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Genetics

Mutations preventing transpositions of yeast mating type alleles

(homothallism/yeast mating type conversion/*Saccharomyces cerevisiae*)

JAMES E. HABER, WALTER T. SAVAGE, SUSAN M. RAPOSA, BARBARA WEIFFENBACH, AND LUCY B. ROWE

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

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ABSTRACT **Homothallic strains of *Saccharomyces cerevisiae* can switch from one mating type to the other as often as every cell division. The conversion of mating type alleles (from *MAT*a to *MAT*α or *vice versa*) depends on other, unexpressed copies of a or α information that can be transposed to *MAT*. Previously, "inconvertible" mutations within *MAT*α and *MAT*a have been described that block the excision of the *MAT* allele. In this paper we describe two *cis*-acting mutations that also impair mating type switching and lie very near, but outside, the *MAT* locus. Both "stuck" mutations, *stk1* and *stk2*, diminish the efficiency of converting *MAT*a to *MAT*α to less than 10% of normal. The *stk1* mutation also slightly reduces conversion of *MAT*α to *MAT*a, whereas *stk2* has no discernible effect. Unlike the inconvertible *MAT*α-*inc* and *MAT*a-*inc* mutations within *MAT*, the *stk* mutations are not replaced by wild-type sequences after the "stuck" cells occasionally switch to the opposite mating type. Because these mutations are not "healed" by mating type conversions, they must lie in sequences outside of the transposable mating type information. These results indicate that the efficient replacement of *MAT* alleles depends on sequences both within and adjacent to the *MAT* locus. Among subclones of homothallic *stk MAT*a strains, approximately 2% show "illegal" transpositions of mating type genes. In these colonies the silent copy of α information at the *HML*α locus has been converted to a, without any change of *MAT*a or the silent a copy at *HMR*a. Such conversions of the unexpressed library genes are not found in wild-type homothallic strains that can switch mating type efficiently, but they are found in *MAT*a-*inc* and *MAT*α-*inc* strains. It appears that all of the *cis*-acting mutations within or adjacent to mating type result in these unusual switches of mating type information at *HML* and *HMR*.**

In the yeast *Saccharomyces cerevisiae* there is a complex genetic system that governs the stability of a mating type (*MAT*) allele and its switching to opposite mating type. In heterothallic strains, mating type alleles are essentially stable, with conversion of one mating type to the other occurring at a frequency of about 1 in 10^6 (1). In homothallic strains, however, the frequency of mating type switching—from *MAT*a to *MAT*α or vice versa—is so frequent that a colony derived from a single haploid cell rapidly becomes composed only of nonmating *MAT*a/*MAT*α diploid cells resulting from the conjugation of a and α cells within the colony (2, 3). The ability of a homothallic haploid cell to switch mating type almost every generation depends on the presence of an allele of a master gene, *HO*, that is dominant to the *ho* allele found in heterothallic strains.

From the experiments of Oshima, Takano, and their coworkers (2, 4, 5) it became clear that the ability of *MAT*a and *MAT*α cells to switch mating type also depended on alleles of two other genes, now designated *HML* and *HMR*. Oshima and Takano proposed that the conversion of one mating type to the other involved the transposition of a genetic element from one of the *HM* loci to *MAT* (4). This proposal was extended by Hicks *et al.* (6) in their "cassette" model. In this model, mating type conversions involve the replacement of the allele at *MAT* with a copy of opposite mating information that is transposed from unexpressed "library" genes, located at *HML* and *HMR* (Fig. 1). Most strains of *S. cerevisiae* carry silent α information at *HML* and a information at *HMR*, but variants carrying *HML*a and *HMR*α have also been found (5). The existence of silent copies made it possible to explain the so-called "healing" of mutants at *MAT*, in which, for example, a *mat*α1 mutant could switch to a normal *MAT*a allele and then to a normal *MAT*α allele (1). The transposition of α information from *HML*α or a information from *HMR*a must occur without deleting or modifying the library copy, because the genetic behavior of *HML*α and *HMR*a is unaffected by the switching process (2, 4).

