EVIDENCE OF CHROMOSOMAL BREAKS NEAR THE MATING-TYPE LOCUS OF *SACCHAROMYCES CEREVISIAE* THAT ACCOMPANY *MAT*α × *MAT*α MATINGS

JOHN H. McCUSKER AND JAMES E. HABER

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

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ABSTRACT

In order for two heterothallic *MAT*α haploids of *Saccharomyces cerevisiae* to mate, one parent must apparently become, at least transiently, an **a**-like cell. Only about 25% of the matings result from an actual transposition of *MAT***a** sequences to replace *MAT*α, and about 1% result from a deletion joining *MAT* to the normally silent *HMR***a** allele. The majority of matings occur after an apparent chromosome break that deletes *MAT*α and all of the known markers more distal on the right arm of chromosome *III*.——The chromosome break occurs at or very near *MAT*, invariably leaving the distal marker *tsm1* hemizygous, but the closely linked proximal marker *cry1* usually is heterozygous. The resulting diploid containing the broken chromosome is mitotically unstable; about 10% of the colonies contain visible sectors in which the rest of the broken chromosome is lost. The region close to the breakpoint (*i.e., cry1*) is unusually active in recombination. About 20% of the intact homologues remaining after chromosome loss were gene-converted for *cry1*. In addition, the broken end participated in reciprocal recombination events that joined the chromosome to the distal portion of the intact homologous chromosome.——The unstable diploids may also become stable and no longer give rise to mitotic segregants. We have found two distinct ways in which stabilization occurs. Most often the diploid becomes euploid by a recombination event that yields a cell homozygous for all markers distal to (and sometimes including) *cry1*. In one of 9 cases so far analyzed, the stable diploid was still hemizygous for *MAT*α and for other markers distal to *MAT*. This last case is similar to the healing of broken chromosomes in maize described by McClintock (1939, 1941, 1951).

DIPLOID strains of the yeast *Saccharomyces cerevisiae* are mitotically stable; they exhibit spontaneous mitotic recombination at frequencies near 10^{-4} and chromosome loss at frequencies of less than 10^{-5} (Liras *et al.* 1978). However, both aneuploid strains and diploids carrying certain mutations display a significant degree of mitotic instability. For example, haploid strains disomic for one chromosome spontaneously lose one of the homologous chromosomes at a frequency greater than 10^{-4} (Campbell, Fogel and Lusnak 1975). Mutations such as *chl1* (Haber 1974; Liras *et al.* 1978) or several cell division cycle (*cdc*) mutations (Kawasaki, personal communication) promote chromosome loss in diploids as frequently as 10^{-2} for some chromosomes.

In all of the cases studied, there seems to be an intimate relationship between chromosome loss and an increase in mitotic recombination events. For example, CAMPBELL, FOGEL and LUSNAK (1975) showed that when chromosome loss occurred in an $n + 1$ haploid disomic for chromosome *III*, nearly 10% of the resulting euploids had undergone an intragenic recombination event at the *leu2* locus. Conversely, if spontaneous intragenic *LEU2* recombinants were selected in these $n + 1$ strains, about 1% had lost one copy of chromosome *III* (CAMPBELL and FOGEL 1977). These same events also occur in diploid strains heteroallelic at the *leu2* and *his4* loci on chromosome *III* (MCCUSKER and HABER, unpublished). Here, nearly 1% of the independently isolated *LEU2* or *HIS4* prototrophic colonies also became $2n - 1$ monosomic diploids.

The relation between recombination and chromosome loss is also found in diploids homozygous for the chromosome-loss mutant, *chl1*. This mutation promotes nonrandom loss of 10 of the 17 chromosomes in *S. cerevisiae*, at frequencies ranging from 5×10^{-2} to 5×10^{-4} (HABER 1974; LIRAS *et al.* 1978; and unpublished). This mutation also increases the frequency of mitotic recombination on all linkage groups. As with chromosome loss in the $n + 1$ disomes, chromosome loss in *chl1* diploids is frequently accompanied by a recombination event. About 10% of the time, the monosomic chromosome remaining after chromosome loss has undergone a recombination event and acquired one or more markers from the lost homologue (LIRAS *et al.* 1978). In none of these studies, however, has it been possible to determine whether recombination must precede chromosome loss or results from some event after a chromosome becomes unstable.

As an alternative way of studying chromosome loss, we have investigated the products of rare matings between two haploid *MAT*α strains. Normally, two strains of the same mating type do not mate, but rare conjugations can be selected by using several auxotrophic markers in both strains. Prototrophic diploids appear at a frequency of about 10^{-6} (HAWTHORNE 1963; STRATHERN 1977; HICKS and HERSKOWITZ 1977). It seems that, for two *MAT*α strains to mate, one haploid must (as least transiently) become an **a**-mater so that normal conjugation between an **a** and an α mater is possible. This can occur in several ways. First, one *MAT*α haploid can be changed to *MAT***a** by the transposition of an **a** allele from the unexpressed "library" gene at *HMR***a** (HICKS and HERSKOWITZ, 1977; HICKS, STRATHERN and KLAR 1979). The conjugation of this *MAT***a** cell with a *MAT*α cell leads to a nonmating, prototrophic diploid. A second type of conversion, noted by HAWTHORNE (1963), has been shown to be a deletion between *MAT* and *HMR***a** (STRATHERN *et al.* 1980; HABER, ROGERS and MCCUSKER 1980). The MAT-*HMR***a** fusion produces an **a**-mating cell carrying a lethal deletion that can, however, be rescued by mating with a *MAT*α haploid. These "HAWTHORNE deletion" strains are nonmating, but hemizygous for markers in the *MAT-HMR* interval.

A third way in which a *MAT*α cell may become an **a**-mater has been deduced from an analysis of the *MAT*α locus itself. Mutations that inactivate both *MAT*α cistrons convert the cells into recessive **a** maters (STRATHERN 1977; HERSKOWITZ *et al.* 1980). Thus, deletions of *MAT*α along with some or all of chromosome *III*

could result in an **a** mater. Indeed, STRATHERN (1977) found diploids arising from *MATα* × *MATα* matings in which one chromosome *III* appeared to contain a deletion of *MATα* and the nearby *CRY1* locus. Similarly, a *MATα* haploid that had entirely lost its chromosome *III* might become an **a** mater and survive long enough to be rescued by conjugation with another *MATα* cell. This possibility appeared to be borne out in our preliminary experiments, where we found that a large proportion of the diploids formed by mating two *MATα* strains were apparently $2n$-1 diploids, monosomic for chromosome *III*. We therefore decided to analyze such matings in detail in order to examine how chromosome loss had occurred.

We used *MATα* strains that were well marked on both sides of the centromere of chromosome *III* (Figure 1). The strains carried a number of other complementary markers to select diploids without selecting against any of the markers on chromosome *III*. In some of the matings, one *MATα* parent carried the *chl1* mutation, to see if this mutation would either increase loss of chromosome *III* or cause other chromosomal rearrangements that would allow a haploid *MATα* cell to mate with another *MATα* cell.

MATERIALS AND METHODS

Strains: Representative strains used in these experiments are listed in Table 1. In some experiments, we used a number of different strains of the same genotypes as those shown in order to reduce the effects of genetic background. These strains were constructed from a variety of haploids, many of which were obtained from the Yeast Stock Center, Berkeley. The *tsm1* and *tsm5* mutations were provided by G. R. FINK.

