Homothallic Mating Type Switching Generates Lethal Chromosome Breaks in *rad52* Strains of *Saccharomyces cerevisiae*

BARBARA WEIFFENBACH AND JAMES E. HABER*

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254

In homothallic cells of *Saccharomyces cerevisiae*, a or α mating type information at the mating type locus (*MAT*) is replaced by the transposition of the opposite mating type allele from *HMLα* or *HMRa*. The *rad52-1* mutation, which reduces mitotic and abolishes meiotic recombination, also affects homothallic switching (Malone and Esposito, Proc. Natl. Acad. Sci. U.S.A. **77**:503–507, 1980). We have found that both *HO rad52 MATa* and *HO rad52 MATα* cells die. This lethality is suppressed by mutations that substantially reduce but do not eliminate homothallic conversions. These mutations map at or near the *MAT* locus (*MATαinc*, *MATa-inc*, *MATa stk1*) or are unlinked to *MAT* (*HO-1* and *swi1*). These results suggest that the switching event itself is involved in the lethality. With the exception of *swi1*, *HO rad52* strains carrying one of the above mutations cannot convert mating type at all. *MATα rad52 HO swi1* strains apparently can switch *MATα* to *MATa*. However, when we analyzed these a maters, we found that few, if any, of them were bona fide *MATa* cells. These a-like cells were instead either deleted for part of chromosome III distal to and including *MAT* or had lost the entire third chromosome. Approximately 30% of the time, an a-like cell could be repaired to a normal *MATa* genotype if the cell was mated to a *RAD52 MATα-inc* strain. The effects of *rad52* were also studied in *mata*/*MATα-inc rad52/rad52 ho/HO* diploids. When this diploid attempted to switch *mata* to *MATa*, an unstable broken chromosome was generated in nearly every cell. These studies suggest that homothallic switching involves the formation of a double-stranded deoxyribonucleic acid break or a structure which is labile in *rad52* cells and results in a broken chromosome. We propose that the production of a double-stranded deoxyribonucleic acid break is the lethal event in *rad52 HO* cells.

In the yeast *Saccharomyces cerevisiae*, mating type is determined by the expression of one of two alternate alleles of the mating type locus, *MATa* or *MATα*, located on chromosome III. *MATa* cells conjugate readily with *MATα* cells to form nonmating sporogenous *MATa/MATα* diploids. Heterothallic strains have a stable mating type that changes from *MATa* to *MATα* or *MATα* to *MATa* only at a frequency of 10^{-6} (13). On the other hand, homothallic strains are able to convert mating type as frequently as every cell division (33). The difference between homothallic and heterothallic strains depends on a single gene, designated *HO*. The recessive *ho* allele is found in heterothallic strains. Haploid cells carrying the dominant *HO* allele will switch *MAT* alleles until a nonmating *MATa/MATα* diploid cell results from the conjugation of cells of opposite mating type. There is no switching in *MATa/MATα HO/HO* diploids.

Homothallic conversion of *MAT* alleles requires the *HML* and *HMR* genes on chromosome III (9, 24). *HML* and *HMR* each contain unexpressed α or a mating type information which can be copied and transposed to the *MAT* locus, where they replace sequences of the opposite mating type and are expressed (11) (Fig. 1). Thus, *HMLa* or *HMRa* is necessary for *MATα* cells to switch to *MATa*, and either *HMLα* or *HMRα* is necessary for *MATa* cells to be converted to *MATα*. Several types of genetic experiments have substantiated this model. Mutations that lie within *MAT* can be "healed" or lost upon mating type switching (13, 16). For example, a *mata1* mutant can be converted to *MATa*, which in turn is converted to a normal

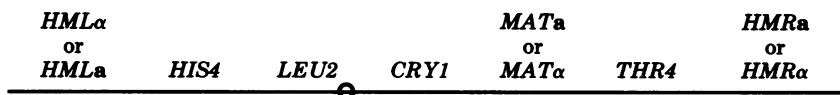| HMLα | | | | MATa | | HMRa |
| or | | | | or | | or |
| HMLa | HIS4 | LEU2 | CRY1 | MATα | THR4 | HMRα |

FIG. 1. *Map of relevant genetic markers on chromosome III (not drawn to scale). Mating type conversions occur by the replacement of an a or α allele at MAT by transposition of a copy of the opposite mating-type allele from the unexpressed HML or HMR genes (11).*

MATα allele by transposition of new *MAT* alleles from *HMLα*. On the other hand, mutations at either *HML* or *HMR* can be repeatedly introduced into *MAT* by the transposition of defective alleles of *HMLα* (15) or *HMRa* (6). These genetic studies have been corroborated by the isolation and characterization of recombinant deoxyribonucleic acid molecules containing *MAT*, *HML*, and *HMR* (12, 23, 29).

A number of components necessary for proper and efficient switching have been described. Sequences both within and immediately adjacent to *MAT* have been identified by *cis*-acting mutations that slow down homothallic switching. *MATα-inc* (32, 31) and *MATa-inc* (21) lie within the *MAT* locus and are healed after infrequent conversions to the opposite mating type. There are also sequences adjacent to *MAT* that are important. The "stuck" mutations, *stk1* and *stk2*, are very closely linked to *MAT* and reduce a to α switching (8). These two mutants are not healed and therefore lie outside of the transposable mating type sequences. A "switch" mutation (*swi1*) unlinked to *MAT* also decreases the efficiency of switching both *MATa* and *MATα* (4).

Another function required for homothallic switching is the *RAD52* gene product. Strains carrying the *rad52* mutation are defective in the repair of γ-irradiation-induced deoxyribonucleic acid damage (2) and show altered frequencies of both mitotic and meiotic recombination (3, 20, 26). Recently, Malone and Esposito (20) showed that the *rad52-1* mutation prevents homothallic *MAT* conversions. *MATa HO rad52* strains did not appear to be able to switch mating type. In the case of *MATα HO* cells, the presence of *rad52* rendered the strain inviable. The *rad52* mutation had no apparent lethal effect on *ho* strains.

We were interested to see whether mutations that reduced homothallic switching altered the survival of *HO rad52-1* cells. In the process of this investigation we found that, in contrast to the results reported by Malone and Esposito (20), *MATa HO rad52* strains are inviable. In addition, all of the mutations that decrease the frequency of homothallic switching allowed survival of *HO rad52* strains. Moreover, *MATα HO swi1 rad52* cells can apparently switch to produce a mating cells; however, most of these

contain a chromosome deletion including *MAT*. Evidence presented here suggests that the lethal event in *HO rad52* strains is the formation of a chromosome break during attempted homothallic switching.

MATERIALS AND METHODS

Strains. Strains used in this report are listed in Table 1. Strains carrying the *rad52-1* mutation were obtained from the Yeast Stock Center, Berkeley, Calif., or from R. E. Malone.

