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Efficient Production of a Ring Derivative of Chromosome III by the Mating-Type Switching Mechanism in *Saccharomyces cerevisiae*

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The mating-type switches in the yeast *Saccharomyces cerevisiae* occur by unidirectional transposition of replicas of unexpressed genetic information, residing at *HML* or *HMR*, into the mating-type locus (*MAT*). The source loci, *HML* and *HMR*, remain unchanged. Interestingly, when the *HM* cassettes are expressed, as in *mar1* strains, the *HML* and *HMR* cassettes can also efficiently switch, apparently by obtaining genetic information from either of the other two cassettes (Klar et al., Cell **25**:517–524, 1981). We have isolated a novel chromosome III rearrangement in heterothallic (*mar1 ho*) strains, which is also produced efficiently in *mar1 HO* cells, presumably the consequence of a recombination event between *HML* and *HMR*. The fusion results in the loss of sequences which are located distal to *HML* and to *HMR* and produces a ring derivative of chromosome III. Cells containing such a ring chromosome are viable as haploids; apparently, no essential loci are located distal to the *HM* loci. The fusion cassette behaves as a standard *HM* locus with respect to both regulation by the *MAR/SIR* control and its role in switching *MAT*.

The **a** and α cell types of the yeast *Saccharomyces cerevisiae* are respectively controlled by the alternate alleles of the mating-type locus (*MAT*), *MAT***a** and *MAT*α. These alleles differ by a DNA substitution lying in the center of the locus: *MAT***a** contains the 642-base pair (bp) Y**a** sequence, whereas *MAT*α contains the nonhomologous, 747-bp Yα sequence (2; Fig. 1). The *MAT***a** and *MAT*α alleles can be interchanged inefficiently at a frequency of $\sim 10^{-6}$ in heterothallic (*ho*) cells (8), but efficiently, as often as every generation, in homothallic strains which contain the *HO* gene (9, 10, 34). The information required for substitution at *MAT* is transposed from the storage loci, *HML* and *HMR*, which contain the unexpressed *MAT* information (Fig. 1) (11, 12, 14, 15, 23, 27, 30). The *HML* and *HMR* loci are maintained unexpressed by the action of at least four loci, variously known as *MAR* (mating type regulator; 16) or *SIR* (silent information regulator; 31) or *CMT* (change of mating type; 5). Hence, this yeast has developed the mechanism to activate the genetic information residing at *HML* and *HMR* by transposing their copies into the expressed *MAT* locus.

The *MAT***a** and *MAT*α cells resulting from the switching process mate to produce the third cell type, the diploid *MAT***a**/*MAT*α. The latter class is unable to mate with either **a** or α cells, is unable to switch further, but can undergo meiosis and sporulation to produce 2**a** and 2α haploid segregants that again are capable of switching and mating.

As diagrammed in Fig. 1, the *HML*, *HMR*, and *MAT* loci share substantial homology with each other and are located in the same orientation on chromosome III (1, 12, 27, 37). Two types of chromosomal rearrangements which result from recombination between the "cassettes" have been described. First, "Hawthorne deletion" is a fusion between *MAT* and *HMR* loci which deletes the chromosome segment lying between these cassettes, whereas fusions between *MAT* and *HML* produce a ring chromosome. Both of these events occur spontaneously at a low frequency ($\sim 10^{-6}$) in *ho* cells (8, 14, 36, 37) but at a relatively high frequency ($\sim 10^{-2}$ in *HO* cultures (6). These chromosomal rearrangements are lethal in the haploid state but can be rescued and propagated by mating to cells containing a normal chromosome III.