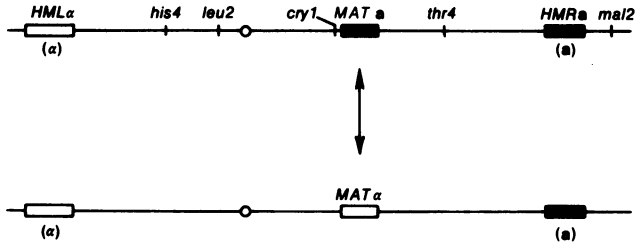

FIG. 1. Mating type conversions in *S. cerevisiae*. According to the cassette model of Hicks *et al.* (6) the mating type locus (*MAT*) on chromosome III can be switched from *MAT*a to *MAT*α by the transposition of a copy of opposite mating type information stored at a distant, unexpressed gene *HML*α. Similarly, *MAT*α can be converted to *MAT*a by the transposition of a information from the unexpressed locus *HMR*a. The positions of several other loci on chromosome III are also indicated. The distance between *MAT* and *cry1* is about 3 centimorgans and between *MAT* and *thr4*, about 20 centimorgans.

The cassette model has been confirmed in a variety of ways, including the isolation of DNA sequences containing *MAT*, *HML* or *HMR* (7, 8). These studies have shown that a and α information, whether at *MAT* or at the *HM* genes, differ in size and contain an approximately 800-base-pair nonhomologous region. The switching of *MAT*α to *MAT*a is accompanied by a physical change in the size of the *MAT* fragment. These studies have also shown that the nonhomologous a- or α-specific regions are flanked by two DNA sequences that are homologous at *MAT*, *HML*, and *HMR*.

The transposition of mating type information depends on other loci besides *HO* and the two *HM* genes. One "switch" mutant that reduced the efficiency of mating type conversions to about 5% of the normal value has been described by Haber and Garvik (9). In addition there are sequences within the *MAT* allele itself that play an important role in switching *MAT*. *cis*-Dominant mutations that prevent the replacement of *MAT*

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Abbreviation: YEPD, 1% yeast extract/2% peptone/2% dextrose.

have been described for both *MAT*α (10, 11) and *MAT***a** (12). These *MAT*α-*inc* and *MAT***a**-*inc* mutations can be shown to lie within the *MAT* locus because these mutations can be "healed." For example, the *MAT*α-*inc* allele can be switched at a low frequency to a normal *MAT***a** allele, which in turn will readily switch to a normal *MAT*α allele (10), as expected if first a normal copy of **a** and then a normal copy of α is transposed from the library genes to replace the defective *MAT* allele (6). Similarly, we have demonstrated the healing of *MAT***a**-*inc* (12).

In this paper we describe two other *cis*-acting mutations that also block mating type conversions, especially from *MAT***a** to *MAT*α. These mutations lie extremely close to *MAT*, but *outside* of the sequences that can be replaced by transposition. Here, a "stuck" *MAT***a** can occasionally be switched to *MAT*α, which in turn can be switched to a stuck *MAT***a**, and so on, without ever being healed. Thus, the replacement of *MAT* alleles depends on DNA sequences outside of the sequences that are transposed.

MATERIALS AND METHODS

Strains. Homothallic (*HO*) and heterothallic (*ho*) strains used frequently in this study are listed in Table 1. Strain N-90-6A was obtained from I. Takano. In addition, we examined a set of UV-sensitive strains (13): JG2, JG3, JG4, JG5, JG6, JG10, JG11, JG12, JG14, JG16, JG19, and JG22, carrying the mutations *rad2*, *rad3*, *rad4*, etc., respectively. These strains were obtained from the Yeast Stock Center (Berkeley, CA).

For the dominance tests of the "stuck" mutants we used the α mating strain U60. This strain has the genotype *mat***a*** *HML*α *HMR*α *cmt* and includes the recessive *mat***a*** allele (14) and the recessive *cmt* mutation that permits the expression of the normally silent copies of mating type at *HML* and *HMR* (15). Because both *HML*α and *HMR*α carry α information and *mat***a*** is recessive, this strain mates as an α, but the resulting diploid, *MAT***a**/*mat***a*** *CMT*/*cmt*, is a-mating and unable to sporulate. In the presence of *HO*, this *MAT***a**/*mat***a*** strain can undergo mating type conversions of one or both *MAT* alleles to *MAT*α (12) and will contain nonmating diploid cells able to sporulate.

Table 1. Strains

Strain	Genotype
Heterothallic†	
JG19	*ho MAT*α *rad19-1 ade2 gal*
A48	*ho MAT*α *ade2 leu2 ura3*
A49	*ho MAT***a** *leu2 his2 his4*
Homothallic‡	
Y55-4	*HO trp3 lys5 can1*
WS34	*HO stk1 ade2 lys2 lys5 tyr1*
DB5-2B	*HO stk2 cry1 leu1 tyr7*
WS36-2D	*HO stk1 lys5 tyr1*
WS64	*HO stk1 cry1 ade2 his1 his4 tyr1*
WS91-4B	*HO stk1 cry1 leu2 tyr1*
N-90-6A	*HO MAT*α*-inc ade1 his4 leu2*
JH201	*HO MAT*α*-inc stk1 cry1 his4 ade1*
SR113-10C	*HO stk2 his4 leu1 his1*
SR124-41D	*HO stk1 cry1 thr4 leu1 his4*
LR112-3A	*HO cry1 thr4 ade1 leu1*
DW202-2C	*HO his4 leu2 thr4*
DW297-3D	*HO MAT*α*-inc trp3 leu2 his4 lysx*
JH209-2B	*HO MAT*α*-inc stk2 HML*α*-inc HMR***a** *cry1 thr4 leu2 ade1*

† All *ho* strains carried *HML*α and *HMR***a**.

