAD* strain (G-99) were both Thr$^+$, all of the preceding crosses (in which the *SAD* mutation presumably occurred) were heterozygous *thr4*/*THR4* and could have generated a duplication like the one shown in Fig. 4A with both *thr4* and *THR4* alleles.

The recombinant proposed in Fig. 4A is the structural reverse of the so-called Hawthorne deletion shown in Fig. 4B (7, 23) in which the recombination is thought to occur in the *X* region. The hybrid cassette generated by the lethal deletion is constitutively expressed. Since the *SAD* hybrid remains under at least partial *SIR* gene control, but the *MAT***a**-lethal hybrid does not, comparison of these two structures suggests that a negative control site for *HMR* lies to the left of the cassette. In addition, a control site in a similar position to the left of the *HML* locus has been inferred from the structure of an intrachromosomal fusion of the *HML*α and *MAT***a** cassettes. This recombination event between *HML* and *MAT*, characterized by Strathern et al. (23), deletes sequences to the right of *MAT* and to the left of *HML* (Fig. 1), yielding a circular chromosome and resulting in a fully α phenotype. Thus, the Yα region originally present at *HML*α is fully expressed, presumably because it lacks the putative control region to the left of *HML*.

**Reversion of *SAD*.** It has been previously reported that the SAD phenotype is very unstable in certain strains, especially diploids (10). We therefore tested for reversion of *SAD* to *sad*$^+$ to correlate the loss of the SAD phenotype with the loss of the extra cassette.

To address this question we evaluated *sad*$^+$ revertants from a haploid strain of genotype *sir2-1 HML*α *MAT*α *SAD HMR*α. This strain, SX48-8D (Table 1), exhibits the nonmating phenotype characteristic of **a**/α strains because the *sir2-1* mutation (also called *marl-1*) (12) increases the expression of *SAD*, yielding a fully functional **a** cassette. This strain segregates α-type cells at a frequency approaching 1% during mitotic growth. Such reversion would be expected to result from reciprocal recombination between sequences in the *MAT-SAD* interval and homologous sequences in the *SAD-HMR* interval, thereby deleting the *SAD* cassette along with one copy of the duplicated chromosomal segment. DNA restriction and blotting analysis performed on six independent revertants is presented in Fig. 5. Consistent

with this interpretation, the *Eco*RI restriction fragment characteristic of *SAD* is missing in each of the α revertants.

**Transcription of *SAD* and the a-lethal deletion.** The leaky **a** character of the *SAD* cassette indicates that the **a** information in it is expressed at a low level. To further quantitate the level of this expression, we have examined the stable RNA population by blotting.

Figure 6A shows duplicate blots of polyadenylated RNA from three strains: a wild-type *MAT***a** strain (lanes a and d), a *MAT*α *SAD* strain (lanes b and e), and a diploid containing the *MAT***a**-lethal deletion on one chromosome and a normal *MAT*α allele on the other (lanes c and f). The blot on the left was probed with a restriction fragment containing the complete *MAT***a** locus, and the blot on the right was probed with a smaller fragment containing only sequences unique to **a** cassettes (**Ya** sequences). The *MAT***a** strain serves as a control for the normal level of expression of the **a***1* and **a***2* transcripts coded for by *MAT***a** (13, 19) (Fig. 1). As expected, this strain exhibits two strong bands corresponding to the **a***1* and **a***2* transcripts when probed with *MAT***a** (lane a) and only the **a***1* transcript when probed with the **a***1*-specific probe Dp393 (lane d). The *SAD* strain, which expresses the α mating phenotype, exhibits a single major band representing the comigrating α*1* and α*2* transcripts coded for by *MAT*α (lane b). The **a***1* and **a***2* regions of this lane are partially obscured by background from the intense α band. With the **a**-specific probe (lane e), however, it can be seen that the *SAD* strain makes no detectable **a***1* transcript. In contrast, the probe shows strong hybridization with the **a***1* transcript from the wild-type *MAT***a** strain, demonstrating that the **a***1* transcript of *SAD* is in fact very reduced relative to *MAT***a**. In contrast to *SAD*, the reciprocal hybrid cassette in the *MAT***a**-lethal diploid is expressed at levels expected for a normal **a**/α diploid (lanes c and f). The three bands corresponding to α*2*, **a***1*, and **a***2* are all present in lane c, and the level of **a***1* in lane f is comparable to that in lane d. Thus, even though *SAD* is leaky and exhibits a characteristic phenotype, it is nearly completely repressed at the level of transcription, indicating that it is subject to *SIR* control.