In this communication, we describe a ring chromosome resulting from the fusion of the *HML* and *HMR* cassettes. This "big ring" chromosome has lost only the DNA sequences distal to *HML* and *HMR*, and in contrast to the Hawthorne deletion and the "small ring" chromosome described above, the *HML-HMR* fusion chromosome can be maintained in the haploid state. The mating-type information

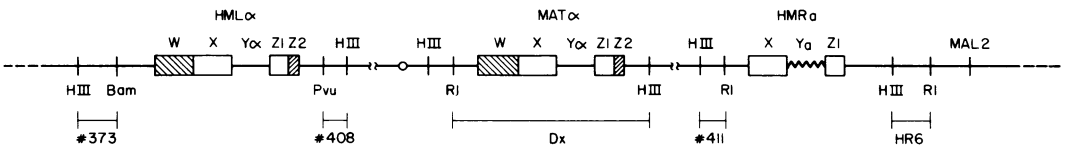

FIG. 1. Organization of mating-type cassettes on chromosome III and sequences used as probes. Heteroduplex and sequence analyses have demonstrated similarities in the structure of *MAT*, *HML*, and *HMR* (1, 12, 27). The **a** and the α alleles differ by an allele-specific DNA substitution: Ya is a unique 642 bp and Yα is a unique 747 bp. Corresponding changes were found in the α and **a** alleles of the silent loci. All of the cassettes share the X region (707 bp) to the left and the Z1 region (239 bp) to the right of the Y region. *HML* and *MAT* share two additional homologies: the W region (723 bp), to the left of X, and the Z2 region (88 bp), to the right of Z1. Switches of *MAT* occur by unidirectional transposition of replicas of the silent *HM* loci to *MAT*, essentially interchanging the Y segments. *HML* and *HMR* are located on opposite arms of chromosome III, at least 100 kbp away from *MAT* (7, 20, 36). The open circle represents the centromere. Various sequences used as probes are indicated. The physical map is not drawn to scale. The indicated probes were constructed in our laboratory. The HR6 probe was kindly provided by C. Michels. HIII, *Hin*dIII endonuclease site; Bam, *Bam*HI; Pvu, *Pvu*II; RI, *Eco*RI.

contained in the *HML-HMR* fusion is regulated by the *MAR/SIR* mechanism.

MATERIALS AND METHODS

***S. cerevisiae* strains.** Strains used and their genotypes are listed in Table 1.

Genetic analysis. All media for growth and sporulation and techniques for micromanipulation and tetrad analysis have been described by Mortimer and Hawthorne (26).

Mutagenesis. Haploid stationary-phase cells of strain K45, at a density of about 3×10^8 cells per ml, were exposed to 3.1% ethyl methane sulfonate in 0.2 M phosphate buffer (pH 8.0) containing 2% glucose (24). After 70 min of treatment, the cells were washed twice with water and then suspended in 6% sodium thiosulfate for 10 min to inactivate any remaining ethyl methane sulfonate. About 15% of the cells survived this treatment.

Biochemical analysis. DNA was prepared by a modification of procedures described by Cryer et al. (3). DNA samples were digested with restriction endonucleases, and the fragments were separated by agarose gel electrophoresis for analysis by Southern blotting (32). Nitrocellulose filters were probed with ^{32}P-labeled pBR322 plasmid containing various *S. cerevisiae* DNA inserts as described previously (12).

RESULTS

Isolation of *HML-HMR* fusions. Strain K45 (*HML*α *MAT*α *HMR***a** *mar1 ho*; see Table 1 for complete genotype) exhibits the sterile "*MAT***a**/*MAT*α" phenotype because the *mar1* mutation allows expression of the mating-type information at the normally silent loci, *HML* and *HMR* (16). K45 cells were mutagenized with ethyl methane sulfonate as described above. Colonies produced from mutagenized cells were screened for those with an α mating type, which appeared at a frequency of about 10^{-3}. At least three classes of mutants were expected: *mar1* reversions; switches of *HMR***a** to *HMR*α; and mutations of *HMR***a**. These studies were originally initiated to isolate mutations of the *HMR***a** locus (14). DNA isolated from the mutants was subjected to Southern (32) blot analysis to determine the genetic content of each cassette. Several mutants resulted from irregular switches of *HMR***a** to *HMR*α (Fig. 2, tracks i, j, and k). The *HMR*α fragment runs slower than *HMR***a** because Yα is about 100 bp larger than Ya (Fig. 1). (Note that a strain with the genotype HMLα *MAT*α *HMR*α *mar1* exhibits an α phenotype because only α cassettes are present.) Such mutants isolated in this fashion have been described before (18). Tracks b through e, g, h, and m represent the *hmr***a** mutants as described earlier (14). Track a represents the parental K45 strain.

