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 ≥ 90 kb from *MAT*. *MATa* cells recombine nearly 90% of the time with *HML*, whereas *MAT α* cells select *HMR*. *MATa* donor preference was examined by deleting *HML* and inserting a donor at other chromosome III locations. *MATa* activated a large (≥ 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, *MAT α 2*; 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 *MAT α* 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 *MAT α* 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, >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 *MAT α* cells and is turned off in *MATa/MAT α* 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 *HML α* , whereas *MAT α* cells selectively recombine with *HMR α* (Strathern and Herskowitz 1979). Donor selection is not dictated by the *Y α* or *Y α* sequences, either in the donors or at *MAT*. For example, a *MATa* cell carrying genetically distinguishable *HML α* and *HMR α* alleles still uses *HML* (Klar et al. 1982). Moreover, when a recessive *mata1* mutant is mated with an *HO MAT α -inc* strain (in which the *inc* mutant cannot be cleaved by *HO*), the resulting α -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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Figure 1. (A) MAT switching in *S. cerevisiae*. HO endonuclease cleaves the *MATa* locus near the Y/Z border and initiates the replacement of *Y_a* sequences with *Y_α* DNA copied from the silent donor *HMLα*. *MATα* encodes two genes, *MATα1* and *MATα2*, as shown. *MATa* encodes a single functional gene, *MATa1*. Other shared regions of homology are indicated. The silent donors *HML* and *HMR* are neither transcribed nor cleaved by the HO endonuclease. Silencing depends on the flanking E and I sites. *HMLα* is preferred over *HMR*, even when *HMR* also carries *Y_α*. (B) Replacement of a 5.4-kb *XhoI* (X) fragment containing *HML* with a cassette including the *HindIII* (H) fragment of *HMRα* adjacent to the 2.2-kb *XhoI*–*SalI* (S) *LEU2* gene segment. This construction is carried in plasmid pXW135. (C) A similar cassette in plasmid pXW145 containing the *LEU2* gene inserted at the *NruI* site (N) of the *HindIII* *HMRα* fragment was inserted at several other chromosomal locations.

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 *MATα*. 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 *MATα2* 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, *HMLα* and *HMRα-B*, containing a single-base-pair substitution in *Y_α* that creates a *Bam*HI site without altering the coded amino acid sequence (see Materials and methods). Thus, a *MATa* cell can switch to *MATα* (from *HMLα*) or to *MATα-B* (from *HMRα-B*). In some experiments (Table 1), switching was induced by dissecting *HO/HO MATa/MATα* diploids to obtain *HO MATa* spores. These spores germinate and usually switch mating type at the four-cell stage producing two nonmating *MATa/MATα* (or two *MATa/MATα-B*) diploids that then grow up into a colony. The *HO* gene is turned off in *MATa/MATα* 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 *Bam*HI-digested DNA, probed with a *Y_α*-specific DNA, distinguishes *MATa/MATα* from *MATa/MATα-B* diploids, and therefore demonstrates which donor was chosen. DNA extracted from eight such nonmating 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. *HMRα-B* in colonies derived from *HO MATa* spores

Strain	Location of donor on left arm	Donor on right arm	Number of colonies analyzed ^a	Percentage using <i>HMRα-B</i>
XW221	<i>HMLα</i>	<i>HMRα-B</i>	32	7.5
XW246	<i>hmlΔ::HMRα</i>	<i>HMRα-B</i>	10	15.0
XW263	<i>HMRα</i> at 91 kb ^b	<i>HMRα-B</i>	32	49.0
XW348	<i>HMRα</i> at 67 kb ^b	<i>HMRα-B</i>	32	49.3

MATa spores were germinated and grown into nonmating colonies, in which *MATa* had switched to either *MATα* or *MATα-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 nonmating *MATa/MATα* diploids, in which there is no more switching.