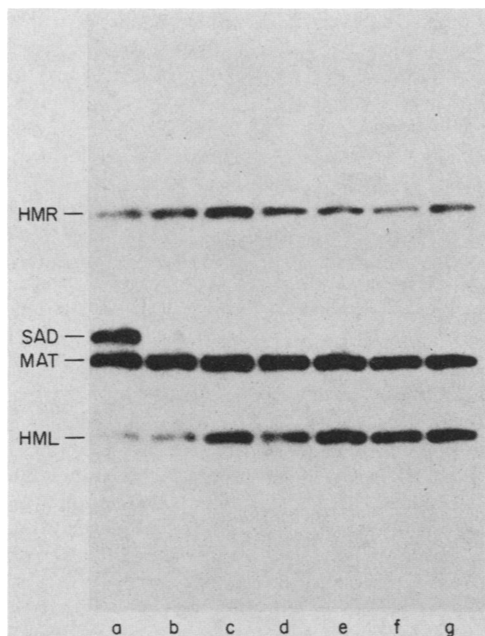


FIG. 5. DNA from the nonmating strain SX48-8D (lane a) and six α mating revertants (lanes b to g) of that strain was digested with *Eco*RI, displayed on a 0.8% agarose gel, and blotted to nitrocellulose. The transfer was probed with labeled plasmid containing the *MAT***a** cassette (p103.1).

The phenotype caused by weak expression of the *SAD* cassette is reminiscent of the phenotype of strains harboring the *sir1-1* defect (20). The *sir1-1* mutation allows partial expression of the *HML* and *HMR* cassettes, such that a strain of genotype *HML*α *MAT*α *HMR***a** *sir1-1* makes a level of **a***1* gene product sufficient for sporulation but not sufficient to induce the **a**/α nonmating phenotype. This correlation raises the possibility that *SAD* and *sir1* mutations are affecting the same control pathway. For example, the *SAD* rearrangement could result in the loss of the site of action of the *SIR1* gene product. A strong implication of this hypothesis is that the two mutations should not be additive in effect. That is, introduction of the *sir1-1* mutation should not cause increased expression of the *SAD* cassette. A genetic study that seems to contradict this model has been published (11). In that study, segregation ratios of the mating and sporulation phenotypes from genetic crosses were used as evidence that the strains of genotype *HML*α *MAT*α *HMR*α *SAD* expressed the α mating type, whereas *HML*α *MAT*α *HMR*α *SAD sir1-1* strains were nonmaters. However, the ratios observed in that cross were not strictly consistent with that interpretation, and the genotypes were not checked at the DNA level. To further test the hypothesis, we made a similar cross to yield haploid segregants of the type described above. Each of 20 meiotic segregants from the cross HX834 (Table 1) was scored for mating type, and the arrangement of mating type cassettes was assayed by Southern blotting (data not shown). The *sir1-2* allele used in this cross was made by insertion of the *LEU2* sequence into the *SIR1* locus (J. Ivy and J. Hicks, unpublished data). The phenotype of the allele is identical in all respects to the *sir1-1* allele used by Kassir et al. (11). Two meiotic segregants of each type (*HML*α *MAT*α *HMR***a** *SAD SIR*$^+$ and *HML*α *MAT*α *HMR*α *SAD sir1-2*) were scored for mating type, and RNA was isolated for Northern blotting analysis. The results confirm the interpretation made by Kassir et al. (11). *MAT*α *SAD SIR1* strains mate as α's, whereas *MAT*α *SAD sir1-2* strains are nonmaters, as if the *sir1* defect raised the level of *SAD* expression to a level comparable to that of *MAT***a**. Gel blotting data (Fig. 6B) further show that the *SAD* cassette, represented by the **a***1* and **a***2* transcripts, is fully expressed in the *sir1-2* strain (lane d) at a level equivalent to that of *MAT***a** in an **a**/α diploid (lane c). For comparison, Fig. 6B, lane e, displays RNA from the *sad*$^+$ *sir1*$^-$ strain DC40 (*HML*α *mat*α*1-5 HMR***a** *sir1-1*), which exhibits the α mating type, showing that the level of **a***1* and **a***2* transcription from *HMR***a** due to *sir1-1* is significant but still not enough to make the strain sterile (20).