Another unexpected novel class, represented by mutants 8 (track f) and 11 (track l), also appeared in which fragments corresponding to *HML* and *HMR* were absent and a new fragment appeared. We hypothesize that these mutants result from recombination between *HML* and *HMR*. As *HML* and *HMR* share substantial homology and are arranged in direct orientation

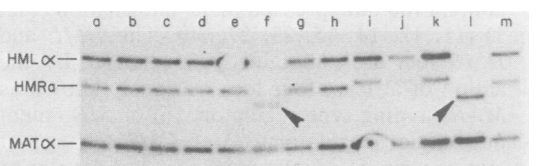

FIG. 2. Southern blot analysis of the K45 parental strain and the isolated α mutants. Lane a, DNA from K45 parental strain; lanes b to m, DNA from the mutants with the α phenotype. The arrows indicate the *HML-HMR* fusion cassette. The ^{32}P-labeled probe used was Dx (see Fig. 1); the restriction enzyme used was *Hin*dIII. Because of the homology between *MAT*, *HML*, and *HMR*, the Dx probe hybridizes to all three mating-type cassettes.

TABLE 1. List of strains

Strain	Genotype
K45	*MATα HMLα HMRa marl ho trpl thrl arg4 lysl ade8 his2 ural*
K243	*MATα HMLα HMRα marl HO MAL2 thr4 ura3 leu2 his4*
K101	*MATα HMLα HMRα MARI HO cryl thr4 leu2 ura3 metx his4 lys2* (or *lysx*) *adel trpl trp3*
K200	*MATa HML-HMRα* fusion *MARI ho cryl leu2 lysl lys2* (?) *ade6* (or *ade8*)
K202	*MATa HML-HMRα* fusion *marl ho cryl leu2 lysl lys2* (?)
K337	*MATα HML-HMRa* fusion *marl ho cryl his4 adel ura3 metl3 trpl*
K338	*MATα-HML-HMRa* fusion *MARI ho his4 leu2 lys2*

(Fig. 1), such a recombination event, as diagrammed in Fig. 3, should produce a ring chromosome.

The **a** cassettes have a unique *Bgl*II restriction site in **Ya**, whereas the α cassettes lack it (37). The structure shown in Fig. 3 predicts that the **Ya** sequence of *HMRa* (and thus the *Bgl*II site) should be lost during formation of the fusion. The new restriction fragment present in mutants 8 and 11 should therefore be resistant to cleavage with *Bgl*II. This prediction is borne out, as shown in Fig. 4.

No sequences distal to *HML* or to *HMR* in mutants 8 and 11. Our *HML-HMR* fusion (Fig. 3) interpretation makes a second specific prediction. That is, strains containing the postulated ring chromosome should lack sequences situated distal to *HML* and to *HMR*. To test this prediction we used *HML*-distal (fragment 373, Fig. 1) and *HMR*-distal (fragment HR6, Fig. 1) sequences as probes to monitor the presence of these sequences in the parental strain, K45, and in the mutants. The top restriction fragment, corresponding to the 373 probe, is present in K45 but is missing in both mutants (Fig. 5). This probe also has strong homology to another (bottom) fragment present elsewhere in the genome. Likewise, the *HMR*-distal sequence, corresponding to the HR6 probe, is also missing in both mutants (top band in Fig. 6). Again, the *HMR*-distal HR6 probe shows weak homology to several other sequences in the genome.

