MATa donor preference in yeast mating-type switching: activation of a large chromosomal region for recombination

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During mating-type gene switching in Saccharomyces cerevisiae, DNA at the MAT locus is replaced by sequences copied from one of two unexpressed donor loci, $HML$ or $HMR$, located near the two ends of the same chromosome and $\approx 90$ kb from $MAT$. $MATa$ cells recombine nearly 90% of the time with $HML$, whereas $MATo$ cells select $HMR$. $MATa$ donor preference was examined by deleting $HML$ and inserting a donor at other chromosome III locations. $MATa$ activated a large ($\approx 40$ kb) region near the left end of chromosome III, such that a donor placed at several sites within this domain was strongly preferred over $HMR$. When inserted outside of this domain, the donor was used equally with $HMR$. $MATa$ donor preference for $HML$ was abolished by the expression of the negative regulator, $MATo2$; however, $HML$ regained its preferred status when the donor was unsilenced. Mating-type-dependent activation of the left end of the chromosome is also observed for other types of recombination that do not involve $MAT$ switching. Spontaneous recombination between two $leu2$ alleles is 20–30 times higher in $MATa$ than in $MATo$ when one of the $leu2$ alleles is inserted in place of $HML$. Transcription in this donor activation region is not affected by mating type. We conclude that $MATa$ donor preference involves a mating-type-regulated change in the accessibility of a large chromosomal domain for recombination.

[Key Words: S. cerevisiae MATa donor preference; mating-type switching; activation; recombination]

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Homothallic switching of the mating-type genes in Saccharomyces cerevisiae occurs by a highly regulated site-specific recombination event (for review, see Strathern 1989; Haber 1992). The difference between $MATa$ and $MATo$ cells resides in the $Y$ region of the mating-type ($MAT$) locus (Fig. 1). Mating type in heterothallic ($ho$) strains is stable. Cells carrying the $HO$ allele express the $HO$ endonuclease, which cleaves $MAT$ in the $MAT-Z$ region, near the $MAT-Y/Z$ border, and initiates $MAT$ switching. The double-strand break (DSB) is repaired by gene conversion with one of two unexpressed donor loci, $HML$ or $HMR$, which are located on opposite arms of the same chromosome, $\approx 90$ kb away from $MAT$ (Fig. 1). The silent $HM$ loci are maintained in a chromatin configuration, different from $MAT$ (Laurenson and Rine 1992), which prevents both transcription and cleavage by the $HO$ endonuclease. However, this altered chromatin structure does not however prevent the ends of the DSB at $MAT$ from invading and copying out new mating-type information to replace the $Y$ region and adjacent sequences at $MAT$. The expression of $HO$ endonuclease is normally confined to the $G_1$ phase of the cell cycle but only in mother cells that have previously divided. $HO$ expression is restricted to haploid $MATa$ or $MATo$ cells and is turned off in $MATa/MATo$ diploids or in haploid cells expressing both mating types. However, cells are able to switch $MAT$ at any time in the cell cycle when the $HO$ gene is expressed from a galactose-inducible promoter (Jensen and Herskowitz 1984; Connolly et al. 1988).

One of the least understood aspects of $MAT$ switching is the phenomenon of donor preference (Klar et al. 1982). $MATa$ cells recombine preferentially (80%–90% of the time) with $HMLa$, whereas $MATo$ cells selectively recombine with $HMRa$ (Strathern and Herskowitz 1979). Donor selection is not dictated by the $Ya$ or $Yo$ sequences, either in the donors or at $MAT$. For example, a $MATa$ cell carrying genetically distinguishable $HMLa$ and $HMRa$ alleles still uses $HML$ (Klar et al. 1982). Moreover, when a recessive $mata1$ mutant is mated with an $HO$ $MATa$-$inc$ strain (in which the $inc$ mutant cannot be cleaved by $HO$), the resulting $a$-mating diploid chooses $HMR$ over $HML$ to replace the $mata1$ locus (Haber et al. 1980). Donor preference does not depend on any other sequences that uniquely define $HML$ or $HMR$, as donor preference is maintained if $HML$ sequences (including its $E$ and $I$ sites) are swapped with the $HMR$ region and vice versa (Weiler and Broach 1992). Donor preference ap-

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Donor preference in yeast mating-type switching

pears to be an active competition between two competent donors, because when HML is deleted, a MATa strain switches efficiently using the normally wrong donor, HMR (Klar et al. 1982).

We have discovered that MATa cells activate a large (≈40 kb) region of the left arm of chromosome III, making this region more available for recombination in MATa cells than in MATe. This activation is not specific for HO-induced mating-type switching but is seen also for general homologous recombination. We show that this activation is under the negative control of the MATa locus and examine the cis-acting sites responsible for this change in accessibility.

Results

To determine the basic rules for MATa donor preference, we have analyzed the competition between two donors, HMLa and HMRA-B, containing a single-base-pair substitution in Ya that creates a BamHI site without altering the coded amino acid sequence (see Materials and methods). Thus, a MATa cell can switch to MATa (from HMLa) or to MATa-B (from HMRA-B). In some experiments (Table 1), switching was induced by dissecting HO/HO MATa/MATa diploids to obtain HO MATa spores. These spores germinate and usually switch mating type at the four-cell stage producing two nonmating MATa/MATa (or two MATa/MATa-B) diploids that then grow up into a colony. The HO gene is turned off in MATa/MATa diploids so that there is usually a single switching event, initiated in the G1 phase of the cell cycle, that gives rise to the nonmating colony (Strathern 1989). A Southern blot analysis of BamHI-digested DNA, probed with a Ya-specific DNA, distinguishes MATa/MATa from MATa/MATa-B diploids, and therefore demonstrates which donor was chosen. DNA extracted from eight such nonmatting colonies was pooled and analyzed together. Four examples of this type of analysis are presented in Figure 2, lanes 1-4, showing that HML is preferred >90% of the time over HMR.

