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Genetics

Deletions and single base pair changes in the yeast mating type locus that prevent homothallic mating type conversions

(yeast mating type switching/*rad52* mutation)

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ABSTRACT **Several *cis*-acting mutations that prevent homothallic mating type conversions in *Saccharomyces cerevisiae* have been examined. Deletions within the mating type (*MAT*) locus were obtained by selecting for survivors among homothallic *MATα* cells carrying the *rad52* mutation. The survivors were unable to switch mating type, even in RAD$^+$ derivatives. The deletions varied in size from fewer than 50 to more than 750 base pairs. All of the deletions removed a *Hha* I site at the border between the α-specific sequences (Yα) and the adjacent Z region. We also examined several spontaneous *inc* mutations that prevent *MAT* switching. Two of these mutations were cloned in recombinant DNA plasmids and their sequences were determined. The *MATα-inc* 3-7 mutation proved to have an altered *Hha* I site at the Yα/Z border, by virtue of a single base pair substitution G·C → A·T in the second base pair of the Z region (Z_2). Restriction fragment analysis showed that two other independently isolated strains with *MATα-inc* mutations had altered the same *Hha* I site. The *MAT*a-*inc* 4-28 mutation contains a single base pair substitution C·G → T·A at position Z_6. A base pair difference at position Z_{11} in two *MAT*a strains does not affect *MAT*a conversions. We conclude that the region near the Y/Z border is essential for the efficient switching of *MAT* alleles and constitutes an enzyme recognition site for a specific nucleolytic cleavage of *MAT* DNA.**

The mating phenotype of *Saccharomyces cerevisiae* is determined by two alternative alleles at the mating type locus, *MAT*a and *MATα*. In heterothallic (*ho*) strains, mating type alleles are essentially stable, whereas in cells carrying the homothallism gene, *HO*, mating type may change as often as every cell division. The homothallic switching of mating type (*MAT*) alleles occurs by the transposition/replacement of an **a** or α sequence at *MAT* with a copy of opposite mating type information found at either of two unexpressed loci, *HMLα* and *HMR***a**, on the same chromosome (1). Heteroduplex and DNA sequence analysis has established the structure of *MAT*, *HML*, and *HMR*. *MAT***a** and *MATα* differ by the presence of **a** or α-specific sequences of 642 and 747 base pairs (bp), designated Ya and Yα, respectively (2). The α-specific (Yα) sequences are also found at *HMLα*, and Ya is also found at *HMR***a**. These unique sequences are flanked by DNA that is found at *MAT*, *HML*, and *HMR*, although the extent of homologous sequences shared between *MAT* and either *HML* and *HMR* differs. *MAT* and *HML* share 1,400 bp to the left of Y (regions designated W and X) plus 320 bp to the right of Y (designated Z1 and Z2); *MAT* and *HMR* share only the X and Z1 regions (see Fig. 2). Analysis of a variety of mating type mutations has shown that *MATα* contains two cistrons, whereas *MAT***a** contains one (3–5).

The precise mechanism by which mating type genes are transposed is not yet known. A variety of experiments have suggested that *MAT* switching occurs by an intrachromosomal recombination event that depends on pairing of sequences at *HML* or *HMR* with homologous sequences at *MAT* (6–8). Several recent experiments have suggested that initiation of mating type switching depends on sequences within and near *MAT*. For example, a number of *cis*-acting mutations that prevent efficient *MAT* conversions have been described. The mutations in one class, designated *inc* (inconvertible), lie within the transposable segments of *MAT* and are "healed" when they undergo a rare switch to the opposite mating type (9–11). The members of a second class, designated "stuck" (*stk*) mutations, are tightly linked to *MAT* but are not "healed" during the switching process (12).