Genetic analysis: Strains were grown on YEPD (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) plates or on minimal medium (0.67% yeast nitrogen base without amino acids, 2%

TABLE 1

*Strains**

Strain	Genotype
JM76-46b	*his4C-864 cdc10-1 cry1 MATα tsm5 mal2 ade1 ade2 lys5 lys9 trp5*
JM76-49c	*his4C-864 cdc10-1 cry1 MATα tsm5 mal2 ade1 lys5 lys9 trp5*
JM78-26a	*his4B-331 leu2-3 MATα thr4 MAL2 ade1 his7 lys2 tyr1 trp1*
JM78-37a	*his4B-331 leu2-3 MATα thr4 MAL2 ade1 lys2 tyr1 trp1*
JM307-146c	*his4B-331 leu2-2 MATα tsm1 thr4 MAL2 ade1 lys2 lys9 trp1*
JHM65-10a	*his4B-331 leu2-1 MATα thr4 ade2 lys5 trp1 ura3 chl1*
JHM65-48a	*his4B-331 leu2-1 MATα thr4 ade2 lys5 trp1 ura3 CHL1*
JHM66-12a	*his4C-864 MATα tsm5 ade1 ade2 lys9 his1 trp5 chl1*
JHM66-16b	*his4C-864 MATα tsm5 ade1 ade2 arg4 lys2 lys9 trp5 CHL1*
JHM72-14b	*his4B-331 leu2-1 MAT***a** *thr4 ade1 ade2 lys2 met13 chl1*
J132	*his4C-864 MAT***a** *tsm5 mal2 ade2 lys9 trp5* *his4C-864 MAT***a** *tsm5 mal2 ade2 lys9 trp5*
J101	*leu2 MAT***a** *ade1* *leu2 MAT***a** *ade1*
J122	*his4B-331 leu2-3 MAT***a** *thr4 MAL2 trp1* *his4B-331 leu2-3 MAT***a** *thr4 MAL2 trp1*

* In most experiments, a set of strains with essentially identical genotypes to those shown here was used to reduce strain-specific effects on mating.

dextrose) supplemented with amino acids or nucleic acid bases as required. Unless otherwise specified, cells were grown at 25° because several of the markers (*cdc10, tsm1* and *tsm5*) are temperature sensitive.

Diploids heterozygous for *his4B/his4C* do not require histidine for growth. Histidine-requiring strains were determined to be *his4B* or *his4C* by complementation testing.

Matings between *MATα* strains were selected by complementation of markers not on chromosome *III* (*ade1, ade2, ura1, lys2, lys9, trp1, trp5, tyr1, tyr7* and *arg4*). Thus, matings were selected at 25° on minimal medium plates supplemented with histidine, leucine, threonine (and adenine or uracil, as the diploids were also homozygous for one other marker).

Genetic analysis of tetraploids was carried out as described by LIRAS *et al.* (1978).

Cryptopleurine resistance was scored on YEPD plates containing 3 μg/ml cryptopleurine (Chemsea Mfg. Pty., Ltd.). The cryptopleurine-resistance mutation, *cry1*, is recessive at this concentration, but exhibits a dosage effect: diploid strains monosomic for chromosome *III* and hemizygous for *cry1* appear to be as sensitive to the antibiotic as *cry1/CRY1* heterozygous diploids (MEADE, RILEY and MANNEY 1977). The presence of *cry1* in *cry1/CRY1* heterozygotes or *cry1* hemizygotes could be scored by the appearance of papillae on YEPD-cryptopleurine plates. In the case of the *cry1/CRY1*, these resistant cells appear to arise by mitotic crossing over to yield homozygous *cry1* cells. In the case of the $2n$-1 cells, resistant cells appear to have undergone an endoduplication of the aneuploid chromosome to become euploid (LIRAS *et al.* 1978).

RESULTS

To study what kinds of events permit the mating of two MATα strains, we used haploid strains that were well marked along the length of chromosome *III*. A map of this chromosome is shown in Figure 1. Two haploid *MATα* strains, one carrying *his4B leu2 MATα thr4 MAL2* and the other carrying *his4C cdc10 cry1 MATα tsm5 mal2* were mixed at a cell density of about 10^7 cells per YEPD plate and grown for two days at 25°. We selected rare matings between the two heterothallic *MATα* strains by replica-plating the cells at 25° to medium that was supplemented with nutrients for all chromosome *III* auxotrophic markers. The selection of diploids required complementation of at least five auxotrophic markers on other chromosomes. In this way, we could isolate diploids without selective

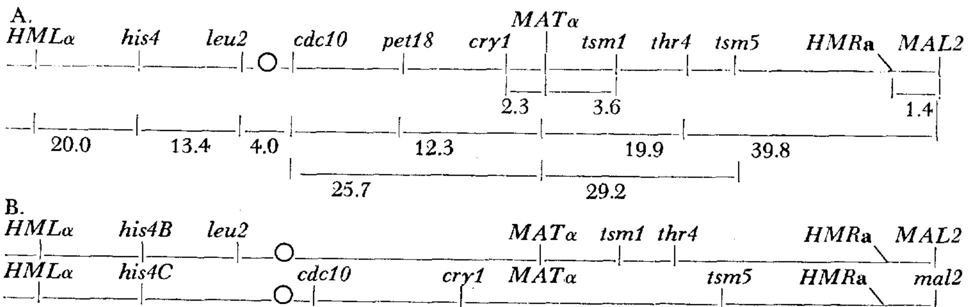

FIGURE 1.—(A) A map of the markers on chromosome *III* used in these experiments. The meiotic map distances (in centiMorgans) were determined from data collected in our laboratory during construction of strains and from MORTIMER and SCHILD (1980). The position of *cdc10* was assigned to the right side of the centromere on the basis of the work of CLARKE and CARBON (1980). (B) Configuration of markers on two chromosome *III* homologues used in the *MATα* × *MATα* matings. Not all markers were used in each experiment.

pressure on any chromosome *III* marker. Although the exact frequency of matings cannot be ascertained from this replica-plating method of colony selection, the number of colonies arising per plate (about 50–200) corresponds approximately to a frequency of 10^{-6}, similar to the results of HAWTHORNE (1963) for *MAT*α × *MAT*α matings. To minimize the contribution of genetic background of any one strain on the proportion of different kinds of diploids that might result, 16 different parents were used in 26 different pairwise crosses. A total of 2921 colonies were examined (Table 2).