Genetic analysis. Cells were grown at 30°C on YEPD medium (1% yeast extract, 2% peptone [Difco Laboratories], 2% dextrose, and 2% agar for plates) or minimal medium (0.67% yeast nitrogen base, 2% dextrose, and 2% agar for plates). Diploids were sporulated

TABLE 1. *List of strains*

Strain[a]	Genotype	Source[b]
J164	HO ade2-1 lys2-1 trp5-20 ura1	Esposito
E8C	HO cry1 his1 his4 ade2	
Y 55-4	HO lys5 trp3 can1	
A108	MATa ho rad52-1 ade1 ade5 arg4 his5 lys7 trp3	Yeast Stock Center
M297	MATα ho rad52-1 ade2-1 lys2-1 ura3 tyr1-2 his7-1	Malone
M298	MATa ho rad52-1 ade2-1	Malone
LR203-10A	MATa ho cry1 ade2 his4 leu2 lys2 thr4	
U60	mata* ho HMLa HMRα cmt leu1 ura3 ade2	
U90	mata* ho leu1 ura3 ade2	Simchen
BW277-15C	mata* ho rad52 ura3 ade2	
U84	MATα-inc HO his4 leu2 thr4 lys5 leu1 trp3 gal	
BW193-43A	MATα-inc HO his4 thr4 lys2 ura3 rad52	
BW193-22A	MATα-inc ho his4 thr4 leu1 ura3 lys5 trpx rad52	
DW39-2A	HO-1 his1	
BW247-18B	MATa-inc HO cry1 arg4 lys2 trp3	
JPG159-9D	MATa HO swi1 ura3 lys5 his5 leu2	
BW330-15B	MATα HO swi1 rad52 lys5 his5 ade2 mal2	
BW330-24B	MATα HO swi1 rad52 lys5 met13 ade2 mal2	
WTS91-4B	MATa stk1 HO cry1 leu2 tyrX	

[a] All strains are *HMLα* and *HMRa* unless noted otherwise. Homothallic (*HO*) diploids are heterozygous for *MATa/MATα*.

[b] Strains without a listed source were constructed in this lab.

by pregrowing on YEPD plates for 2 days and then replica plating onto KAc plates (2% potassium acetate, 0.05% dextrose, 0.22% yeast extract, 2% agar, and required amino acids). Asci were digested with 10% Glusulase, and tetrads were dissected. The presence of the *rad52* mutation was detected by the inability of cells to grow on YEPD plates after exposure to 50 krad of γ-irradiation using a ^{60}Co Atomic Energy of Canada Gamma Cell 200 irradiator.

Mating type tests were performed as follows. Strains carrying at least one auxotrophic marker were replica plated to YEPD plates and cross-stamped with *MAT*a or *MAT*α tester cells carrying complementary auxotrophic markers. This plate was incubated overnight at 30°C, and prototrophic diploids were selected by replica plating to minimal medium. By this method, homothallic cells were nonmating. Strains carrying the switching mutation *swi1* showed unequal bisexual mating (4).

The presence of *HO* in Rad$^-$ segregants was assessed in the following manner. Haploid cells containing the *mata** mutation are **a** maters, but *mata**/ *MAT*α *ho* diploids are α mating and asporogenous (14). *mata**/*MAT*α *HO* diploids are capable of sporulation because they are able to switch the defective *mata** allele to a normal *MAT*a (5, 28). Thus, *MAT*α *rad52* colonies were tested for the presence of *HO* by mating to the *mata** *ho* strain, U90, and then observed for the ability of this diploid to sporulate. *MAT*a *rad52* segregants were mated to a *mata** *HML*α *HMR*α *cmt* *ho* strain, U60. The recessive *cmt* mutation permits expression of mating type information at *HML* and *HMR* (5), making U60 an α mater. This α mating strain can mate with a *MAT*a strain, forming a *mata**/ *MAT*a *cmt*/+ diploid which is a-mating. *MAT*a/ *mata** diploids are asporogenous if they contain *ho*. If *HO* is present, *mata** can be switched to a normal *MAT*α, allowing this diploid to sporulate. Thus, *MAT*a/*mata** diploids were tested for the presence of *HO* by their ability to sporulate.

The *mata** *rad52* *ho* strain, BW277-15C, was constructed by mating the *mata** strain, U90, with a *MAT*α *rad52* *ho* strain, M297. One zygotic diploid was sporulated by the method of Klar (17) and dissected to obtain an **a** mating *rad52* segregant, BW277-15C.

RESULTS

*MAT*a and *MAT*α *rad52 HO* spores are inviable.
In normal diploids constructed by crossing a heterothallic *MAT*a or *MAT*α haploid with spores of a homothallic diploid, one expects to find an equal number of homothallic (*HO*) and heterothallic (*ho*) meiotic segregants. Because both *HO MAT*a and *HO MAT*α spores will grow into nonmating colonies, we would expect 50% of all segregants to be homothallic and therefore nonmating. The remaining segregants should be 25% heterothallic **a** maters and 25% heterothallic α maters. These expectations were borne out in data collected from tetrads dissected from several control crosses (Table 2A).

The effect of *rad52* on the viability of *HO*

strains is evident among the meiotic segregants of several diploids constructed by mating *ho rad52* strains with spores of several different homothallic strains (Table 2B). Among the Rad$^+$ segregants, the ratio of **a** maters, α maters, and nonmaters was approximately 1:1:2, as expected. In contrast, there was only one nonmating Rad$^-$ segregant. All of the Rad$^-$ segregants were tested for the presence of *HO*, using tests described above. Of 90 *rad52* segregants, only 5 carried *HO*, and further analysis of one such segregant chosen at random (data not shown) revealed that it carried a new *HO* mutation which lowered homothallic conversions. The low frequency of viable *HO rad52* colonies indicated that *MAT*a *HO rad52* spores, as well as *MAT*α *HO rad52* spores, were inviable.

There also seemed to be a general lethal effect of *rad52* on both *ho* and *HO* segregants in some of these crosses, because the number of *ho rad52* colonies was less than half that of *ho RAD52* segregants. However, it is clear that the lethality is much more pronounced in *HO* segregants. To eliminate the lethality of *rad52* in *ho* cells we back-crossed *ho rad52* segregants of strains BW-209 with spores of the *HO* parent J164. (Because diploids homozygous for *rad52* yield only inviable spores [2, 25] we could not construct homozygotes for this analysis.) When these diploids were sporulated and dissected, essentially the same results as before were obtained, except that the number of *ho rad52* segregants more nearly approached the number of *ho RAD52* segregants in two of the three cases (Table 2C). Here again, it was clear that virtually all *HO rad52* segregants were dead. There were 2 *HO rad52* nonmaters, as compared to 99 *HO RAD52* nonmaters.

These results are clearly different from those of Malone and Esposito (20), who found that *MAT*a *HO rad52* strains survived as **a** maters. Since we were using the same *rad52* allele, we thought that the difference between our results and those of Malone and Esposito might be due to a difference in strain background. Two *rad52* strains sent by Malone, M297 (*MAT*α *ho rad52-1*) and M298 (*MAT*a *ho rad52-1*), were mated to spores of the homothallic strain J164 (diploids BW208 and BW221, respectively; Table 2D). Dissection of asci from BW208 gave no viable *MAT*a *HO rad52* segregants, in agreement with our previous results. In contrast, about half of the viable *MAT*a *rad52* segregants from BW221 carried *HO*.