^aRatio of donor usage determined by densitometry of Southern blots of *Bam*HI-digested DNA that distinguish between *MATα* and *MATα-B* (see Fig. 2). Except for XW246, 32 or more nonmating 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 *HMRα-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/MATα* or *MATa/MATα-B* cells. DNA was pooled from eight such nonmating colonies, cut with *Bam*HI and *Hind*III and separated by gel electrophoresis. The Southern blot was probed with a γ -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 γ probe hybridizes to the 4.4-kb *MATα* or 3.1-kb *MATα-B* *Bam*HI 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 G_1 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). *Bam*HI-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 *MATα* or *MATα-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, *MATa* 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 G_1 phase of the cell cycle or when the galactose-inducible *HO* gene is expressed throughout the cell cy-

cle. 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 MATa-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 *MATa* 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 *MATa* cells activate not simply the region around *HML* but a large (≥ 40 kb) chromosomal region to be especially recombinogenic during *MATa* 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. 3J), the donor at *HIS4* was *HMLα-H*, carrying a *Hind*III site in γ . Among the *MATa* 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 *MATa* 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

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 *Bam*HI site that permits the two sequences to be distinguished (A). Except for XW246, all strains were analyzed with a minimum of 26 independent α -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 *MATα-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 *MATα-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 con-

structed 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* (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 XW486 (J) is deleted for *HMR* and carries *HMLα* at its normal location and the 6.6-kb *Bam*HI fragment of *HMLα-H* inserted at *HIS4* (67 kb). Strain XW526 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).

(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 (XW526) 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. 3J, strain XW486) 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 a new telomere sequence (Fig. 3I). Despite the removal of ~ 10 kb of the sequences normally found on either side of *HML*, *MATa* donor preference remained unchanged. Thus, any cis-acting sequences responsible for establishing *MATa* donor preference are located far from the location of the normal *HML* locus. Larger deletions of this region are haploid-inviable, so that further analysis will require that essential genes in this interval be duplicated elsewhere.

MATa donor preference is regulated by *MATα2*

An early study of *MAT* switching (Tanaka et al. 1984) suggested that the repressor of α -specific gene expression, *MATα2*, might play a decisive role in regulating donor preference. An *HO hmlα2 mata2 HMRα* cell switched only rarely to *MATa*, 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 *mat α 2* mutation itself reduced the frequency of HO cleavage and switching, which would have yielded a similar result. Here we show that *MAT α 2*, expressed in *trans*, does regulate donor preference. A *GAL::HO* strain, XW431, of genotype *MAT α HML α HMR α -B* was transformed with either the *MAT α 1* gene or the *MAT α 2* gene inserted at *URA3* on chromosome V (Fig. 4A,B). The *MAT α 1* gene carries an *inc* mutation so that it is not cut by HO. The ectopic expression of either *MAT α 1* or *MAT α 2* causes a *MAT α* cell to become nonmating, but for different reasons. In the case of *MAT α 1*, both *a*-specific and α -specific genes are expressed simultaneously, whereas in the case of *MAT α 2*, both *a*-specific and α -specific genes are turned off. We induced the *HO* gene and recovered α -mating switched cells that were either *MAT α* (from *HML*) or *MAT α -B* (from *HMR*). In *MAT α* [*MAT α 1*] cells, donor preference remained in favor of *HML*, but in *MAT α* [*MAT α 2*] cells donor preference shifted to that expected for *MAT α* cells (Fig. 4). Thus, the expression of *MAT α 2* prevents the activation of *HML*. Whether *MAT α 2* 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 struc-

