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Mechanism of *MAT*α Donor Preference during Mating-Type Switching of *Saccharomyces cerevisiae*

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During homothallic switching of the mating-type (*MAT*) gene in *Saccharomyces cerevisiae*, a- or α-specific sequences are replaced by opposite mating-type sequences copied from one of two silent donor loci, *HML*α or *HMR*a. The two donors lie at opposite ends of chromosome III, approximately 190 and 90 kb, respectively, from *MAT*. *MAT*α cells preferentially recombine with *HMR*, while *MAT*a cells select *HML*. The mechanisms of donor selection are different for the two mating types. *MAT*a cells, deleted for the preferred *HML* gene, efficiently use *HMR* as a donor. However, in *MAT*α cells, *HML* is not an efficient donor when *HMR* is deleted; consequently, approximately one-third of *HO HML*α *MAT*α *hmr*Δ cells die because they fail to repair the HO endonuclease-induced double-strand break at *MAT*. *MAT*α donor preference depends not on the sequence differences between *HML* and *HMR* or their surrounding regions but on their chromosomal locations. Cloned *HMR* donors placed at three other locations to the left of *MAT*, on either side of the centromere, all fail to act as efficient donors. When the donor is placed 37 kb to the left of *MAT*, its proximity overcomes normal donor preference, but this position is again inefficiently used when additional DNA is inserted in between the donor and *MAT* to increase the distance to 62 kb. Donors placed to the right of *MAT* are efficiently recruited, and in fact a donor situated 16 kb proximal to *HMR* is used in preference to *HMR*. The *cis*-acting chromosomal determinants of *MAT*α preference are not influenced by the chromosomal orientation of *MAT* or by sequences as far as 6 kb from *HMR*. These data argue that there is an α-specific mechanism to inhibit the use of donors to the left of *MAT*α, causing the cell to recombine most often with donors to the right of *MAT*α.

Homothallic mating-type gene switching depends on the presence of the two silent mating-type cassettes, *HML*α and *HMR***a**, located on chromosome III near the left and right telomeres, respectively (reviewed in references 9, 16, and 32) (Fig. 1A). Expression of the HO endonuclease initiates a site-specific gene conversion event in which the *MAT* locus is cut and then the double-strand break is repaired by recombining with homologous DNA sequences (X and Z1) in one of the donor loci (Fig. 1A). HO fails to cut the equivalent sequences in *HML* and *HMR*, as these regions are maintained in a different chromatin structure that also prevents their transcription (reviewed in reference 18). During the repair of the double-strand break at *MAT*, the Y**a** or Yα sequences that specify mating type are normally replaced by the opposite mating information encoded by one of the two donors. Although the double-strand break at *MAT* could be repaired by using homologous sequences from either donor, the process is regulated in such a way that *MAT*α cells preferentially recombine with *HMR* 80 to 90% of the time, even when both *HML* and *HMR* carry Yα sequences (14). Similarly, *MAT***a** cells use *HML*α about 90% of the time (14).

The mechanisms of *MAT***a** and *MAT*α donor preference are apparently not simple mirror images of each other. *MAT***a** donor preference involves an activation mechanism to regulate the accessibility of a large region (about 40 kb surrounding the position of *HML*) at the end of the left arm of chromosome III (42). In *MAT***a** cells, when a cloned *HMR*α gene was inserted at three different sites within this donor activation region, it was preferentially used in competition with the normal *HMR*α locus. But when this donor was inserted at several other chromosome III sites outside this region, it was not utilized preferentially. The expression in *MAT***a** cells of the negative regulator Matα2 (3, 31) abolishes this activation mechanism, presumably by repression of one or more **a**-specific genes (42). Surprisingly, the increased recombination of this donor activation region in *MAT***a** cells is independent of mating-type sequences. Spontaneous recombination between two *leu2* alleles is 20 to 30 times higher in *MAT***a** cells than in *MAT*α cells when one of the *leu2* alleles is inserted in place of the *HML* locus (42). Thus *HML* and other DNA sequences inserted in *HML* locus are more accessible in *MAT***a** cells, so that *HML* is selected as a preferred donor in competition with *HMR*. However, *HMR* is apparently not actively excluded as a donor, because *MAT***a** cells can switch mating type efficiently, using *HMR* as a donor, when *HML* is deleted (14) (see below).

As we show in this report, in contrast to *MAT***a** cells, a significant fraction of *MAT*α cells die when their preferred donor, *HMR*, is deleted. Moreover, there is no significant difference in the rate of *leu2* recombination in *MAT*α and *MAT***a** cells when one of the recombining *leu2* alleles is inserted in place of *HMR* (42). Very little is known about the mechanism controlling the *MAT*α donor preference. Previous work showed that *MAT*α donor preference was not changed by swapping the alleles (Y**a** and Yα) resident at the donor loci (14). In addition, inversion of the centromere did not influence donor selection (39). Since donor preference is mating-type dependent, the factors encoded by the *MAT* locus may be directly or indirectly involved in the regulation of donor preference. Rine et al. (26) showed that *matα1* sterile cells still chose *HMR* over *HML*, suggesting that α1 was not involved in the regulation of *MAT*α donor preference and also demonstrating that the expression of a full α phenotype was not required for *MAT*α donor preference. On the other hand, pedigree analysis showed that *HO hmlα2 matα2 HMR***a** cells

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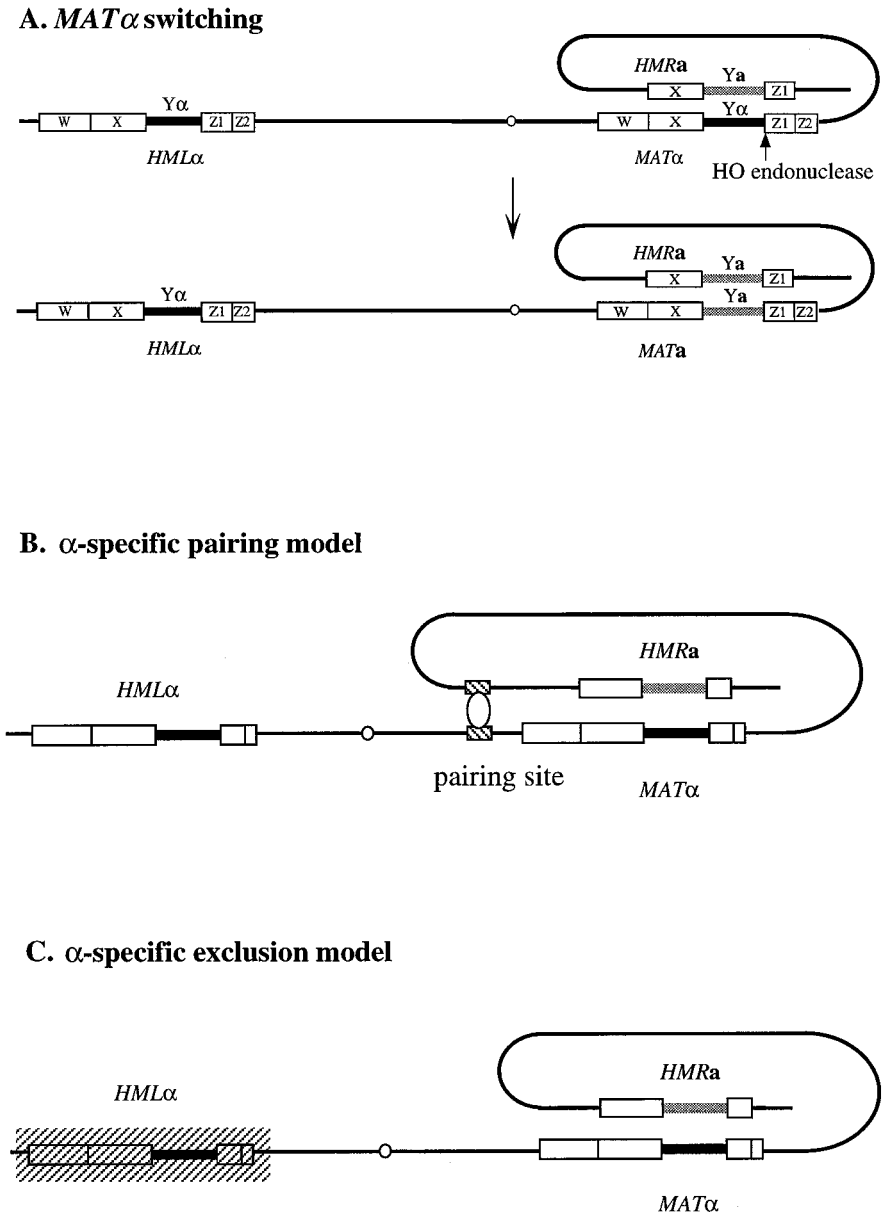

FIG. 1. HO-induced *MAT*α switching and models for *MAT*α switching. (A) Diagram of *MAT*α switching with *HMR*. After HO endonuclease cleavage at the Yα/Z1 border of *MAT*α, the Yα sequences are replaced with the Y**a** sequences of *HMR***a** by a gene conversion mechanism. The relative positions of the silent-copy donor loci, *HML*α and *HMR***a**, with respect to the *MAT* locus and the centromere of chromosome III are indicated. The homologous regions (W, X, Z1, and Z2) shared between *MAT* and the two donor loci are also indicated. (B) Model illustrating an α-specific pairing exclusively between *MAT*α and *HMR*. The model predicts the existence of α-specific proteins that bind at *cis*-acting sites to pair and align *MAT*α with the *HMR* donor. (C) Model illustrating exclusion of *HML* from interactions with *MAT*α by a mechanism which alters the chromatin structure around *HML* to prevent its participation as a donor.

rarely switched to *MAT***a**, implying that the donor preference in such cells was changed (34). In addition, we have shown that by expressing Matα2 in the *MAT***a** cells, *MAT***a** donor preference is shifted to *HMR* (42).