Hybrid *HML-HMR* cassette in mutants 8 and 11. To establish that the new cassette present in mutants 8 and 11 resulted from fusions between *HML* and *HMR*, their DNA was subjected to Southern (32) analysis. The model shown in Fig. 3 predicts that the fusion cassette should harbor both *HML*-proximal (probe 408, Fig. 1) and *HMR*-proximal (probe 411, Fig. 1) sequences on

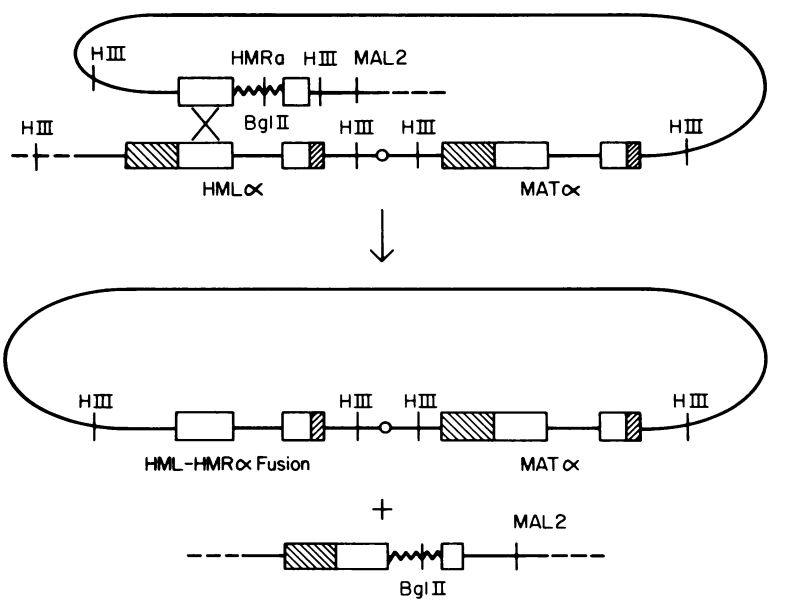

FIG. 3. Postulated recombination event between the X region of the *HML* and *HMR* to produce a ring derivative of chromosome III. Note the production of an acentric fragment which is expected to be lost during growth. The open circle represents the centromere.

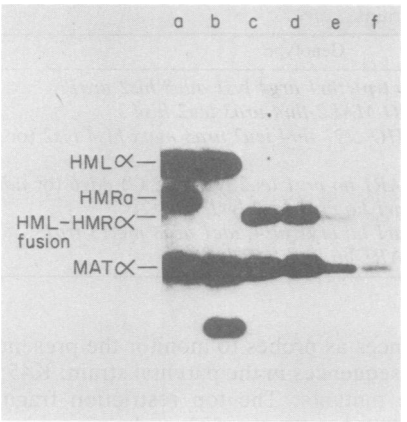

FIG. 4. Southern blot analysis of strain K45 and the mutants 8 and 11, demonstrating the absence of Ya information in the fusion cassette. Lanes a, K45 DNA, *Hin*dIII digested; b, K45 DNA, *Hin*dIII + *Bgl*II digested; c, mutant 8 DNA, *Hin*dIII digested; d, mutant 8 DNA, *Hin*dIII + *Bgl*II digested; e, mutant 11 DNA, *Hin*dIII digested; f, mutant 11 DNA, *Hin*dIII + *Bgl*II digested. Only the *HMR***a** sequence present in K45 (lane a) is cut with *Bgl*II (lane b), producing the proximal *Hin*dIII-*Bgl*II fragment; the distal *Hin*dIII-*Bgl*II fragment was run off the gel. The probe used was Dx.

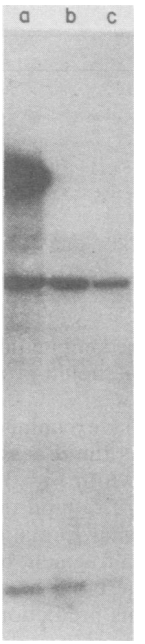

FIG. 6. Southern blot analysis demonstrating the absence of *HMR* distal sequences in mutants 8 and 11. The probe used was *HR6*; the rest of the details are the same as those in the legend to Fig. 5.

a single *Hin*dIII restriction fragment (see Fig. 3). Three sets of *Hin*dIII-digested DNA, isolated from the K45 parent and both mutants, were run in parallel on a single gel. The Southern blot strips were then probed with Dx (Fig. 7A), 408 (Fig. 7B) and 411 (Fig. 7C) sequences. Figure 7A displays all of the cassette sequences present in these strains. Figures 7B and C demonstrate that, as predicted, the *HML*-proximal and *HMR*-proximal sequences reside on a single *Hin*dIII fragment present in each mutant.