Because strains M297 and M298 are closely related, it appeared likely that the viability of *MAT*a *HO rad52* segregants from BW221 was due to a variant closely linked to *MAT*a in the strain M298. This was confirmed by finding that

TABLE 2. *Mating phenotypes of rad52 and RAD52 spores*

Diploid[a]		Genotype	rad52 segregants[b]					RAD52 segregants with mating phenotype			Bisexual maters[c]	
			a Maters		α Maters							
			HO[d]	ho	HO	ho	N	a	α	N	rad52	RAD52
A												
BW279	BW187-28A / J164	$\frac{MAT\alpha\ ho}{MATa\ HO}$						50	43	90		
BW280	BW187-32B / J164	$\frac{MATa\ ho}{MAT\alpha\ HO}$						40	37	75		
B												
BW203	BW187-4B / E8C	$\frac{MATa\ ho\ rad52}{MAT\alpha\ HO\ +}$	1[e]	9	0	11	0	24	25	45		
BW204	BW187-6C / Y55-4	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	1	15	0	17	0	16	16	27		
BW205	BW187-6C / E8C	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	2	10	0	12	1	16	10	20		1
BW209	BW187-4B / J164	$\frac{MATa\ ho\ rad52}{MAT\alpha\ HO\ +}$	0	6	0	5	0	15	17	15		2
C												
BW215	BW209-26B / J164	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	0	27	0	22	2	20	33	53		
BW216	BW209-15C / J164	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	0	2	0	3	0	13	11	27		1
BW217	BW209-7A / J164	$\frac{MATa\ ho\ rad52}{MAT\alpha\ HO\ +}$	0	7	0	11	0	14	11	19		
D												
BW208	M297 / J164	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	0	9	0	9	0	15	9	25		1
BW221	M298 / J164	$\frac{MATa\ ho\ rad52}{MAT\alpha\ HO\ +}$	20	16	0	17	0	20	19	45	1	2
BW218	BW208-20A / J164	$\frac{MAT\alpha\ ho\ rad52}{MATa\ HO\ +}$	0	7	0	9	1	9	7	22		1

[a] The diploid number as well as the parental strains are noted.
[b] Nonmating segregants are designated by N. These presumably carry *HO*.
[c] Homothallic colonies with reduced efficiency of switching are designated as bisexual maters.
[d] The presence of *HO* was detected as described in the text.
[e] This one *MATa rad52* strain (BW203-43B) carries a mutation at *HO*.

four heterothallic or homothallic *MATa rad52* segregants from diploid B221 yielded viable *MATa HO rad52* segregants when back-crossed to J164, whereas three *MATα ho rad52* segregants from the same cross did not yield viable *HO rad52* segregants (Table 3).

Healing of the *MATa* allele from M298 which renders viable *HO rad52* strains. To see whether the variant that protected *MATa* segregants lay within the *MAT* locus, we carried out a healing experiment to determine whether a new *MATa* allele, transposed from *HMRa*,

·

TABLE 3. *Back-crosses of BW221 rad52 segregants*

Segregant	Diploid[a]	Genotype	rad52 segregants					RAD52 segregants with mating phenotype			Bisexual maters[c]	
			a Maters		α Maters		N[b]	a	α	N	rad 52	RAD 52
			HO	ho	HO	ho						
A. MATa rad52	BW228 $\dfrac{\text{BW221-3A}}{\text{J164}}$	$\dfrac{MATa \quad rad52 \quad ho}{MAT\alpha \quad + \quad HO}$	4	7	0	6	0	3	10	9		5
	BW232 $\dfrac{\text{BW221-9A}}{\text{J164}}$	$\dfrac{MATa \quad rad52 \quad ho}{MAT\alpha \quad + \quad HO}$	11	10	0	9	1	9	12	14		6
	BW234 $\dfrac{\text{BW221-5A}}{\text{J164}}$	$\dfrac{MATa \quad rad52 \quad HO}{MAT\alpha \quad + \quad HO}$	14	0	0	0	0	1	0	22		·2
	BW235 $\dfrac{\text{BW221-12A}}{\text{J164}}$	$\dfrac{MATa \quad rad52 \quad HO}{MAT\alpha \quad + \quad HO}$	16	0	0	0	0	0	0	25	1	3
B. MATα rad52	BW233 $\dfrac{\text{BW221-6B}}{\text{J164}}$	$\dfrac{MAT\alpha \quad rad52 \quad ho}{MATa \quad + \quad HO}$	0	15	0	10	0	9	10	29		
	BW237 $\dfrac{\text{BW221-21B}}{\text{J164}}$	$\dfrac{MAT\alpha \quad rad52 \quad ho}{MATa \quad + \quad HO}$	0	3	0	4	0	4	7	9		
	BW238 $\dfrac{\text{BW221-34A}}{\text{J164}}$	$\dfrac{MAT\alpha \quad rad52 \quad ho}{MATa \quad + \quad HO}$	0	13	0	3	0	6	6	9		

[a] Diploid number and their parent haploids are noted.
[b] Homothallic nonmaters are designated N.
[c] Homothallic colonies with reduced efficiency of switching are designated as bisexual maters.

was still resistant to the lethal effect of *HO* and *rad52* (Fig. 2). A nonmating colony derived from a *MATa HO RAD52* spore consists of *MATa/MATα* diploids, where the *MATα* is the result of switching *MATa* to *MATα*. These *MATα* spores were mated with LR203-10A (*MATa cryl ho*). The *cryl* mutation maps very close to *MAT*, and allows one to follow the segregation of the adjacent *MAT* locus. When the diploid was sporulated and dissected, approximately 25% of these segregants were nonmating and *CRY1*; these must have come from *MATα HO* spores which had switched to *MATa* and conjugated. We then tested these newly converted *MATa* alleles for their viability in association with *HO* and *rad52* by mating spores of the *CRY1* nonmater to M297 (*MATα ho rad52*).

The newly converted *MATa* allele is different from the *MATa* allele of M298. Only 6 of 103 a mating *rad52* meiotic segregants contain *HO* in those diploids carrying the new healed *MATa* allele (Table 4). In contrast, we previously have shown that half of the *MATa rad52* segregants from diploids in which one parent contained the *MATa* allele from M298 are homothallic (Table 2D and 3A). Clearly, this variant in M298 which renders *MATa HO rad52* cells viable lies within the transposable mating type sequences, since it can be healed.

Mutations that lie within *MAT* suppress the lethality of *HO rad52*. We investigated known mutations that decrease the efficiency of homothallic mating type conversions to see whether they would make *HO rad52* spores viable. One such mutation, *MATα-inc*, is located within *MAT* and slows down switching about 1,000-fold, so that a *MATα-inc HO* colony is α mating (31, 32). A diploid heterozygous for *MATα-inc, HO,* and *rad52* was therefore constructed for tetrad analysis (BW193, Table 5A). When the *rad52* segregants were tested for the presence of *HO* (see above), 8 of the 23 *MATα-inc rad52* segregants were *HO*, whereas none of the 16 *MATa rad52* segregants were *HO*. Furthermore, when one *MATα-inc HO rad52* segregant (BW193-23C) was mated to spores of the homothallic strain J164 (diploid BW278, Table 5A), *MATα-inc HO rad52* segregants were again obtained.