We envision two general classes of models to explain *MAT*α donor preference. In one class, the preferred donor *HMR* is actively recruited to recombine with *MAT*. This could occur by a selective pairing (Fig. 1B), in which one or more α-specific gene products interact with specific pairing sites to bring *MAT* and *HMR* together. Alternatively, *HMR* could be made more recombinogenic, by an alteration of chromosome structure or some change in its arrangement within the nucleus, similar to what we have observed for *MAT***a** donor preference (42). An alternative class of models is exemplified by Fig. 1C, in which there is selective exclusion of *HML*, whereby the wrong donor is directly prevented from interacting with *MAT*. This could arise by alterations in chromatin structure of the wrong donor, so that the HO-cleaved *MAT* DNA cannot invade the intact donor sequences to permit copying of new information. Recall that HO endonuclease cannot cleave *HML* or *HMR* (7, 33) even though the ends of *MAT* can invade and recombine with the same recognition site; therefore, it would not be difficult to imagine ways in which access even to the cut DNA ends could be restricted. Alternatively, the wrong donor could be somehow sequestered so that it cannot pair efficiently with *MAT*.

In this report, we show that chromosome III is arranged in

a way that a donor inserted in most regions to the left of *MAT*α, on either side of the centromere, is excluded from being used efficiently. However, when a donor is located to the right of *MAT*, it is efficiently recruited. These data support an exclusion model, except that the exclusion region is not limited to the *HML* locus but extends to most of the region to the left of *MAT*.

MATERIALS AND METHODS

Strains and plasmid constructions. All *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Strains NR226-7B and NR238-7C are segregants of a series of five or more backcrossings of an unrelated *HO* strain with the well-characterized strain DBY745, obtained by Norah Rudin. All experiments reported here were carried out with derivatives of NR226-7B, NR238-7C, or DBY745 modified only by gene transplacements, with the exceptions described below.

To test the effect of the *matα2-38* mutation (35) on donor preference, we first isolated strain JKM5, a Sir^+ Leu^- **a**>α segregant with strong **a** mating and weak α mating from a cross between strains 1523 and XW121. Strain 1523 (provided by I. Herskowitz) contains an *hmlα2-38* replacement of *HML*α (35). Strains that are *HO hmlα2-38 MAT***a** *HMR***a** will switch to produce *matα2-38* (sterile) cells but on rare occasions will produce a functional *MAT*α, depending on the extent of strand transfer and subsequent mismatch repair of an *hmlα2-38* mutation (21). If this infrequent switch occurs, these *MAT*α cells will mate to form normal diploids that sporulate. Strain JKM7 was isolated as a segregant from a cross between XW119 and JKM5, a segregant of a cross between strain 1523 and XW121. This segregant was screened for a phenotype that was Leu^+ **a**>α and able to sporulate.

Molecular biological methods for plasmid constructions were as described by Sambrook et al. (28). The *HMR*α sequences were copied from the genome of strain LR387-1A (Table 1) by gap repair (25). The gapped template plasmid was prepared by first removing the *URA3* gene from pURA3-9 (pJH162) (2) by *Xho*I digestion and then performing plasmid dephosphorylation. This gapped plasmid, with *HMR* flanking sequences forming the gap, also carries *ARS1*, *CEN3*, and a selectable marker, *TRP1*. After transformation of strain LR387-1A with the gapped template, Trp^+ transformants were selected and plasmids were recovered from yeast cells (23). The successful gap-repaired plasmid, pXW134, was shown by restriction analysis to contain *HMR*α sequences. In pJH455 (40), the *Xho*I *HML*α fragment of the original cloned *Bam*HI piece was replaced with the *Xho*I-to-*Sal*I *LEU2* gene. In the *Xho*I site of pJH455, a *Hin*dIII *HMR*α fragment was inserted to yield pXW135.

The *Xho*I-to-*Sal*I *LEU2* fragment was inserted into the *Nru*I site of the *Hin*dIII *HMR*α fragment in pXW145 (Fig. 2B). This *LEU2*-marked *Hin*dIII *HMR*α piece was inserted at different regions along chromosome III. These regions were first cloned by PCR amplification by using oligonucleotide primers designed according to the published DNA sequence of chromosome III (24). The *HMR*α cassette was inserted into a unique *Xho*I site of the PCR fragment at nucleotide (nt) 67682 near the *HIS4* gene (pXW200), a unique *Xho*I site at nt 90286 near the *LEU2* gene (pXW162), a unique *Pst*I site at nt 133090 (pXW212), a unique *Xba*I site at nt 162589 (pXW185), a unique *Xba*I site at nt 233174 (pXW161), a unique *Nru*I site at nt 248900 (pXW187), and a unique *Xba*I site at nt 302187 (pXW183). The *HMR*α sequence was also inserted at kb 276 by replacing a 231-bp fragment between the two *Eco*RV sites (nt 275993 and 276224) with the *HMR*α::*LEU2* sequence. pXW216-3 is a pBR322-based plasmid containing 17 kb of λ sequence, a *URA3* gene, and a 3.4-kb sequence corresponding to the 190-kb region of chromosome III.

pURA3-9 contains DNA from the *HMR* locus in which the *HMR* cassette marked by *Xho*I linker inserts has been deleted and replaced with a *Xho*I-linked *URA3* gene. DNA sequence analysis showed that the *HMR* deletion extends from a site 57 bp proximal to the X border to a site 166 bp distal from the Z1 border (41). Additionally, an *Xho*I site, originally 500 bp distal from *HMR*, has been deleted in this plasmid (2). Plasmid pWAC4U extended the *HMR* deletion in pURA3-9 to a site 248 bp proximal to the X border and a site 310 bp distal from the Z1 border. This larger deletion was made by limited *Bal*31 digestion of *Xho*I-digested pURA3-9 followed by the reintroduction of an *Xho*I linker and the *Xho*I-linked *URA3* gene. The *URA3* gene in pWAC4U was replaced by an *Xho*I-to-*Sal*I *LEU2* gene to produce pXW103. In pXW107, *HMR* along with flanking sequences 1.7 kb proximal and 166 bp distal was replaced by a 1.1-kb *URA3* gene. In pXW139, *HMR* along with flanking sequences 57 bp proximal and 166 bp distal was replaced by a 1.1-kb *URA3* gene, and a 5.4-kb *Xho*I fragment of *HML*α from pJH285 (40) was inserted into a unique *Xho*I site.

pXW123 was obtained by deleting the 5.4-kb *Xho*I fragment spanning the *HML* locus from pJH285, which contains a 6.5-kb *Bam*HI *HML*α fragment, and replacing the deleted *HML* cassette with an *Xho*I site-flanked *ADE1* gene (22). A 7.3-kb *Hin*dIII *HMR*α fragment marked with the *LEU2* gene from pXW145 was used to replace the *Xho*I piece of *HML*α in pJH285 to produce pXW202.

Terminal deletions of the right arm of chromosome III were constructed by placing a gene targeting sequence adjacent to 0.3 kb of *Tetrahymena* T_2G_4 repeats that serve to create a new yeast telomere (36). In pXW152 (Fig. 2A), a 1-kb fragment about 6 kb proximal to *HMR* was placed next to a *LEU2* gene and *Tetrahymena* T_2G_4 repeats. The 5.1-kb *Hin*dIII *HMR*α sequence was inserted between the 1-kb fragment and the *LEU2* gene in pXW152 to produce pXW164. Similarly, a 5.4-kb *Xho*I *HML*α sequence was inserted at the same site as *HMR*α in pXW164 to create pXW153.

The *HMR*α-*Bam*HI allele was created by oligonucleotide-directed mutagenesis of C to A at position 658 in Yα in pXW142 to create a *Bam*HI site as described previously (42). The *Bam*HI site was introduced into the *MAT*α locus by switching *MAT***a** *HMR*α-*Bam*HI strains to *MAT*α-*Bam*HI. Strains XW496, XW551, and XW572 were obtained by crossing *MAT*α-*Bam*HI *HMR*α-*Bam*HI strains with appropriate *MAT***a** *HMR***a** strains and dissecting the resulted diploid strains.

A 71-kb chromosome III inversion in strain XW430 was created by inserting a *leu2K* allele (19) at kb 233 (from pXW225) and integrating an oppositely oriented *leu2R* allele (19) with a *URA3* gene and a DNA fragment of the kb 162 region on a pBR322 vector (from pXW227) at kb 162. $Leu2^+$ strains that contained a crossover between two *leu2* alleles were identified by their failure to produce 5-fluoroortic acid-resistant papillae, because the kb 162 region sequences that flank the *leu2* allele become translocated to different chromosomal regions, and thus these regions cannot easily recombine to "pop out" the originally intervening *URA3* gene (4, 10). The inversion was confirmed by Southern analysis. pXW225 was constructed by inserting an *Xho*I-to-*Sal*I *leu2K* allele, which contains an *ADE1* gene at the *Ase*I site of *leu2K*, at the *Xba*I site of a 0.83-kb fragment corresponding to kb 233 on chromosome III, which was previously cloned in the pGEM3Zf(+) vector. pXW227 was constructed by inserting a *URA3* gene, a 0.76-kb piece of the kb 162 region of the chromosome, and a *leu2R* allele in pGEM3Zf(+).

The following plasmids were generously provided by others. Plasmid pSL1469 contains a *STE3* deletion marked by *URA3* (30a). pDJ154 contains a *STE4* deletion marked by *LEU2* (11). pSUL16 contains a *STE12* deletion marked by *LEU2* (8). pDH90 contains a *STE7* deletion marked by *LEU2* (12a). pSM86 contains an *MF***a***1* deletion marked by *LEU2*, and pSM35 contains an *MF***a***2* deletion marked by *URA3* (21a).