About 43% of the colonies were prototrophic for all markers on chromosome *III*. Approximately 26% of all events were apparently *MAT***a**/*MAT*α nonmating diploids resulting from the conversion of *MAT*α to *MAT***a**. As expected, when diploids from class A were sporulated and dissected, there were 2 **a** and 2 α maters in each tetrad. The other prototrophic diploids recovered from MATα × MATα matings (Class B) were α mating, presumably resulting from the direct fusion of two *MAT*α cells or, alternatively, when one *MAT*α allele was inactivated or deleted. To see if the α maters were homozygous or hemizygous for *MAT*α, three such colonies were mated with a *MAT***a**/*MAT***a** diploid (J132) to produce a tetraploid that could be sporulated for tetrad analysis (Table 3). From a normal *MAT*α/*MAT*α/*MAT***a**/*MAT***a** tetraploid, we would expect to find primarily

TABLE 2

Phenotypes of different class of diploids arising from MATα × MATα *matings**

Class	Mating type	Growth phenotype *his4C*	*his4B*	*leu2*	*cdc10*	*thr4*	*tsm5*	*MAL2*	Number of colonies
A	nm	+	+	+	+	+	+	+	859
B	α	+	+	+	+	+	+	+	408
C	nm	+	+	+	+	—	+	+	7
D	nm	+	+	+	+	+	—	+	17
E	α	+	—	—	+	—	+	+	493
F	α	—	+	+	—	+	—	—	195
G	α	+	+	+	+	—	+	+	557†
H	α	+	+	+	+	+	—	—	263
I	α	+	—	+	+	—	+	+	39
J	α	—	+	+	+	+	—	—	22
K	α	+	—	+	+	+	—	—	2‡
L	α	+	+	+	—	+	—	—	2
M	α	—	+	+	+	—	+	+	12
N	α	+	+	—	+	+	—	—	4
O	α	+	+	—	+	—	+	+	11
P	α	—	+	+	—	—	+	+	13
Q	α	+	—	—	+	+	—	—	11
R	α	+	—	—	—	+	—	—	3
S	α	—	+	—	+	—	+	+	3

* Twenty-six previous crosses between heterothallic haploid strains, including JM76–46b and JM78–26a, were performed.

† Fifty-two of the 557 colonies were sectored, with half the colony expressing *his4B* and/or *leu2*.

‡ One of the 2 colonies was sectored, with half expressing *leu2*.

TABLE 3

Segregation of mating type in tetraploids where one parent is either hemizygous or homozygous for MATα*

α-mating parent in tetraploid		Mating phenotypes in tetrad† 4N 0a 0α	2N 1a 1α	0N 2a 2α	1N 2a 1α	2N 2a 0α	0N 3a 1α
A. Class B	#4	20	18	2			
	#52	6	10	4			
	#63	6	5	1			
B. Class E	#1	4	3	4	19	10	7
	#33			3	10	8	
	#88		1	3	6	6	
C. Class P	#1	3	5	2	3	1	3
	#44	3	2	1	1	3	
	#52	7	10	4			
Class Q	#1	5	8	1	1		

* Representatives of α-mating diploids of various phenotypic classes (Table 2) were crossed with *MAT*a/*MAT*a diploid strains J122 or J132.

† Nonmating segregants are designated by N.

three types of tetrads: those with 4 nonmaters; those with 2 nonmaters, 1 **a**, and 1 α mater; and those with 2 **a** and two α maters. All three colonies gave rise to tetrads whose mating types indicated that the α-mating diploids of class B did indeed contain two copies of *MATα* (Table 3A). These conclusions are also borne out by the segregation of *MAL2* and *TSM5* in these tetraploids. In all three cases, the wild-type alleles (on opposite chromosome *III* homologues) segregated 2+:2−, as expected if both wild-type alleles were heterozygous in the Class B diploids.

Among the diploids formed by mating two *MATα* strains were a small number (1%) of apparent "HAWTHORNE deletions," *i.e.*, non-maters that exposed either the recessive marker *thr4* or *tsm5* (Classes C and D). Because the class D diploids were still able to ferment maltose, the deletion did not extend to *MAL2* (HAWTHORNE, 1963; STRATHERN *et al.* 1980; HABER, ROGERS and MCCUSKER 1980). Furthermore, when these Class D nonmaters were sporulated, all of the viable spores were α mating and nearly all were *mal2*, as expected if the new *MAT*a locus was associated with a recessive lethal deletion that extended from *MAT* to *HMR* (data not shown). Southern blotting analysis that confirmed that one chromosome *III* contains a *MAT/HMR*a fusion was previously presented for one class C and one class D strain isolated in this study (HABER, ROGERS and and MCCUSKER 1980). Furthermore, when these Class D nonmaters were sporulated, all of the viable spores were α mating and nearly all were *mal2*, as expected if the new *MAT*a locus was associated with a recessive lethal deletion that extended from *MAT* to *HMR* (data not shown). Southern blotting analysis that confirmed that one chromosome *III* contains a *MAT/HMR*a fusion was previously presented for one class C and one class D strain isolated in this study (HABER, ROGERS and MCCUSKER 1980).

Examples of chromosome loss in MATα $\times$ MATα *matings*: Nearly 24% of all *MAT*α $\times$ *MAT*α matings expressed all the recessive markers on one of the parental chromosome *III* homologues (although the cells were clearly not haploid, because of the complementation of at least five other markers on other chromosomes). These diploids, classes E and F in Table 2, appear to be monosomic ($2n-1$) for chromosome *III*. We have confirmed that these colonies were monosomic for *MAT*α by crossing representatives of class E with a *MAT***a**/*MAT***a** diploid (J132) and analyzing tetrads from the tetraploids (Table 3B). In a tetraploid that is trisomic for chromosome *III* (or deleted for the *MAT* region on one homologue), we would expect to find that most of the tetrads would fall into tetrad types different from those found with true tetraploids. For example, there would be tetrads with 1 nonmater, 1 α mater and 2 **a** maters; other tetrads would have 2 nonmaters and 2 **a** maters (SHAFFER *et al.* 1971; LIRAS *et al.* 1978). A third prevalent type of tetrad, with 2 **a** and 2 α maters, would be expected both in true tetraploids and in those trisomic for chromosome *III*. Quite often, however, the analysis is complicated by the fact that $2n-1$ strains monosomic for chromosome *III* quite frequently become euploid, so that one may find tetrads characteristic of both normal tetraploids and those still trisomic for chromosome *III* (LIRAS *et al.* 1978). Nevertheless, in all three cases tested, many of the tetrads clearly came from a *MAT*α/ *MAT***a**/*MAT***a** $4n-1$ tetraploid (Table 3B). In these same tetrads, we could examine the segregation of *MAL2*. By looking at the tetrads that must have come from trisomic segregation of *MAT*, we found that all of these also exhibited the pattern of segregation for *MAL2* that would be expected if that marker, to the right of *MAT*, were also monosomic. Of 75 tetrads from the three Class E tetraploids, 68 exhibited 2+:2— segregation for *MAL2*, six were 1+:3—, and one was 3+:1—. These results are consistent with there being only one *MAL2* allele in a trisomic (+/—/—) tetraploid. Similarly, by looking at the segregation of *leu2* or the *his4* alleles, we could also show that these class E tetraploids were also monosomic for the left arm of chromosome *III* (data not shown).

There were other groups of colonies shown in Table 2 that appeared to be the result of chromosome loss. In these cases, however, (for example, classes P and Q) there must have been a recombination event accompanying chromosome loss, similar to that observed in other studies of chromosome loss (CAMPBELL and FOGEL 1977; LIRAS *et al.* 1978). By tetraploid analysis of the type described above, we showed that at least two of the three representatives of class P were monosomic for chromosome *III*, as *MAT*α segregated in a manner consistent with there being only one copy in the Class P diploid (Table 3C). Among the tetrads were *MAT* segregation must have been trisomic, we also found that *MAL2* segregated as if there were only one wild-type allele (data not shown). One representative of class Q was also analyzed by constructing a diploid with strain J122. Essentially, all of the tetrads were of the type expected for a tetraploid carrying two copies of *MAT*α; however, this may be a case where the colony entirely reverted from aneuploidy. Thus, at least five of the seven apparently monosomic diploids (Tables 3B and 3C) have been shown to be diploids hemizygous for one or both arms of the chromosome.