Table 1. Competition between a donor on the left arm of chromosome III vs. HMRA-B in colonies derived from HO MATa spores

| Strain | Location of donor on left arm | Donor on right arm | Number of colonies analyzed | Percentage using HMRA-B |
|--------|-------------------------------|--------------------|----------------------------|-------------------------|
| XW221  | HMLa                          | HMRA-B             | 32                         | 7.5                     |
| XW246  | hmlΔ::HMRA                    | HMRA-B             | 10                         | 15.0                    |
| XW263  | HMRA at 91 kb"                | HMRA-B             | 32                         | 49.0                    |
| XW348  | HMRA at 67 kb"                | HMRA-B             | 32                         | 49.3                    |

MATa spores were germinated and grown into nonmatting colonies, in which MATa had switched to either MATa or MATa-B, depending on which donor was used. Most homothallic switching occurs at the four-cell stage, in which two cells change to the opposite mating type and then mate with the two cells of the original genotype to produce nonmatting MATa/MATa diploids, in which there is no more switching.

aRatio of donor usage determined by densitometry of Southern blots of BamHI-digested DNA that distinguish between MATa and MATa-B (see Fig. 2). Except for XW246, 32 or more nonmatting colonies were analyzed in mixtures of DNA from eight colonies, and the results averaged. For XW246, 10 colonies were scored in a single mixture, confirming previously published results (Weiler and Broach 1992).

bA 5.4-kb region containing HML was deleted by and replaced by the ADE1 gene.
Figure 2. Competition between mating-type donors in MATa homothallic switching. HO MATa spores carrying HMRa-B and another donor located on the left arm of chromosome III were germinated and grown into nonmating colonies. Each colony results from one, or occasionally more than one, switching event to yield either MATa/MATa or MATa/MATa-B cells. DNA was pooled from eight such nonmating colonies, cut with BamHI and HindIII and separated by gel electrophoresis. The Southern blot was probed with a Ya-specific probe. [Lanes 1–4] Four sets of eight pooled colonies of strain XW221 showing a strong preference of HMLα over HMRα-B (Table 1). [Lanes 5–8] Sets of eight pooled colonies of strain XW263 exhibiting nearly equal usage of HMRα-B and LEU2::HMRα, inserted 91 kb from the left end of chromosome III. The Ya probe hybridizes to the 4.4-kb MATa or 3.1-kb MATa-B BamHI fragments that are produced by switching MATa (which does not hybridize). The probe also hybridizes with the donor sequences, which are all approximately the same size (HMLα = 5.17 kb, HMRα-B = 4.98 kb, and LEU2::HMRα = 5.08 kb). When the left arm donor is far from HML, it is used about as often as HMRα-B, whereas the HMLα donor itself is used nearly all the time.

In many other experiments reported here, ho MATa cells in liquid culture were induced to switch mating type after a brief galactose induction of a plasmid-borne GAL::HO gene (Jensen and Herskowitz 1984; White and Haber 1990). Cells are induced to switch at different points of the cell cycle, as opposed to normal HO expression, which is confined to the G1 phase of the cell cycle (Jensen and Herskowitz 1984; Connolly et al. 1988). About 60%–80% of ho MATa HMLα HMRα-B cells changed mating type after a 1.5-hr induction (White and Haber 1990). BamHI-digested DNAs from individual α-mating colonies were analyzed by Southern blots or by PCR amplification [see Materials and methods]. Most cells gave rise to either MATa or MATa-B, but ~5%–10% of the colonies were mixed, presumably because switching was initiated in cells that had already replicated their chromosomes and each chromosome acted independently (Connolly et al. 1988). The results obtained by galactose induction of HO were the same as those obtained with the normal HO gene: In both strains XW221 and XW431, MATα switched >90% of time using HML as the donor [Fig. 3A; Table 1]. In itself, this result is important because it shows that donor preference is the same when the normal HO gene is expressed in the G1 phase of the cell cycle or when the galactose-inducible HO gene is expressed throughout the cell cycle. These two different methods of HO induction also give similar results in cases where normal donor preference was altered (see below).

**Competition between donors defines a large MATα-activated region at the left end of chromosome III**

To understand which DNA sequences around HML or HMR are important to establish donor preference, we created a large set of strains in which the 5.4-kb region, including the HML locus and all of the surrounding silencer sequences, were deleted and HMRα and its adjacent silencers were inserted at different locations [Fig. 3]. In agreement with Weiler and Broach (1992), we found that inserting HMRα directly in place of HMLα [Fig. 1B] gave the same donor preference as HMLα (Table 1, cf. strains XW221 and XW246). We then created strains in which HML was deleted and HMRα was inserted at four different locations on the left arm of chromosome III. When HMR was inserted near HIS4 or LEU2 (67 or 91 kb from the left end), there was no preference in the use of the donor on the left chromosome arm over HMRα-B [Fig. 3E,F]. In these situations, the two donors, located approximately equidistant from MAT on two different chromosome arms, are used equally [Fig. 3, Table 1]. One example, for the competition between LEU2::HMRα and HMRα-B, is shown by the Southern blot in Figure 2, lanes 5–8. The results were the same whether HO was induced by galactose [Fig. 3, XW453, XW452] or by its natural expression [Table 1, XW348, XW263]. These results rule out models in which all donor sites to the left of the centromere or to the left of MATα would be preferred over HMR. Also excluded are mechanisms in which HMR is specifically sequestered or inactivated so that it cannot act efficiently in competition with another donor.