Genetic methods and media. Complex media, synthetic media with amino acid supplements, and sporulation medium, as well as all general methods for growth and sporulation of yeast strains, were as described by Sherman et al. (30). Galactose induction of the *HO* gene was performed as follows. Yeast cells were incubated in dropout medium with glucose to maintain the *GAL-HO* plasmid overnight. Cells were then washed with water and diluted in YEP-lactate medium so that the concentration of the culture would reach 1×10^6 to 5×10^6/ml after overnight incubation. Galactose was added to a concentration of 2%, and growth was induced for 1.5 h. Subsequently, cells were diluted and spread on YEPD plates.

Transformations were carried out by using the method of Ito et al. (13) as modified by Schiestl and Gietz (29). Specific gene deletions were made by gene transplacement methods described by Rothstein (27), using linear plasmid fragments which resulted in a deletion of the gene marked by a nutritional marker.

DNA analysis. PCR amplification of the *MAT* locus was performed by using primers KK200 (CGACCACTCAAGAAAGA) and JK735 (ATGTGAACCG CATGGGCAGT) to amplify a 769-bp *MAT*α-specific DNA fragment. *MAT*α-*Bam*HI contains a *Bam*HI site; after *Bam*HI digestion of the PCR products, two fragments of 582 and 187 bp are obtained.

RESULTS

An *HO MAT*α strain fails to recombine efficiently with an *HML*α strain when *HMR* sequences are deleted. To test how strongly *MAT*α cells are excluded from using *HML*α as a donor, we constructed a homothallic *MAT*α/*MAT***a** diploid strain deleted for *HMR* and carrying *HML*α. When this diploid (XW179) was sporulated and tetrads were dissected, the two *MAT***a** segregants could switch efficiently, still using their preferred *HML* donor, to form nonmating *MAT***a**/*MAT*α colonies. The consequences of this deletion for *MAT*α segregants deprived of *HMR* are illustrated in Fig. 3A. Here, *MAT*α cells should switch from *MAT*α to *MAT*α and grow into α-mating colonies. Apparently, *HML* cannot act as an efficient backup donor in *MAT*α cells, as the two *MAT*α segregants form very tiny colonies. It appears that the double-strand break at *MAT*α often cannot be repaired, leading to the death of the cell. This result is in contrast to what is seen in segregants of an *HO*/*HO MAT***a**/*MAT*α diploid homozygous for *HMR***a** but deleted for *HML* (Fig. 3B). Here, all four segregants form large colonies, confirming previously published results that *MAT***a** cells can use *HMR* as a backup donor, continuously switching from *MAT***a** to *MAT***a**, when *HML* is deleted (14).

TABLE 1. *S. cerevisiae* strains used

Strain	Genotype	Source
NR226-7B	*HO HML*α *MAT*α *HMR***a** *leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *HMR***a** *leu2-3,112 lys5 ura3-52*	This laboratory
NR238-7C	*HO HML*α *MAT*α *HMR***a** *ade1 leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *HMR***a** *ade1 leu2-3,112 lys5 ura3-52*	This laboratory
XW119	*HO HML*α *MAT*α *hmr*Δ*::LEU2 leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *hmr*Δ*::LEU2 leu2-3,112 lys5 ura3-52*	Segregant of NR226-7B (pXW103)[a]
XW121	*HO hml*Δ*::LEU2 MAT*α *HMR***a** *ade1 leu2-3,112 lys5 ura3-52* *HO hml*Δ*::LEU2 MAT***a** *HMR***a** *ade1 leu2-3,112 lys5 ura3-52*	Segregant of NR238-7C (pJH455)[a]
XW133	*HO HML*α *MAT*α *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52*	Segregant of NR226-7B (pXW107)[a]
XW153	*HO hml*Δ*::ADE1 MAT*α *HMR***a** *ade1 leu2-3,112 lys5 ura3-52* *HO hml*Δ*::ADE1 MAT***a** *HMR***a** *ade1 leu2-3,112 lys5 ura3-52*	Segregant of NR238-7C (pXW123)[a]
XW155	*HO HML*α *MAT*α *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52 ste7*Δ*::LEU2* *HO HML*α *MAT***a** *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52 STE7*	XW133 (pDH90)[b]
XW157	*HO hml*Δ*::ADE1 MAT*α *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *HO hml*Δ*::ADE1 MAT***a** *HMR***a** *ade1 leu2-3,112 lys5 ura3-52*	XW153 (pURA3-9)[b]
XW177	*HO hml*Δ*::ADE1 MAT*α *hmr*Δ*::(HML*α *URA3) ade1 leu2-3,112 lys5 ura3-52* *HO hml*Δ*::ADE1 MAT***a** *hmr*Δ*::(HML*α *URA3) ade1 leu2-3,112 lys5 ura3-52*	Segregant of XW133 (pXW139)[a]
XW179	*HO HML*α *MAT*α *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *hmr*Δ*::URA3 leu2-3,112 lys5 ura3-52*	Segregant of NR226-7B (pWAC4U)[a]
XW186	*HO HML*α *MAT*α *hmr*Δ*::(HMR*α *LEU2) leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *hmr*Δ*::(HMR*α *LEU2) leu2-3,112 lys5 ura3-52*	Segregant of XW110 (pXW145)[a]
XW189	*HO hml*Δ*::ADE1 leu2::(HML*α *LEU2) MAT*α *hmr*Δ*::URA3 ade1 leu2 lys5 ura3-52* *HO hml*Δ*::ADE1 leu2::(HML*α *LEU2) MAT***a** *hmr*Δ*::URA3 ade1 leu2 lys5 ura3-52*	Segregant of XW157 (pXW148)[a]
XW200	*HO HML*α *MAT*α (6 kbΔ *hmr*Δ*::LEU2* all distal Δ) *leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** (6 kbΔ *hmr*Δ*::LEU2* all distal Δ) *leu2-3,112 lys5 ura3-52*	XW110 (pXW152)[b]
XW221	*HO HML*α *MAT*α (*HMR*α*-Bam*HI *URA3*) *leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** (*HMR*α*-Bam*HI *URA3*) *leu2-3,112 lys5 ura3-52*	Segregant of XW111 (pXW172)[a]
XW227	*HO HML*α *MAT*α kb 285::(*HMR*α *LEU2*) 6-kb *HMR* proximal Δ, all *HMR* distal Δ *HO HML*α *MAT***a** kb 285::(*HMR*α *LEU2*) 6-kb *HMR* proximal Δ, all *HMR* distal Δ *leu2-3,112 lys5 ura3-52* *leu2-3,112 lys5 ura3-52*	Segregant of XW110 (pXW164)[a]
XW230	*HO hml*Δ*::ADE1 MAT*α kb 233::(*HMR*α *LEU2*) *hmr*Δ*::URA3 ade1 leu2-3,112* *HO hml*Δ*::ADE1 MAT***a** kb 233::(*HMR*α *LEU2*) *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *lys5 ura3-52*	Segregant of XW157 (pXW161)[a]
XW234	*HO hml*Δ*::ADE1 leu2::(HMR*α *LEU2) MAT*α *hmr*Δ*::URA3 ade1 leu2 lys5 ura3-52* *HO hml*Δ*::ADE1 leu2::(HMR*α *LEU2) MAT***a** *hmr*Δ*::URA3 ade1 leu2 lys5 ura3-52*	Segregant of XW157 (pXW162)[a]
XW236	*HO hml*Δ*::ADE1 MAT*α *hmr*Δ*::URA3* kb 302::(*HMR*α *LEU2*) *ade1 leu2-3,112* *HO hml*Δ*::ADE1 MAT***a** *hmr*Δ*::URA3* kb 302::(*HMR*α *LEU2*) *ade1 leu2-3,112 lys5 ura3-52* *lys5 ura3-52*	Segregant of XW157 (pXW183)[a]
XW238	*HO hml*Δ*::ADE1* kb 162::(*HMR*α *LEU2*) *MAT*α *hmr*Δ*::URA3 ade1 leu2-3,112* *HO hml*Δ*::ADE1* kb 162::(*HMR*α *LEU2*) *MAT***a** *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *lys5 ura3-52*	Segregant of XW157 (pXW185)[a]
XW240	*HO hml*Δ*::ADE1 MAT*α kb 249::(*HMR*α *LEU2*) *hmr*Δ*::URA3 ade1 leu2-3,112* *HO hml*Δ*::ADE1 MAT***a** kb 249::(*HMR*α *LEU2*) *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *lys5 ura3-52*	Segregant of XW157 (pXW187)[a]
XW246	*HO hml*Δ*::(HMR*α *LEU2) *MAT*α (*HMR*α*-Bam*HI *URA3*) *ade1 leu2-3,112 lys5* *HO hml*Δ*::(HMR*α *LEU2*) *MAT***a** (*HMR*α*-Bam*HI *URA3*) *ade1 leu2-3,112 lys5 ura3-52* *ura3-52*	Segregant of XW221 (pXW135)[a]
XW251	*HO hml*Δ*::ADE1 his4::(HMR*α *LEU2*) MAT*α *hmr*Δ*::URA3 ade1 leu2-3,112 lys5* *HO hml*Δ*::ADE1 his4::(HMR*α *LEU2*) MAT***a** *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *ura3-52*	Segregant of XW157 (pXW200)[a]
XW253	*HO hml*Δ*::HMR*α *LEU2 MAT*α *hmr*Δ*::URA3 ade1 leu2-3,112 lys5* *HO hml*Δ*::HMR*α *LEU2 MAT***a** *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *ura3-52*	Segregant of XW157 (pXW202)[a]
XW265	*HO hml*Δ*::ADE1 leu2::(HMR*α *LEU2) *MAT*α (*HMR*α*-Bam*HI *URA3*) *ade1* *HO hml*Δ*::ADE1 leu2::(HMR*α *LEU2*) *MAT***a** (*HMR*α*-Bam*HI *URA3*) *ade1 leu2-3,112 lys5 ura3-52* *leu2-3,112 lys5 ura3-52*	Segregant of XW233 × XW221
XW305	*HO hml*Δ*::ADE1* kb 133::(*HMR*α *LEU2*) *MAT*α *hmr*Δ*::URA3 ade1* *HO hml*Δ*::ADE1* kb 133::(*HMR*α *LEU2*) *MAT***a** *hmr*Δ*::URA3 ade1 leu2-3,112 lys5 ura3-52* *leu2-3,112 lys5 ura3-52*	Segregant of XW157 (pXW212)[a]