Further insight into *MAT* switching comes from the study of the lethal effect of the *rad52* mutation in homothallic cells (13, 14). Cells carrying the *rad52* mutation are defective in the repair of double-strand breaks in DNA and are also defective in mitotic and meiotic gene conversion events (15–17). *rad52* cells that attempt to switch mating type suffer a lethal chromosome break at or very close to the *MAT* locus (14). The *inc* and *stk* mutations protect homothallic cells carrying the *rad52* mutation from dying (14). These data suggested that *MAT* conversions were initiated by a site-specific cut in the *MAT* locus whose recognition site may be defined by the *inc* or *stk* mutations (14). Direct evidence for such a double-strand cut has come from examination of DNA from Rad$^+$ cells that continually undergo mating type switching (18). A small proportion of DNA from these strains contains a double-strand cut near the border between **a**- or α-specific DNA sequences (Ya or Yα) and the adjacent Z region.

During the course of studying the lethality of the *rad52* mutation in homothallic strains, Weiffenbach and Haber noted that a small proportion of *MATα* cells that attempted to switch mating type did not die (14). The survivors proved to be nonmating strains that still carried *HO* and *rad52*. In this paper we demonstrate that these nonmating subclones carry deletions within the *MAT* locus that simultaneously render them unable to switch mating type and defective in the *MATα1* functions. Furthermore, we show that these deletions coincide with the location of single base pair changes in the sequences of the *inc* mutations.

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Abbreviations: bp, base pair(s); kb, kilobase(s).

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Table 1. Strains

Strain	Genotype
T1851-2D 4-28	*MAT***a**-*inc* (4-28) *HMLα HMR***a** *HO ade1 arg4 his4 leu2 lys2 trp1*
T1851-2D 3-7	*MATα-inc* (3-7) *HMLα HMR***a** *HO ade1 arg4 his4 leu2 lys2 trp1*
A393	*MATα HMLα HMR***a** *ho ade8-10 arg4-17 his4-4 met13 trp1-1 lys2 MAL2*
BW330-26A	*MATα HMLα HMR***a** *HO swi1 rad52 met13 his5 ade2 mal2*
BW539-8A	*matα1* (deletion BWd3) *HMLα HMR***a** *HO ade2 met10 met13 trp1 his5 his3 ura3*
BW541-6B	*matα1* (deletion BWd6) *HMLα HMR***a** *HO ade8 met10 met13 his3 his5 ura3*
BW542-3A	*matα1* (deletion BWd9) *HMLα HMR***a** *HO his3 his5 leu2 ura3 ade2*
U111	*MATα HMLα HMRα ho his5 lys1*
U112	*MATα HMLα HMRα ho his5*
U180	*MATα HMLα HMRα HO lys1 ade1*
U181	*MAT***a**-*inc* (4-28) *HMLα HMR***a** *cry1 HO ade1 arg4 his4 leu2 lys2 trp1*
U189	*MATα-inc* (*Saccharomyces diastaticus*) *HMLα HMR***a** *HO ade1 ura3 leu2 cry1*

MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. Genetic analysis of mutants affecting mating type switching has been described (14).

Restriction Site Analysis. DNA was isolated as described (6) and digested with restriction endonucleases (New England Biolabs). The fragments were separated by agarose gel electrophoresis and analyzed according to the method of Southern (19). Three different recombinant plasmids were used to probe Southern blots (Fig. 3A): pJH3 [3.5-kilobase (kb) *Eco*RI/*Hin*dIII *MAT***a** fragment], pJH4 (300-bp *Alu* I/*Alu* I fragment containing part of the Yα region [bp 1,546–1,845 (2)], and pCSH414 [a 400-bp fragment from an artificial *Xho* I site inserted by Tatchell *et al.* (5) into the *Hin*dIII site distal to *MAT*].