Two groups of unstable diploids arising from MATα × MATα *matings*: Nearly one-third of the *MATα* × *MATα* matings shown in Table 2 fell into other classes that we had not anticipated. The vast majority of these colonies were α maters, prototrophic for *cdc10* very close to the right side of the chromosome *III* centromere and for all three markers to the left of the centromere, but homozygous or hemizygous for markers to the right of *MAT* (Classes G and H). These diploids were clearly different from the *MAT–HMR* deletions, both because they were α mating and unable to sporulate and also because the *tsm5* strains were all *mal2*. Thus, they were homozygous or hemizygous for a marker beyond *HMR***a**, as well as for *tsm5*. In addition, these diploids were also mitotically quite unstable. Fifty-two of the 557 *thr4* colonies of class G were sectored for *leu2* and/or *his4B*. Because the *tsm5* colonies of class H were already temperature sensitive, no sectors involving *cdc10* could be detected, but no colonies appeared to be sectored for *his4C*.

In addition to the sectored class G colonies, we found that some of the apparently unsectored diploids in both classes G and H were mitotically unstable and gave rise to subclones expressing other recessive markers (Table 4). We analyzed 26 class G and 26 class H colonies, each from a different mating. Approximately 100 subclones from each colony were tested. Eleven of the 26 class G colonies gave no evidence of instability, as all of the subclones were still *His*$^+$ *Leu*$^+$ *MATα thr4 MAL2*; however, 15 of the 26 colonies gave rise to *his4B leu2 MATα thr4 MAL2* subclones that appeared to be identical to the $2n - 1$ colonies (Class E) found in the initial survey of *MATα* × *MATα* matings (Table 2). They represented about 5% of all the subclones. In addition, there were a number of other, less frequent, types, either hemizygous or homozygous for some of the

TABLE 4

Mitotic instability of G and H classes

	Number of subclones	Number of original diploids where subclone type observed
A. Subcloning of class G colonies*		
His$^+$ *Leu*$^+$ *Cdc*$^+$ *MATα thr4 Tsm*$^+$ *MAL2*	2301	26
his4B leu2 Cdc$^+$ *MATα thr4 Tsm*$^+$ *MAL2*	125	15
his4B Leu$^+$ *Cdc*$^+$ *MATα thr4 Tsm*$^+$ *MAL2*	2	1
his4C Leu$^+$ *cdc10 MATα thr4 Tsm*$^+$ *MAL2*	34	2
his4C Leu$^+$ *Cdc*$^+$ *MATα thr4 Tsm*$^+$ *MAL2*	1	1
His$^+$ *leu2 cdc*$^+$ *MATα thr4 Tsm*$^+$ *MAL2*	1	1
His$^+$ *Leu*$^+$ *cdc10 MATα thr4 Tsm*$^+$ *MAL2*	1	1
B. Subcloning of class H colonies†		
His$^+$ *Leu*$^+$ *Cdc*$^+$ *MATα Thr*$^+$ *tsm5 mal2*	2989	26
his4C Leu$^+$ *cdc10 MATα Thr*$^+$ *tsm5 mal2*	100	6
his4C Leu$^+$ *Cdc*$^+$ *MATα Thr*$^+$ *tsm5 mal2*	87	2

* Twenty-six class G colonies (each from a separate *MATα* × *MATα* cross) were subcloned, and 2467 colonies tested.

† Twenty-six independent class H colonies were subcloned, and 3176 colonies tested.

markers to the left of *MAT*α. These recombinant types were found among the subclones of only one or two of the 26 original class G colonies. They appear to be identical to some of the less frequent types of diploids observed in Table 2 (Class I-S). Thus the most frequent mitotic segregants of the unstable class G colonies were apparently the $2n - 1$ diploids hemizygous for all markers on the *thr4* parental chromosome. This suggests that some or all of the class G diploids were unstable intermediates that could completely lose one homologue of chromosome *III*.

A similar picture of instability emerged for class H (Table 4), although there were fewer unstable colonies. There may be some selective disadvantage for diploids that are hemizygous for *tsm5* even at 25°, as there were fewer diploids in all classes expressing *tsm5*, as opposed to *thr4*. This may also explain why we did not find any sectored class H colonies. (We reached the same conclusion from another, independent set of experiments described in Table 9). Twenty of the 26 class H colonies tested gave rise only to *His*$^+$ *Cdc*$^+$ *MAT*α *tsm5 mal2* subclones. In six of the 26 diploids, there were subclones that appeared to be identical to the *his4C cdc10 MAT*α *tsm5 mal2* colonies (Class F) observed in the initial screening of rare matings. The remaining group appeared to be the same as class J in Table 2. Although there were 87 such subclones, 85 were found among the subclones of only one of the 26 colonies. Thus, among class H diploids, the most frequently observed mitotic segregant was apparently a $2n - 1$ colony having lost all of one chromosome *III* homologue.

The mitotic instability of the class G and H diploids was not a feature of all diploids isolated from mating two *MAT*α strains. There was very little, if any, instability among the prototrophic nonmating diploids (class A), α-mating diploids (class B), or the nonmating HAWTHORNE deletion diploids (classes C and D). For example, there were no cases of chromosome loss among 6568 subclones from 25 class A colonies or 5188 subclones from 37 class B colonies. There were six apparent chromosome losses among 4717 subclones of 18 representatives of the "Hawthorne deletions" in classes C and D. These diploids, with a large internal deletion from *MAT* to *HMR*, are approximately 100 times more stable than the unstable diploids in classes G and H. Thus, it is unlikely that the unstable diploids carry a slightly larger internal deletion extending from *MAT* beyond *HMR*.

It seems likely that the unstable diploids contain one chromosome *III* that has been broken at or near *MAT* and has lost all of the right arm of chromosome *III* beyond this point (Figure 1). This would also explain why we have not found any cases where there was recombination between any of the markers distal to the putative break point (*thr4*, *tsm5* or *MAL2*) although we found recombinants in all of the intervals to the left of *MAT* (see Table 2). We imagine that these unstable diploids would be formed by the mating of a normal *MAT*α strain with an **a**-like strain that had lost *MAT*α and all of the other chromosome *III* markers distal to *MAT*. The partial aneuploid would be mitotically unstable because of the broken chromosome end. The next series of experiments was designed to test this hypothesis.

Position of the initial event in the formation of unstable partial aneuploids: One parent in each cross carried the recessive marker *cry1*, which maps about 2 cM to the left of *MAT*. We could therefore ask if the *cry1* locus remained heterozygous in diploids that appeared to have lost one copy of *MAT*α and other markers to the right. Diploids heterozygous for *cry1* give rise to papillae after 3–4 days when colonies are replica-plated to medium containing cryptopleurine. We examined 95 of the 557 class G colonies that we had initially isolated. All of the 95 unstable α-mating, *His*$^+$ *thr4* colonies were of wild-type genotype for *cdc10* near the centromere. Nearly half (41/95) gave rise to cryptopleurine-resistant papillae and therefore were still heterozygous for *cry1*. We believe this to be a minimal estimate of the number of colonies that retained *cry1* (see DISCUSSION). If the breakpoint occurred at random in the 25 cM interval between *cdc10* and *MAT*, we would have expected only a small fraction to have retained *cry1*, which is only 2 cM to the left of *MAT*. This seems to indicate that there is a preferential site in the interval between *MAT* and *cry1* that leads to the formation of the unstable diploids.