We conclude from these experiments that defects in *SIR1* increase the expression of the *SAD* cassette to a level comparable to that of the *MAT***a** cassette. Therefore, despite the similarity in phenotype between the *sir1-1* and *SAD* mutations, the defects are independent and additive.

## DISCUSSION

The results presented here indicate that the *SAD* mutation represents a fusion of the Z region and right-hand flanking sequences of the *MAT* cassette with the **Ya**, X, and left-hand flanking sequences of the cassette at *HMR*. One likely mechanism for generating such a structure is a mitotic recombination between the *MAT*α locus on one chromo-

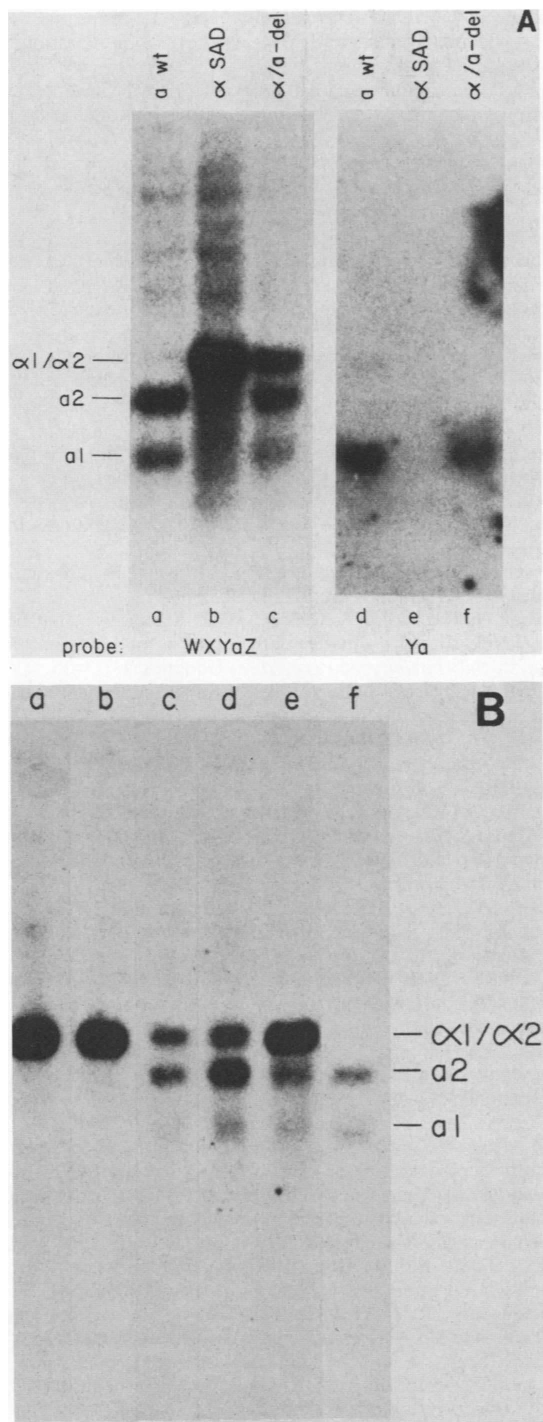


FIG. 6. (A) RNA transcripts from a normal *MAT*a haploid (lanes a and d), a *MAT*α *SAD* haploid (lanes b and e), and a diploid containing *MAT*α and the *MAT*a-lethal known as the Hawthorne deletion (lanes c and f). The locations of the transcripts are diagrammed in Fig. 1. The α*1* and α*2* transcripts migrate as a single band at ca. 750 bases, whereas the **a***1* and **a***2* transcripts migrate faster at 400 and 600 bases, respectively. Actual transcript endpoints have been mapped by Nasmyth et al. (19). Lanes a to c are probed with a complete *MAT*a cassette (plasmid p103.1) which hybridizes to all four transcripts (13). Lanes d to f are probed with a fragment unique to the Ya region of *MAT*a (plasmid p393), obtained by subcloning a segment from the *Bgl*II site (Fig. 1) to an *Xho*I site inserted in vitro by Tatchell et al. (25). This fragment hybridizes only to the **a***1* transcript and shows that whereas the **a***1* transcript is produced from the **a**-lethal cassette, there is no detectable transcription from the *SAD* cassette. (B) Comparison of *SAD* transcription in Sir$^+$ and Sir$^-$ strains: lane a, YD106-15A (Sir$^+$ SAD$^+$); lane b, JH834-7D (Sir$^+$ Sad); lane c, HX834 (**a**/α diploid); lane d, JH834-10C (Sir$^-$ SAD); lane e, DC40 (Sir$^-$ Sad$^+$); lane f, *MAT*a (Sir$^+$ Sad$^+$). Probe is p1031.

some and the *HMR***a** locus on the homologous chromosome in the *MAT*α/*MAT*α *thr4*/*THR4* diploid constructed by V. MacKay (9). Such an event, occurring in the Z1 region of each locus, would account for the observed restriction map of the *SAD* cassette as well as for the genetic data indicating that only one *THR4*$^+$ allele appears to be present in haploid *SAD* isolates (10).