‡ Unless otherwise noted, all *HO* strains contained *MAT***a**/*MAT*α diploids homozygous for all markers indicated. All strains carried *HML*α and *HMR***a**, unless otherwise designated. Both *MAT***a** and *MAT*α haploids could be obtained from these strains either by mating (**a**>α) or (α>**a**) colonies or by matings of spores.

Genetic Analysis. Cells were grown, sporulated, and dissected by using standard methods (9, 12, 15). Cryptopleurine resistance was tested on 1% yeast extract/2% peptone/2% dextrose (YEPD) plates containing cryptopleurine (Chemsea, Sydney, Australia) at 0.4 μg/ml.

Isolation of "Stuck" Mutants. Normal homothallic haploid spores grow into nonmating diploid colonies, whereas spores carrying mutations that block homothallic conversions give rise to colonies containing many haploid cells of one mating type and a few cells of opposite mating type (9, 12). Thus a colony displaying a distinctly asymmetric dual mating type—either (**a**>α) or (α>**a**)—is indicative of the presence of a mutation preventing normal conversions of *MAT*. Two "stuck" (*stk*) mutants were found in different searches for mutants blocked in efficient switching of *MAT* alleles.

The *stk1* mutant was discovered during a screening of a number of radiation-sensitive yeast strains that were being examined on the assumption that defects in DNA repair might also affect homothallic switching. Each heterothallic radiation-sensitive strain was crossed with spores of the normal homothallic strain Y55-4 and meiotic segregants were then examined to see if there were dual mating phenotypes characteristic of mutations affecting switching. Only in the case of strain JG19 (*rad19*) were there segregants with unusual mating behavior; among 15 colonies there were 4 weak (α>**a**) colonies, as well as 4 nonmaters, 3 **a**-maters, and 4 α-maters. The (α>**a**) phenotype was not linked to *rad19*, because 2 of the 4 (α>**a**) colonies had normal radiation sensitivity. The (α>**a**) colonies contained nonmating cells that could be sporulated and dissected. Each tetrad contained 2 weak (α>**a**) colonies and 2 much more pronounced (**a**>α) segregants. One of the (**a**>α) segregants was again backcrossed with Y55-4 and an (**a**>α) segregant, designated JH19/55-5D, was selected for further genetic analysis.

The *stk2* mutant was found by selecting cells that had not become nonmating diploids after homothallic spores were germinated and grown overnight to allow nearly all cells to become nonmating (9). Cells of the homothallic diploid strain JPG80-6D were treated with UV light at 2 J/m^2. After growth, the cells were sporulated and digested with Glusulase (Endo Laboratories, New York) to produce single spores (15). Spores were spread on YEPD plates at a density of about 10^4 cells per plate, grown overnight, and replica plated to a fresh YEPD plate that had been spread with a lawn of spores of a second homothallic strain, JH77-18C. After overnight growth, matings between JPG80-6D cells that had not become nonmaters and JH77-18C cells were selected on minimal medium plates supplemented with tryptophan. These diploids were then sporulated and dissected to see if any segregated colonies defective in homothallic switching. Among three diploids analyzed, one yielded two nonmating and two (**a**>α) colonies per tetrad. One (**a**>α) segregant, designated DB5-2B, was chosen for subsequent analysis.

RESULTS

When normal homothallic spores germinate and grow, the frequency of mating type switching is sufficient to produce a colony of nonmating *MAT***a**/*MAT*α cells. Mutations that block efficient switching result in mosaic colonies, containing many haploid cells of the initial mating type, a few cells of opposite mating type, and some nonmating cells (9, 11). We have found two independently isolated "stuck" mutants that gave rise to (**a**>α) colonies characteristic of *MAT***a** cells unable to switch

mating type efficiently. The degree to which these strains could still switch from *MAT***a** to *MAT*α could be assessed by examining subclones of (**a**>α) colonies (8). Cells that had not yet switched from *MAT***a** again gave rise to (**a**>α) colonies, whereas cells that had switched to *MAT*α might have mated or subsequently switched to become nonmating (*MAT***a**/*MAT*α) diploids. From the data in Table 2, it is clear that the majority of cells in (**a**>α) colonies of both *stk1* and *stk2* strains had not switched. From the frequency of nonmaters, it appears that *stk1* strains switch 10–20% as efficiently as a normal strain, whereas *stk2* *MAT***a** strains switch about 20–30% as efficiently.