Continued on following page

TABLE 1—*Continued*

Strain	Genotype	Source
XW312	*HO HML*α *MAT*α *hmr*Δ::*ADE1 mf***a***1*Δ::*LEU2 mf***a***2*Δ::*URA3 leu2-3,112 lys5 ura3-52* *HO HML*α *MAT***a** *hmr*Δ::*ADE1 mf***a***1*Δ::*LEU2 MF***a***2 leu2-3,112 lys5 ura3-52*	Segregant of XW203 (pSM86+pSM35)[a]
XW330	*HO hml*Δ::*ADE1* kb 133::(*HMR*α *LEU2*) *MAT*α (*HMR*α-*Bam*HI *URA3*) *ade1* *HO hml*Δ::*ADE1* kb 133::(*HMR*α *LEU2*) *MAT***a** (*HMR*α-*Bam*HI *URA3*) *ade1 leu2-3,112 lys5 ura3-52*	Segregant of XW305 × XW221
XW336	*HO hml*Δ::*ADE1* kb 162::(*HMR*α *LEU2*) 190 kb::(25-kb *URA3* insert) *MAT*α *HO hml*Δ::*ADE1* kb 162::(*HMR*α *LEU2*) 190 kb::(25-kb *URA3* insert) *MAT***a** *hmr*Δ::*URA3 ade1 leu2-3,112 lys5 ura3-52*	Segregant of XW238 (pXW216-3)[a]
XW348	*HO hml*Δ::*ADE1 his4*::(*HMR*α *LEU2*) *MAT*α (*HMR*α-*Bam*HI *URA3*) *ade1* *HO hml*Δ::*ADE1 his4*::(*HMR*α *LEU2*) *MAT***a** (*HMR*α-*Bam*HI *URA3*) *ade1 leu2-3,112 lys5 ura3-52*	Segregant of XW251 × XW221
DBY745	*ho HML*α *MAT*α *HMR***a** *ade1 leu2-3,112 ura3*	
LR387-1A	*HO HML*α *MAT***a**-*inc(4-28) HMR*α *ade1 ade2 lys1 lys9 trp1 can*^{s}	
XW424	*ho HML*α kb 162::(*leu2R URA3*) *MAT*α-*Bam*HI 233 kb::(*leu2K ADE1*) *HMR***a** *ade1 leu2*Δ *ura3-52* pJH283 (*Gal-HO TRP1*)	XW551 (pXW225, pXW227)
XW430	*ho HML*α 71-kb inversion (from kb 162 to 233 including *MAT*α-*Bam*HI *ADE1 LEU2 URA3*) *HMR***a** *ade1 leu2*Δ *ura3-52* pJH283 (*Gal-HO TRP1*)	XW424 (inversion)
XW496	*ho hml*Δ::*ADE1 his4*::(*HMR*α *LEU2*) *MAT*α-*Bam*HI *HMR***a** *leu2-3,112 lys5 ura3-52* pJH283 (*Gal-HO TRP1*)	Materials and Methods
XW551	*ho HML*α *MAT*α-*Bam*HI *HMR***a** *ade1 leu2*Δ *trp1 ura3-53* pJH283 (*Gal-HO TRP1*)	Materials and Methods
XW572	*ho hml*Δ::*ADE1 MAT*α-*Bam*HI *HMR***a** *ade1 leu2 trp1 ura3-53*	Materials and Methods
XW586	*ho hml*Δ::*ADE1 MAT*α-*Bam*HI kb 276::(*HMR*α *LEU2*) *HMR***a** *ade1*, *leu2-3,112 trp1 ura3-52* pJH283 (*Gal-HO TRP1*)	XW572 (pXW252)[c]
JKM5	*HO hml*α*2-38 MAT*α *HMR***a** *leu2 his4 ura3-52* *HO hml*α*2-38 MAT***a** *HMR***a** *leu2 his4 ura3-52*	Materials and Methods
JKM7	*HO hml*α*2-38 MAT*α *hmr*Δ::*LEU2 leu2 his4 ura3-52* *HO hml*α*2-38 MAT***a** *hmr*Δ::*LEU2 leu2 his4 ura3-52*	Materials and Methods
JKM26	*HO HML*α *MAT*α *hmr*Δ::*URA3 leu2-3,112 lys5 ura3-52* *ste4*Δ::*LEU2* *HO HML*α *MAT***a** *hmr*Δ::*URA3 leu2-3,112 lys5 ura3-52* *STE4*	XW133 (pDJ154)[b]
JKM27	*HO HML*α *MAT*α *hmr*Δ::*LEU2 leu2-3,112 lys5 ura3-52* *ste3*Δ::*URA3* *HO HML*α *MAT***a** *hmr*Δ::*LEU2 leu2-3,112 lys5 ura3-52* *STE3*	XW119 (pSL1469)[b]
JKM28	*HO HML*α *MAT*α *hmr*Δ::*URA3 leu2-3,112 lys5 ura3-52* *ste12*Δ::*LEU2* *HO HML*α *MAT***a** *hmr*Δ::*URA3 leu2-3,112 lys5 ura3-52* *STE12*	XW133 (pSUL16)[b]

[a] Homozygous segregants from dissections of the strains indicated after gene transplacements utilizing the plasmid indicated.
[b] The strain and plasmid used for gene transplacements are indicated. For descriptions of plasmids used for gene transplacements, see Materials and Methods.
[c] The strain and plasmid used for gene transplacements are indicated. In addition, a *GAL-HO* plasmid was transformed into the strain.

The conclusion that the lethality of *HO HML*α *MAT*α *hmr*Δ segregants resulted from unsuccessful attempts to switch is supported by several lines of evidence. First, the apparent lethality of *HO HML*α *MAT*α *hmr*Δ segregants depended on HO-mediated events. The same disruption in an *ho* haploid strain had no effect (data not shown). Second, *MAT*α lethality is not simply a consequence of continuous switching in which Yα is replaced by another copy of Yα. To establish this point, we replaced *HMR***a** with *HMR*α by using plasmid pXW145 (Fig. 2B and Materials and Methods) and demonstrated that *HO HML*α *MAT*α *HMR*α spores gave rise to normal-size, α-mating colonies (Fig. 3C). All four segregants of diploid XW186 were large, in sharp contrast to the tiny *MAT*α segregants in strains carrying *hmr*Δ. Thus, continuous switching to Yα is not lethal in these strains; rather, the deletion of *HMR* causes lethality.

Third, pedigree analysis showed that mother cells that attempted to switch frequently died. Normally, *HO* cells show a lineage-dependent pattern of switching (12) in which a cell that has previously divided (the mother cell) can express *HO* and give rise to two switched progeny, while a newly formed daughter cell does not switch and gives rise to two cells of the original mating type. Subsequently, the first daughter, now a mother, can switch. To examine the lethality of *HO HML*α *MAT*α *hmr*Δ cells, strain XW119 was sporulated and the four members of each tetrad were placed close to a source of α factor, a streak of *MAT*α cells. The two *MAT***a** derivatives formed shmoos, while the two *MAT*α spores began to divide. As soon as the first cell division was complete, the mother and daughter *MAT*α cells were separated from the pheromone and allowed to continue growing. Most cells produced the same tiny colonies characteristic of *HO HML*α *MAT*α *hmr*Δ cells, but approximately one-third of the time (10 of 31 cases), one of the two cells in a mother-daughter pair failed to grow into a visible colony. These cells apparently failed to repair the double-strand break created in the next cell division. In keeping with previous observations of such cells (15), they produced microcolonies of between two and eight cells.

Finally, we could demonstrate that at least some cells in the tiny colonies carry a broken chromosome as a result of the failure to repair the double-strand break at *MAT*. *HO HML*α *MAT*α *hmr*Δ::*URA3* tiny colonies show weak **a** mating as well as strong α-mating behavior (α>**a** mating), although there is no Y**a** sequence in the cell. The weak **a** phenotype is a consequence of the disruption of *MAT*α1 and *MAT*α2 expression by an unrepairable *MAT* locus undergoing a DNA degradation

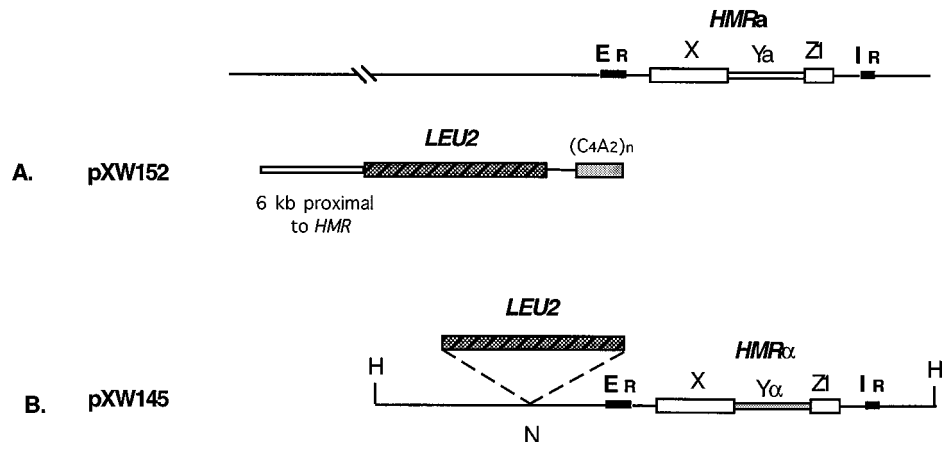

FIG. 2. Plasmid constructs. (A) pXW152 contains a 1-kb fragment, corresponding to the region 6 kb proximal to *HMR* on chromosome III. This fragment was placed next to a *LEU2* gene and a 0.3-kb *Tetrahymena* T_2G_4 repeat. (B) In plasmid pXW145, a *LEU2* gene was inserted into the *Nru*I site (N) of the *Hin*dIII *HMR*α fragment. This fragment was inserted at several different locations on chromosome III. E_R and I_R refer to *HMR* E and I silencers.

which, transiently on the way to death, yields an **a**-like (*matα1*$^-$ *α2*$^-$) phenotype (17, 37). These **a**-like cells can be rescued by mating with an *ho MAT*α-*inc* (an uncuttable allele of *MAT*α) *ura3* strain. The resulting diploids were almost all α mating, as expected if the **a**-mating cells were only **a**-like and the broken chromosome was repaired by recombining with the *MAT*α-*inc* locus (17, 37).