DNA Sequence Analysis. Recombinant DNA plasmids carrying *MAT-inc* mutants were constructed by inserting *Eco*RI restriction fragments of DNA from different strains into the *Eco*RI site of vector RB8. Ampicillin-resistant transformants of *Escherichia coli* strain HB101 were screened (20) for the presence of *MAT* DNA by using ^{32}P-labeled phage λ DNA containing the *Eco*RI fragment of *MAT***a**. Plasmid DNA was purified (21), digested with restriction endonuclease(s), and then labeled at the 3′ end (22). Fragments were purified by acrylamide gel electrophoresis and then their sequences were determined (23). The sequence of the region containing the Y/Z boundary in *MAT***a**-*inc* was determined from the *Bgl* II site in the 1.4-kb *Bgl* II/*Hin*dIII fragment. A 140-bp *Msp* I/*Hin*cII fragment labeled at the *Msp* I site was used to determine the sequence of the analogous region in *MATα-inc*. An additional 0.9 kb of DNA sequence was determined in *MAT***a**-*inc*, corresponding to the following nucleotides in wild-type *MAT***a** (2): 520–632, 559–648, 773–703, 1,030–900, 1,393–1,585, 2,007–1,876, and 2,007–2,154. Furthermore, the sequence of approximately 300 bases from the 0.8-kb *Xba* I/*Xba* I fragment containing DNA to the left of the W region was determined and compared with that from the wild-type *MAT***a** (C. Astell, personal communication).

RESULTS

Homothallic Switching of *rad52 swi1* Strains Generates Lesions in *MAT*. The lethality of the *rad52* mutation in homothallic strains was studied in cells that also carried a second mutation (*swi1*) that slowed down the rate of *MAT* conversions (14). When *HO rad52 swi1 MATα* cells attempted to switch, they gave rise predominantly to transiently viable "a-like" cells that did not express *bona fide MAT***a** function and apparently contained a double-strand chromosome break within the *MATα* locus (14). [The double-strand break apparently causes a disruption in the expression of the two *MATα* cistrons and, like mutations that inactivate the two cistrons, produces an a-like phenotype (4).] In addition, we also recovered a small number of viable cells that were no longer able to switch. Most of these derivatives were nonmating and no longer gave rise to **a**-like cells. We suspected that these nonmating cells might have arisen by mutations either in *MATα1* or in *MATα2*, because such mutations have been shown to make *MATα* strains nonmating or sterile (3, 4). We therefore analyzed eight independently isolated nonmating subclones from the *HO rad52 swi1 MATα* strain BW330-26A to see if they contained mutations in the *MATα* locus. We also examined one stable **a**-like subclone that was able neither to switch mating type nor to express actual *MAT***a** information.

DNA from each of the nonmating and **a**-mating subclones as well as DNA from a Rad$^+$ *MATα* strain (A393) was digested with *Hin*dIII and the Southern blot was probed with ^{32}P-labeled plasmid, pJH3, containing the *MAT***a** region. Because *MAT*, *HML*, and *HMR* contain homologous sequences, the plasmid hybridized to three different restriction fragments (1) (Fig. 1). A comparison of the *MAT* restriction fragments showed that these nonmaters did not contain a normal-sized 4.1-kb fragment containing *MATα* but had smaller *MATα*-containing fragments ranging in size between 3.3 and 4.0 kb (Fig. 1). The smallest fragment was found in the **a**-mating subclone BWd1. The sizes of the *HML* and *HMR* fragments were unchanged in all cases.