ture 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 *MAT α* preference is abolished. As noted above, in strain XW425, a *MAT α* cell that also expresses *MAT α 2* shifts its donor preference to *HMR*. We constructed a related strain, XW490, in which the 5.4-kb region, including *HML α* and its adjacent silencer sequences, was replaced with a 6.4-kb *EcoRI* fragment of *MAT α -inc*, which lacks the adjacent E and I silencer sites (Fig. 4C). This (*E*⁻ *I*⁻) *hml Δ ::MAT α -inc MAT α HMR α -B* strain is nonmating because both *MAT α* and *MAT α 2* (from *hml Δ ::MAT α -inc*) are expressed. *MAT α 1* is not expressed in diploids expressing both *MAT α* and *MAT α 2* (Herskowitz 1989); thus, this cell is phenotypically equivalent to XW425. Only *MAT α* can be cleaved by HO, and thus the cell can either use the expressed *hml Δ ::MAT α -inc* or the silent *HMR α -B* as a donor. Unlike strain XW425, where donor preference shifted to *HMR α -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 MAT α than in MAT α

The activation of *HML* in *MAT α* 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 *MAT α* 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

Figure 4. Ectopic expression of the *MAT α 1* or *MAT α 2* genes in *MAT α* strains. A *URA3*-marked plasmid carrying either the *MAT α 2* gene (A) or the *MAT α 1* (B) gene was inserted by homologous integration at the *ura3-52* locus on chromosome V. The *MAT α 1* gene was PCR amplified from a *MAT α -inc* strain and thus contains a single base-pair mutation that prevents HO cutting (Weiffenbach et al. 1983). The *MAT α 2* gene is expressed along with *MAT α* in strain XW490, in which *MAT α -inc* is inserted in place of *HML* (C); thus, this strain has the same effective genotype as strain XW425 (A).

different allele, *leu2-K*, was inserted between two tandem repeats of either *MATa* or *MATα* (Fig. 5B). The rate of spontaneous recombination to *Leu*⁺ prototrophy was 30 times higher in *MATa* than in *MATα* 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*]/ [*MATα*–(*leu2-K*)–*MATα*] 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 *MATα* strain, 50/55 (91%) became *Leu*[–], indicating that the *leu2-K* allele adjacent to *MAT* 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-R* 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* oc-

curs 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 *MATα* (Table 2). Recombination between *hmlΔ::leu2-R* on chromosome III and *leu2-K* integrated near *URA3* on chromosome V was again much higher in *MATa* (Fig. 5D; Table 2). We also note that there is an intrinsically higher rate of intra-versus interchromosomal 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 *MATα*. 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 *MATα2*

To determine whether *MATα2* 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*)–*MATα*, which could be compared with those carrying two tandem copies of *MATa* (XW499) or *MATα* (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 *NruI*–*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 *MATα*, when it recombines with a *leu2-K* allele adjacent to *MAT*. The ratio of the rates of recombination in *MATa* vs. *MATα* cells is presented.

Table 2. Effect of mating type on the rate of LEU2 prototroph formation between *leu2* alleles in different chromosomal locations

Strain	Mating type ^a	Location of		Rate of Leu ⁺	Ratio	
		<i>leu2-R</i>	<i>leu2-K</i>		a/α	nm/α ^a
XW499	a	HML	MAT	6.72×10^{-6}	32	
XW500	α	HML	MAT	2.13×10^{-7}		
XW498	nm	HML	MAT	1.34×10^{-6}		6.3
XW440	a	HML	LEU2	5.82×10^{-6}	19	
XW421	α	HML	LEU2	3.01×10^{-7}		
XW485	a	HML	URA3	3.06×10^{-7}	28	
XW456	α	HML	URA3	1.10×10^{-8}		
XW529	a	MAT	LEU2	2.15×10^{-7}	2.3	
XW530	α	MAT	LEU2	9.20×10^{-8}		
XW531	nm	MAT	LEU2	3.38×10^{-7}		3.7
H334	a	MAT	LEU2	2.37×10^{-7}	1.8	
H333	α	MAT	LEU2	1.31×10^{-7}		
XW412a	a	162 kb*	233 kb*	6.12×10^{-7}	0.88	
XW412	α	162 kb*	233 kb*	6.93×10^{-7}		
XW508	a	HMR	MAT	6.79×10^{-7}	0.42	
XW506	α	HMR	MAT	1.62×10^{-6}		

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 *hmlΔ::leu2-R* and 249 kb::(*leu2-K*) insertions on chromosome III.