The nonmating subclones derived from the (**a**>α) colonies must be homozygous at all loci except *MAT*. Thus, by sporulating and dissecting those diploids we could examine the effect of the *stk* mutations on the switching of both *MAT***a** and *MAT*α spores. When *stk1* diploids were dissected, each tetrad contained two (**a**>α) and two weaker (α>**a**) colonies. When subcloned, the (α>**a**) colonies gave rise mostly to nonmaters, consistent with the conclusion that *stk1* blocks switching of *MAT***a** much more than the switching of *MAT*α. With *stk2* nonmaters, each tetrad contained two (**a**>α) and two nonmating segregants. Thus, the *stk2* mutation appears to affect switching of *MAT***a** spores without any discernible effect on *MAT*α.

Are the "Stuck" Mutations Healed? Especially in the case of *stk2* strains, in which there was no effect on *MAT*α switching, it was possible that the mutation was within the *MAT***a** locus—as is *MAT***a**-*inc* (11)—and would be healed in switching from *MAT***a** to *MAT*α and then back to a normal *MAT***a**. However, this was not the case for either *stk1* or *stk2*. Both the weak (α>**a**) *stk1* colonies and the nonmating *stk2* segregants (derived from *MAT*α spores) contained nonmating *MAT*α/*MAT***a** diploid cells in which the *MAT***a** allele was produced by a switch from *MAT*α. These were new *MAT***a** alleles copied from *HMR***a**. When such nonmating colonies were sporulated and dissected, the (**a**>α) phenotype reappeared. In the case of *stk1* diploids, each tetrad contained two (**a**>α) diploids and two weak (α>**a**) colonies, whereas the *stk2* diploids again gave rise to two (**a**>α) and two nonmating colonies. Thus, the (**a**>α) phenotype was not healed by successive conversions of *stk* *MAT***a** to *stk* *MAT*α to *stk* *MAT***a**.

As a further demonstration that these mutations were not healed, we followed *stk1* and *stk2* through a series of crosses with heterothallic *MAT***a** and *MAT*α strains. A schematic illustration of these experiments is shown in Fig. 2*A*, and is contrasted with a simlar experiment demonstrating the healing of a *MAT***a**-*inc* mutation (Fig. 2*B*). For example, when the a-mating cells from a weakly (α>**a**)-mating *stk1* segregant, WS34, were mated with the *ho* *MAT*α strain A48, the (**a**>α) phenotype appeared in 15/60 meiotic segregants. In contrast, when α-maters in the same (α>**a**) colony, WS34, were crossed

Table 2. Mating phenotype of subclones of (**a**>α) colonies from homothallic strains carrying *stk1* or *stk2*

Strain	No. of subclones (**a**>α)	Non-mating	**a**
WS36-2D (*stk1*)	114	20	4
WS86-7B (*stk1*)	155	40	10
SR113-10C (*stk2*)	55	37	8
LR112-3A (*stk2*)	140	57	3
DW202-2C†	0	100	0

† One hundred spores of the normal *HO* strain were germinated and tested for mating type, to illustrate the normally very efficient switching of *MAT* alleles.