The novel phenotype of the *SAD* mutation appears to be the result of very limited expression of this particular hybrid **a** cassette. The ability to sporulate is normally associated with expression of both **a** and α mating type genes and is concomitant with the absence of mating. The *SAD* cassette is unusual in that it provides enough **a** gene function to make *MAT*α/*MAT*α *SAD*/*sad*$^+$ strains sporulate efficiently but not enough **a** gene function to turn off α mating ability. It is clear that the *SAD* locus contains an intact **a** cassette: in a sir$^-$ background, *SAD* produces the phenotype associated with a fully expressed, wild-type **a** cassette (see above) (11). In a Sir$^+$ background, however, we have shown that transcription of the *SAD***a** cassette is very much reduced compared with transcription of a normal *MAT***a** allele. We estimate that the level of stable mRNA is reduced at least 20-fold. Thus, the *SAD* phenotype is correlated with reduced levels of normal **a** gene product.

A phenotype similar to that associated with *SAD* has been previously observed in *MAT*α *HMR***a** *sir1-1* strains (20). The *sir1-1* mutation causes partial expression of the *HMR* and *HML* loci, resulting in enough **a** gene product to allow sporulation but not enough to completely suppress α mating ability. We have recently observed that this leaky phenotype is not unique to the *sir1-1* allele but is characteristic of all *sir1* mutations including deletions (Ivy and Hicks, unpublished data). This observation led to the hypothesis that a control site for *HMR*, missing in the *SAD* fusion, was the sole site of action for the *SIR1* gene product. A strong prediction based on this hypothesis is that introduction of a defect in *SIR1* should cause no additional expression of the *SAD* cassette. Our results on segregants of cross HX834 (see above) are in conflict with that prediction and, in fact, confirm the observation of Kassir et al. (11) that *sir1* mutations bring expression of the *SAD* cassette up to normal constitutive levels equivalent to expression at *MAT*. Furthermore, these studies show directly that transcription of the *SAD* cassette is correlated with its phenotype.