^a(nm) Nonmating.

obtained where the *leu2* alleles are at *leu2* locus itself and MAT. The results shown in Table 2 show a four- to sixfold increase of recombination in nonmating derivatives over MATα, whether the *leu2* sequences are at the

HML site or not. This is expected in view of other studies showing that nonmating (*MATa/MATα*) 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 MATα cells, but there is no longer any positional effect as observed in MATa cells. We conclude that expression of MATα2 represses the MATa-dependent high level of *leu2* heteroallelic recombination for *hmlΔ::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 MATα 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 MATα 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

Figure 6. Mating-type dependence on the formation of Leu⁺ recombinants. A diploid of genotype [*MATa-leu2-K*]-*MATa*/*MATα-leu2-K*]-*MATα* and carrying *hmlΔ::leu2-R* was constructed (see Fig. 5B). The diploid was sporulated, and the four spores of each tetrad were dissected. Each row represents the two MATa and two MATα colonies derived from one tetrad, which were replica-plated from rich medium to synthetic medium lacking leucine. The frequency of Leu⁺ prototrophs is much higher in two of the four segregants, both of which are MATa. In the first row, e.g., the first and fourth segregants are MATα and the middle two are MATa. In the second row, the two patches on the left are from MATα and the two on the right are from MATa.

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 *MATa* and *MATα* isogenic strains carrying *hmlΔ::LEU2* and deleted for the normal *LEU2* locus [these strains were the precursors of strains XW499 and XW500 that showed *MATa*-dependent elevation of *leu2* recombination (Table 2)]. There was no significant difference in the abundance of *LEU2* mRNA (data not shown).

Discussion

When *MATa* 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 *MATa* 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. *MATa* 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 *MAT* 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 *MATa* cells.

Another model that might have explained donor preference would be the existence of pairing sites adjacent to *HML* and *MAT* that permit a prealignment of the donor when an *a*-specific gene product is expressed. However, the idea of specific pairing sites between *MAT* and *HML* seems less likely given that *MATa* 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 *MATa* cells even when the other *leu2* is on a different chromosome.

MATa 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 *MATa* 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 se-

quences located elsewhere in the genome but that this constraint is released in *MATa* cells. For example, the DAR could be anchored to the nuclear envelope, similar to the *SIR* gene-dependent tethering of telomere regions (Palladino et al. 1993), in *MATα* cells but released in *MATa*. If there is such a release of this region of the chromosome, it does not apparently involve inactivation of the *SIR* genes, as *HML* remains silent in *MATa* cells and physical analysis of *MATa* switching has demonstrated that the *HML* locus remains refractory to HO cleavage (Connolly et al. 1988). Nevertheless, the state of silencing does influence donor preference. As we showed above, the repression of *HML* recombination in a *MATa* cell expressing *MATα2* can be overcome by providing an unsilenced *hmlΔ::MATα-inc* donor at the position of *HML*. Thus, there seems to be a hierarchy of structural features that dictate donor preference: Normally, a silenced *HML* is efficient in *MATa* switching, but an unsilenced donor at the same position can overcome the loss of the normal activation mechanism. The identification of a *cis*-acting sequence responsible for activating the donors in *MATa* cells should provide some indication of the way in which these sequences may be used.