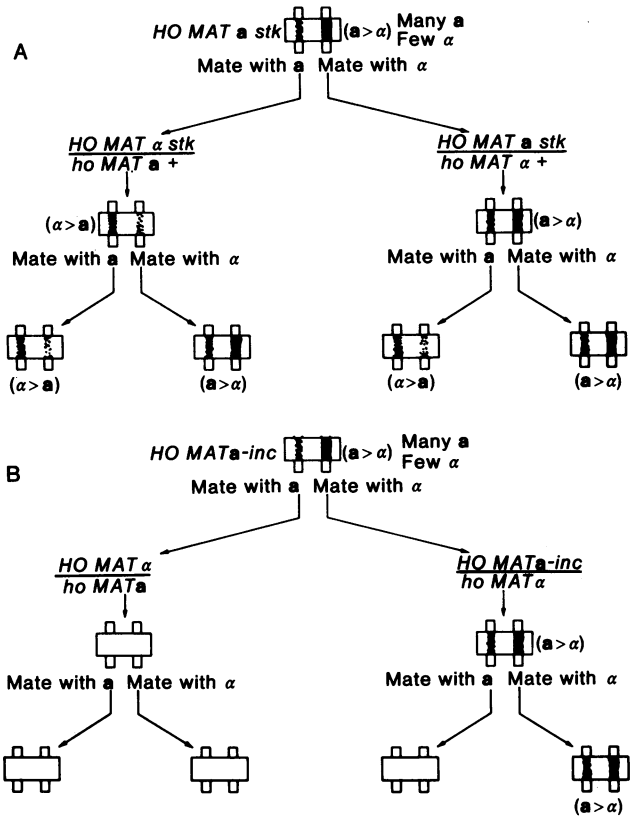

FIG. 2. (*A*) Inheritance of (**a**>α) and (α>**a**) mating phenotypes of *stk1* strains crossed with *ho* *MAT*α or *ho* *MAT***a** strains. An (**a**>α) *stk1* *MAT***a** *HO* colony (depicted by a complementation assay for mating type) (8) was mated with heterothallic *MAT*α and *MAT***a** strains. When the new diploids were dissected, homothallic segregants displayed an (**a**>α) or weaker (α>**a**) colony, respectively. This same procedure was followed for another generation. (*B*) Healing of a *MAT***a**-*inc* mutation (12). An (**a**>α) colony of a *MAT***a**-*inc* *HO* strain was mated with heterothallic *MAT***a** and *MAT*α strains. When the new diploids were sporulated and dissected, the diploid containing the original *MAT***a**-*inc* allele gave rise to (**a**>α) colonies, whereas the other diploid [containing a *MAT*α allele from the original (**a**>α) colony] gave rise to a nonmating colony. The same procedure was followed for another generation, by mating spores of the nonmating colony as well as the (**a**>α) colony. The (**a**>α) phenotype was inherited only by strains inheriting the original *MAT***a**-*inc* allele.

with an *ho* *MAT***a** strain, A49, none of the segregants exhibited (**a**>α) mating, but 12/64 segregants were weak (α>**a**)-maters. This same pattern was reproduced in two further rounds of matings: whenever (**a**>α) or (α>**a**) colonies were crossed with *MAT***a** cells, only the (α>**a**) phenotype appeared. These results suggested that *stk1* must be closely linked to *MAT* and that either the (**a**>α) or the (α>**a**) phenotype will appear, depending on the construction of a diploid by mating with either *MAT*α or *MAT***a**. Unlike *MAT***a**-*inc*, which lies within *MAT*, the *stk1* mutant is not healed.

Virtually identical results were obtained when the (**a**>α) *stk2* *MAT***a** strain was crossed with heterothallic *MAT***a** and *MAT*α strains. Whenever an (**a**>α) colony was mated with a *MAT*α strain, all of the *HO* *MAT***a** segregants showed an (**a**>α) phenotype, but whenever the same colony was mated with a *MAT*α strain, all homothallic segregants were nonmating. However, the (**a**>α) phenotype could be recovered from half of the nonmating colonies (i.e., from those carrying *stk2*) upon further dissection. In these cases, there were two (**a**>α) and two nonmating colonies per tetrad. Here again, *stk2* must be closely linked to *MAT*.

***stk* Mutations Are Closely Linked to MAT.** To assess the linkage between the *stk* mutations and *MAT* we constructed strains heterozygous for *cryl* and *thr4*, which flank the *MAT* locus. We crossed the (a>α) strain WS91-4B (*MAT*a *stk1 cryl HO*) with the α-mating *MAT*α-*inc thr4 HO* strain N-90-6A. If *stk1* were very close to *MAT*a, nearly every tetrad would contain two (a>α)- and two α-mating colonies (because *MAT*α-*inc* cannot switch). A recombination event separating *MAT*a from *stk1* would yield a nonmating colony. This approach was complicated by the fact that, about 5% of the time, a *stk1 MAT*a spore colony switches efficiently enough to grow into a nonmating colony. However, if a nonmater of this sort is sporulated and dissected, the (a>α) phenotype reappears. Among 105 tetrads analyzed, none contained a *bona fide* recombinant.