However, the region immediately around HML is not the only preferred domain. When HML was deleted and the donor on the left arm was placed at locations 22 or 41 kb from the left end of chromosome III [strains XW426 and XW427], the donor on the left arm was clearly preferred, just as at HML itself [Fig. 3C,D]. This suggests that MATα cells activate not simply the region around HML but a large (~40 kb) chromosomal region to be especially recombinogenic during MATα switching.

Donor preference was also seen when the normal HMR locus was deleted and the strain carried two donors on the left arm, one at HML and a second at HIS4 (67 kb). In this strain [XW486] [Fig. 3I], the donor at HIS4 was HMLα-H, carrying a HindIII site in Ya. Among the MATα cells that switched, 75% used the HMLα locus and 25% used the donor at HIS4. This confirms that the donor preference mechanism can discriminate between two locations on the left arm of chromosome III.

Finally, to assess whether the proximity of either HML or HMR to a telomere plays an important part in the regulation of donor preference, we constructed a MATα strain in a circular chromosomal derivative of chromosome III that contains a single, recombined HMR/HMLα locus and lacks all sequences distal to both donors.
[Haber et al. 1984]. A second donor, HMLα-H, was then inserted at the HIS4 locus, outside of the region of preferential donor activation (Fig. 3K). After GAL::HO induction, >80% of the switches of this strain (XW26) came from HMR/HMLα, suggesting that donor preference does not involve a telomere-dependent activation of HML in MATa cells. These results are essentially the same as those for the linear chromosome version of this experiment (Fig. 3), strain XW46) described above. They also suggest that sequences needed to activate HML are proximal to HML (as all HML-distal sequences are missing); moreover, there are no sites proximal to HMR that prevent the use of an adjacent donor in MATa cells.

Cis-acting regions responsible for MATa donor preference lie ≫10 kb from HML

The activation of HML and the rest of a large chromosomal domain in MATa cells must involve cis-acting sites on the left arm of chromosome III. We have constructed a number of deletions to identify those sites. We created deletions of 12 kb to the left of HML (Fig. 3G) or 8 kb to the right of HML (Fig. 3H), neither of which had any significant effect on MATa donor preference. To construct a larger deletion we began with the strain illustrated in Figure 3D, where the donor is located 41 kb from the chromosome end, which is still in the region where the donor is activated in MATa cells. A terminal deletion of 22 kb was then constructed by the insertion of sequences to the right of HML (nucleotide 11,294) to nucleotide 21,974 with an insert containing LEU2 and HMRα (see Fig. 1), thus effectively removing the 8 kb that normally lies to the right of HML. Strain XW46 (I) is deleted for HMR and carries HMLα at its normal location and the 6.6-kb BamHI fragment of HMLα inserted at HIS4 (67 kb) (Fig. 3I). Strain XW26 carries a circular chromosome (K) containing a fusion of HMR and HML (Haber et al. 1984) and also the insertion of HMLα-H at HIS4 (67 kb).

Donor preference in yeast mating-type switching

| A | HMLα | MATa | HMRα-B |
|---|------|------|--------|
| B | ΔHML | MATa | HMRα-B |
| C | ΔHML | MATa | HMRα-B |
| D | ΔHML | MATa | HMRα-B |
| E | ΔHML | MATa | HMRα-B |
| F | ΔHML | MATa | HMRα-B |
| G | ΔHML | MATa | HMRα-B |
| H | ΔHML | MATa | HMRα-B |
| I | ΔHML | MATa | HMRα-B |
| J | HMLα | MATa | HMLα-H |
| K | HMLα-H | MATa | HMLα-H |

Figure 3. Effect of position of a donor located to the left of MATa on donor preference. Normal switching involves a competition between HMLα and HMRα-B, carrying a BamHI site that permits the two sequences to be distinguished (A). Except for strain XW246, all strains were analyzed with a minimum of 26 independent a-mating colonies (up to a maximum of 72 colonies) produced after induction of MATa switching using the GAL::HO gene. A small minority of colonies gave evidence of mixed events, in which both MATa and MATa-B cells were present. These presumably arose when HO was induced in a cell that had completed DNA replication and thus could give rise to two independent switching events (Connolly et al. 1988). The proportion of cells using HMRα-B as the donor was then determined by counting these events as two independent events and adding half their number to the totals for both MATa and MATa-B. Results for strain XW246 are based on the experiment described in Table 1. When HML and its surrounding sequences are deleted and replaced by HMRα, donor preference still favors the donor on the left chromosome arm (B). A series of strains were constructed in which HML was deleted and the HMRα locus and an adjacent LEU2 gene were inserted at several other chromosomal locations: (C) 22 kb from the left chromosome end; (D) 41 kb; (E) 67 kb; and (F) 91 kb. Terminal deletions of chromosome III were constructed by the gene targeting Tetrahymena telomere sequences to create a new telomeric end. A deletion of the terminal 12 kb (G) and 22 kb (I) were constructed. An internal deletion of sequences to the right of HML (H) was constructed by homologous recombination/replacement of the region from the left of HML (nucleotide 11,294) to nucleotide 21,974 with an insert containing LEU2 and HMRα (see Fig. 1), thus effectively removing the 8 kb that normally lies to the right of HML. Strain XW46 (I) is deleted for HMR and carries HMLα at its normal location and the 6.6-kb BamHI fragment of HMLα inserted at HIS4 (67 kb). Strain XW26 carries a circular chromosome (K) containing a fusion of HMR and HML (Haber et al. 1984) and also the insertion of HMLα-H at HIS4 (67 kb).