There are other indications that the left-most 40 kb of chromosome III is unusual in its structure. For example, Collins and Newlon (1994) have surveyed the activity of 14 DNA sequences found on the left two-thirds of chromosome III, which were shown to act as origins of replication on plasmids [so-called autonomously replicating sequences (ARSs)]. However, only six of these ARS sequences appear to have bona fide origin of replication activity on the chromosome. Most of the inactive origins are in the 40-kb region identified by our study. This may suggest that the region has an unusual chromosome structure where accessibility to replication (and recombination) machinery may be constrained. Whether more of these origins would be active in diploids, where *a*- and *α*-specific genes are repressed, is not known. The *MATa*-dependent activation of DAR reminds us of X chromosome dosage compensation in *Drosophila* males (Bone et al. 1994), where transcription is increased over that in females, presumably because male-specific gene products bind to activation sites on the X chromosome. In yeast, the activation of DAR apparently requires the action of an *a*-specific gene product whose expression is turned off by *MATα2*. We imagine that this *a*-specific protein binds to one or more *cis*-acting sites in DAR. The activation of DAR is sufficient to increase spontaneous *leu2* recombination by 20- to 30-fold. However, the change in accessibility of the donor must be accomplished without unsilencing the donor. Unlike the *Drosophila* case, there is no mating-type-dependent change in the level of transcription of a gene that we showed was activated for recombination.

MATa donor preference operates differently from *MATα* donor selection

In this paper we have focused our attention on *MATa* donor preference. How *MATα* cells choose *HMR* is not

yet clear, but several lines of evidence argue that *MAT α* donor preference for *HMR* is not simply the mirror image of how *DAR* is activated in *MAT α* . For example, we have observed that *HO MAT α* cells deleted for *HMR* are not efficient in using *HML α* as a "backup" donor, whereas *HO MAT α* cells switch efficiently using *HMR α* when *HML* is deleted. About one half of *MAT α* 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, *MAT α* cells do not show a significant activation of *leu2* heteroallelic recombination when one of the alleles is situated in place of *HMR*. Finally, we have shown recently that, whereas *HML α* on chromosome III can act efficiently to switch *MAT α* inserted on chromosome V, *HMR α* fails to repair a DSB at *MAT α* 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 *MAT α* 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 *MAT α* 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 MAT α 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 heterothallic derivatives from Y55 crossed once with DBY745. The genotypes of the strains used are given in Table 3.