To avoid the problem of false nonmaters we constructed a strain in which *stk1* was linked to *MAT*α-*inc* and then looked for recombinants in which *stk1* was recombined adjacent to *MAT*a to give an (a>α) colony. Even though *stk1* was very close to *MAT*, we could easily obtain the *stk1 MAT*α-*inc* strain by transposing an α-*inc* allele from *HML*α-*inc* to replace *MAT*a adjacent to *stk1* (16–18). The resulting *MAT*α-*inc stk1 cryl HO* strain JH201 was mated with *MAT*a *thr4 HO* spores of strain DW202-2C. Among 69 tetrads examined all but one contained two α and two nonmaters, the parental ditype. The remaining tetrad consisted of two α, one nonmater, and one (a>α) colony. Here, the (a>α) colony must have arisen by recombination to form a *stk1 MAT*a spore. When sporulated and dissected this (a>α) colony gave rise to two (a>α) and two very weak (α>a) colonies per tetrad. Crosses with heterothallic *MAT*a and *MAT*α strains confirmed that the (a>α) phenotype was very closely linked to *MAT*. This (a>α) segregant, JH203-5A, was both *cryl* and *thr4*, whereas the parental *MAT*a strain was *CRY1 thr4*. Hence the crossover between *stk1* and *MAT*a also recombined *cryl*. The *cryl* locus recombined away from *MAT*α-*inc* in 7 of 69 tetrads. Thus, we could place *stk1* between *cryl* and *MAT*, less than 1 centimorgan from *MAT*.

A similar analysis was carried out with *stk2* by crossing *MAT*a *stk2 cryl thr4 HO* cells of strain SR124-41D with the *MAT*α-*inc HO* strain DDW297-3D. Among 111 tetrads, there were 21 containing two α, one (a>α), and one nonmater; however, all but two of these resulted from poor penetrance. Both of the *bona fide STK MAT*a recombinants were still *cryl* and *thr4*, the parental configuration of outside markers. To avoid the problems of penetrance, we also constructed a *stk2 MAT*α-*inc HO cryl thr4* strain (JH209-2A) and crossed it with *MAT*a *HO* spores of strain JH302-3D. From 143 tetrads we found 5 (a>α) recombinants, but in all five cases, they had the parental configuration of flanking markers (i.e., they were cryptopleurine sensitive and prototrophic for threonine). There were, however, reciprocal recombinations separating *cryl* and *MAT* in 7 of 143 cases, and 54 of 143 tetrads were recombinant for *MAT* and *thr4*. Here again it seems that all of the recombinants arose without reciprocal recombination of flanking markers. Thus we can conclude that *stk2* lies very close to *MAT*, but we cannot assign it a position on one side or the other.

***STK* Mutations Are *cis*-Dominant.** Diploid strains homozygous for *MAT*a do not sporulate; however, in the presence of *HO*, a *MAT*a/*MAT*a diploid can readily substitute mating type alleles to form *MAT*a/*MAT*α cells that can sporulate. Similarly, a *MAT*a/*mat*a* diploid (*mat*a* is a recessive *MAT*a allele) cannot sporulate (14, 15). Under the action of *HO*, a *MAT*a/*mat*a* cell can switch either *MAT*a or *mat*a* to *MAT*α and generate nonmating sporulating cells of several genotypes, including *MAT*a/*MAT*α diploids and *MAT*a/*mat*a*/*MAT*α/*mat*a* tetraploids (14, 15). We could ask if *stk* mutants prevented switching of an adjacent *MAT*a allele without affecting the switching of *mat* a*.

We therefore constructed *cryl stk1 MAT*a/*CRY1 STK1 mat*a* HO/ho* diploids by crossing the *stk1* strain WS64 with the *mat*a* strain U60. Forty zygotes were isolated, allowed to grow into colonies, and sporulated. By examining subclones of each of the 40 colonies, we found that each colony consisted almost entirely of nonmating cells, able to sporulate. Thus, the presence of the *stk1* mutation did not prevent high frequency of homothallic switching to convert the initial *MAT*a/*mat*a* cells into a nonmating diploid or tetraploid. From each colony, cryptopleurine-resistant spores were isolated by germinating sporulated colonies on YEPD plates containing cryptopleurine, and then the colonies were tested for mating type. In all 40 cases, the heterothallic *cryl* segregants were a-mating and homothallic *cryl* cells grew into (a>α) colonies. Thus, the *MAT* locus adjacent to *cryl* and *stk1* had not switched from *MAT*a to *MAT*α. If *stk1* were recessive and not *cis*-acting, we would have expected the *MAT*a allele to switch to *MAT*α about half of the time, as we have in similar dominance tests previously observed for *MAT*a/*mat*a* diploids not carrying a *stk* mutation (12). *stk1* did not prevent the switching of *mat*a* to *MAT*α but did prevent the conversion of the adjacent *MAT*a allele.

Identical results were found for equivalent diploids carrying *stk2*. This mutation, too, appears to be *cis*-acting.