Efficient production of *HML-HMR* fusions in *HO marl* cells. In standard strains transposition of mating-type cassettes is unidirectional, that is, the information moves only from the *HM* loci to *MAT*. The *MAT* locus switches while the *HM* loci remain unaltered. Recently we have shown that the *HM* loci may also switch efficiently in *marl* strains (17) where they are transcriptionally active (22, 28). The *HML-HMR* fusions described above were isolated from a *marl ho* (K45) strain. It is likely that the low-frequency switching observed in *ho* cells may cause this recombination event. Do such events occur efficiently in *HO marl* cells? To determine this,

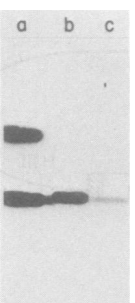

FIG. 5. Southern blot analysis demonstrating the absence of *HML* distal sequences in mutants 8 and 11. Lanes: a, K45 DNA, *Hin*dIII digested; b, mutant 8 DNA, *Hin*dIII digested; c, mutant 11 DNA, *Hin*dIII digested. The probe used was no. 373 (see Fig. 1).

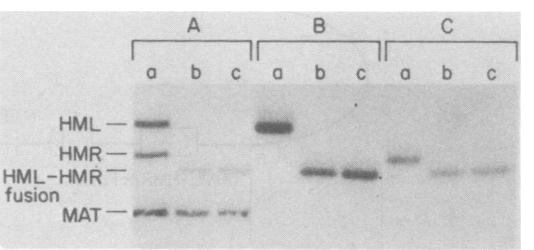

FIG. 7. Southern blot analysis demonstrating the presence of *HML-HMR* fusion cassette in mutants 8 and 11. Lanes a, K45 DNA; lanes b, mutant 8 DNA; lanes c, mutant 11 DNA. Digestions were done with *Hin*dIII. (A) Dx probe; (B) *HML*-proximal 408 probe; (C) *HMR*-proximal (411) probe.

strain K243 with the genotype *HMLα MATα HMRα MAL2 HO marl* was constructed. The *MAL2* marker confers ability to grow on media containing maltose as the sole carbon source. It is located about 1 map unit distal to *HMR* (7, 8, 20). (We have shown that the *HMLα MATα HMRα HO* cells efficiently produce homologous *MATα*-to-*MATα* switches [19]). The strain K243, being *marl*, is expected to switch the *HM* loci as well as the *MAT* locus. In this strain, any event which causes fusions of *HML* with *HMR* should lead to the loss of the *MAL2* marker (see Fig. 3). Nearly 7% of the K243 subclones tested were observed to be incapable of fermenting maltose, designated Mal$^-$ phenotype. The resulting Mal$^-$ clones maintained their phenotype, whereas the Mal$^+$ (capable of fermenting maltose) clones again segregated Mal$^-$ clones. Three of the Mal$^-$ isolates were subjected to Southern (32) blot analysis. Indeed, these cells had only the two cassettes expected of the *HML-HMRα MATα* genotype (Fig. 8). Clearly, the *HML* fragment has been lost in the Mal$^-$ isolates. The observation that the *HML-HMR* fusion fragment runs with a mobility similar to the *HMR* fragment in K243, unlike that shown in Fig. 2, is attributed to a restriction site polymorphism in these strains. This is supported by the observation that the *HMR*-distal sequences (HR6 in Fig. 1) have been deleted in these strains (Fig. 9, top band).

In contrast, the *MAR*$^+$ strain K101 (*HMLα MATα HMRα MAL2 HO*) which is expected to switch only *MAT*, failed to produce Mal$^-$ colonies (0 of 400). Thus, we presume that the *HML-HMR* fusions originate because of the ability of the *HM* loci to switch in *marl* strains.