MATa-inc, like *MATα-inc*, lies within *MAT* and slows down homothallic switching but to a lesser degree than *MATα-inc*. Thus, a *MATa-inc HO* colony has an a>α phenotype (21). A diploid heterozygous for *MATa-inc, HO,* and *rad52* was constructed (BW247). When BW247 was sporulated and dissected, spore viability was very low, even among Rad⁺ segregants. To improve general viability, an a>α *RAD52* segre-

A $MAT\text{a}^{M298}$ HO spore

↓   homothallic conversion of $MAT\text{a}$ to $MAT\alpha'$ and self diploidization

$$\frac{MAT\text{a}^{M298}}{MAT\alpha'} \quad \frac{HO}{HO} \quad \text{spores} \qquad \times \qquad MAT\text{a}\ cry1\ ho\ \text{(LR203-10A)}$$

↓

B

$$\frac{MAT\text{a}}{MAT\alpha'} \quad \frac{cry1}{CRY1} \quad \frac{ho}{HO}$$

↓   sporulate and dissect

$MAT\alpha'\ CRY1\ HO$ segregant

↓   homothallic conversion of $MAT\alpha'$ to $MAT\text{a}'$ and self-diploidization

$$\frac{MAT\alpha'}{MAT\text{a}'} \quad \frac{CRY1}{CRY1} \quad \frac{HO}{HO} \quad \text{spores} \quad \times \quad MAT\alpha\ rad52\ ho\ \text{(M297)}$$

↓

C

$$\frac{MAT\alpha}{MAT\text{a}'} \quad \frac{rad52}{RAD52} \quad \frac{ho}{HO}$$

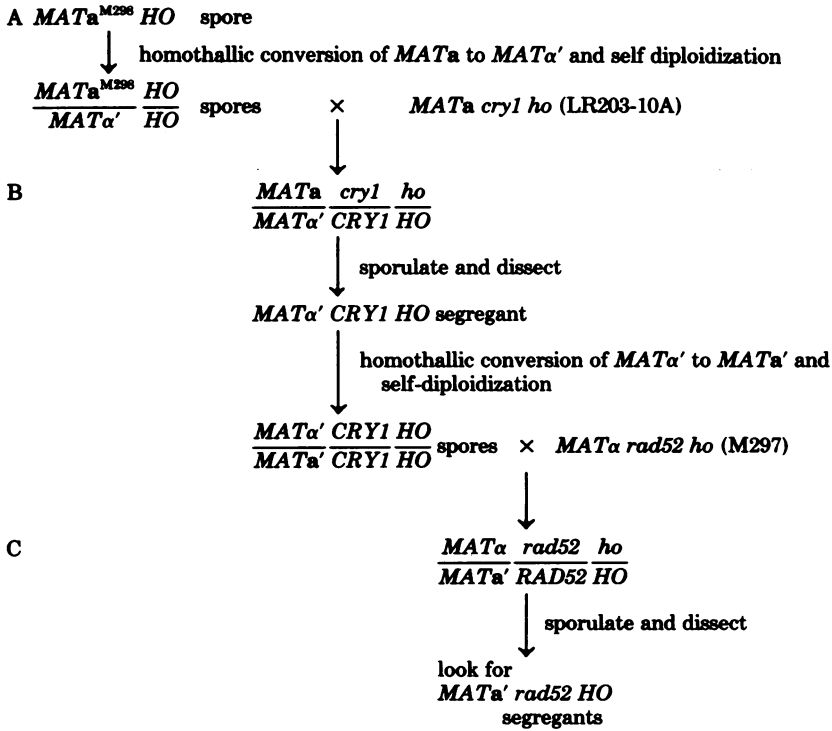↓   sporulate and dissect

look for
$MAT\text{a}'\ rad52\ HO$
segregants

FIG. 2. *Healing the MATa allele of M298. (A) A CRY1 MATa HO rad52 segregant from an outcross of M298 (BW221-10A) was mated to a cry1 MATα HO RAD52 strain to obtain colonies that arose from a CRY1 MATa HO RAD52 meiotic spore. Such segregants are nonmating, having converted the MATa allele from M298 to MATα. This newly introduced MATα is designated MATα'. MATα' spores were mated to a MATa strain (LR203-10A) which contains the cry1 allele which is closely linked to the MAT locus and was used to follow MAT. (B) The cry1 MATa/CRY1 MATα' diploid was sporulated and dissected to obtain nonmating CRY1 segregants. These should have arisen from MATα' HO spores which converted MATα' to MATa followed by conjugation. The newly introduced MATa allele is designated MATa'. These nonmating diploid segregants were sporulated and MATa' spores were mated to a MATα rad52 ho strain (M297). (C) MATα/ MATa' rad52/rad52 diploids were sporulated, dissected and analyzed for the presence of MATa' rad52 HO segregants (Table 4).*

TABLE 4. *Healing the MATa-specific defect in M298 that renders viable MATa HO rad52 cells*

Diploid[a]	rad52 segregants						RAD52 segregants with mating phenotype			
	a Maters		α Maters			Bisexual maters	a (ho)	α (ho)	N[b] (HO)	Bisexual maters
	HO	ho	HO	ho	N[b]					
BW290	2	19		33			22	20	51	
BW291		12		13			22	15	32	
BW292		18		16		3	24	18	37	
BW293	1	10		27			25	24	54	4
BW294	1	14		20			27	23	38	4
BW295	2	24	1	32	2	3	26	24	48	5

[a] These diploids are described in Fig. 2C.
[b] Homothallic nonmaters are designated N.

gant was back-crossed to M297. When tetrads from this diploid (BW250b) were analyzed (Table 5B), no a>α rad52 segregants were seen, yet about half of the MATa rad52 segregants were HO and a-mating. This indicates that MATa-

inc allows HO rad52 spores to live, but they apparently cannot switch to MATα.

Switching mutations that lie close to MAT render HO rad52 cells viable. The stk1 mutation lies very close to MAT but outside the

TABLE 5. *Mutations that decrease the efficiency of switching suppress the lethality of rad52 HO*

Diploid	Genotype	rad52 segregants — a Maters: HO	ho	a>α	rad52 segregants — α Maters: HO	ho	α>a	RAD52 segregants: a	α	N[b]	a>α	α>a
A. *MATα-inc*												
BW193 *BW187-4B / U87*	*MATa rad52 ho / MATα-inc + HO*		16		8	15		15	46	18	2	
BW278 *BW193-23C / E8C*	*MATα-inc rad52 HO / MATa + HO*	1			18				20	15		
B. *MATa-inc*												
BW250b *BW247-18B / M297*	*MATa-inc + HO / MATα rad52 ho*	7	12		1	12		14	9	6	23	4
C. *stk1*												
BW306 *WTS91-4B / BW193-43A*	*stk1 MATa + HO / + MATα-inc rad52 HO*	41			35			1	41		3	26
BW319 *WTS91-4B / BW250-4D*	*stk1 MATα + HO / + MATa-inc rad52 HO*	45	1		3		1	12			47	25
D. *HO-1*												
BW262 *DW39-2a / M297*	*MATa + HO-1 / MATα rad52 ho*	5	10		6	5	1	6	8	0	7	11

[a] Diploid numbers as well as parental strains are noted.
[b] Homothallic nonmaters are designated N.

mating type sequences excised upon switching. It reduces switching in *MATa* cells such that *stk1 MATa HO* cells are a>α (8). To determine what effect *rad52* has on *stk1 MATa HO* cells, a diploid was constructed that was heterozygous for *stk1* and *rad52* and homozygous for *HO* (BW306, Table 5C). In the presence of *stk1*, *MATa HO rad52* segregants were obtained and all were a mating. Thus, like *MATa-inc*, *stk1* allows *MATa HO rad52* spores to live, but they do not switch to *MATα*.