Secondary Mutations Induced by *stk* Mutations. When homothallic *MAT*a *HMR*a *HML*α strains carrying *stk1* or *stk2* were subcloned, about 1% of the subclones were only a-mating instead of (a>α) (Table 2). In contrast to *stk MAT*a cells that can switch inefficiently to *MAT*α, these a-maters were apparently completely unable to switch from *MAT*a to *MAT*α. For example, when these colonies were transferred to sporulation plates, there was no sporulation (<0.1%), whereas the parent *stk MAT*a (a>α) colonies sporulated at least 10%. When these a-mating colonies were crossed with normal *HO MAT*a *HML*α *HMR*a spores, and the resulting diploids were dissected, every tetrad contained two nonmating colonies and two segregants that were either (a>α)- or a-mating (Table 3). Because about half of the *stk MAT*a segregants were (a>α) and the other half only a-mating, we concluded that the a-only phenotype segregated independently of *MAT*a and *stk*. By subsequent crosses, with *HO MAT*α *HML*a *HMR*a spores and with *HO MAT*α *HML*α *HMR*α cells, we could show that the second mutation mapped at *HML* and was actually a conversion of *HML*α to *HML*a. For example, by crossing an a-mating *MAT*a *stk HML*$^-$ *HMR*a *HO* strain with a *MAT*α *HML*α *HMR*a *HO* strain, we could recover *MAT*α *HO* segregants carrying the mutant *HML*$^-$ and *HMR*α. All such segregants could switch to *MAT*a and form nonmating colonies (data not

Table 3. Segregation of a mutation preventing *MAT*a strains from switching mating type

Diploid†	No. of diploids of tetrad mating type: a a nm nm	a (a>α) nm nm	(a>α) (a>α) nm nm
WS91-104/JH159	18	37	10
SR113-10C/DDW202-2C	10	20	7

† nm, Nonmating. An a-mating subclone from the *stk1* strain WS91-104 and another from the *stk2* strain SR113-10C were crossed with spores of wild-type *HO HML*α *HMR*a strains JH159 or DDW202-2C.

shown). Thus, the *HML*$^-$ mutant must have been able to provide a functional copy of **a** information, and the *HML*$^-$ mutations were conversions of *HMLα* to *HMR***a**.

DISCUSSION

We have described two *cis*-acting *stk* mutations that block homothallic switching, especially of *MAT***a** to *MATα*. One of the *stk* mutations also causes a diminished efficiency in converting *MATα* to *MAT***a**. These results, coupled with our characterization of *MAT***a**-*inc* (12) and the study of *MATα*-*inc* by Takano *et al.* (10, 11) lead to the conclusion that the efficient replacement of mating type alleles depends on DNA sequences both within and adjacent to *MAT*.

Differences in Switching *MAT*a and *MATα*. We do not yet understand why *stk1* and *stk2* affect the conversion of *MAT***a** to *MATα* so much more than switching *MATα* to *MAT***a**. It may mean that the sequences used in the excision and replacement of *MAT***a** are not identical with those used to remove *MATα*. These differences are not confined to the *stk* mutants. For example, we have previously described a defective allele of *HO* that blocks the conversion of *MATα* more than of *MAT***a** (12). In addition, recent work by Malone and Esposito (19) has shown that the DNA repair mutation *rad52* is lethal in *MATα HO* strains but has less effect on *MAT***a** *HO* cells.

Recent heteroduplex analysis of cloned DNA segments of *MAT*, *HML*, and *HMR* by Nasmyth and Tatchell (8) has suggested a possible physical basis for these differences. Each copy of mating-type information at *MAT*, *HML*, or *HMR* is flanked on each side by sequences homologous at all three loci; however, *HML* and *MAT* share additional homologous sequences not found at *HMR*. It is possible that the *stk* mutations lie in the sequences near *MAT* shared by *HML* and not by *HMR*. Consequently, switching of *MAT***a** from *HMLα* might be affected without any effect on transpositions from *HMR***a** to replace *MATα*.

Relationship of MAT Switching to Transpositions in Bacteria. In some ways, the conversions of *MAT* are reminiscent of the highly specific integration and excision of phage λ in *Escherichia coli* (20). By that analogy, the *stk* mutations might be similar to *attB* mutations, whereas *MAT***a**-*inc* and *MATα*-*inc* might act like *attP* mutations (21).