There is still no clear explanation for the unusual phenotypes associated with *SAD* and with *sir1*$^-$ *HMR***a** strains, that is, sporulation without the nonmating phenotype. However, the ability to separate the two components of the **a** phenotype by simply reducing the level of expression of the **a** cassette is not entirely surprising. It is possible, on the basis of previous genetic analyses, that the **a** gene product, like the *araC* protein of *E. coli*, functions both as an activator and as a repressor. That is, the **a** gene product is required in concert with α*2* to activate expression of genes involved with **a**/α-specific functions—which include sporulation functions—as well as to repress expression of genes required for manifestation of the α mating phenotype. As

Metzenberg (16) has pointed out, a lower concentration of an activator is required to promote reasonable expression of a gene under its control when compared with the concentration required for a repressor to completely shut off expression of a gene which it regulates. Thus, it is not unreasonable to assume that a low cellular concentration of the **a***1* gene product would not be adequate for complete repression of the α-specific genes but would be sufficient for obtaining some expression from the **a**/α-specific genes, thereby conferring the ability to sporulate.

The level of transcription of the hybrid cassette formed by the *SAD* fusion sheds some light on the mechanism of regulation of silent mating type cassettes. Whereas the mating type cassette at *MAT* is transcribed, the mating type cassettes resident at *HML* and *HMR* are not, even though nucleotide sequence analysis of the cloned *MAT* and *HML* cassettes and part of the cloned *HMR* cassette indicates that these regions are exactly homologous at all three sites (2). It is unlikely, therefore, that repression of expression of the silent cassettes is effected through sequences lying within these regions. Directing our attention to the flanking sequences, then, we observe that cassette fusions which delete the regions to the left of *HMR* and *HML* (7, 23) and fuse the Y and Z regions of the silent genes to the *MAT* locus result in constitutive phenotypic expression of the fused cassette. In fact, in this paper we have demonstrated that one of these fusions, the so-called Hawthorne deletion, exhibits the normal level of **a** gene transcripts (Fig. 6). On the other hand, the *SAD* cassette, in which the right-hand side of *HMR* has been deleted, shows no detectable transcription in Sir$^+$ cells, although full genetic expression and transcription are obtained in Sir$^-$ strains. Thus, the deletion associated with the *SAD* cassette does not significantly alter the transcriptional regulation of the silent cassette. We therefore infer that an essential site for Sir control lies to the left of the *HMR* cassette. Nonetheless, the fact that some expression of the *SAD* cassette occurs, as determined by its ability to support sporulation, suggests that complete repression of the *HMR* locus requires sequences lying to the right of the cassette as well. Recent in vitro mutagenesis of cloned *HMR* and *HML* sequences, conducted in our laboratories, has confirmed this interpretation (1; Abraham et al., in press; Feldman et al., in press). We have designated the left-hand control site at each silent cassette (the solid boxes in Fig. 4) the essential or E site and the right-hand control sequence (the dashed boxes in Fig. 4) the important or I site (1).

As described above, mating type cassettes are transcribed divergently from the center of a region of DNA that is precisely homologous over an extended distance at all mating type loci. Results presented in this paper and elsewhere (19, 23) suggest that repression of transcription at the *HM* loci effected by *SIR* gene products is accomplished by interactions with sequences outside the cassette, that is, at a site or sites at least 800 bp removed from the apparent site of transcription initiation. One likely explanation for this action at a distance is that the various *SIR* products orchestrate the nature of chromatin organization, either in its density or precise positioning, over the region of the silent cassettes. Indeed, experimental evidence indicating a difference in chromatin structure at the *HM* loci as a function of expression has recently been obtained (17).

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