MATα donor preference is regulated by MATα2

An early study of MATα switching (Tanaka et al. 1984) suggested that the repressor of a-specific gene expression, MATα2, might play a decisive role in regulating donor preference. An HO hmlα2 mata2 HMRα cell switched only rarely to MATα, suggesting that mata2...
cells were a-like in their donor preference. However, the pedigree studies on which this was based could not rule out the possibility that the mata2 mutation itself reduced the frequency of HO cleavage and switching, which would have yielded a similar result. Here we show that MATa2, expressed in trans, does regulate donor preference. A GAL::HO strain, XW431, of genotype MATa HMLa HMRa-B was transformed with either the MATa1 gene or the MATa2 gene inserted at URA3 on chromosome V (Fig. 4A,B). The MATa1 gene carries an inc mutation so that it is not cut by HO. The ectopic expression of either MATa1 or MATa2 causes a MATa cell to become nonmating, but for different reasons. In the case of MATa1, both a-specific and a-specific genes are expressed simultaneously, whereas in the case of MATa2, both a-specific and a-specific genes are turned off. We induced the HO gene and recovered a-mating switched cells that were either MATa [from HML] or MATa-B [from HMR]. In MATa [MATa1] cells, donor preference remained in favor of HML, but in MATa [MATa2] cells donor preference shifted to that expected for MATa cells (Fig. 4). Thus, the expression of MATa2 prevents the activation of HML. Whether MATa2 also increases the accessibility of HMR is not established by this experiment.

Role of silencing in regulating HML donor accessibility

One of the most interesting aspects of the mating-type system in yeast is that the chromatin structure of the donors make them inaccessible to cleavage by HO endonuclease, yet the HO-cleaved ends of MAT are able to invade the same site in the donor and begin to copy the donor DNA. Thus, one simple way to control donor preference would be to regulate a donor’s degree of accessibility to strand invasion by MAT DNA. For example, the wrong donor could be hypersilenced, so that MAT DNA would be less able to invade it. Alternatively, the structure of the preferred donor could be somewhat relaxed to be more available (though not to the extent that the silent donor would be cut by HO or be transcribed). We have tested an extreme version of this idea by asking if an unsilenced donor would be used preferentially even when normal MATa preference is abolished. As noted above, in strain XW425, a MATa cell that also expresses MATa2 shifts its donor preference to HMR. We constructed a related strain, XW490, in which the 5.4-kb region, including HMLα and its adjacent silencer sequences, was replaced with a 6.4-kb EcoRI fragment of MATα-inc, which lacks the adjacent E and I silencer sites (Fig. 4C). This [E⁻ I⁻] hmlΔ::MATα-inc MATa HMRα-B strain is nonmating because both MATa and MATa2 (from hmlΔ::MATα-inc) are expressed. MATa1 is not expressed in diploids expressing both MATa and MATa2 (Herskowitz 1989), thus, this cell is phenotypically equivalent to XW425. Only MATa can be cleaved by HO, and thus the cell can either use the expressed hmlΔ::MATα-inc or the silent HMRα-B as a donor. Unlike strain XW425, where donor preference shifted to HMRα-B, this nonmating strain continues to prefer the donor located at HML. We conclude that donor preference can be significantly altered by “opening up” a silent donor.

A LEU2 gene in place of HML recombines more frequently in MATa than in MATα

The activation of HML in MATa cells is not restricted to mating-type sequences or to recombination initiated by the HO endonuclease. We show below that spontaneous recombination between leu2 heteroalleles to yield Leu⁺ recombinants is also increased in MATa cells when one of the two alleles is inserted in place of HML. A 5.4-kb XhoI segment including HML and its surrounding E and I silencer sites was replaced with the 2.2-kb fragment containing the mutant allele of the LEU2 gene, leu2-R. A

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Figure 4. Ectopic expression of the MATa1 or MATa2 genes in MATa strains. A URA3-marked plasmid carrying either the MATa2 gene (A) or the MATa1 (B) gene was inserted by homologous integration at the ura3-52 locus on chromosome V. The MATa1 gene was PCR amplified from a MATα-inc strain and thus contains a single base-pair mutation that prevents HO cutting (Weiffenbach et al. 1983). The MATa2 gene is expressed along with MATa in strain XW490, in which MATa-inc is inserted in place of HML (C), thus, this strain has the same effective genotype as strain XW425 (A).
Donor preference in yeast mating-type switching

different allele, leu2-K, was inserted between two tandem repeats of either MATa or MATa [Fig. 5B]. The rate of spontaneous recombination to Leu + prototrophy was 30 times higher in MATa than in MATa cells (Table 2). Thus, a spontaneous recombination event that does not involve mating-type sequences exhibits the same sort of mating-type-controlled preference as MAT switching itself. This difference is easily visualized when a [MATa–leu2-K–MATa]/[MATa–leu2-K–MATa] diploid homozygous for hmlΔ::(leu2-R) was sporulated and dissected [Fig. 6]. In every tetrad there were two segregants [both MATa] that had a much higher level of Leu + papillae when the colonies were replica-plated to leucine dropout medium.