Unlike *MATa*, *stk1* has very little effect on the ability of *MATα* strains to switch to *MATa*, so that *stk1 MATα HO* colonies are weakly α>a or nonmating (8). The effect of *rad52* on *stk1 MATα HO* was determined by mating an a>α *stk1 HO* strain with a *MATa-inc HO rad52* strain (BW319, Table 5C). Sporulation and dissection of BW319 revealed that only 4 of 134 viable segregants were derived from *stk1 MATα HO rad52* spores. The differential survival of *stk1 MATa HO rad52* and *stk1 MATα HO rad52* strains correlates well with their switching efficiency. *stk1 MATa HO* strains, which switch slowly, are viable in the presence of *rad52* and *HO*. In contrast, *stk1 MATα HO* strains, which switch almost as efficiently as *STK1 MATα HO* strains, die in the presence of *rad52* and *HO*.

rad52 cells survive in the presence of a defective allele of HO. The *HO* mutation, *HO-1*, reduces the efficiency of homothallic conversions in *MATa* and *MATα* cells (21). Thus, *MATa HO-1* cells are a>α mating, and *MATα HO-1* cells are α>a mating. To determine whether *HO-1 rad52* cells are viable, a diploid was constructed that was *HO-1/ho* and *rad52/RAD52* (BW262, Table 5D). Out of 27 *rad52* segregants, 12 were homothallic, and 11 of the 12 homothallic segregants were a or α maters and thus appeared unable to convert mating type. *HO-1* suppressed the lethality of *rad52*, but there was no switching when both mutations were present.

swi1 suppresses the lethality of HO rad52 spores. The *swi1* mutation partially blocks the switching of both *MATa* and *MATα HO* strains. When these cells grow into colonies, they have a distinctive unequal bisexual mating type, reflecting the fact that most cells in the colony are of one haploid mating type, but a few cells of opposite mating type are continually produced (4). Thus, a *MATa HO swi1* colony has an a>α phenotype, and a *MATα HO swi1* strain is α>a. To determine whether *swi1* would alter the lethality of *rad52* segregants, a diploid was constructed which was heterozygous for *HO, swi1,*

and *rad52* (BW199, Table 6). Ten α>a *HO rad52* segregants were obtained when spores from BW199 were dissected, suggesting that *swi1* prevented the lethality of *rad52*. There were no a>α *rad52* colonies.

To facilitate a more detailed analysis, a diploid homozygous for both *HO* and *swi1* and heterozygous for *rad52* was constructed (BW222). Among the segregants of this diploid, there were essentially equal numbers of four types of segregants: (a>α) *RAD52*, (α>a) *RAD52*, a *rad52*, and (α>a) *rad52* (Table 6). We concluded that *swi1* did indeed prevent the lethality of *MATα HO rad52* strains, resulting in colonies that could apparently switch mating type as efficiently as a *MATα HO swi1 RAD52* strain. Furthermore, it seemed that *MATa HO swi1 rad52* strains were all viable but did not switch at all to *MATα*, as do *RAD52 MATα swi1 HO* cells.

It seemed paradoxical that the *swi1* mutation that slows down *MAT* conversion should not only rescue *MATα HO rad52* spores, but should allow them to switch mating type. It was possible that the a mating cells were not bona fide conversions of *MATα* to *MATa*. Several recent studies have shown that haploids deleted or defective for *MATα* become a maters even though they do not express the *MATa* functions necessary for sporulation (J. Strathern, Ph.D. thesis, University of Oregon, Eugene, 1977; J. H. McCusker and J. E. Haber, submitted for publication). Diploids resulting from conjugation of these a-like cells with *MATα* are α mating because no actual *MATa* functions are expressed.

We therefore asked whether the a maters in an α>a *HO swi1 rad52* colony were actually *MATa* or only a-like, by examining subclones. If *MATa* cells had been produced, we would expect to find both nonmaters (arising from conjugation of *MATa* and *MATα* cells within the colony) and a mating colonies (as we have shown above; *MATa HO swi1 rad52* spores grow into a mating colonies).

We examined 1,470 subclones from 12 differ-

ent *MATα HO swi1 rad52* colonies (Table 7). The results were significantly different from those found when *HO swi1 RAD52* colonies are subcloned (4), where about 30% of the colonies are nonmaters. With these subclones from α>a *HO swi1 rad52* colonies only 4 of the 12 segregants gave rise to any nonmaters. Because the relative strengths of α- and a mating in the α>a *HO swi1 rad52* colonies are identical by visual comparison to those in α>a *HO swi1 RAD52* colonies, we would have expected about 30% nonmaters. The low proportion of nonmating subclones (1.3%) suggested that not all a maters were in fact *MATa*. Among the subclones summarized in Table 7, there were also only 3 a mating colonies, all coming from 1 of the 12 α>a original segregants. Again, we would have expected an average of 1 to 2% of the colonies to be a mating, based on subclonings of *RAD52* α>a *HO swi1* colonies (4).

The nonmating subclones we obtained could not be sporulated and subjected to tetrad analysis because diploids homozygous for *rad52* produce inviable spores (2, 25). Therefore, we could not show directly that the nonmating colonies

TABLE 7. *Subclones of α>a swi1 HO rad52 segregants*

Segregant	Mating phenotype			
	α>a	α	a	N[a]
2C	72	2		14
5A	130	3		3
7A	136			
8B	44	2		
10A	185		3	
11C	123			1
15B	81	55		
17A	128			
18A	123	4		1
19C	120			
25B	48	68		
27B	124			

[a] Homothallic nonmaters are designated N.

TABLE 6. *swi1 suppresses the lethality of HO rad52*

Diploid[a]	Genotype	Mating phenotype								
		rad52 segregants				*RAD52* segregants				
		a	a>α	α	α>a	a	α	N[b]	a>α	α>a
BW199	BW197-6C *MATα rad52 ho +* / JPG-159-9D *MATa + HO swi1*	18	0	20	10	21	23	23	4	7
BW222	BW199-8B *MATa + HO swi1* / BW199-17C *MATα rad52 HO swi1*	45	0	0	48	0	0	1	42	39

[a] Diploid number as well as parental strains are noted.
[b] Homothallic nonmaters are designated N.

were indeed *MATα/MATa* diploids. From the data presented below and from subsequent experiments (Weiffenbach and Haber, manuscript in preparation), we have concluded that α>a *HO swi1 rad52* colonies contain few, if any, bona fide *MATa* cells. Recently, we have found that at least some of the nonmaters were in fact haploids carrying a deletion of part of the *MATα1* cistron (*matα1* mutants have a "sterile" nonmating phenotype [19; Strathern, Ph.D. thesis]).

Recovery of a-like cells by mating with *MATα-inc* strains. Apparently only a very small proportion of the a maters in α>a *HO swi1 rad52* colonies could be actual conversions of *MATα* to *MATa*. We have used a second approach to demonstrate that most of the a maters were only a-like, rather than *MATa*. We could "rescue" the a maters by mating cells of an α>a colony with an α mating *MATα-inc HO his4 leu2 thr4* strain, U84. Even if an a mater contained a large deletion of *MAT* and other portions of chromosome III, the resulting diploid would be at least hemizygous and therefore viable. The parental strains were allowed to mate on YEPD plates for 5 h, and diploids were then selected by spreading for single colonies on minimal media supplemented with threonine, histidine, and leucine. If the a mater was deleted for markers on chromosome III, some of the recessive markers on that chromosome (*thr4*, *leu2*, and/or *his4*) would become hemizygous, and

thus the colony would require these amino acids for growth. For this analysis, we compared the a maters in α>a *HO swi1 RAD52* colonies with those from α>a *HO swi1 rad52* colonies.