We then carried out another set of experiments to establish a more accurate location for the position of this chromosomal site that allows one *MAT*α cell to mate with another. We performed mating experiments between sets of two *MAT*α strains, one of which had the chromosomes *III* markers *his4B leu2 MAT*α *tsm1 thr4 MAL2* and the other *his4C cdc10 cry1 MAT*α *tsm5 mal2*. The *tsm1* mutation is a temperature-sensitive marker that lies about 3.6 cM distal to *MAT*; *cry1* lies about the same distance proximal to *MAT* (see Figure 1 and Table 5). Haploid parents of these types were mixed and allowed to grow together on YEPD plates for two days at 25° and then were replica-plated to selective medium lacking histidine and leucine, but supplemented with threonine, in order to select diploids analogous to those in class G. A total of 82 independently isolated α-mating His$^+$ Leu$^+$ Thr$^-$ colonies were recovered (Table 5). All 82 diploids were *tsm1* by complementation testing. Therefore, all of the events that uncovered *thr4* (more than 20 map units from MAT) also uncovered *tsm1* (very close to *MAT*).

TABLE 5

Position of the initial genetic event giving rise to HIS$^+$ thr4 *diploids arising from* MATα $\times$ MATα *matings**

Strains mated	Colony phenotype†	Number
JM76-49c $\times$ JM307-146c	His$^+$ Cry$^{+/-}$ *tsm1 thr4*	13
	His$^+$ Cry^S *tsm1 thr4*	6
JM76-46b $\times$ JM307-146c	His$^+$ Cry$^{+/-}$ *tsm1 thr4*	51
	His$^+$ Cry^S *tsm1 thr4*	12

* Pairs of *MAT*α haploid strains were mated as described in the text, and diploids were selected on minimal medium supplemented with threonine and adenine, at 25°. In each experiment, one parent had the chromosome *III* genotype *his4C cdc10 cry1 MAT*α *tsm5* and the other carried *his4B leu2 MAT*α *tsm1 thr4*. Approximately 1/4 of all colonies that appeared were *His*$^+$, *thr4*. These were tested for cryptopleurine resistance and temperature-sensitive colonies were also shown to be *tsm1* and not *tsm5* by complementation testing.

† Colonies heterozygous for *cry1*/+ show papillae on cryptopleurine plates after 3 to 4 days.

In contrast, 64 of the 82 diploids were still heterozygous for *cry1*, as evidenced by the appearance of papillae when these same colonies were replica-plated to plates containing cryptopleurine. Thus, it seems that the prevalent event that permitted the two *MATα* cells to mate was a chromosomal break that occurred at or just to the left of *MAT*, resulting in the loss of *MAT*, *tsm1*, *thr4*, *MAL2* and other markers distal to *MAT*. Other markers on the same side of the centromere, but proximal to *MAT* (*i.e.*, *cdc10* and *cry1*) are usually not affected.

Very high gene conversion frequencies associated with chromosome loss: A further indication that the region near *cry1* was near the breakpoint came from further examination of class E colonies, which had become hemizygous for markers on both sides of the centromere. Forty-five of the 493 *his4B leu2 MATα thr4 MAL2* colonies were tested for cryptopleurine resistance. Ten of these 45 colonies had become *cry1* instead of carrying the *CRY1* marker that was on the parental chromosome. Thus, more than 20% of the chromosome losses were associated with a gene conversion of *cry1*. This is a much more frequent event than the relatively rare cases where some other recessive marker on chromosome *III* was apparently converted during chromosome loss. For example, there were no cases among the more than 700 examples of chromosome loss in Table 2 where a colony had become converted to the *his4C his4B* double mutant. Similarly, out of more than 500 cases where the monosomic diploids were *thr4 MAL2*, there were only three colonies (class S) where *his4B* has been replaced by *his4C*. Furthermore, there were no examples of a colony of genotype *his4B leu2 cdc10 MATα thr4 MAL2* that would be expected if *CDC10* were converted to *cdc10*. We did find 39 examples of *his4B* Leu+ Cdc+ *MATα thr4 MAL2* colonies (class I) that may represent examples of gene conversions of *leu2* during chromosome loss; however, as we will show in the next section, at least some of the colonies in class I are still unstable diploids, heterozygous for *leu2*/+. An estimate of the frequency of gene conversion events at different chromosome *III* loci that accompany chromosome loss is presented in Table 6A. Gene conversion events involving *cry1* are much more frequent than for any other interval.

This conclusion is further substantiated by looking at those classes where there appears to have been a reciprocal recombination event before or during chromosome loss. For example, Class P, some of whose members we have shown to be monosomic for chromosome *III* (Table 3C), appears to have arisen by a recombination event between *cdc10* and *MATα*, to produce a *his4C cdc10 MATα thr4 MAL2* colony. We could more precisely identify the crossover point by looking at the cryptopleurine resistance of the colonies (Table 6B). Half of the 13 cases appear to have occurred in the very narrow interval between *cry1* and *MATα* (*i.e.*, seven of the colonies were cryptopleurine resistant).

Further analysis of unstable diploids: In addition to the unstable diploids of classes G and H, we found that several other classes of diploids from the *MATα* × *MATα* matings were also mitotically unstable. We subcloned representatives of classes I, J, O and M, all of which were auxotrophic for some markers on the left arm of chromosome *III* (Table 7). We found that at least some colonies of each type gave rise to mitotic segregants that appeared to be the result of a chromo-

TABLE 6

Recombination or gene conversion accompanying chromosome loss

A. Conversion of one marker*	Marker converted or recombined	Number of examples	Frequency
Class E	*cry1*	10/45	0.222
	cdc10	0/560	0.000
Class I	*leu2*	39/560†	0.070
Class S	*his4C*	3/560	0.005

B. Recombination of two markers	Recombination interval	Number
Class P	*cry1–MATα*	7
	cdc10–cry1	6
Class Q	*cry1–MATα*	1
	cdc10–cry1	7
Class M	*leu2–cdc10*	12‡
Class R	*leu2–cdc10*	3

* Conversions of single loci were determined only for cases where the monosomic diploid carried *thr4* and *MAL2*.

† The number of examples of conversion of *leu2* is overestimated by Class I, since the one member that has been tested was apparently still heterozygous for *leu2/+* and gave rise to *leu2* subclones (Table 8).

‡ The number of examples of recombination in Class M may be overestimated, as one colony (of 6 tested) was apparently heterozygous for *leu2/+* and gave rise to *leu2* subclones (Table 7).

some-loss event. These unstable diploids apparently became homozygous for one marker on the left arm of chromosome *III*, and later became hemizygous or homozygous for the remaining chromosome *III* markers. Thus, it seems that there are at least some cases where recombination occurs some generations prior to chromosome loss.