The increased recombination of hmlΔ::(leu2-R) in MATa cells could either be because there is a more efficient donor or a more avid recipient. We could determine which allele was converted in strains XW499 and XW500 by “popping out” the MAT–URA3–LEU2–MAT duplication, selecting for Ura– colonies on 5-fluoro-orotic acid (5-FOA) medium [Boeke et al. 1987]. If the duplication carried the converted Leu + locus, then the Ura– derivatives would become also Leu–. If hmlΔ::(leu2-R) had been converted, the strain would remain Leu+. In the MATa strain, 50/55 (91%) became Leu–, indicating that the leu2-K allele adjacent to MATa had been converted. In the MATa strain, this proportion did not change: 50/54 (93%) converted the leu2-K allele. In previous experiments we have found that leu2-K is converted more frequently to wild type than is leu2-R when they are at the same chromosomal location; however, when the two alleles are in different locations, the proportion of leu2-K alleles that is converted to wild type can be increased if it is situated in the site that is most often the recipient in the recombination event (Lichten et al. 1987; Lichten and Haber 1989). In the present case, it appears that the mating-type-dependent activation of hmlΔ::(leu2-R) occurs without making it the recipient. Instead, hmlΔ::(leu2-R) locus is activated to be a more efficient donor during these spontaneous recombination events, as it is in MAT switching.

The high level of Leu + recombination in MATa cells does not require pairing sites that might be postulated to align HML and MAT, because similar results were obtained when hmlΔ::leu2-R recombined with a leu2-K allele situated at its normal location, ~100kb from MAT [Fig. 5C]. Leu + recombinants arose 20 times more frequently in MATa cells than in MATa (Table 2). Recombination between hmlΔ::(leu2-R) on chromosome III and leu2-K integrated near URA3 on chromosome V was much higher in MATa ([Fig. 5D; Table 2]. We also note that there is an intrinsically higher rate of intrachromosomal recombination, as expected from previous studies of recombination between these same two alleles [Lichten and Haber 1989].

In general, MATa cells do not have higher levels of leu2 recombination than MATa. Strains with leu2-K at its normal location and leu2-R at MAT displayed no mating-type effect on the rate of Leu + recombination [Fig. 5E; Table 2]. A similar result was obtained when the two leu2 alleles were inserted at two other chromosome III locations, 162 and 233 kb from the left end of the chromosome, respectively [Fig. 5F; Table 1].

Elevated recombination of hmlΔ::leu2 is repressed in MATa cells expressing MATa2

To determine whether MATa2 also regulates the activation of hmlΔ::(leu2-R) recombination, as it does MATa donor preference, we constructed the nonmating strain XW498 of genotype MATa–URA3–(leu2-K–MATa), which could be compared with those carrying two tandem copies of MATa [XW499] or MATa [XW500]. A similar set of strains [XW529, XW530, and XW531] were

![Figure 5. Mating-type-dependent recombination of leu2 heteroalleles. An XhoI fragment containing HML and its adjacent silencer E and I regions was deleted and replaced with a 2.2-kb fragment containing the leu2-R allele. This allele can recombine with a leu2-K that was inserted at other chromosomal locations on chromosome III (B,C) or chromosome V (D). Except when the normal leu2 locus itself was tested for recombination, it was deleted. The strong mating-type effect on the rate of Leu+ recombination is not seen when leu2-K is inserted at its normal location and leu2-R is adjacent to MAT [E]. No mating-type effect is seen when the two leu2 alleles are placed at two other chromosome III locations (F). A similar replacement by leu2-R of the Nitr–XhoI fragment of HMR, including sequences 250 bp proximal and 300 bp distal, was constructed (G). This shows only a small mating-type effect, in favor of MATa, when it recombines with a leu2-K allele adjacent to MAT. The ratio of the rates of recombination in MATa vs. MATa cells is presented.](link-to-figure)
Table 2. Effect of mating type on the rate of LEU2 prototroph formation between leu2 alleles in different chromosomal locations

| Strain | Mating type | Location of leu2-R | Ratio of Rate of Leu+ | Location of leu2-K | Ratio of Rate of Leu+ |
|--------|-------------|-------------------|----------------------|-------------------|----------------------|
| XW499  | a           | HML               | 6.72 x 10^-6         | MAT               | 32                   |
| XW500  | a           | HML               | 2.13 x 10^-7         | MAT               | 19                   |
| XW498  | nm          | HML               | 1.34 x 10^-6         | MAT               | 6.3                  |
| XW440  | a           | HML               | 5.82 x 10^-6         | LEU2              | 19                   |
| XW421  | a           | HML               | 3.01 x 10^-7         | LEU2              | 28                   |
| XW485  | a           | HML               | 5.06 x 10^-7         | URA3              | 2.3                  |
| XW529  | a           | MAT               | 2.15 x 10^-7         | LEU2              | 28                   |
| XW530  | a           | MAT               | 9.20 x 10^-8         | LEU2              | 3.7                  |
| XW531  | nm          | MAT               | 3.38 x 10^-7         | LEU2              | 1.8                  |
| H334   | a           | MAT               | 2.37 x 10^-7         | LEU2              | 1.8                  |
| H333   | a           | MAT               | 1.31 x 10^-7         | LEU2              | 0.88                 |
| XW412a | a           | 162 kb*           | 6.12 x 10^-7         | 233 kb*           | 0.42                 |
| XW412  | a           | 162 kb*           | 6.93 x 10^-7         | 233 kb*           | 0.42                 |
| XW508  | a           | HMR               | 6.79 x 10^-7         | MAT               | 0.42                 |
| XW506  | a           | HMR               | 1.62 x 10^-6         | MAT               |                       |