The appearance of smaller restriction fragments in place of

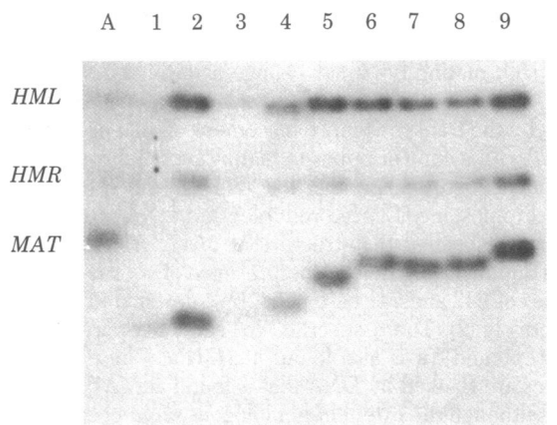

FIG. 1. Deletions in the *MAT* region in **a**-mating and nonmating derivatives of an *HO rad52 swi1 MATα* strain. DNA from Rad$^+$ strain A393 (lane A) and from nine derivatives of *HO rad52 swi1 MATα* strain BW330-26A (lanes 1–9) were digested with *Hin*dIII restriction endonuclease. The Southern blot was probed with ^{32}P-labeled plasmid pJH3, which contains the *Hin*dIII *MAT***a** fragment. The bands homologous to the probe are *HML*, *HMR*, and *MAT*. In the **a**-mating derivative, BWd1, and in the eight nonmaters, BWd2–9, the *MAT* band was absent and replaced by a smaller restriction fragment.

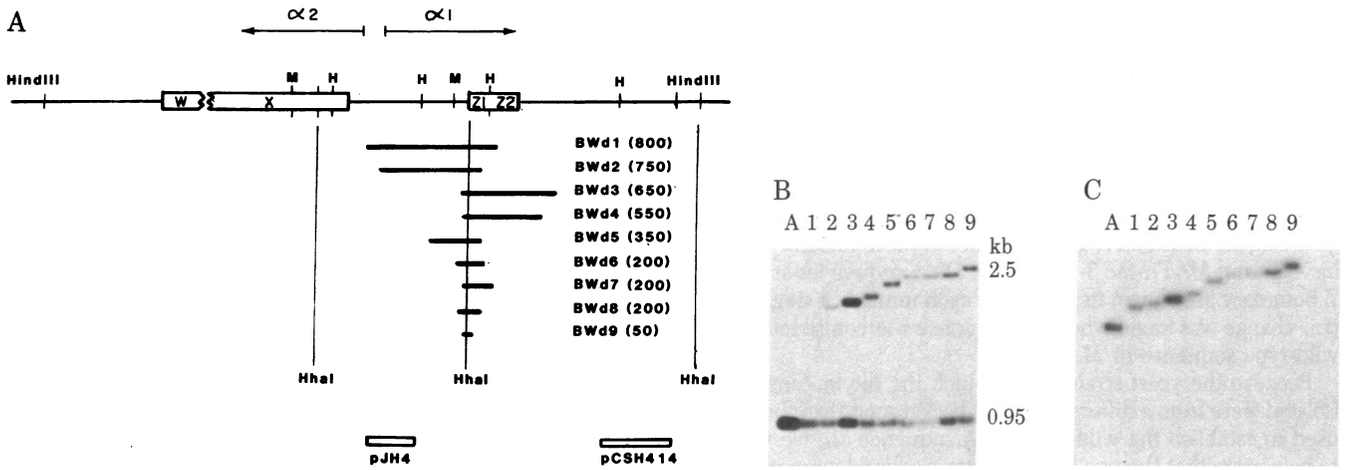

FIG. 2. Restriction site analysis of deletions within *MATα*. (*A*) Location of restriction sites within *MATα* that were used in this analysis. In addition to *Hin*dIII and *Hha* I sites, *Msp* I sites (M) and *Hae* III sites (H) were also used. The positions of the two *MATα* transcripts are also shown. Restriction digests were probed with plasmid pJH4 or plasmid pCSH414, which contain the *MATα* sequences as shown. The positions and extents (in bp) of the deletions analyzed in this paper are also shown. All of the deletions eliminated a *Hha* I site at the Y/Z border. (*B*) Southern blot of a *Hha* I digest of a wild-type strain (lane A) and nine BWd deletion strains (lanes 1–9) probed with the Yα probe, pJH4. In the wild type, both *MATα* and *HMLα* give rise to a 0.95-kb fragment that hybridizes to the probe. In the deletion strains, the 0.95-kb fragment is still present (from *HMLα*) but a new larger fragment is also found. Note that in the largest deletion, BWd1, no additional band was seen. (*C*) Same Southern blot as in *B*, except probed with the *MAT*-distal probe, pCSH414. Only the larger restriction fragment is homologous to this probe. In this case, fragments from all nine deletion strains, including BWd1, hybridize to the probe.