The presence of **a**-like cells in the tiny colonies contributes to the tiny-colony phenotype of *HO HML*α *MAT*α *hmr*Δ cells. The **a**-like cells produce MF**a** pheromone, which apparently can inhibit the growth of neighboring *MAT*α cells. This was demonstrated by constructing diploid XW312 (Table 1) and obtaining *HO HML*α *MAT*α *hmr*Δ segregants with deletions of both copies of the *MF***a** gene (*mf***a***1*Δ::*LEU2* and *mf***a***2*Δ::*URA3*). The strain is homozygous for a deletion of *MF***a***1* but heterozygous for *MF***a***2*. *MAT***a** or **a**-like cells that are unable to produce MF**a** have a nonmating (sterile) phenotype. Hence, these *HO HML*α *MAT*α *hmr*Δ cells now were only α mating instead of α>**a** mating. Dissection results for XW312 are shown in Fig. 3D. Both *MAT*α and *MAT***a** spores of genotype *HO HML*α *hmr*Δ *mf***a***1*Δ *mf***a***2*Δ eventually form *HML*α *MAT*α *hmr*Δ *mf***a***1*Δ *mf***a***2*Δ colonies. The first switching event in *MAT***a** colonies usually happens at the four-cell stage, but the two *MAT*α cells produced after switching cannot mate with the other two sterile *HO HML*α *MAT***a** *hmr*Δ *mf***a***1*Δ *mf***a***2*Δ unswitched cells; therefore, switching continues until all cells in the colony switched to α. All Leu$^+$ and Ura$^+$ cells gave rise to small colonies instead of tiny colonies (Fig. 3D). *MAT*α segregants that still carried *MF***a***2* produced, as expected, tiny colonies. *MAT***a** segregants carrying *MF***a***2* form normal, large nonmating colonies. This result confirmed that sometimes *MAT*α cells were able to use *HML*α as a donor when *HMR* was deleted, but not efficiently.

We have obtained essentially identical results by constructing *HO HML*α *MAT*α *hmr*Δ strains that are deleted for one of the mating pheromone signal transduction genes, *STE3*, *STE4*, *STE7*, and *STE12*. In each case, the *MAT*α segregants grew into small, as opposed to tiny, colonies (data not shown). Thus, the apparent lethality in *HO HML*α *MAT*α *hmr*Δ strains is exaggerated, in a useful way, by the autoinhibition of growth within the colony when **a**-like cells are created, but the frequent lethality in these strains is apparent even when **a**-like cells are prevented from inhibiting their neighbors. This distinctive tiny-colony phenotype is a convenient and reliable indicator of whether an alternative donor is efficient when *HMR* is deleted. We refer to this effect as the exclusion, or discouragement, of *HML*, although we do not mean to imply that *HML* is completely prevented from acting as a donor in *MAT*α *hmr*Δ strains.

HMR*α sequences replacing *HML* behave as if they were *HML* sequences, and vice versa.** Although *HML* and *HMR* are both capable of being used as donors, the two regions are not identical. *HML* shares more homology with *MAT* than does *HMR* (Fig. 1A). Moreover, the E and I silencer regions around *HML* and *HMR*, which are essential to transcriptionally silence these regions, are significantly different in DNA sequence and in the ability to silence adjacent regions (1, 20). Finally, the sequences flanking the two donors are not the same. We therefore asked if the failure to use *HML* as an efficient donor was position dependent or donor sequence dependent. Could *HMR*α, inserted in place of *HML*, act as an efficient donor, or would it, too, be excluded from efficient *MAT* repair? To answer this question, a 5.4-kb *Xho*I fragment, including all of the *HML* sequences and its adjacent silencer sites, was removed and replaced with 5.0 kb of *HMR* and its adjacent silencer sequences (see Materials and Methods). Diploid XW253, of genotype *MATa**/*MAT*α and homozygous for *HO*, *hmr*Δ::(*HMR*α *LEU2*), and *hmr*Δ::*URA3*, was sporulated and dissected. All of the *HO hml*Δ::(*HMR*α *LEU2*) *MAT*α *hmr*Δ::*URA3* spores gave the same tiny-colony phenotype as did segregants that carried *HML*α (Fig. 3E). All *MAT***a** segregants gave normal size, nonmating diploid colonies, as expected if *hml*Δ::*HMR*α could act as an efficient donor for *MAT***a**. These results lead to the conclusion that the basis of excluding *HML* or including *HMR* is not found in the sequence differences between *HML* and *HMR* or in the immediately surrounding vicinity that was inserted into another location. This observation is consistent with the results of Weiler and Broach (39) and shows that our strains do not give different results. These results are important for the experiments described below, in which *HMR*α was moved to other chromosomal locations.

We also asked whether *HML*α would still be excluded as a donor if it were moved to the normal position of *HMR*. *HMR* was deleted and replaced with the 5.4-kb *Xho*I *HML*α fragment marked with *URA3*. Diploid strain XW177 homozygous for *HO*, *hml*Δ::*ADE1*, and *hmr*Δ::(*HML*α *URA3*) was sporulated and dissected. As shown in Fig. 3F, all four segregants were large, similar to the dissection pattern of strain XW186

[*HML*α *hmr*Δ::*(HMR*α *LEU2)*] discussed above (Fig. 3C). Thus, none of the sequences in the *Xho*I-*Xho*I region including *HML*α are responsible for excluding *HML*α as a donor when it is moved to a different location. Therefore, like Weiler and Broach (39), we conclude that donor preference in *MAT*α cells depends not on the difference between *HML* and *HMR* or their flanking sequences but on their locations.

Flanking sequences near *HMR* are not required for *MAT*α donor preference. As described in the introduction, either the *HMR* pairing/activation model or the *HML* exclusion model could account for donor preference in *MAT*α cells. The pairing/activation model requires that some sequences around *HMR* and *MAT* be used to bring these loci into conjunction and thus exclude *HML*. This might occur even if *HMR* itself were deleted but if pairing sites were left intact. A large deletion around *HMR* was constructed to determine whether the lethality of *HO HML*α *MAT*α *hmr*Δ cells could be rescued by preventing *MAT* and *hmr*Δ pairing. This large deletion removed *HMR* and all surrounding sequences from a point 6 kb proximal to *HMR* to the end of the chromosome and was constructed by integrating a 200- to 300-bp *Tetrahymena* $(T_2G_4)_n$ sequence to serve as an artificial telomere (36) (Fig. 2A). This deletion failed to rescue the tiny-colony phenotype of the *HO HML*α *MAT*α *hmr*Δ strain (data not shown). Therefore, if pairing sites exist, they reside more than 6 kb proximal to *HMR*.

Donor position on chromosome III dictates its use in *MAT*α switching. Another way to establish why one donor is preferred is to move *HMR*α to another location on chromosome III, far from either *HML* or *HMR*. If the failure to use *HML*α as an efficient donor reflects some sort of local exclusion of *HML*, then if a donor is inserted at a different site, far from *HML*, it should be efficiently recruited. On the other hand, if the pairing/activation model is correct, insertion of a donor far away from the *HMR* locus will separate the pairing site or activation site from the donor and the alternative donor will not be used efficiently. From the results of moving *HMR* in place of *HML*, we presumed that the 5-kb *Hin*dIII fragment containing *HMR*α did not carry any putative pairing or activation sites. We therefore created plasmid pXW162 to insert a 5-kb *Hin*dIII fragment containing *HMR*α at the *LEU2* locus (91 kb from the left end of chromosome III) (Fig. 4A, construct IV), which is located approximately equidistant between the *HML* locus (12 kb from the left end) and the *MAT* locus (199 kb from the left end). Beginning with a heterozygous transformant of strain XW157 with this construct, we obtained homozygous *HO*/*HO MAT***a**/*MAT*α segregants deleted for both normal donors (*hml*Δ::*ADE1* and *hmr*Δ::*URA3*) but carrying *LEU2*::*HMR*α. When this strain, XW234, was sporulated and dissected, we found that *MAT*α segregants had the same tiny-colony phenotype indicative of inefficient use of the alternative donor (Fig. 4B). When the 5.4-kb *Xho*I *HML*α fragment was inserted in the *LEU2* locus (XW189), the same results were obtained. These results seem in favor of models in which there is an activation or pairing site linked to *HMR* rather than an exclusion site near *HML*. However, additional results argue for a more complex mechanism (see below).