The leu2-K and leu2-R alleles (Lichten et al. 1987) were inserted into different chromosomal locations on chromosome III. (162 kb* and 233 kb*) Locations from the left end of chromosome III as determined by Oliver et al. (1992) [see Materials and methods]. Except when the normal LEU2 locus was specifically involved in recombination, the locus was deleted by the removal of the XhoI-EcoRV segment including both the leu2-K and leu2-R sites [E.J. Louis and J.E. Haber, unpubl.]. The rates of LEU2 prototroph formation was determined by a fluctuation test {Lea and Coulson 1949) based on a minimum of five independent cultures of each strain, initiated from 20 to 80 cells and grown to saturation. The LEU2 genes in these constructions are all oriented with the 5' -> 3' direction being left to right on the chromosome, as shown in Figure 5, except for the hmlA::(leu2-R) and 249 kb::(leu2-K) insertions on chromosome III. (nm) Nonmating.

The leu2-K and leu2-R alleles at leu2 locus itself and MAT. The results shown in Table 2 show a four- to sixfold increase of recombination in nonmating derivatives over MATa, whether the leu2 sequences are at the HML site or not. This is expected in view of other studies showing that nonmating (MATa/MATa) diploids exhibit higher levels of spontaneous recombination in general over strains homozygous for one mating type (Friis and Roman 1968; Durand et al. 1993). Thus, nonmating cells have an elevated level of recombination relative to MATa cells, but there is no longer any positional effect as observed in MATa cells. We conclude that expression of MATa represses the MATa-dependent high level of leu2 heteroallelic recombination for hmlA::(leu2-R) in the same way that donor preference in mating-type switching is repressed.

A LEU2 gene in place of HMR does not show strong mating-type preference

A similar experiment was carried out to determine whether MATa cells would preferentially activate recombination of a leu2-R allele inserted in place of HMR [Fig. 5G]. Here, we found a 2.5-fold preference for MATa over MATa [Table 2], which is a much more modest effect, though statistically significant.

Activation of the region around HML for recombination is not accompanied by a change in the level of transcription

One general type of model to explain the mating-type-dependent activation of the region including HML for...
recombination is that there is also a general increase in transcription, as it has been shown previously that recombination is increased when a gene is transcribed than when it is not [Thomas and Rothstein 1989]. This does not seem to be the case here. We used Northern blots to examine the level of LEU2 mRNA in MATα and MATα isogenic strains carrying hmlA:: LEU2 and deleted for the normal LEU2 locus [these strains were the precursors of strains XW499 and XW500 that showed MATα-dependent elevation of leu2 recombination (Table 2)]. There was no significant difference in the abundance of LEU2 mRNA (data not shown).

Discussion

When MATα is cut by HO endonuclease, the DSB must be repaired by recombination. There is a competition between alternative donors located on the same chromosome [Klar et al. 1982]. The preferential use of HML over HMR does not result from inactivating or sequestering HMR, as HMR is used equivalently to donors located elsewhere on the chromosome, but outside of the donor activation region (DAR) that includes HML. The observations that we have presented suggest that MATα donor preference results from the activation of HML to be more available for recombination than HMR or than donors located in many other positions. However, the region immediately around HML is not unique in being activated. MATα cells activate donors at [at least] two other positions in a large region ≥40 kb at the left arm of chromosome III so that they also recombine more efficiently with MATα than do donors at other chromosomal locations. The activation of this region does not depend specifically on sequences surrounding HML, as HMR will work just as efficiently in place of HML [Weiler and Broach 1992] or at other sites in this 40-kb region (our data). The activation of this region does not depend on any of the sequences defining mating-type donors, as leu2 sequences situated in the DAR are also activated for recombination in MATα cells.

Another model that might have explained donor preference would be the existence of pairing sites adjacent to HML and MATα that permit a prealignment of the donor when an a-specific gene product is expressed. However, the idea of specific pairing sites between MATα and HML seems less likely given that MATα cells prefer donors located at several positions within a 40-kb region at the left end of chromosome III. Moreover, the leu2 recombination experiments show that a leu2 allele in place of HML is activated in MATα cells even when the other leu2 is on a different chromosome.

MATα donor preference is preserved even if the terminal 22 kb of the left arm of chromosome III is deleted. Thus, there are cis-acting sequences, sufficient to ensure MATα donor preference, that lie at least 10 kb proximal to the original HML site. Further analysis of this region will require complementation of essential genes lying between 22 and 41 kb [X. Wu, unpubl.].

Another possibility is that the terminal 40 kb of chromosome III is prevented from interacting with DNA sequences located elsewhere in yeast mating-type switching

Donor preference in yeast mating-type switching

In this paper we have focused our attention on MATα donor preference. How MATα cells choose HMR is not
yet clear, but several lines of evidence argue that MATα donor preference for HMR is not simply the mirror image of how DAR is activated in MATa. For example, we have observed that HO MATα cells deleted for HMR are not efficient in using HMLα as a “backup” donor, whereas HO MATα cells switch efficiently using HMRα when HML is deleted. About one half of MATα cells die when they are provided only with the “wrong donor” because they fail to repair the DSB [X. Wu, J.K. Moore and J.E. Haber, unpubl.]. Furthermore, MATα cells do not show a significant activation of leu2 heterallelic recombination when one of the alleles is situated in place of HMR.