the normal *MATα* band suggested that the nonmating derivatives contained deletions within the *MATα* locus. This conclusion was supported by finding the same size differences in *MAT* restriction fragments when DNA was digested with *Eco*RI, which also cuts the DNA outside of the W and Z homology regions (data not shown). Furthermore, we showed that some other restriction sites within *MAT* had been lost. We have used three restriction enzymes (*Hha* I, *Hae* III, and *Msp* I) whose normal cleavage sites are shown in Fig. 2*A*. All of the nonmating strains were missing the *Hha* I site that lies very close to the border between α-specific (Yα) and common (Z) regions. Fig. 2*B* shows an autoradiograph of *Hha* I-digested DNA hybridized with an α-specific probe, pJH4, that normally hybridizes to a 0.95-kb fragment found in both *MATα* and *HMLα*. In most of the deletion strains, we found two restriction fragments, one 0.95 kb (which came from *HMLα*) and another whose size was proportional to the size of the deletions found with the *Hin*dIII digest. DNA from the strain with the largest deletion, BWd1, hybridized only to the *HMLα* band. When these same digests were probed with a *MAT*-specific probe, pCSH414, containing DNA that lies to the right of the Z region, only the larger restriction fragment hybridized (Fig. 2*C*). These results argued that the larger *Hha* I restriction fragment in these nonmating strains was formed by a deletion that (*i*) removed the *Hha* I site near the Y/Z border and (*ii*) created a new restriction fragment that contained DNA sequences complementary both to the Yα region (pJH4) and to sequences distal to the *MAT* locus (pCSH414). The fact that all of these strains contained deletions in this region also demonstrated why they were nonmating: all of them contained mutations in the *MATα1* cistron.

Similar analyses using the restriction endonucleases *Hae* III and *Msp* I have shown that the deletions do not all remove the same regions. The results of these experiments (data not shown) are summarized in Fig. 2*A*. The largest of the deletions, BWd1, which makes the strain a-like, apparently deleted not only most of the *MATα1* cistron but also extended into the *MATα2* region (and therefore did not hybridize with the Yα probe, pJH4). This deletion fragment did, however, hybridize to the *MAT*-specific probe containing sequences to the right of Z. Thus, this a-like cell is apparently mutant in both *MATα* cistrons.

Genetic Analysis of *matα1* Deletion Mutants. The deletion strains were apparently unable to switch mating type, because none of them gave rise to transiently viable a-like cells. This failure to switch mating type was further studied in Rad$^+$ derivatives, in which switching would not be lethal. For this analysis we selected rare matings between the nonmating *HO rad52 swi1 matα1* deletion strains and a Rad$^+$ *HO MAT*a-*inc* strain, U181. In all eight cases we recovered the original *matα1* deletions and showed that they could not switch even in Rad$^+$ strains. The analyses of three examples (deletions BWd6, -9, and -10) are shown in Table 2. The results of tetrad analysis, expressed as random spores, showed that there were an approximately equal number of *rad52* and *RAD52* segregants. There were also approximately equal numbers of a-mating and nonmating segregants (Table 2). Some *HO MAT*a-*inc* segregants switched very slowly to *MATα*, leading to a colony with an (a>α)-mating phenotype (10). These results are what would be expected if the diploid was heterozygous for *rad52* and heterozygous for *MAT*a-*inc* and the *matα1-inc* deletion (i.e., an approximately 1:1:1:1 ratio of *rad52 MAT*a-*inc*, *rad52 matα1* deletion, *RAD52 MAT*a-*inc*, and *RAD52 matα1* deletion). The Rad$^+$ *matα1* deletion segregants were all unable to switch mating type and therefore were unable to yield diploids able to sporulate. We concluded that these strains still contained the original *matα1* deletion and

Table 2. Mating type* of meiotic segregants from rare diploids arising from crossing nonmating *HO swi1 rad52* isolates with *MAT*a-*inc HO SWI1 RAD52* strain U181