Healing of unstable diploids: As mentioned before (Table 4), only about half of the class G and class H colonies gave rise to any phenotypically different sub-

TABLE 7

Mitotic segregants found among various colonies arising from mating two MATα *strains*

Class	Original phenotype	Mitotic segregant found	Fraction of subclones
I	*his4B Leu+ MATα thr4 MAL2*	*his4B leu2 MATα thr4 MAL2*	19/342
J	*his4C Cdc+ MATα tsm5 mal2*	*his4C cdc10 MATα tsm5 mal2*	2/60
O	*His+ leu2 MATα thr4 MAL2*	*his4B leu2 MATα thr4 MAL2*	20/81
		his4B leu2 MATα thr4 MAL2	3/266
			0/351
M	*his4C Leu+ MATα thr4 MAL2*	*his4C leu2 MATα thr4 MAL2*	8/62
			0/254
			0/348
			0/231
			0/226
			0/41

clones. Subsequent analysis has shown that these diploids remained stable even after further subclonings. We also found that diploids that initially gave rise to mitotic segregants also became stable when single colonies were again subcloned (McCUSKER and HABER, manuscript in preparation). Thus, it appears that unstable diploids can not only undergo subsequent chromosome loss, but can also become stable. We have examined nine independent colonies that were derived from class G and class H colonies. Each diploid was mated with the *MAT***a**/*MAT***a** strain J132 for tetraploid analysis (Table 8). Only one diploid from class G (#6–40) gave rise to tetrads whose mating phenotypes suggested they were exclusively from a *MATα/MAT***a**/*MAT***a** trisomic tetraploid. This is certainly a case where there was only one functional copy of *MATα* in an unstable diploid. There was also only one *MAL2* allele, as this marker segregated 2+:2– in 87 of 98 tetrads we examined. This diploid was, however, still heterozygous for markers on the left arm of chromosome *III* and for *cdc10* on the right arm (data not shown). Unlike the tetrads we analyzed before involving a $2n - 1$ strain monosomic for chromosome *III* (see Table 3), few if any of the tetrads showed segregation of *MAT* or *MAL2* that would be consistent with the idea that this diploid had become euploid. Thus, this derivative of a class G diploid appears to carry a stable terminal deletion of the right arm of chromosome *III* including *MAT*.

The other eight diploids from classes G and H analyzed in this manner yielded tetrads whose segregation pattern for mating type and other markers was an indication that these diploids had become completely disomic for chromosome *III*. They were homozygous for markers distal to *MATα*, but heterozygous for *his4B/his4C*, *leu2* and *cdc10*. It seems that, in these cases, the region to the right of the breakpoint has been restored by a recombination event, similar to what apparently occurred in the generation of a monosomic diploid such as class P (*his4C cdc10 cry1 MAT α thr4 MAL 2*). This subject will be treated in more detail in another paper (McCUSKER and HABER, in preparation).

Effect of the chromosome loss mutation (chl) *on the* MATα × MATα *matings*:

TABLE 8

Tetraploid analysis of α-mating unstable class G and class H diploids

α-mating parent in tetraploid		Mating phenotypes in tetrad* 4N 0a 0α	2N 1a 1α	0N 2a 2α	1N 2a 1α	2N 2a 0α	0N 3a 1α
Class G	#1	5	5	2			
	#2	18	18	10			
	#5	4	11	0			
	#13	22	24	3			
	#21	13	15	4			
	#40		1	12	40	40	11
Class H	#1	27	23	3			
	#2	34	20	6	1		
	#115	6	3	1			

* Nonmating segregants are designated by N.

The chromosome loss mutation *chl* induces a high frequency of spontaneous chromosome *III* loss in diploids (LIRAS *et al.* 1978). If this were also true in haploids, we might detect such chromosome loss by mating two *MAT*α strains together. If one of the two strains lost chromosome *III*, it should become transiently an **a**-like cell, because it should not express any *MAT* function. If such a cell, lacking chromosome *III*, could survive long enough to mate with another *MAT*α strain, it should form a $2n - 1$ diploid, monosomic for chromosome *III*. We know from our previous experiments (Table 2) that a haploid containing a large internal deletion from *MAT* to *HMR***a** does survive long enough to be rescued by mating (classes C and D). We therefore carried out another series of mating experiments, using a new set of haploid *MAT*α strains that had been derived from diploids heterozygous for *chl*. In half of the matings, neither parent carried *chl*; in the other matings one of the two parents was *chl*. The results of these matings are summarized in Table 9. They indicate that there was no significant effect of *chl1* on the relative frequencies of different types of diploids. Furthermore, although the procedures used for mating do not permit accurate quantification of the frequency of mating, there was no obvious difference in the number of matings that appeared on the selective plates (data not shown).

We also asked if the process of chromosome loss in *chl* diploids involved, as a first step, the formation of a partial aneuploid strain, as we found to be the case for the unstable diploids in the *MAT*α matings. For this experiment, we constructed diploids well-marked along chromosome *III* and homozygous for *chl*. One parent carried a chromosome *III* marked with *his4C MAT*α *tsm5* and the other carried *his4B leu2 MAT***a** *thr4*. A total of 16 different diploids from related haploid strains were constructed. The diploids were subcloned at 25° on medium supplemented with all of the nutritional requirements specified by chromosome

TABLE 9

Different classes of diploids arising from MATα × MATα *matings**

Class	Mating† type	Growth phenotype				Stability	Number of colonies‡		Percent total colonies	
		his4	*leu2*	*thr4*	*tsm5*		*CHL1* × *CHL1*	*chl1* × *CHL1*	*CHL1* × *CHL1*	*chl1* × *CHL1*
A	nm	+	+	+	+	stable	123	79	0.048	0.052
B	α	+	+	+	+	stable	339	201	0.131	0.132
C	nm	+	+	—	+	stable	13	11	0.005	0.007
D	nm	+	+	+	—	stable	7	12	0.003	0.008
E	α	—	—	—	+	stable	609	277	0.236	0.182
F	α	—	+	+	—	stable	404	299	0.157	0.196
G	α	+	+	—	+	unstable	568	317	0.220	0.208
H	α	+	+	+	—	unstable	458	301	0.178	0.197
Other	α	+	+	+	—		50	29	0.019	0.019
TOTAL							2581	1526		

* 24 different pairwise matings where both parents were wild type for the *CHL1* gene were used; 17 pairwise crosses where one parent carried *chl1* were also analyzed.
† nm = nonmating.
‡ Unstable colonies gave rise to sectored colonies for *leu2* or *his4*.

III markers. Among 43,726 subclones examined, nearly 1% (413) appeared to have undergone a chromosome loss, leaving a diploid expressing all of the markers on one or the other chromosome *III* (Table 10). There were also seven cases where it appears that chromosome loss was accompanied by a recombination event (*e.g.*, classes 4–9). Finally there were 19 cases that might reflect the occurrence of a chromosome break near *MAT* or might simply be examples of reciprocal recombination in the interval between the centromere and *MAT* (classes 10 and 11). We favor the latter interpretation, because there were also an equivalent number of other diploids that had become homozygous without chromosome loss (classes 12–21). There were also no sectored colonies, where half had the phenotype of a complete loss of one homologue of chromosome *III* and half were still prototrophic for one or more markers. All these observations suggest that a chromosome broken at or near *MAT* is not a frequent intermediate in *chl*-mediated chromosome loss.

DISCUSSION

Our analysis of the diploids resulting from rare matings between two heterothallic *MAT*α haploid strains has revealed that a large proportion of these diploids

TABLE 10

Chromosome loss and mitotic recombination in MATa/MATα chl/chl *diploids**

Colony type	Growth phenotype†						Number
	his4C	*his4B*	*leu2*	*MAT*	*thr4*	*tsm5*	
1	+	+	+	**a**/α	+	+	43,244
2	+	—	—	**a**	—	+	303
3	—	+	+	α	+	—	110
4	—	+	+	**a**	—	+	1
5	+	—	—	α	+	—	1
6	+	—	—	**a**	—	—	1
7	+	+	—	**a**	—	+	1
8	—	—	—	**a**	—	+	2
9	—	—	+	α	+	—	1
10	+	+	+	**a**	—	+	14
11	+	+	+	α	+	—	5
12	+	+	+	**a**	+	+	7
13	+	+	+	α	+	+	4
14	+	+	+	**a**/α	—	+	4
15	+	+	+	**a**/α	+	—	3
16	+	—	+	**a**/α	+	+	5
17	—	+	+	**a**/α	+	+	5
18	+	+	—	**a**/α	+	+	4
19	+	—	—	**a**/α	+	+	2
20	—	—	+	**a**/α	+	+	3
21	—	—	—	**a**/α	+	+	2

* A diploid homozygous for *chl* and carrying *his4C MAT*α *tsm5* on one chromosome *III* (JHM66–12a) and *his4B leu2 MAT***a** *thr4* on the other (JHM72–14b) was subcloned to look for mitotic segregants.