Finally, we have shown recently that, whereas HMLα on chromosome III can act efficiently to switch MATα inserted on chromosome V, HMRα fails to repair a DSB at MATα on chromosome V >95% of the time [C. Wu, X. Wu, N. Rudin, and J.E. Haber, unpubl.]. Further experiments are under way to analyze MATα donor preference.

### Relation of S. cerevisiae donor preference to programmed chromosomal rearrangements in other organisms

Preferential recombination between alternative DNA segments involved in gene activation by chromosomal rearrangement has been observed in organisms ranging from bacteria to humans. In the mammalian immune system, for example, there is a clear preference for which of many possible V regions become involved in recombination with a distant J sequence [Blackwell and Alt 1990], Knight and Becker 1990]. Mechanisms of donor preference also appear to be important in the ordered expression of VSG genes in trypanosomes [Thon et al. 1990]. In Schizosaccharomyces pombe, mating-type gene switching also involves an expressed mat locus and two unexpressed donors, though the DNA sequences and many aspects of the mechanism of switching are entirely different from S. cerevisiae [Klar 1992]. mat switching in S. pombe also exhibits donor preference, which can be altered by a swi6 mutation that also apparently changes the chromatin structure in the 15-kb interval between the two silent donors [Thon and Klar 1993]. A thorough understanding of the way in which MATα activates a chromosomal region for recombination will prove to be of great importance in understanding these other types of programmed chromosomal rearrangements.

### Materials and methods

#### Strains

Most strains were isogenic derivatives of strain DBY745 (ho MATα ade1-100 leu2-3,112 ura3-52) or of HO strains NR226-7B, NR238-7C, and NR272-19A, derived from an HO strain (Y55) and backcrossed at least six times against DBY745. Strain XW526, carrying a ring chromosome III, was also derived from the same genetic background [Haber et al. 1984]. Strains H333 and H334 are heteroallelic derivatives from Y55 crossed once with DBY745. The genotypes of the strains used are given in Table 3.

| Strain | Genotype |
|--------|----------|
| DBY745 | ho HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW425  | ho HMLα MATα HMRα BamHI::ura3 ade1-100 leu2-3,112 ura3-52 |
| XW427  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW429  | ho (C4A 2, LEU2, all HMLα dist. seq.A) HMLα ade1-100 leu2-3,112 ura3-52 |
| XW441  | ho (from the end to 23kb; ade1-100; LEU2, HMRα) MATα ade1-100 leu2-3,112 ura3-52 |
| XW452  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW453  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW490  | ho HO HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW492  | ho HO HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW526  | ho HMR-HMLα fusion [HMLα HindIII (URA3)] MATα circular chromosome pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |

*HMRα* was cloned from yeast by gap repair [Orr-Weaver and Szostak 1983] of a centromeric plasmid containing the regions flanking HMR separated by an XhoI site that was used to linearize the plasmid [Abraham et al. 1984]. The recovered *HMRα*

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**Table 3. Yeast strains**

| Strain | Genotype |
|--------|----------|
| DBY745 | ho HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW425  | ho HMLα MATα HMRα BamHI::ura3 ade1-100 leu2-3,112 ura3-52 |
| XW427  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW429  | ho (C4A 2, LEU2, all HMLα dist. seq.A) HMLα ade1-100 leu2-3,112 ura3-52 |
| XW441  | ho (from the end to 23kb; ade1-100; LEU2, HMRα) MATα ade1-100 leu2-3,112 ura3-52 |
| XW452  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW453  | ho HMLα MATα HMRα BamHI::ura3 pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |
| XW490  | ho HO HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW492  | ho HO HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52 |
| XW526  | ho HMR-HMLα fusion [HMLα HindIII (URA3)] MATα circular chromosome pBH312[GAL-HO URA3] ade1-100 leu2-3,112 ura3-52 |

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gene was cloned as a HindIII fragment into a pGEM3Z[+ ] vector for further manipulation. The HMRA-B allele was created by oligonucleotide-directed mutagenesis [Kolodiej and Young 1991] of C→A at position 658 in Yc to create a BamHI site. HMRA-B was introduced into the chromosome in place of HMRa by the one-step gene replacement method [Rothstein 1983] of a HindIII fragment containing the URA3 gene inserted at the NruI site. The URA3 marker was then made Ura- by selection with 5-FOA [Bocke et al. 1987]. Similar mutagenesis was performed to create HMLα-H in which position 379 of Yc was mutated A→T to create a HindIII site. A cassette containing HMRA and LEU2 was constructed in plasmid pXW145 [see Fig. 1C] and was integrated at other chromosomal locations by inserting it into a unique site of a previously cloned chromosomal segment. These regions were carried in a pGEM3Z[+ ] vector as ~500-bp PCR-amplified fragments, obtained by using oligonucleotide primers determined from the complete DNA sequence of chromosome III [Oliver et al. 1992]. Thus, the cassette was inserted at a unique Apol site nucleotide 21,974, at a unique Clal site at nucleotide 41,404, at a unique Xhol site at nucleotide 67,682 near HIS4, and at a unique Xhol site at nucleotide 90,286 near LEU2. The fragments containing the target sequences flanking LEU2 and HMRA were then transformed into yeast [Rothstein 1983]. The HMLα-H gene, plus an adjacent URA3 gene, was inserted at the Xhol site near HIS4 [nucleotide 67,682] in a similar fashion.