	No. of *rad52* segregants			No. of *RAD52* segregants				
Diploid	a	α	N	a	a>α	α	α>a	N
BWd9/U181	8	0	18	14	8	0	0	17
BWd6/U181	14	0	22	21	0	0	0	23
BWd10/U181	20	1	27	23	7	0	0	21

*Nonmaters are designated N. Colonies containing mutations such as *MAT*a-*inc* that impair switching exhibit an unequal, dual mating phenotype (a>α) or an a-mating phenotype, depending on the presence of *swi1*.

were unable to switch mating type, even when they were Rad$^+$ segregants.

Sequence Analysis of Two *MAT-inc* Mutations. The observation that all of the nine nonswitching derivatives of *HO rad52 swi1 MATα* strain had deleted a common region raised the possibility that previously isolated *inc* mutations that prevented *MAT* conversion but did not alter *MAT* function might also fall in this vicinity. We tested this possibility by cloning and sequencing the *Eco*RI *MAT* regions from two *inc* mutations, *MAT***a***-inc* 4-28 and *MATα-inc* 3-7 (11). The DNA sequences at the Y/Z boundary are shown in Fig. 3. In each mutant, a single base pair change was found when the sequence was compared to the wild-type sequence of *MATα* (2).

Because the yeast strains from which the *inc* mutants were isolated were from a different genetic background than the strain used to establish the wild-type DNA sequence (2), we wished to be certain that the two base changes we found were not simply the result of random DNA polymorphisms. We therefore determined the sequence of an additional 1.2 kb from the *MAT***a***-inc* 4-28 strain. This sequence included both the W/X and X/Ya borders, as well as DNA in the W, X, and Ya regions. There was only one other polymorphism between the *MAT***a***-inc* 4-28 sequence and that of the previously determined *MAT***a** sequence. Eleven base pairs from the Y/Z border (Z_{11}) the *MAT***a***-inc* sequence has a T·A pair, just as do the *MATα-inc* and *MATα* sequences. However, the original *MAT***a** sequence (2) contained an A·T pair at this position. It appears that the T·A base pair found in *MAT***a***-inc* 4-28 is not related to the *inc* mutation, because *MAT***a** strains carrying the A·T variant are not defective in *MAT* conversion (unpublished data).

The *MATα-inc* 3-7 mutation alters the *Hha* I site at the Yα/Z border by a mutation (C-G-C-A) that does not alter the amino acid sequence of the *MATα1* gene. We have also found two other *MATα-inc* mutations by this approach. Both the *MATα-inc* 5-22 mutation derived by Oshima and Takano (11) in the same genetic background as *MATα-inc* 3-7 and a *MATα-inc* variant introduced from *S. diastaticus* (9) also have lost this *Hha* I site.

Effect of *inc* Deletions and Point Mutations on Double-Strand Breaks at *MAT*. Strathern *et al.* (18) have observed that a double-strand break within *MAT* is detected in a small proportion of the DNA from homothallic cells that continually switch mating type alleles. On a Southern blot of *Hin*dIII-digested DNA from an *HO MATα HMLα HMRα* strain, probed with a labeled pBR322 plasmid containing *MAT*, one finds two fragments, 2.9 and 1.2 kb, in addition to the more strongly hybridizing fragments containing *MATα*, *HMRα*, and *HMLα* (Fig. 4, lane 3). As shown by Strathern *et al.* (18), the position of the double-strand break lies very close to the Y/Z border. The two fragments indicative of a nucleolytic cleavage within *MAT* were not found in heterothallic (*ho*) cells of the same genotype (Fig. 4, lanes 1 and 2).