Regulation of the *HML-HMR* fusions. *HML* and *HMR* are normally kept unexpressed by a negative control exerted by the function of at least four *MAR/SIR* loci (5, 16, 31). Recently Abraham et al. (1) have conducted in vitro deletion analyses of *HMR* and *HML* locus to define the *cis*-acting sequences involved in keeping the *HM* loci unexpressed. These studies implicate sequences lying on either side of the cassettes as being needed for repression. We wondered whether a hybrid cassette resulting from *HML-HMR* recombination would be regulated normally with respect to (i) expression and (ii) its ability to act as a donor for switching *MAT*. By standard genetic crosses, several strains containing the **a** or α forms at *MAT* and the *HML-HMR* fusion were constructed. The *HML-HMR***a** allele was obtained by selecting rare switches of *HML-HMRα* to *HML-HMR***a** in a fashion analogous to the switches of *HMR***a** to *HMRα* in strain K45 as discussed above.

Results presented in Table 2 demonstrate that the fusion cassettes are unexpressed in *MAR*$^+$ but expressed in a *marl*$^-$ background. Thus, the fusion cassette is apparently regulated normally. In addition, we have recently documented that such fusions act as efficient donors for switching *MAT* (19).

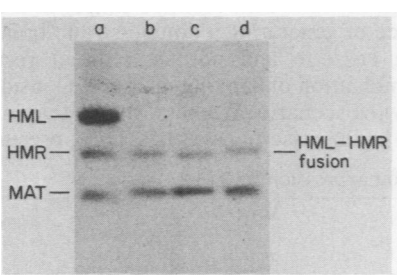

FIG. 8. Southern blot analysis of strain K243 and three Mal$^-$ derivatives. Lane a, K243 DNA; lanes b through d, DNA from independently derived Mal$^-$ derivatives of K243. *Hin*dIII-digested DNA was probed with Dx.

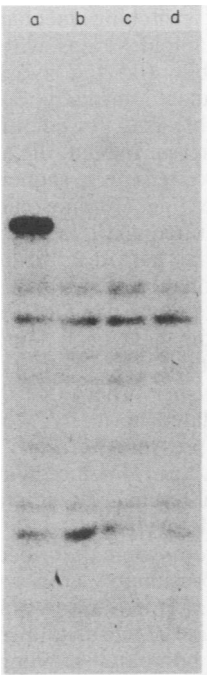

FIG. 9. Southern blot analysis demonstrating the absence of *HMR*-distal sequences in the Mal$^-$ derivatives of strain K243. The probe used was HR6; the rest of the details are the same as in the legend to Fig. 8.

DISCUSSION

We have shown that: (i) *HML-HMR* fusions produce a ring derivative of chromosome III; (ii) these rearrangements are produced efficiently

by the *MAT* switching mechanism in *mar*$^-$ strains; (iii) haploid strains with this chromosome are viable; (iv) the hybrid *HML-HMR* cassette is regulated normally. Such a ring chromosome can be used for identifying chromosome III telomeres. Indeed, the absence of one, or possibly two, telomeric sequences in a strain containing the ring chromosome has recently been demonstrated (38). In addition, this ring chromosome has helped us to define the basis for the directionality of switching (19).

By deletion analysis, sequences flanking both the *HM* cassettes have been demonstrated to be required for their regulation (1). By genetic tests, we have shown here that the *HML-HMR* fusion is regulated normally; it is repressed in *MAR*$^+$ but expressed in a *mar*$^-$ genetic background. Thus, the *HMR* regulatory sites can apparently be interchanged with the corresponding *HML* regulatory sites.

The ring chromosome described here is viable as a haploid; presumably no essential genes are located distal to *HML* and to *HMR*. However, as the *HML*- and *HMR*-distal probes used here showed some homology residing elsewhere in the genome (Fig. 5 and 6), the presence of essential but functionally duplicate loci cannot be ruled out. To date, only a single locus, *MAL2*, has been mapped distal to *HMR* (7, 8, 20), and no markers have been mapped distal to *HML*.