To confirm the results that we obtained with strains XW189 (*HML*α at *LEU2*) and XW234 (*HMR*α at *LEU2*), *HMR*α was also inserted into a location 233 kb (relative to the left telomere) close to the *RAD18* locus (pXW161) in a donorless diploid strain (Fig. 4A, construct VIII). Surprisingly, only large-spore colonies were obtained after dissection of strain XW230 (Fig. 4C). This result was fundamentally different from that for XW234, in which the *HMR*α donor is near *LEU2*. To clarify this ambiguity, *HMR*α was inserted into several more

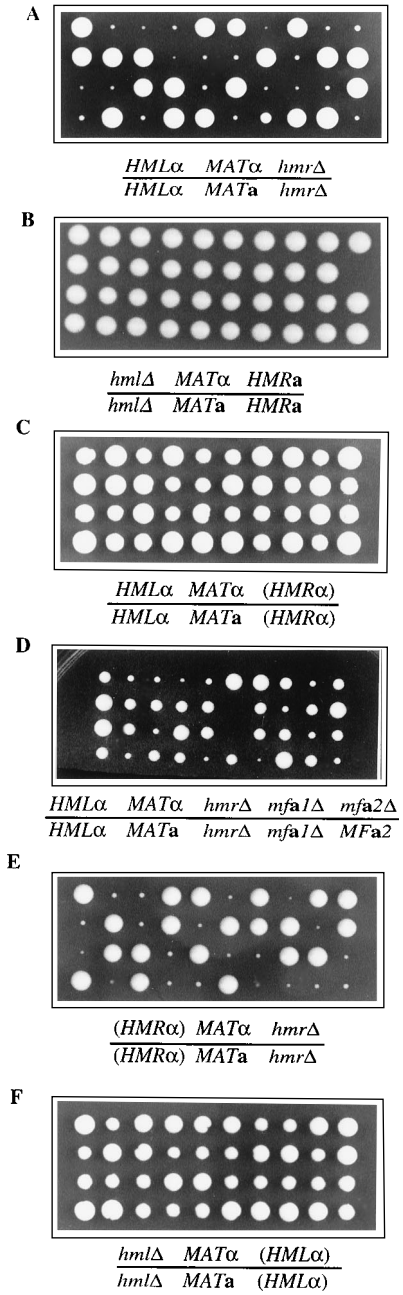

FIG. 3. Tetrad dissections of strains with altered mating cassettes. Each column shows four spore colonies derived from one tetrad. (A) Diploid strain XW179 is homozygous for a deletion of the *HMR* locus. (B) Diploid strain XW153 is homologous for a deletion of the *HML* locus. (C) Diploid strain XW186 is homozygous for a replacement of the *HMR***a** cassette with an *HMR*α cassette (in parentheses). (D) Diploid strain XW312, deleted for the *HMR* locus, is homozygous for an *MFa1* deletion and heterozygous for an *MFa2* deletion. (E) Diploid strain XW253 is homozygous for a replacement of the *HML*α cassette with an *HMR*α cassette (in parentheses) and is homozygous for a deletion of the *HMR* locus. (F) Diploid strain XW177 is homozygous for a deletion of the *HML* locus and homozygous for a replacement of the *HMR* locus with an *HML*α cassette.

locations on the chromosome III (Fig. 4A). Approximately 500 bp of the chromosome III sequence were cloned from genome by PCR amplification using primers designed according to the published chromosome III sequence (24) (see Materials and Methods). Then *HMR*α marked with *LEU2* (the *Hin*dIII frag-

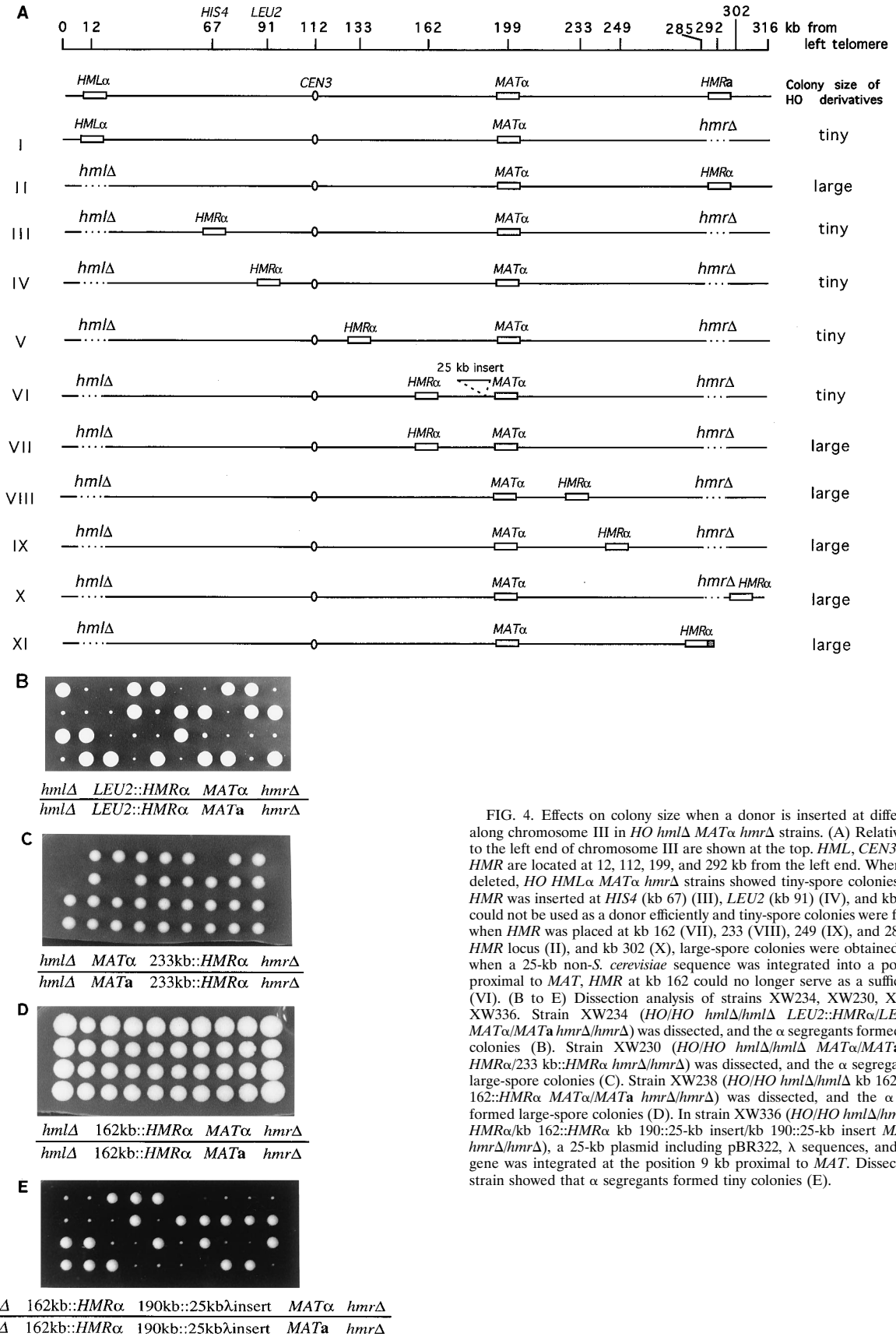

FIG. 4. Effects on colony size when a donor is inserted at different places along chromosome III in *HO hml*Δ *MAT*α *hmr*Δ strains. (A) Relative positions to the left end of chromosome III are shown at the top. *HML*, *CEN3*, *MAT*, and *HMR* are located at 12, 112, 199, and 292 kb from the left end. When *HMR* was deleted, *HO HML*α *MAT*α *hmr*Δ strains showed tiny-spore colonies (I). When *HMR* was inserted at *HIS4* (kb 67) (III), *LEU2* (kb 91) (IV), and kb 133 (V), it could not be used as a donor efficiently and tiny-spore colonies were formed. But when *HMR* was placed at kb 162 (VII), 233 (VIII), 249 (IX), and 285 (XI), the *HMR* locus (II), and kb 302 (X), large-spore colonies were obtained. However, when a 25-kb non-*S. cerevisiae* sequence was integrated into a position 9 kb proximal to *MAT*, *HMR* at kb 162 could no longer serve as a sufficient donor (VI). (B to E) Dissection analysis of strains XW234, XW230, XW238, and XW336. Strain XW234 (*HO*/*HO hml*Δ/*hml*Δ *LEU2*::*HMR*α/*LEU2*::*HMR*α *MAT*α/*MAT***a** *hmr*Δ/*hmr*Δ) was dissected, and the α segregants formed tiny-spore colonies (B). Strain XW230 (*HO*/*HO hml*Δ/*hml*Δ *MAT*α/*MAT***a** 233 kb::*HMR*α/233 kb::*HMR*α *hmr*Δ/*hmr*Δ) was dissected, and the α segregants formed large-spore colonies (C). Strain XW238 (*HO*/*HO hml*Δ/*hml*Δ kb 162::*HMR*α/kb 162::*HMR*α *MAT*α/*MAT***a** *hmr*Δ/*hmr*Δ) was dissected, and the α segregants formed large-spore colonies (D). In strain XW336 (*HO*/*HO hml*Δ/*hml*Δ kb 162::*HMR*α/kb 162::*HMR*α kb 190::25-kb insert/kb 190::25-kb insert *MAT*α/*MAT***a** *hmr*Δ/*hmr*Δ), a 25-kb plasmid including pBR322, λ sequences, and the *URA3* gene was integrated at the position 9 kb proximal to *MAT*. Dissection of this strain showed that α segregants formed tiny colonies (E).