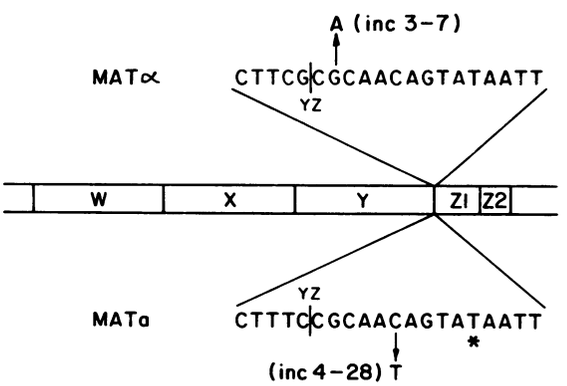

FIG. 3. Nucleotide changes in *MATα-inc* and *MAT***a***-inc* 4-28 near the Y/Z border. The two sequences were compared both with each other and with wild-type *MATα* and *MAT***a** sequences (2). An additional base pair change at position Z_{11} (*) (a T instead of an A) was found in comparing the *MAT***a***-inc* sequence with the previously determined *MAT***a** sequence. This base pair change does not affect *MAT***a** switching.

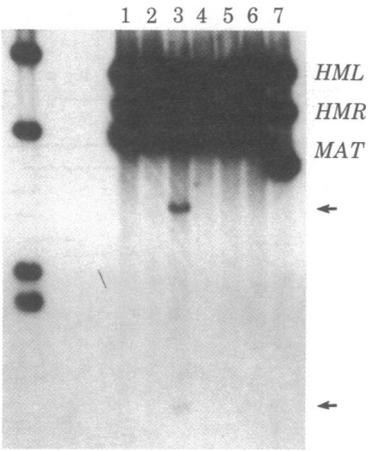

FIG. 4. Effect of *MATα-inc* point mutations and deletions on the formation of a double-strand break within *MAT*. A Southern blot of *Hin*dIII-digested DNA from homothallic and heterothallic strains was probed with ^{32}P-labeled plasmid pJH3 containing the *MAT* region. Fragments of *Hin*dIII-digested phage λ DNA were also included as size markers; bands shown on the left of the figure are at 6.4, 4.2, 2.2, and 1.8 kb. Lanes 1 and 2, *ho MATα HMLα HMRα* strains U111 and U112; lane 3, *HO MATα HMLα HMRα* strain U180; lane 4, *HO MATα-inc HMLα HMR***a** strain U189; lanes 5, 6, and 7, *HO matα1* (deletion) *HMLα HMR***a** strains BW542-3A, BW541-6B, and BW539-8A. Two restriction fragments, 2.9 and 1.2 kb, smaller than *MATα* (4.1 kb) are observed in lane 3 but are not found in DNA either from a heterothallic strain or from homothallic strains containing *inc* mutations. The autoradiogram has been overexposed to display the minor bands.

These fragments were also not found in *Rad*$^+$ *HO* cells carrying *MATα-inc* from *S. diastaticus* (9) or in Rad$^+$ *HO* derivatives of three of the *matα1* deletions, BWd3, -6, and -9 (lanes 5, 6, and 7). We conclude that both the *MATα-inc* mutation and the *rad52*-induced *matα1* deletion mutations interfered with homothallic switching at a step prior to or during nucleolytic cleavage of *MAT*.

DISCUSSION

Several lines of evidence have suggested that the initiation of *MAT* switching involves the formation of a double-strand break near the Y/Z border. In homothallic *rad52* strains that cannot repair double-strand breaks, cells that attempt to switch mating type suffer a chromosome break at the *MAT* locus (14). Mutations such as *inc* mutations that prevented *MAT* conversions also prevented *HO rad52*-induced lethality (14). The lethality of switching in *HO rad52 swi1* strains provided a strong selection for mutants that could no longer switch *MAT* and therefore survived. We have now analyzed nine such strains, all of which proved to have deletions that, though variable in size, removed a region including the *Hha* I site at the Yα/Z border. It should be pointed out that such deletions have not been found in *HO RAD52 MATα* strains that carried *swi1* (24), nor have they been found in heterothallic strains carrying *rad52* or in *HO rad52 MATα-inc* strains (unpublished data). Thus, these deletions arose in *rad52* cells attempting to switch mating type. The common sequences deleted in these different strains therefore define a site that is essential for efficient switching.