In addition, the results bear on the resolution of the recombination intermediate. The *MAT* switching is formally considered a proces of gene conversion (6, 12, 21). Two types of observations have suggested physical interaction between the recipient *MAT* locus and the donor *HM* loci during transposition. First, we have demonstrated that, under the influence of the *HO* gene, the *MAT***a**$^+$ recombinants can be efficiently produced in a strain which contains defective **a** cassettes, at *MAT* and *HMR*, marked with nonidentical mutations (21). Second, the fusions between *MAT* and *HMR* and between *MAT* and *HML* are found at a frequency of $\sim 10^{-6}$ in *ho* cells and at 10^{-2} in *HO* cells (6, 8, 14, 18, 36, 37). These observations at best are only suggestive of physical interaction between the donor and the recipient loci; other interpretations will be discussed below. If indeed a physical interaction occurs, then we may imagine the production of a Holliday structure (13; see also references 25 and 33) between the recipient and the donor loci. The Holliday structure may be resolved with equal probability in either of two ways allowing recombination of flanking markers in 50% of the cases (4). As the mating-type cassettes are arranged in the same orientation (Fig. 1), such a recombination of flanking markers in this intrachromosomal recombination event should efficiently lead to the fusions of *MAT* with *HML* or *HMR*. As discussed above, such events are found only rarely during *MAT* switching. Thus, in this system some constraint exists so that efficient recombination of flanking markers is not allowed to occur in standard *MAR*$^+$ cells.

Recently, we have demonstrated that the *HM* loci also can switch, as efficiently as *MAT*, when they are allowed to express as in *mar1* (17) or in *mar2* strains (Klar, unpublished data; A. Commeau and J. Haber, personal communication). In such strains each locus can act as a donor as well as a recipient. Because of this property, a physical interaction between *HML* and *HMR* may occur and thus provide the opportunity for producing the *HML-HMR* fusions. To account for the increased rate of fusions in *mar1* cells we may propose that the constraint for lack of recombination of flanking markers during transposition in *MAR*$^+$ strains may be relaxed in *mar*$^-$ strains, resulting in recombination between *HML* and *HMR*. As drawn in Fig. 3, such an event would produce the ring chromosome described here. Such an hypothesis predicts that the *MAT-HMR* and *MAT-HML* fusions may also be produced at an increased rate in *mar*$^-$ strains. Exact quantitation of such events is difficult because these events, unlike the *HML-HMR* fusions, lead to lethality. Nonetheless, these events are easily observed in *mar*$^-$ strains (Klar, unpublished data).

However, there is a caveat in the interpretation proposed above. It is equally likely that the fusions in *mar*$^-$ cells resulted simply as a consequence of generating a double-strand break at a given cassette and not as a direct result of recombination of flanking markers. Consider the following scenario. We have shown that in *mar*$^-$ cells each cassette switches (17), presumably

TABLE 2. Control of *HML-HMR* fusion expression by *MAR1*

Strain	Cassette genotype	*MAR1* or *mar1*	Phenotype
K200	*MAT***a** *HML-HMR*α, fusion	*MAR1*	**a**
K202	*MAT***a** *HML-HMR*α, fusion	*mar1*	Sterile[a]
K338	*MAT*α *HML-HMR***a**, fusion	*MAR1*	α
K337	*MAT*α *HML-HMR***a**, fusion	*mar1*	Sterile

[a] The sterile phenotype results from expression of both **a** and α cassettes in *mar1* strains.

because each cassette can experience a double-strand break (35; Klar, unpublished data). Since the broken ends are reported to be highly active in recombination (29), such an end could pair with homology existing elsewhere in the genome and therefore fuse with that cassette. Thus, these fusions may be only a secondary consequence of a double-strand break and not a direct result of recombination of flanking markers during switching. Likewise, the fusions observed at a frequency of about 1 to 2% in MAR^+ cells (6) may also be explained only as a consequence of a double-strand break at *MAT* or at the donor.

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