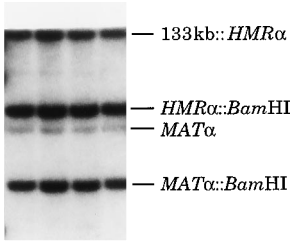

FIG. 5. Southern blotting analysis of *MAT*α donor preference. DNA was extracted from four α segregants of XW330 (*HO*/*HO hml*Δ/*hml*Δ *MAT***a**/*MAT*α 133 kb::*HMR*α/133 kb::*HMR*α *HMR*α-*Bam*HI/*HMR*α-*Bam*HI) and digested with *Hin*dIII and *Bam*HI. The blot was hybridized with a Yα-specific probe. *MAT*α and *MAT*α-*Bam*HI bands have sizes of 4.4 and 3.1 kb, respectively. The other two bands are *HMR*α-*Bam*HI (5.0 kb) and 133 kb::*HMR*α (11.0 kb).

ment from pXW145; Fig. 2B) was inserted at unique restriction sites of these cloned sequences (see Materials and Methods) and introduced into yeast cells by gene transplacement. Results are shown in Fig. 4A. The insertion positions are represented as the distance from the left telomere. When *HMR* was inserted at kb 67 (near *HIS4*) and at kb 133, the dissection patterns were the same as for XW234 (*HMR* in *LEU2*): *MAT*α segregants yielded tiny-spore colonies. Thus, when a donor was at *HML*, or at three other positions to the left of *MAT* (on either side of the centromere), it was not used as an efficient donor when the original *HMR* locus was deleted. However, when *HMR* was inserted at kb 162 (Fig. 4D), kb 249, and kb 302, large colonies were formed from *hml*Δ *MAT*α *hmr*Δ spores, the same as when *HMR* was inserted at kb 233 (Fig. 4C). Similar results were obtained when *HMR* was located at kb 285 in a strain in which all more distal sequences were deleted and replaced by a new telomere (XW227). This result indicates that sequences distal to this point are not needed to ensure that *HMR* is used instead of *HML*.

The positions kb 133 and kb 162 are both at the left side of *MAT*, but the results for these two cases were quite different. One possibility was that *HMR* at kb 162 might be too close to *MAT* and might not be under normal regulation of donor preference. To test this possibility, we increased the distance between kb 162 and the *MAT* locus. In strain XW336, a 25-kb segment including phage λ sequences, pBR322 sequences, and the *URA3* gene was integrated into a position 9 kb proximal to *MAT*, thus increasing the distance between *HMR* at kb 162 and the *MAT* locus from 37 to 62 kb, a distance similar to that of kb 133 with respect to *MAT*. Interestingly, this strain [*hml*Δ kb 162::*HMR*α kb 190::(25-kb λ insert) *MAT*α *hmr*Δ] produced the same tiny colonies as *HMR*α with kb 133 (Fig. 4E). This result suggests that the increase of use of *HMR* with kb 162 reflects some proximity effect that overrides normal donor preference.

On the right side of *MAT*, a donor inserted at kb 233 may also be too close to *MAT* (34 kb away), similar to the results with *HMR* at kb 162 on the left side of *MAT*. However, the observation that the efficient use of a donor at kb 249 as well as at kb 285 and kb 302 (Fig. 4) supports the idea that the donor can be used when it is situated anywhere to the right side of *MAT*. Taken together, the results indicate that when the *HMR* donor was inserted on the left side of *MAT*, beyond a distance of about 50 kb, *HMR* could not be used as an efficient donor, but when *HMR* was located on the right side of *MAT*, it could serve as a good donor during mating-type switching.

HMR*α inserted to the left of *MAT* is used rarely in competition with *HMR* at its normal location.** As described above, when *HMR*α was at kb 12 (*HML* locus), kb 67 (*HIS4*), kb 91 (*LEU2*) or kb 133, in the absence of normal *HML* and *HMR* donors, it was not an efficient donor to repair a double-strand break at *MAT*. We now show that *HMR* at these loci is used rarely when it is in competition with *HMR* present in its normal location. For these experiments, we modified the normal *HMR*α gene by a single base pair substitution in Yα that creates a *Bam*HI site without changing the coded amino acid sequences; this mutation is designated *HMR*α-B (42). Strain XW330 (a *MATa**/*MAT*α diploid homozygous for *HO hml*Δ kb 133::*HMR*α *HMR*α-*Bam*HI) was dissected. *MAT*α segregants grew into normal-size colonies, containing cells derived from *HO hml*Δ 133 kb::*HMR*α *MAT*α *HMR*α-*Bam*HI spores that had undergone many events of switching, since *HO* was activated all of the time. Thus, the ratio of *MAT*α and *MAT*α-*Bam*HI cells in one such colony should reflect the percentage of the time that cells used *HMR*α at kb 133 or *HMR*α-*Bam*HI at its normal locus. A Southern blot of DNA from α segregants, digested with *Bam*HI and *Hin*dIII and probed with a Yα-specific probe, is shown in Fig. 5. *MAT*α yields a band of 4.3 kb, while the *MAT*α-*Bam*HI band is 3.1 kb. *HMR*α inserted at kb 133 was used only about 15% of the time, similar to the results for a strain of genotype *HO HML*α *MAT*α *HMR*α-*Bam*HI (Table 2). The same results were obtained for XW246 (*HMR*α at *HML*), XW265 (*HMR*α at kb 91), and XW348 (*HMR*α at kb 67) (Table 2). These results show that the tiny-colony phenotype that we had used is an accurate reflection of the poor use of a donor in such unfavorable locations as *HML* (kb 12), kb 67, kb 91, and kb 133.

Similar results were obtained when an *ho* haploid strain with a plasmid-borne *GAL*::*HO* gene was used. In such an analysis, cells in liquid culture were induced with galactose to allow the expression of *HO*. About 60 to 80% of the cells switched mating type after 1.5 h of induction. Strain XW551 (*ho HML*α *MAT*α-*Bam*HI *HMR***a** *GAL-HO*) and strain XW496 [*ho hml*Δ::*ADE1 his4*::(*HMR*α *LEU2*) *MAT*α-*Bam*HI *HMR***a** *GAL*::*HO*] were tested. After galactose induction, cells were spread on YEPD and mating types of each colony were checked. The **a**-mating colonies resulting from switching must have used *HMR***a** as the donor. The α-mating colonies were analyzed by PCR amplification and subsequent *Bam*HI digestion (Materials and Methods) to distinguish switched *MAT*α cells from unswitched *MAT*α-*Bam*HI cells. *MAT*α colonies must have switched by using *HML*α (XW551) or *HMR*α in *his4* (XW496) as a donor, whereas *MAT*α-*Bam*HI colonies did not undergo mating-type switching. Similar to results for *HO* strains, *HMR* inserted at kb 67 was used only about 10% of the time in the presence of *HMR*, the same as *HML* in its normal locus (Table 3).

***MAT*α cells use a donor 16 kb proximal to the *HMR* locus more efficiently than normal *HMR*.** Data presented above sup-

TABLE 2. Competition between a donor on the left arm of chromosome III and *HMR*α-B in *MAT*α cells: switching of *HO MAT*α spores, using *HMR*α-*Bam*HI or the donor in other locations[a]

Strain	Donor location on the left arm	% Using *HMR*α-*Bam*HI
XW221	*HML*α	90
XW246	*HMR*α at *HML*	89
XW265	*HMR*α at kb 91 (*LEU2*)	88
XW348	*HMR*α at kb 67 (*HIS4*)	88
XW330	*HMR*α at kb 133	85

[a] For all strains, the initial *MAT* locus was *MAT*α and the *HMR* locus was *HMR*α-*Bam*HI.

TABLE 3. Competition between a donor on the left arm of chromosome III and *HMR*α-B in *MAT*α cells: *GAL*::*HO* induction to switch *MAT*α-B, using *HMR*α or the other donor[a]

Strain	Donor location on the left arm	No. of colonies			% using *HMR***a**
		Switched to *MAT***a**	Switched to *MAT*α	Not switched *MAT*α-B	
XW551	*HML*α	57	7	12	90
XW496	*HMR*α at kb 67 (*HIS4*)	21.7[b]	2	38	92
XW586	*HMR*α at kb 276	5.6[b]	34	17	14

[a] For all strains, the initial *MAT* locus was *MAT*α-*Bam*HI.

[b] Seventy-four Trp^+ colonies of XW496 were analyzed; 26 were **a** mating, and the remaining 48 were α mating. Among the 48 α-mating colonies, 40 were analyzed by PCR analysis along with *Bam*HI digestion. The number of **a**-mating colonies was therefore multiplied by 40/48 to yield 21.7 **a**-mating colonies. Similarly, 61 Trp^+ colonies of XW586 were analyzed; 6 were **a** mating, and remaining 55 were α mating, among which 51 were analyzed. The calculated value of 5.6 **a**-mating colonies was obtained by multiplying 6 by 51/55.

port the idea that the left arm of chromosome III relative to the *MAT* locus is under suppression, and so a donor inserted there is used inefficiently. It is less clear if the region around *HMR* is also especially activated. We know that donors to the right of *MAT* are used well enough to yield a normal-size colony, but this tells us only that the donor is not excluded; it does not tell us if the normal *HMR* locus is used much more efficiently than a donor at another site to the right of *MAT*. To address this question, we examined a competition between two donors located to the right of *MAT*. We constructed strain XW586 (*hml*Δ *MAT*α-*Bam*HI 276 kb::*HMR*α *HMR***a** *GAL*::*HO*), in which *HML* is deleted and *HMR*α is inserted at a position 16 kb proximal to the *HMR***a** locus. After galactose induction and analysis of the switched colonies by phenotype and restriction analysis, we found that *HMR*α at kb 276 was used 86% of the time and that *HMR***a** at its normal locus esd used only 14% of the time (Table 3). This finding suggests that the *HMR* locus is not specially activated for *MAT*α switching. These data do not allow us to determine whether the entire 100-kb region to the right of *MAT*α is activated, similar to what happens for the 40-kb region around *HML* in *MAT***a** cells (42), or if sites closer to *MAT*α on the right side are used preferentially. We have previously shown that proximity does increase spontaneous intrachromosomal *leu2* heteroallelic recombination in *MAT*α cells along chromosome III (42). It is possible that the use of *HMR*α at kb 276 is greater than use of *HMR***a** at kb 292 simply because *MAT*α-B shares homology with *HMR*α in the Y region. However, in other competition experiments (e.g., when *HMR*α was at kb 133 or 162), there was no evidence that the Yα region significantly biased the outcomes. In any case, it seems clear that the original *HMR***a** locus is not favored over other sites to the right of *MAT*.