However, it may be more useful to consider the transpositions of transposable elements in bacteria. A growing body of evidence suggests that these sequences can be replicated and transposed to a new site without excision of the original sequence (22, 23). This seems to be an important aspect of the *S. cerevisiae* system, too, because conversions of *MAT* normally occur without change or loss of the copy at *HMLα* or *HMR***a** (5). There is also evidence that at least some bacterial transposons display a marked site specificity for insertion (24), so that a common mechanism might account for both bacterial transposition and the conversions of yeast mating types. Mating type conversions could involve a pairing of homologous sequences adjacent to or within *MAT* and *HMLα* and *HMR***a**, followed by a nonreciprocal gene conversion in which the *MAT* locus would be replaced by a copy from *HMLα* or *HMR***a**. In this model, the *stk* mutants might prevent proper pairing of *MAT* and *HML* or *HMR*, or they might alter a site necessary to remove the *MAT* locus.

"Illegal" Transpositions of Mating Type Genes. All of the *cis*-acting mutations, both within and adjacent to *MAT*, give rise to conversions of silent copies of mating type allele at *HML* and *HMR*. In wild-type homothallic strains, these silent copies are quite stable. For example, in one study, the *HMLα* allele was not switched to *HML***a** in any of 39,000 spores (25). In contrast, 2% of the subclones of *HO MAT***a** strains carrying *stk1* or *stk2* had been converted from *HMLα* to *HML***a**, and more than 1% of subclones of *HO MAT***a**-*inc* strains had been converted from *HMLα* to *HML***a**-*inc* (12). Similarly, about 1% of *MATα*-*inc* subclones became changed from *HMR***a** to *HMRα*-*inc* (25). Thus all of the mutations interfere with the normal transposition process to cause "illegal" conversion of *HML* and *HMR*. Thus, any model of mating type conversion must account not only for the normal unidirectional transfer of new alleles from *HML* or *HMR* to *MAT* but also for these "illegal" events when normal switching is impaired.

In summary, we have described two mutations that lie in sequences adjacent to *MAT* but are not healed by transpositions. These mutations identify one or more regions that are essential for the efficient excision and replacement of mating type alleles, especially the conversion of *MAT***a** to *MATα*.

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1. Hicks, J. B. & Herskowitz, I. (1977) *Genetics* **85**, 12–47.
2. Takano, I. & Oshima, Y. (1970) *Genetics* **65**, 421–427.
3. Hicks, J. B. & Herskowitz, I. (1976) *Genetics* **83**, 245–258.
4. Oshima, Y. & Takano, I. (1971) *Genetics* **67**, 327–335.
5. Harashima, S., Nogi, Y. & Oshima, Y. (1974) *Genetics* **77**, 639–650.
6. Hicks, J., Strathern, J. & Herskowitz, I. (1977) in *DNA Insertion Elements, Plasmids and Episomes*, eds. Bukhari, A. I., Shapiro, J. A. & Adhya, S. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 457–462.
7. Hicks, J., Strathern, J. & Klar, A. J. S. (1979) *Nature (London)* **282**, 478–483.
8. Nasmyth, K. & Tatchell, K. (1980) *Cell* **19**, 753–774.
9. Haber, J. E. & Garvik, B. (1977) *Genetics* **87**, 33–50.
10. Takano, I., Kusumi, T. & Oshima, Y. (1973) *Mol. Gen. Genet.* **126**, 19–28.
11. Takano, I. & Arima, K. (1979) *Genetics* **91**, 245–254.
12. Mascioli, D. W. & Haber, J. E. (1980) *Genetics*, in press.
13. Game, J. & Cox, B. (1971) *Mutat. Res.* **12**, 328–331.
14. Kassir, Y. & Simchen, G. (1976) *Genetics* **82**, 187–206.
15. Haber, J. E. & George, J. P. (1979) *Genetics* **93**, 13–35.
16. Sherman, F., Fink, G. R. & Lukins, H. B. (1970) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Haber, J. E. & Mascioli, D. W. (1979) *J. Supramol. Struct. Suppl.* **3**, 57.
18. Haber, J. E., Mascioli, D. W. & Rogers, D. T. (1980) *Cell*, in press.
19. Malone, R. E. & Esposito, R. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 503–507.
20. Campbell, A. (1962) *Adv. Genet.* **11**, 101–145.
21. Shulman, M. & Gottesman, M. (1973) *J. Mol. Biol.* **81**, 461–482.
22. Lungquist, E. & Bukhari, A. I. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3143–3147.
23. Shapiro, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1933–1937.
24. Kleckner, N., Steele, D., Reichardt, K. & Botstein, D. (1979) *Genetics* **92**, 1023–1040.
25. Herskowitz, I., Blair, L., Forbes, D., Hicks, J., Kassir, Y., Kushner, P., Rine, J., Sprague, G., Jr. & Strathern, J. (1980) in *Molecular Genetics of Development*, eds. Loomis, W. & Leighton, T. (Academic, New York), in press.