In some of these constructions, the Xhol fragment embracing HML was deleted and replaced by a 1.9-kb ADE1 gene [plasmid pXW123]. Terminal deletions of the left arm of chromosome III were constructed by targeting a DNA fragment carrying 0.3 kb of Tetrahymena Cα3 repeats that serve to create a new yeast telomere [Dunn et al. 1984]. The linearized transforming fragment contained the telomere sequences, a selectable LEU2 gene, and ~500 bp of homology to a particular site on chromosome III.

**LEU2 recombination**

In experiments involving leu2 heterallelic recombination, leu2-R was inserted into a hmlA::LEU2 deletion by the integration and popping-out of a URA3–leu2-R plasmid, as described previously [Lichten et al. 1987]. In strains H333 and H334, introduction of leu2-R at LEU2 [91 kb from the left telomere] was accomplished in the same fashion, as was the creation of hmlA:leu2-R. Insertions at MAT [199 kb] were done by the integration of a MAT–URA3–leu2-R plasmid, as described previously [Lichten et al. 1987], to create a MAT–URA3–leu2-R–MAT duplication. A URA3–leu2-R plasmid was integrated into the ura3-52 locus on chromosome V to create ura3-52–pBR322–(leu2-R)–URA3 duplications. In strains XW440 and XW421 the same plasmid was inserted into a previously constructed partial deletion of leu2 to yield leu2A–pBR322–URA3–leu2-R. PCR-amplified DNA from the regions of 162 kb on chromosome III was cloned into a URA3–leu2-R plasmid and this was integrated at nucleotide 162,870 to create a "162"–leu2-R–pBR322–URA3–"62" insertion. A PCR-amplified fragment of the 233-kb region was cloned into a pGEM plasmid, and the Xhol–SaII leu2-K fragment that also carried an ADE1 insert at the AflII site [W.Y. Leung and J.E. Haber, unpublished] was inserted at nucleotide 233,174, to create the "233" insertion. The LEU2 genes in these constructions are all oriented with the 5’→3’ direction being left to right on the chromosome in its standard presentation [Oliver et al. 1992], except for the hmlA:leu2-R and 249 kb:leu2-K insertions on chromosome III. Strains XW529, XW530, and XW531 are meiotic segregants of a cross between H333 and H334, in which a crossover in the interval containing the duplicated MAT loci produces a nonmating [MATa–MATa] segregant carrying both MATα and MATa.

In strains XW499 and XW500, carrying hmlA:leu2-R and MAT–URA3–leu2-K–MAT, the two leu2 alleles are in opposite orientation. The proportion of gene conversion events at leu2-K could be determined by placing Leu+ recombinants on 5-FOA to select Ura- derivatives that had popped out the duplication at MAT and became Leu+. A small fraction of the gene conversion events were associated with a reciprocal crossing-over, such that a very large pericentric inversion was created. These could be recognized because the cells failed to yield many Ura- papillae on 5-FOA plates, as we have documented previously [Harris et al. 1993]. Altogether, 4/196 Leu+ derivatives of the MATa strain XW500 and 1/196 of the MATa strain XW499 contained inversions. These were not counted in the analysis of which allele had converted.

**DNA analysis**

Preparation of yeast genomic DNA and Southern blot analysis was carried out as described previously [White and Haber 1990]. A Yc-specific DNA probe was derived from plasmid pH315.

PCR amplification of MATα or MATα-B derivatives, after switching from MATa, was accomplished by using primers KK200 (5’-CGACCACTCAAGAAAGA-3’) and JK735 (5’-ATGTGAACCGATGGCGAGT-3’) to amplify a 769-bp MATα-specific DNA fragment. MATα-B contains a BamHI site that when cut, yields DNA fragments of 582 and 187 bp. Equivalent analysis was carried out when one donor carried HMLα-H, to yield MATα-H containing an HindIII site, after switching.

**Cell growth and induction MAT switching**

Cells were grown and sporulated at 30°C. Induction of HO endonuclease was carried out in two ways. In some experiments, meiotic segregants carrying MATα, HO, and the donors indicated were dissected by micromanipulation and allowed to germinate and grow into nonmating colonies. These nonmating colonies result from the switch of MATα mother cells in the first several cell divisions to MATa or MATα-B and their mating with remaining MATα daughter cells. The proportion of switches using MATα or MATα-B was determined from densitometry of Southern blots of DNA cut with BamHI.

In most experiments a ho MATα strain carrying the URA3-marked GAL::HO plasmid pH132 [Nickoloff et al. 1989] or the similar LEU2-marked plasmid pH727 was grown in YEP–lactate medium to which 2% galactose was added for 1.5 hr to induce expression of HO endonuclease, as described previously [White and Haber 1990]. The haploid MATα or MATα-B derivatives were then analyzed by Southern blots or PCR amplification and BamHI cleavage.

**Northern blot analysis**

Ten-milliliter cultures of cells carrying hmlA::LEU2 and deleted for the normal leu2 locus were grown in logarithmic phase to a density of 1×10⁸ cells/ml in YEPD. RNA was extracted according to Cross and Tinkelenberg [1991]. In some experiments cells were first shifted from YEPD medium to minimal growth medium to allow greater expression of LEU2 and HIS4 mRNA. The Northern blots were probed with a 32P-labeled EcoRV–SalI fragment of LEU2 to measure the effect of mating type on transcription of a gene located near HML, and with a 32P-labeled PvuII–ClaI fragment of HIS4, which was used to estimate the abundance of mRNA. The blots were counted by PhosphorImaging, as described above.
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1932 GENES & DEVELOPMENT
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