Inversion of *MAT*α and 71 kb of flanking sequence does not change donor preference. The region surrounding *MAT* appears to serve as a landmark to distinguish the left arm from the right, since the results described above lead to the conclusion that the donors inserted to the left side of *MAT* cannot be used efficiently but the donors inserted on the right side of *MAT* are selected. Therefore, *MAT* was inverted to determine whether its orientation provided signals to differentiate the chromosomal arms on its left or right side. A 71-kb inversion from kb 162 to 233 (Fig. 6) was created by inserting a *leu2K* allele at kb 233, distal from *MAT*, and integrating an oppositely oriented *leu2R* with *URA3* and a piece of kb 162 region sequences on a pBR322 vector at kb 162 (see Materials and Methods). The inversion construct was confirmed by Southern analysis. In this strain (XW493), the *MAT* locus carried a silent mutation creating a *Bam*HI site, while the donors were *HML*α and *HMR***a**. The plasmid-borne *GAL*::*HO* gene was used to induce switching. *HMR***a** was preferentially used in this inversion (Fig. 6). Therefore, inversion of *MAT*α and 71 kb of flanking sequences does not change donor preference.

MAT*α*2* is required for *MAT*α donor preference.** Previous study showed that a *mat*α*2* mutant changed the donor preference to *HML* instead of *HMR* (34). We confirmed this conclusion by analyzing segregants of strain JKM7 (*HO*/*HO hml*α*2-38*/*hml*α*2-38 MATa**/*MAT*α *hmr*Δ::*LEU2*/*hmr*Δ::*LEU2*). The only donor that either *MAT***a** or *MAT*α segregants can use for switching is *hml*α*2-38*, which is sterile, so that after many generations, the colony consists almost entirely of *mat*α*2-38* cells. All of these segregants formed large colonies, suggesting that *MAT*α*2* was required to impose normal donor preference and the formation of tiny colonies when only a donor at *HML* was available. In this experiment, we ruled out the possibility that there was a strain difference between the R18 strain originally carrying *hml*α*2-38* and strains from this laboratory by showing that all *HO HML*α *MAT*α *hmr*Δ segregants emanating from the construction of JKM7 all gave a tiny-colony phenotype (data not shown).

DISCUSSION

The mechanisms controlling the donor preference in *MAT*α cells and in *MAT***a** cells are different. In *MAT***a** cells, there is a competition mechanism between the two donors and *HML* is selected as a preferred donor because of the activation of an approximately 40-kb region, including *HML*, for recombination (42). If *HML* is deleted, the wrong donor, *HMR*, is able to serve as an efficient donor. As suggested by Klar et al. (14), the two donors are in competition, but *HML* is activated to be used

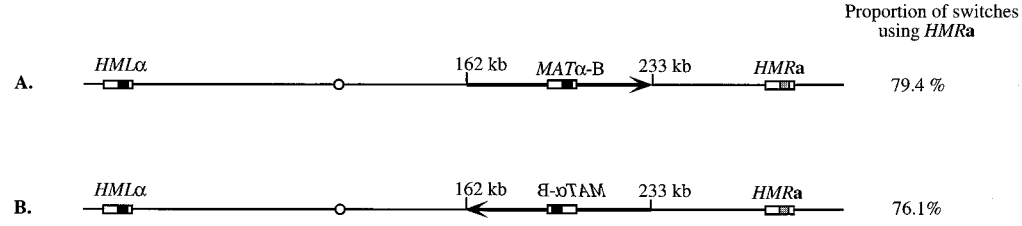

FIG. 6. Effect of an inversion of *MAT*α and 71 kb of flanking sequences on *MAT*α donor preference. Switching was induced by galactose induction of a plasmid-borne *GAL*::*HO* gene, and individual colonies were analyzed to determine if they had switched from *MAT*α-B to *MAT***a** (using *HMR***a**) or were still α mating. Then these α-mating cells were analyzed by *Bam*HI digestion of *MAT* DNA to distinguish cells that had switched to *MAT*α (using *HML*α) from those *MAT*α-B cells that had not switched. (A) Strain XW424 is the parent strain, with no chromosomal inversion. (B) Strain XW430 carries a 71-kb inversion from kb 162 to 233 on chromosome III.

more often. But in *MAT*α cells, when the preferred *HMR* is deleted, a significant number (one-third) of cells die, presumably after making repeated attempts to switch. Cell death most likely occurred because cells harboring an unrepaired double-strand break at *MAT* eventually initiated DNA replication and entered mitosis even though they carried a broken chromosome. We note that the lethality of *HO HML*α *MAT*α *hmr*Δ strains is apparently much less pronounced in some strain backgrounds, as this was not reported by Klar et al. (14), although we have seen the same tiny-colony phenotypes in several different strains. Even when the autoinhibition of **a**-like cells is eliminated, *HO HML*α *MAT*α *hmr*Δ cells still have a distinctively smaller colony size compared with cells containing a normal *HMR*α donor. This is not the case for *MAT***a** cells in which *HML* is deleted. Thus, there is also a competition between *HMR* and *HML* in *MAT*α cells, but *HML* is inherently inefficient as a donor.

A second difference between *MAT*α and *MAT***a** donor preference is that *MAT***a** switching involves an activation of donors in the first 40 kb of the left arm of chromosome III (42), but there does not seem to be an equivalent activation of *HMR* and its surrounding sequences for *MAT*α switching. For example, when *HML* is replaced with a *leu2* allele and a different *leu2* allele is inserted elsewhere on chromosome III or even on another chromosome, the frequency of Leu^+ recombination is 20 to 30 times higher for *MAT***a** cells than for *MAT*α cells (42). In contrast, when *HMR* is replaced by the *leu2* allele, its ability to recombine with another *leu2* allele is only twofold greater in *MAT*α cells than in *MAT***a** cells (42). This finding suggests that *HMR* is used preferentially simply because *HML* has been discouraged.

In this report, we present evidence that the left arm of chromosome III, and most of the *MAT*-proximal part of the right arm, is organized in such a way that a donor inserted anywhere in this region is prevented from recombining efficiently with *MAT*α. This mechanism is similar to the exclusion model proposed in the introduction except that now the excluded region is not a small locus surrounding *HML* but 150 kb of DNA. This discouragement of recombination could result from physically immobilizing these regions on the nuclear envelope or creating a more tightly folded chromatin structure. Weiler and Broach (39) suggested that the centromere of chromosome III might define a left/right distinction that regulated donor choice. Our data support the idea that there is such a distinction, but the boundary seems to be close to the *MAT* locus itself. We do not know the locations and number of *cis*-acting sites that are necessary to enforce this exclusion mechanism. Interestingly, a donor inserted at kb 162 on the left side of *MAT* is used efficiently, but when the same sequence is pushed further away, by the insertion of additional DNA at another site between the donor and *MAT*, the proximity effect is lost and the donor is again poorly used. It appears that an approximately 30- to 40-kb region to the left (and perhaps to the right) side of *MAT* is arranged differently from the rest of the sequences to the left; for example, perhaps this region is part of the same tethered loop of chromatin that contains *MAT* and facilitates interactions within the same domain. If this is so, the boundaries of that region are defined not by chromosome III sequences themselves but by actual distance, since inserting additional DNA changes the relationship between *MAT* and the donor.

Our studies of both *MAT*α and *MAT***a** donor preference have demonstrated that there is a complex regulation of the accessibility for recombination of a large region of chromosome III. The 40-kb region near the left telomere is especially activated for recombination, but not transcription, in *MAT***a** cells (42), but the entire left arm and part of the right arm proximal to *MAT* are also rendered unavailable for recombination in *MAT*α cells.

How *MAT*α finds a donor to its right is not yet clear. One possibility is that *HMR* is not specially activated for recombination but is used preferentially simply because *HML* has been partially excluded from competing effectively with *HMR*. This idea is supported by the observation that *MAT*α will recombine efficiently with a donor located anywhere to the right of *MAT* and in fact favors a donor closer to *MAT*. This choice does not depend on the orientation of *MAT* or on the sequences surrounding *HMR*, which is again consistent with the idea that *HML* is discouraged from recombination.

It is also possible that there is, in addition to the inactivation of *HML*, an activation of *HMR* for recombination. The activated region could be the entire 100-kb region to the right of *MAT*. However, this possibility is not supported by experiments replacing *HMR* by a *leu2* allele and measuring *leu2* recombination with another *leu2* allele located elsewhere (42), as discussed above. Whatever the mechanism, it appears that donor preference in *MAT*α cells is more strongly dependent on the donor being on the same chromosome than is the case for *MAT***a**. We have recently examined the ability of a *MAT* sequence located on chromosome V to recombine with *HML* or *HMR* on chromosome III. *MAT*α cells, using *HMR***a** on chromosome III, are at least five times less efficient in this transaction than are *MAT***a** cells, using *HML*α, suggesting that *MAT*α is much more constrained in finding even its preferred donor. There is no mating-type difference when the same construct containing *MAT* is integrated in place of the normal *MAT* locus (43). These results suggest that *MAT*α uses a much more *cis*-acting mechanism to find its donor than does *MAT***a.**

Further experiments are under way to identify the *cis*- and *trans*-acting factors that inactivate donors to the left of *MAT*α and to determine how donors to the right are chosen. We are intrigued by the possibility that chromosome III, the yeast sex chromosome, exhibits changes of chromosome structure over long regions that seem to bear some resemblance to the mechanisms of X chromosome dosage compensation in *Drosophila melanogaster* (the X chromosome in males is activated for a higher level of transcription [5]) or in *Caenorhabditis elegans* (the two X chromosomes of females are apparently partially condensed to reduce transcription [6]). In *S. cerevisiae*, the effect seems to be not on transcription (42) but on recombination.

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