We have also found that the two *inc* mutations whose se-

quences we have determined lie in the Z region, within 7 bp of the Y/Z border. Two other *MATα-inc* mutants also exhibited the loss of the *Hha* I site spanning the Yα/Z border. These mutations both inhibit homothallic switching and prevent *HO rad52*-induced lethality.

The conclusion that homothallic switching is initiated by a double-strand break at *MAT* is also supported by several other recent observations. First, Strathern *et al.* (18) have observed a double-strand break near the Y/Z border in a small proportion of homothallic cells undergoing repeated switching events. As we showed in Fig. 4, this double-strand break is not found in DNA from *HO MATα-inc* or from several *HO matα1* deletion strains. More direct evidence for the site-specific cleavage of *MAT* DNA during switching has come from R. Kostriken, J. Strathern, A. Klar, J. Hicks, C. Moomaw, and F. Heffron (personal communication), who have identified an endonuclease in extracts of homothallic cells. This endonuclease specifically cleaves *MAT* DNA in the Z region with a 4-bp staggered cleavage 3–7 bp from the Y/Z border

C-G-C-A-A-C-A G-T-A
G-C-G T-T-G-T-C-A-T

The two *inc* mutations whose sequences we have determined alter base pairs at positions Z_2 and Z_6.

The deletions and point mutations near the Y/Z border define a site that is (*i*) required for *MAT* switching and (*ii*) at or close to the site of a double-strand cut. Apparently this region contains a recognition sequence for a site-specific enzymatic cleavage of *MAT* DNA during switching. From the sequences of the two mutations, we cannot precisely define the recognition sequence; however, it seems probable that the site includes all of the base pairs extending at least from the Y/Z border to the more distal point mutation (i.e., C-G-C-A-A-C). We also know that a change in the sequence 11 bp from the Y/Z border (Z_{11}) does not affect switching. Thus, if any single base change in a recognition site would prevent switching (as do the changes in the two *inc* mutations), we might conclude that position Z_{11} does not seem to be part of the essential recognition sequence.

The fact that *HO rad52* cells are viable if they contain an *inc* or *stk* mutation at *MAT* suggests there is no other site in the yeast genome that is efficiently cleaved in homothallic cells. Although statistically a unique recognition sequence in a genome of the size of yeast should contain about 13 bp (25), it is possible that the 10 bp from the Y/Z boundary to the polymorphic site at Z_{11} are unique. However, an attractive possibility is that the nucleotide sequence required for switching is an interrupted one with two specific sequences. The *inc* mutants would correspond to one part of the specific recognition site and the *stk* mutants to the other part.

Mechanism of *MAT* Switching. The data presented here support our suggestion (26, 27) that *MAT* conversions are initiated at *MAT* by a site-specific nucleolytic cleavage. A double-strand break can initiate this nonreciprocal recombination event with *HML* or *HMR* as the donor in a fashion similar to the more general recombination mechanism proposed by Orr-Weaver *et al.* (28). Mechanisms specific for the features of *MAT* conversions have recently been proposed (18, 27).

Formation of Deletions in *HO rad52* Strains. In *HO rad52* strains a double-strand chromosome break apparently cannot be repaired efficiently, and nearly all cells that attempt to switch *MAT* die (14). However, approximately 1% of *HO rad52 swi1 MATα* cells were recovered as *HO rad52*-resistant derivatives that had apparently repaired the double-stranded break by forming a deletion. It is interesting to note that deletions are rarely found among mutations selected at many loci in Rad^+ strains (29). Our mapping of the restriction sites of these deletions has shown that they were variable in size and that deletions of one size did not remove all the same sequences. An analysis of the DNA sequences involved in the formation of these deletions should provide insight into the mechanism by which deletions are generated in a *rad52* background. It may also be possible to use *rad52* strains to generate many deletions of various sizes at any locus.

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