† A colony that exhibited a bisexual mating phenotype characteristic of the parent diploid has a mating phenotype designated as **a**/α.

were partial aneuploids that were mitotically quite unstable. We believe our data demonstrate that, in these unstable diploids, one of the chromosome *III* homologues has been deleted for *MAT* and all of the markers distal to *MAT*. Thus, the cells were hemizygous for *MAT, tsm1, thr4, tsm5* and *mal2* on the right arm, but still heterozygous for a marker closely proximal to *MAT* (*cry1*), for *cdc10* on the right arm near the centromere and for markers on the left arm (*his4* and *leu2*). These unstable diploids give rise to sectored colonies, half of which have the genotype of the unstable parent cell and half of which are completely aneuploid, monosomic for chromosome *III*. Thus, the partial aneuploid strains appear to be an intermediate of chromosome loss.

The initial event must occur in a haploid *MAT*α strain prior to mating. We believe that this event is a double-stranded chromosome break that occurs at, or very close to, the *MAT* locus. The chromosome break event would initially generate a haploid strain carrying an acentric portion of chromosome *III* distal to *MAT*. This fragment would be lost at the next mitotic division. It is possible that this chromosome break event results from an incomplete attempt to convert *MAT*α to *MAT***a**, as we will discuss in more detail below. In the haploid, the loss of this large chromosomal region would be lethal, but the cell can apparently continue to grow for at least part of another cell division. The loss of the *MAT*α locus converts the cell into an **a**-like mater and promotes mating with an intact *MAT*α haploid. We believe that our data support the idea that the end of this chromosome is actually broken, rather than representing a large internal deletion covering the interval from *MAT* to beyond the last known marker, *MAL2*. We draw this conclusion from a comparison of these unstable diploids with the other types of diploids produced by the rare matings of *MAT*α cells. For example, those in classes C and D (Table 2) contain large recessive lethal deletions of one chromosome *III* in which *MAT* has been fused to *HMR*. These deletion strains still contain the *MAL2* region and presumably all of the more distal portion of the right arm of chromosome *III*. These diploids are approximately 100 times more stable mitotically than are the diploids of classes G and H. Thus, the unstable diploids do not seem simply to carry a large internal deletion, as they behave entirely differently from these known internal deletion cases.

It is also possible that some of the unstable diploids hemizygous for markers beyond *MAT*α contained a circular chromosome resulting from the fusion of homologous sequences at *MAT*α and *HML*α (STRATHERN *et al.* 1979, 1980; HABER, ROGERS and MCCUSKER 1980). We have previously shown that diploids carrying such a circular chromosome are mitotically unstable (HABER, ROGERS and MCCUSKER 1980). It is unlikely that such circular chromosomes account for more than a small proportion of the unstable diploids. First, their proportion of the total should be approximately the same as HAWTHORNE (*MAT–HMR*) deletions, which were only about 1% of the total events. Second, such a circular structure would not account for the high incidence of recombination between *cry1* and *MAT* found among $2n-1$ monosomic mitotic segregants from these diploids.

A broken chromosome end does account for the surprising observation that a very high proportion of the chromosome loss events from the unstable diploids

involve a gene conversion of the *cry1* marker. Mitotic gene conversion events accompanying chromosome loss have also been seen in chromosome loss both in diploids homozygous for *chl* (LIRAS *et al.* 1978) and in haploid strains disomic (*MAT***a**/*MAT*α) for chromosome *III* (CAMPBELL and FOGEL 1977; CAMPBELL, FOGEL and LUSNAK 1975). We do find examples of recombination accompanying chromosome loss in other intervals of this chromosome, but at frequencies at least 10 times lower than in the *cry1* interval. We believe that the very high frequency of recombination in the *cry1* region can be accounted for by the fact that it would be close to the end of the broken chromosome. A single-stranded end of the chromosome might very well participate in D-loop formation as the first step in the formation of a recombination structure (MESELSON and RADDING 1975).

The stimulation of recombination between the broken end and the homologous region on the intact chromosome *III* also seems to account for most of the other kinds of stable colonies that were derived from the unstable class G and class H cells. Some of these events are illustrated schematically in Figure 2. In Figure 2A the broken end initiated a recombination event, which was resolved as a gene conversion without reciprocal recombination, to convert only the *cry1* locus. Subsequently the broken chromosome is lost. In Figure 2B and Figure 2C, the initial formation of a heteroduplex is followed by a reciprocal recombination event, leading to an intact, recombined chromosome *III*. Depending on subsequent mitotic segregation, the cell will become either monosomic (Figure 2B) or euploid (Figure 2C). These represent the predominant kinds of events (other than loss of the broken chromosome without recombination) that we have observed. We therefore believe that essentially all of the monosomic and stable euploid diploids that we found (Table 2) were derived from initially unstable diploids of classes G and H.

Origin of the chromosome break: The creation of a chromosome break appears to be related to mating-type switching events. The conversion of *MAT* alleles appears to occur by the same mechanism in both homothallic and heterothallic cells, although as much as 10^5 more frequently in homothallic cells (HABER, ROGERS and MCCUSKER 1980). The mechanism of switching appears to involve a specialized gene conversion event in which the donor sequence at *HMR* or *HML* pairs with the sequence at *MAT*, following which there is a gene conversion event that replaces the sequence at *MAT*. More recent experiments have indicated that one step in the switching of mating-type genes is the formation of a labile structure at *MAT*. First, in homothallic *HML*α *MAT*α *HMR*α cells, which cannot actually switch from *MAT*α to *MAT***a**, abortive attempts to switch mating type result in the appearance of **a**-like cells, with properties exactly analogous to those described here (HABER unpublished). Second, in homothallic cells that attempt to switch mating-type alleles, the *rad52* mutation is lethal (MALONE and ESPOSITO 1980). By using diploid strains and mutations that slow down or prevent switching, we have been able to identify the lethal event in *HO rad52* cells as a chromosome break at or near *MAT* producing an unstable chromosome that is readily lost in diploids (WEIFFENBACH and HABER 1981). Thus, a *MAT*α cell will attempt to switch and produce a transiently viable **a**-like cell. The properties of diploids formed by mating these **a**-like homothallic cells with a *MAT α-inc* strain (which