When three α>a *HO swi1 RAD52* segregants were mated to U84, 766 of 767 diploids analyzed were normal *MATa/MATα* nonmating colonies. The one exception was an α mating Thr⁻ Leu⁺ His⁺ colony which, upon subcloning, yielded only α Thr⁻ Leu⁺ His⁺ colonies. This diploid could have arisen by either a mitotic crossover event between the centromere and *MAT* or loss of the entire right arm of chromosome III distal to *MAT*. The stability of this diploid suggests that it arose by a mitotic crossover event.

In contrast, the diploids formed by mating 12 α>a *HO swi1 rad52* segregants with U84 were strikingly different from those generated by Rad⁺ strains (Table 8). Only 32% of the 811 diploids were nonmating and able to sporulate. Nearly all of the rest were α mating, asporogenous, and either hemizygous or homozygous for recessive markers on one or both arms of chromosome III.

The nonmating diploids we recovered appeared to be normal *MATa/MATα-inc* diploids, just as we had found when α>a *HO swi1 RAD52* cells were tested. When asci were dissected, we recovered some tetrads with four viable spores. There were also some tetrads with fewer viable spores, but these could be inferred to carry *MATa HO swi1 rad52* and therefore to be invi-

TABLE 8. *Classes of diploids obtained from mating α>a swi1 rad52 HO segregants of BW222R with strain U84 (HO MATα-inc his4 thr4 RAD52)*

BW222R segregant	No. of diploids in class						
	A (*HIS4* N^d *THR4*)	B^a (*HIS4* α *thr4*)	C^b (*his4* α *thr4*)	D (*HIS4* α *THR4*)	E^c (*his4* α *THR4*)	F (*HIS4* N *thr4*)	G (*his4* N *THR4*)
7A	15	8	17	2	1	1	
8A	15	15	46	3			
8B	14	23	35	6	1		
10A	118	2		1		1	
11C	12	14	25	3			
15B	6	20	6	6	1		
18A	3	7	26	3		1	
18B	15	6	20	4	2	1	
19C	8	18	36	2	1		
25B	15	18	30	2	1		
27B	26	16	36	7	1		1
27D	15	21	49	2		1	
Percent	32	21	40	5	1	0.6	0.1

ᵃ Segregants 8A, 8B, 15B, and 18B could also be tested for *leu2*, and all diploids were found to be *LEU2* except one diploid each from 8A and 8B.

ᵇ Segregants 8A, 8B, 15B, and 18B could also be tested for *leu2*, and all diploids were *leu2* except three colonies each from 8A and 18B.

ᶜ Segregants 8A, 8B, 15B, and 18B could also be tested for *leu2*, and one diploid each from 8B and 18B were *leu2*. All other diploids were *LEU2*.

ᵈ Nonmaters are designated N.

able (data not shown). Thus, we could recover actual conversions of *MAT*α to *MAT*a from an α>a *HO swi1 rad52* colony.

However, most of the diploid colonies we recovered by mating an α>a *HO swi1 rad52* colony with a *MAT*α-*inc HO RAD52* strain were not *MAT*a/*MAT*α-*inc*. Some diploids appeared to have lost all of chromosome III from the *rad52* parent (Table 8, class C). Other diploid colonies were heterozygous for markers on the left arm of chromosome III but either homozygous or hemizygous for *MAT*α-*inc* and *thr4* on the right arm (Table 8, class B). Similar types of diploids have been found among the products of rare matings between two *ho MAT*α strains (McCusker and Haber, submitted for publication). In that study, they found that diploids with the phenotype of class B were in fact unstable partial aneuploids for the right arm of chromosome III. These unstable diploids frequently lost the remaining portion of that chromosome to become *2n-1* monosomic diploids, similar to class C. We therefore wished to know if the class B diploids in this study were indeed unstable. Five α mating Thr⁻ colonies were subcloned (Table 9). Each was apparently unstable, as more than 10% of the subclones had become homozygous or hemizygous for markers on the left arm of chromosome III. Thus, a significant fraction of the a mating cells in an α>a *MAT*α *HO swi1 rad52* colony must not have been bona fide MATa haploids. Rather, they appear to have been partial aneuploids lacking some or all of chromosome III distal to and including *MAT*. These a-like cells can be rescued by mating with a *MAT*α haploid.

The same kind of partial aneuploids might also arise in *MAT*a *HO swi1 rad52* cells, except that the a-like cells would be masked by normal *MAT*a cells. Several *MAT*a *HO swi1 rad52* colonies were mated with the *MAT*α-*inc HO thr4 his4 leu2* strain, U84, to see whether any of the diploids exposed any of the three nutritional markers on chromosome III. Of 323 zygotic diploids formed, 321 were normal *MAT*a/*MAT*α

TABLE 9. *Subclones of HIS4 α thr4 diploids*

Sub-clone	No. of diploids in class				
	A (*HIS4 LEU2* α *thr4*)	B (*his4 leu2* α *thr4*)	C (*his4 leu2* N^a *thr4*)	D (*HIS4 LEU2* N^a *thr4*)	E (*his4 LEU2* α *thr4*)
2	74	10	1	1	
7	7	59			1
16	53	10			
28	19	60	1		
43	1	91			

^a Homothallic nonmaters are designated N.

colonies. There were two exceptional colonies. One diploid was α mating Thr⁻ Leu⁺ His⁺ and could have arisen by a mitotic crossover event or loss of part of the right arm of chromosome III. When this colony was subcloned, it was unstable, generating α mating Thr⁻ Leu⁻ His⁻ colonies. This colony was most likely generated by the loss of part of the right arm of chromosome III. The other diploid was α mating Thr⁻ Leu⁻ His⁻, which was more likely to have been generated by a loss of an entire chromosome III than by mitotic crossovers involving both the right and left chromosome arms. In conclusion, it appears as if *MAT*a *HO swi1 rad52* strains, like *MAT*α *HO swi1 rad52* strains, generate a-like cells. The frequency of a-like cells generated by both strains is similar. In *MAT*α *HO swi1 rad52* colonies, 1 to 10% of the cells were a maters, and two-thirds of the a maters were a-like cells. Likewise, the occurrence of a-like cells in *MAT*a *HO swi1 rad52* colonies was approximately 1%.

Repair of a-like cells to *MAT*a requires the *RAD52* gene product. The two methods used to analyze the a maters in an α>a *HO swi1 rad52* colony gave conflicting results. Subcloning showed that there are few if any viable *MAT*a cells in an α>a colony. On the other hand, the percentage of nonmating *MAT*a/*MAT*α-*inc* diploids (32%) obtained when α>a segregants were mated to a *MAT*α-*inc RAD52* strain suggested that there are a large number of *MAT*a cells in the α>a colonies. One possible explanation is that, in the mating experiment, the diploid was initially a-like/*MAT*α-*inc rad52/+ swi1/+ HO/HO*. Since the wild-type *RAD52* gene product is present in the zygote, this diploid could be converted to *MAT*a/*MAT*α-*inc*. This healing, if it occurs, should not be seen if the *MAT*α-*inc* parent is *rad52*. Thus, we mated two α>a *HO swi1 rad52* segregants (BW330-15B and BW330-24B) with a *MAT*α-*inc ho rad52 his4 thr4* strain (BW193-22A). The classes of zygotic colonies obtained are listed in Table 10. Unlike the previous experiment, there were no *MAT*a/*MAT*α-*inc* diploids. All diploids were hemizygous or homozygous for that portion of the right arm of chromosome III including *MAT* and *thr4*. Some lost the entire chromosome. Others seemed to be unstable His⁺ Thr⁻ diploids that gave rise to many His⁻ Thr⁻ mitotic segregants. These unstable diploids also occur frequently with the mating of two *MAT*α cells (8a). Thus, all of the a maters in an α>a *HO swi1 rad52* colony are a-like, but can be repaired to *MAT*a if mated to a *MAT*α-*inc RAD52* parent (Table 8).

Chromosome III breaks and losses also

TABLE 10. *Repair of a-like cells in rad52/rad52 diploids*[a]

Diploid	Genotype	No. of diploids in class			
		A (*his4* α *thr4*)	B[b] (His^+ α *thr4*)	C (His^+ α *thr4*)	D (His^+ α Thr^+)
A. BW330-24B / BW193-22A	α>a *rad52 swi1 HO* / *MATα-inc rad52* + *ho*	144	17	5	
BW330-15B / BW193-22A	α>a *rad52 swi1 HO* / *MATα-inc rad52* + *ho*	150	19	5	
Percent		87	11	2	
B. BW227-15C / BW193-43A	*mata* rad52 ho* / *MATα-inc rad52 HO*	272			1

[a] The diploids in part A were constructed by mating the a-like cells from an α>a *HO rad52 swi1* colony with an α mating *MATα-inc HO rad52* strain. The diploids in part B were isolated as zygotes formed between a *mata* ho rad52* strain and a *MATα-inc HO rad52* strain.

[b] Class B diploids were not truly $\frac{His^+}{His^+}$ but contained many papillae.

occur in *mata*/MATα-inc rad52/rad52* diploids. If chromosome III deletions and losses are the lethal events in *rad52 HO* strains, they should not only be seen in *swi1* strains but also in *SWI1 rad52* strains. To test this, we took advantage of the ability of a *mata*/MATα-inc ho/HO* diploid to convert *mata** to *MATa* at a high frequency (5). Thus, we mated a *mata* HIS4 THR4 ho rad52* strain (BW277-15C) with a *MATα-inc his4 thr4 HO rad52* strain (BW193-43A) on YEPD and selected zygotic clones on minimal media supplemented with threonine, histidine, and uracil. If the *mata** was converted to *MATa*, then one would expect to find non-mating *MATa/MATα-inc* His^+ Thr^+ diploids. If chromosome III deletions or losses accompany an attempt to convert *mata** to *MATa*, then diploids should be found which are either α mating His^+ Thr^- (if part of the right arm of chromosome III is lost) or α mating His^- Thr^- (if the entire homolog is lost). Of 274 independent zygotic colonies examined (Table 10B), 272 were α mating His^- Thr^-, one was α mating His^+ Thr^-, and one was α mating His^+ Thr^+. The α mating His^+ Thr^- colony was unstable; subcloning on nonselective media resulted in 17/81 His^- subclones. This colony appeared to have lost part of the right arm of chromosome III. Thus, no simple conversions of *mata** to *MATa* were seen. When switching was attempted, it led to the deletion or loss of the *mata** chromosome III.

DISCUSSION

We have shown that both *MATa* and *MATα* *HO rad52* spores are inviable due to a lethal event which probably occurs during the homothallic conversion process. This lethality can be suppressed by the presence of mutations that reduce the efficiency of *MAT* conversions. These include mutations within or near the *MAT* locus

itself (*MATα-inc*, *MATa-inc*, and *stk1 MATa*) as well as the unlinked *HO-1* and *swi1* mutations. These results suggest that the wild-type *RAD52* gene product is necessary at the same time or later than the steps identified by these switching mutations.

Although all of the switching mutations allowed *rad52 HO* colonies to survive, the *MATα HO swi1 rad52* cells were unique in apparently allowing switching to occur. However, the data presented here demonstrate that most of the a mating cells in an α>a mating colony of these *MATα HO rad52 swi1* cells were not bona fide *MATa* cells, but rather were a-like cells. These a-like cells appear to lack all of chromosome III or at least that part of the right arm extending from *MAT* to *THR4*. We have also shown that chromosome III losses occur in every *mata*/MATα-inc ho/HO rad52/rad52* zygotic clone (where *mata** switches readily to *MATa*).

The formation of a-like cells from *MATα* strains can occur in several possible ways. In addition to bona fide conversions of *MATα* to *MATa* by transposition, an intrachromosomal recombination event between *MATα* and the silent copy at *HMRa* will also create an a-mating cell (7, 10, 30). Such "Hawthorne deletions" are haploid lethal because of the deletion of all of the part of chromosome III between *MAT* and *HMR*; however, the a maters can be rescued by mating with a *MATα* strain. These *MAT/HMRa* fusions express functional a information and are therefore different from the a-like cells generated by *MATα HO rad52 swi1* cells described in this paper. The generation of a-like strains that are both deleted for markers to the right of *MAT* and do not express normal a functions have previously been found in studies of rare matings between heterothallic *MATα* strains (8a); McCusker and Haber, submitted for publication). In fact, more than 60% of the mat-

ings between two *ho MATα* strains occurred after one parent had become a-like by a chromosome break that removed *MATα* and the more distal part of chromosome III. In that study, we showed that such chromosome breaks occurred at or very close to the *MATα* locus. A very similar picture has also emerged from the study of a-like cells that are produced by homothallic *HMLα MATα HMRα* strains that have no copies of a information but suffer chromosome breaks at *MATα* to produce transiently viable a-like cells (Haber, unpublished data). Thus, it seems most likely that the a-like cells arising in *HO rad52 swi1 MATα* strains are also produced by such chromosome breaks. As expected, when these cells are rescued by mating with a *MATα-inc* strain, the resulting diploid contains an unstable, broken chromosome that is frequently lost.

We believe that the lethal event in *HO rad52* cells is the formation of a double-stranded deoxyribonucleic acid break near *MAT*. This must occur in virtually every cell that attempts to switch *MAT* alleles. Thus, those cells that escape the inhibition of the *swi1* mutation and attempt to switch from *MATα* to *MATa* become instead transiently viable, a-like cells by virtue of a chromosome break in which the distal part of chromosome III, including the *MAT* locus, is lost. If the a-like cells are mated to a *RAD52 MATα-inc* strain they can be repaired (or switched) to *MATa*. No such repair was found when the a-like cells were crossed with a *rad52 MATα-inc* strain. This suggests that the initial event in the formation of an a-like cell may be a single-stranded lesion or a double stranded break where the two broken ends are held in close proximity.

Role of *rad52* in homothallic mating type conversions. The *rad52* mutation reduces mitotic and abolishes meiotic recombination (3, 20, 26) as well as eliminating homothallic switching (20). This suggests that the mechanism by which the *rad52* mutation affects recombination is similar to that operating in homothallic switching (20). A body of evidence has been accumulating which supports a gene conversion mechanism for homothallic conversion.