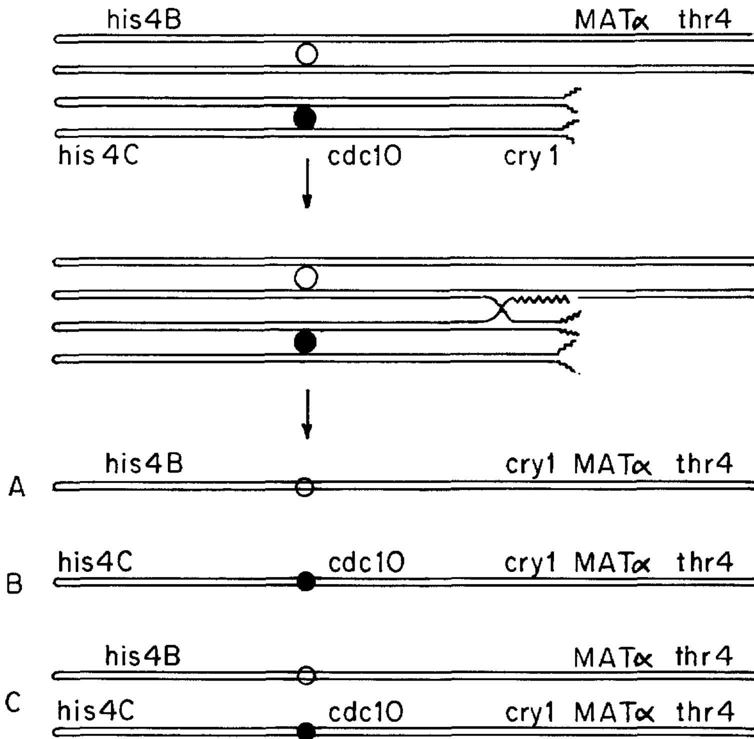

FIGURE 2.—Mitotic recombination events initiated by a broken chromosome end. The initial break-point is between *cry1* and *MATα*. The broken chromosome end can initiate the formation of a heteroduplex structure in the region including *cry1*. Subsequent isomerization and mismatch repair events can lead to a conversion of *CRY1* to *cry1* either with or without recombination of flanking markers. The event is depicted as occurring during chromosome replication, but may also occur during G1 (ESPOSITO 1978) with similar consequences. Some possible mitotic segregants are shown. In examples A and B, we presume that the mitotic cell receives one intact and one broken chromosome (which is then lost). In C, a cell becomes a stable diploid by inheriting two intact homologues, one of which arose by recombination. These three cases represent the most frequently recovered types of stable mitotic segregants from an initially unstable diploid carrying a broken chromosome.

cannot switch mating type) are essentially identical to those described in this paper for rare heterothallic *MATα* × *MATα* matings. These results seem to demonstrate that in both homothallic and heterothallic cells, a double-stranded chromosome break may be produced in some fraction of cells attempting to switch mating type.

Relation of the chromosome loss mutation to MATα × MATα *unstable diploids*: We may also ask whether the chromosome loss events that arise under other circumstances also depend on the same chromosome break mechanism that seems to be almost always used in the rare matings we have described here. For example, do the chromosome *III* losses that occur in diploids homozygous for the *chl1* mutation also give rise to unstable intermediates, hemizygous for markers distal

and including *MAT*? This does not appear to be the case. We constructed diploids homozygous for *chl1* and heterozygous for markers along chromosome *III* and screened subclones of these diploids for the appearance of auxotrophic markers. Although nearly 1% of the 44,000 colonies examined showed chromosome loss, there were at most 0.03% cases consistent with the formation of an unstable intermediate by a break on one chromosome proximal to *MAT*. The number of these events, however, was about the same as for other reciprocal recombination events in other places on chromosome *III*. Unlike the many colonies in the *MAT*α $\times$ *MAT*α crosses (Table 2), there were no examples of a sectored *chl1/chl1* colony where both sides of the sector was *thr4* or *tsm5*, but one half has His$^+$ and the other half His$^-$. Thus, there seems to be no requirement that chromosome loss induced by the *chl1* mutation should initially generate a chromosome break at or near *MAT*.

Our conclusion that *chl1* does not generate chromosome breaks is also supported by our finding that *chl1* had no effect on the frequency of rare matings between *MAT*α haploids. In addition, these results also raise the possibility that *chl1* may act only in diploids. If *chl1* promoted chromosome loss in a *MAT*α haploid, we might expect this haploid to become a transient **a**-like cell, in the same way that the haploids that had lost part of the right arm could continue to grow for a short while, even with the deletion of genes for many essential functions. That we did not find an increase in *MAT*α $\times$ *MAT*α matings when one of the parents carried *chl1* suggests that this mutation may not promote chromosome loss in a haploid strain. However, we cannot rule out the possibility that a haploid that has lost all of chromosome *III* might not be able to grow, even briefly, in the way that the partially deleted haploids apparently can.

There is one other mutation in yeast that may promote the kind of chromosome breakage at *MAT*α that we have described. MELNICK and BLAMIRE (1978) described a dual mating-type (*dmt*) mutation that confers on *MAT*α cells a weak **a**-mating type. The weak **a** maters, when crossed to another *MAT*α strain, appear to lack part or all of chromosome *III*. Second, *dmt/dmt* diploids exhibit apparent loss of several chromosomes, including chromosome *III*. We have found that the two phenotypes are genetically separable (MCCUSKER, unpublished) and that the chromosome loss phenotype is caused by a mutation other than *chl1*. There is no evidence that *dmt* causes chromosome breaks, however.

Similar effects of broken ends of chromosomes in maize: Unstable chromosomes were first described in maize by MCCLINTOCK (1939, 1940). Her studies were carried out on unstable chromosomes generated by the breakage-fusion-bridge (BFB) cycle, in which mechanical rupture of a dicentric chromosome during meiosis produces a chromosome with an apparently broken end. After chromosomal replication, the two broken chromatids may fuse and the resulting dicentric chromosome may again break during the next mitosis. In this way a varying portion of the chromosome may be broken and lost from a daughter cell. During this BFB cycle, a variety of dominant markers that were initially closer to the centromere than the break point can be lost, as the broken chromosome becomes shorter and uncovers these regions. In some cells, the broken-ended chromosome appears to be lost entirely.

Broken-ended chromosomes in maize can appear in other ways besides mechanical rupture during meiosis. In later work, McClintock (1951) showed that the BFB cycle can be initiated by a broken chromosome arising at or near the site of a transposable controlling element, *Ds*.

It seems possible that a similar sequence of events may occur in the formation and growth of the unstable diploids in yeast. Initially, a chromosome break must occur at or near *MAT*, but certainly to the right of *cry1*. In subsequent rounds of mitotic division, however, a BFB cycle would generate shorter broken chromosomes, some of which would reveal the *cry1* locus on the intact homologue. In other cases, the broken chromosome appears to be lost completely. As in maize, broken chromosomes can apparently be healed. We have found one case (Class G #40, Table 8) where the broken chromosome is now stable and the diploid is hemizygous for *MAT* and *MAL2*. More recent analysis has indicated that the deletion now includes all but about 15 map units of the right arm of the chromosome (that is, about 10 map units to the left of *cry1* and *MAT*) (McCusker and Haber, in preparation).

The other type of healed chromosome that we have found, where the deleted portion has been restored by a copying or recombination event with the intact homologue, seems to have arisen by a different mechanism. The fact that this event is quite frequent may indicate that homologous pairing and mitotic recombination is much more prevalent in yeast than in maize.

All of these similarities lead us to suggest that we are observing the consequences of a double-stranded chromosome break and possibly the BFB cycle in *Saccharomyces cerevisiae*. In the absence of good cytological methods, however, we do not have direct evidence for the formation of dicentric chromosomes. It is also possible that the shortening of the broken chromosome occurs by progressive exonucleolytic digestion of the broken ends. In any case, we have clearly shown that apparently broken chromosomes in yeast are mitotically unstable and that the broken end itself is especially active in recombination.

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