(i) Intrachromosomal rearrangements occur in approximately 1% of homothallic *MAT* conversions. These fusions of *MAT* with *HML* or *HMR* can be understood as a recombinational event with exchange of flanking markers that occurs during *MAT* switching (7). Such recombinations are frequently found accompanying both mitotic and meiotic gene conversion events (1).

(ii) Normal *MATa* recombinants can be obtained from homothallic strains carrying a defective a allele at *MAT* and a different defective a allele at *HMR* (18). A high proportion of these conversions are accompanied by a recombination even joining the left part of *MAT* with the right part of *HMR* (Haber, manuscript in preparation).

These data favor a model involving a pairing between homologous sequences at *MAT* and *HML* or *HMR* followed by an asymmetric gene conversion which results in the replacement of sequences at *MAT* with those at *HML* or *HMR* (7). A vareity of molecular models for the sequence of events in such gene conversions have been proposed by Meselson and Radding (22) and Stahl (27).

These studies demonstrate that the *RAD52* gene product is necessary for maintaining chromosome integrity in homothallic switching. The production of a chromosome with double-stranded breaks may be a part of the switching process. Alternatively, a structure might be generated during *MAT* conversions which is labile in *rad52* strains and results in a double-stranded DNA lesion. We are investigating the possibility that the absence of meiotic recombination in strains containing the *rad52* mutation is due to a lethal event similar to that in homothallic switching.

LITERATURE CITED

1. Fogel, S., R. Mortimer, K. Lusnak, and E. Tavares. 1978. Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **38**:1325–1341.
2. Game, J., and R. Mortimer. 1974. A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24**:281–292.
3. Game, J. C., T. J. Zamb, R. J. Braun, M. Resnick, and R. M. Roth. 1980. The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94**:51–68.
4. Haber, J. E., and B. Garvick. 1977. A new gene affecting the efficiency of mating-type interconversions in homothallic strains of *Saccharomyces cerevisiae*. Genetics **87**:33–50.
5. Haber, J. E., and J. P. George. 1979. A mutation that permits the expression of normally silent copies of mating type information in *Saccharomyces cerevisiae*. Genetics **93**:13–35.
6. Haber, J. E., D. W. Mascioli, and D. T. Rogers. 1980. Illegal transpositions of yeast mating-type genes. Cell **20**:519–528.
7. Haber, J. E., D. T. Rogers, and J. H. McCusker. 1980. Homothallic conversions of yeast mating-type genes occur by intrachromosomal recombination. Cell **22**:277–289.
8. Haber, J. E., W. T. Savage, S. M. Raposa, B. Weiffenbach, and L. B. Rowe. 1980. Mutations preventing

transposition of yeast mating-type alleles. Proc. Natl. Acad. Sci. U.S.A. 77:2824–2828.

8a. Haber, J. E., B. Weiffenbach, D. T. Rogers, J. Mc-Cusker, and L. R. Rowe. 1981. Chromosomal rearrangements accompanying yeast mating type switching: evidence for a gene conversion model. Cold Spring Harbor Symp. Quant. Biol. 45:991–1002.

9. Harashima, S., Y. Nogi, and Y. Oshima. 1974. The genetic system controlling homothallism in Saccharomyces yeasts. Genetics 77:639–650.

10. Hawthorne, D. C. 1963. A deletion in yeast and its bearing on the structure of the mating type locus. Genetics 48:1727–1729.

11. Hicks, J., J. Strathern, and I. Herskowitz. 1977. The cassette model of mating-type conversion, p. 457–462. In A. J. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

12. Hicks, J., J. N. Strathern, and A. J. S. Klar. 1979. Transposable mating type genes in Saccharomyces cerevisiae. Nature (London) 282:478–483.

13. Hicks, J. B., and I. Herskowitz. 1977. Interconversion of yeast mating types. II. Restoration of mating ability to sterile mutants in homothallic and heterothallic strains. Genetics 85:373–393.

14. Kassir, Y., and G. Simchen. 1976. Regulation of mating and meiosis in yeast by the mating-type region. Genetics 82:187–206.

15. Klar, A., and S. Fogel. 1979. Activation of mating type genes by transposition in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U.S.A. 76:4539–4543.

16. Klar, A., S. Fogel, and D. N. Radin. 1979. Switching of a mating-type mutant allele in budding yeast Saccharomyces cerevisiae. Genetics 92:759–776.

17. Klar, A. J. S. 1980. Mating type functions for meiosis and sporulation in yeast act through cytoplasm. Genetics 94:597–605.

18. Klar, A. J. S., J. McIndoo, J. N. Strathern, and J. B. Hicks. 1980. Evidence for a physical interaction between the transposed and the substituted sequences during mating type gene transposition in yeast. Cell 22:291–298.

19. MacKay, V., and T. Manney. 1974. Mutations affecting sexual conjugation and related processes in Saccharomyces cerevisiae. Genetics 76:255–271.

20. Malone, R. E., and R. E. Esposito. 1980. The RAD52 gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in

yeast. Proc. Natl. Acad. Sci. U.S.A. 77:503–507.

21. Mascioli, D. W., and J. E. Haber. 1980. A cis-dominant mutation within the MATa locus of Saccharomyces cerevisiae that prevents efficient homothallic mating type switching. Genetics 94:341–360.

22. Meselson, M., and C. Radding. 1975. A general model for genetic recombination. Proc. Natl. Acad. Sci. U.S.A. 72:358–361.

23. Nasmyth, K., and K. Tatchell. 1980. The structure of transposable yeast mating type loci. Cell 19:753–764.

24. Oshima, Y., and I. Takano. 1971. Mating type in Saccharomyces. Their convertibility and homothallism. Genetics 67:327–335.

25. Prakash, L., and S. Prakash. 1977. Isolation and characterization of MMS-sensitive mutants of Saccharomyces cerevisiae. Genetics 86:33–55.

26. Prakash, S., L. Prakash, W. Burke, and B. Montelone. 1979. Effects of the RAD52 gene on recombination in Saccharomyces cerevisiae. Genetics 94:31–50.

27. Stahl, F. W. 1979. Genetic recombination: thinking about it in phage and fungi. W. H. Freeman and Co., San Francisco.

28. Strathern, J. N., L. C. Blair, and I. Herskowitz. 1979. Healing of mat mutations and control of mating type interconversion by the mating type locus in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U.S.A. 76:3425–3429.

29. Strathern, J. N., L. C. Blair, E. Spatola, C. McGill, and J. B. Hicks. 1980. Structure and organization of transposable mating type cassettes in Saccharomyces yeasts. Proc. Natl. Acad. Sci. U.S.A. 77:2839–2843.

30. Strathern, J. N., C. S. Newlon, I. Herskowitz, and J. B. Hicks. 1979. Isolation of a circular derivative of yeast chromosome III: implications for the mechanism of mating type interconversion. Cell 18:309–319.

31. Takano, I., and K. Arima. 1979. Evidence of the insensitivity of the α-inc allele to the function of the homothallic genes in Saccharomyces yeasts. Genetics 91:245–254.

32. Takano, I., T. Kusumi, and Y. Oshima. 1973. An α mating-type allele insensitive to the mutagenic action of the homothallic gene system in Saccharomyces cerevisiae. Mol. Gen. Genet. 126:19–28.

33. Takano, I., and Y. Oshima. 1970. Mutational nature of an allele-specific conversion of the mating type by the homothallic gene HOα in Saccharomyces. Genetics 65:421–427.