Table 3. Yeast strains

Strain	Genotype
DBY745	<i>ho HMLα MATα HMRα ade1-100 leu2-3,112 ura3-52</i>
XW425	<i>ho HMLα MATα HMRα-BamHI::ura3 ura3::α2::URA3 pJH727(GAL-HO LEU2) ade1-100 leu2-3,112</i>
XW426	<i>ho hmlΔ::ADE1 41kb::(LEU2,HMRα) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW427	<i>ho hmlΔ::ADE1 22kb::(LEU2,HMRα) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW429	<i>ho (C$_4$A$_2$ LEU2, all HMLdist. seq.Δ) HMLα MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW431	<i>ho HMLα MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112</i>
XW441	<i>ho (hmlΔ::LEU2 8kb HML prox. seq.Δ) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW448	<i>ho HMLα MATα HMRα-BamHI::ura3 ura3::α1::URA3 pJH727(GAL-HO LEU2) ade1-100 leu2-3,112</i>
XW452	<i>ho hmlΔ::ADE1 LEU2::HMRα MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW453	<i>ho hmlΔ::ADE1 his4::(LEU2,HMRα) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW490	<i>ho hmlΔ::(MATα-inc,LEU2) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW533	<i>ho (from the end to 22kbΔ::ura3) 41kb::(LEU2, HMRα) MATα HMRα-BamHI::ura3 pJH132(GAL-HO URA3) ade1-100 leu2-3,112 ura3-52</i>
XW486	<i>ho HMLα his4::(HMLα-HindIII,URA3) MATα hmrΔ::ADE1 pJH727(GAL-HO LEU2) ade1-100 leu2-3,112 lys5 ura3-52</i>
XW526	<i>ho HMR-HMLα fusion his4::(HMLα-HindIII,URA3) MATα circular chromosome pJH727(GAL-HO LEU2) ade1-100 leu2-3,112 lys2 or lys5 ura3-52</i>
NR226-7B	<i>HO HMLα MATα HMRα leu2-3,112 lys5 ura3-52</i>
NR238-7C	<i>HO HMLα MATα HMRα ade1 leu2-3,112 lys5 ura3-52</i>
NR272-19A	<i>HO HMLα MATα HMRα ade1 leu2-3,112 lys5 ura3-52</i>
XW153	<i>HO hmlΔ::ADE1 MATα HMRα ade1 leu2-3,112 lys5 ura3-52</i>
XW221	<i>HO HMLα MATα HMRα-BamHI leu2-3,112 lys5 ura3-52</i>
XW246	<i>HO hmlΔ::(HMRα,LEU2) MATα HMRα-BamHI leu2-3,112 lys5 ura3-52</i>
XW265	<i>HO hmlΔ::ADE1 LEU2::HMRα MATα HMRα-BamHI leu2-3,112 lys5 ura3-52</i>
XW348	<i>HO hmlΔ::ADE1 his4::(LEU2,HMRα) MATα HMRα-BamHI leu2-3,112 lys5 ura3-52</i>
XW499	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ) [MATα--URA3 leu2K pBR322--MATα] HMRα ade1-100 ura3-52</i>
XW500	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ) [MATα--URA3 leu2K pBR322--MATα] HMRα ade1-100 ura3-52</i>
XW498	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ) [MATα--URA3 leu2K pBR322--MATα] HMRα ade1-100 ura3-52</i>
XW440	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ)::(pBR322 URA3 leu2K) MATα HMRα ade1-100 thr4 ura3-52</i>
XW421	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ)::(pBR322 URA3 leu2K) MATα HMRα ade1-100 thr4 ura3-52</i>
XW485	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ) MATα HMRα ura3-52::(URA3 pBR322 leu2K) ade1-100 thr4</i>
XW456	<i>ho hmlΔ::leu2R leu2Δ(XhoI-EcoR5Δ) MATα HMRα ura3-52::(URA3 pBR322 leu2K) ade1-100 thr4</i>
H334	<i>ho HMLα leu2K [MATα--URA3 leu2R pBR322--MATα] HMRα ade1-100 his6 lys2 met13 ura3-52</i>
H333	<i>ho HMLα leu2K [MATα--URA3 leu2R pBR322--MATα] HMRα ade1-100 lys2 met13 trp5 ura3-52</i>
XW529	<i>ho HMLα leu2K [MATα--URA3 leu2R pBR322--MATα] HMRα ade1 lys2 met13 trp5 ura3-52</i>
XW530	<i>ho HMLα leu2K [MATα--URA3 leu2R pBR322--MATα] HMRα ade1 lys2 met13 ura3-52</i>
XW531	<i>ho HMLα leu2K [MATα--URA3 leu2R pBR322--MATα] HMRα his6 lys2 met13 ura3-52</i>
XW412a	<i>ho HMLα leu2Δ(XhoI-EcoR5Δ) 162kb::(leu2R pBR322 URA3) MATα 233kb::(leu2K ADE1) HMRα ade1-100 trp1 ura3-52</i>
XW412	<i>ho HMLα leu2Δ(XhoI-EcoR5Δ) 162kb::(leu2R pBR322 URA3) MATα-BamHI 233kb::(leu2K ADE1) HMRα ade1-100 trp1 ura3-52</i>
XW508	<i>ho HMLα leu2Δ(XhoI-EcoR5Δ) [MATα--URA3 leu2K pBR322--MATα] Δhmr::leu2R ade1-100 lys5$^+$ ura3-52</i>
XW506	<i>ho HMLα leu2Δ(XhoI-EcoR5Δ) [MATα--URA3 leu2K pBR322--MATα] Δhmr::leu2R ade1-100 lys5$^+$ ura3-52</i>

HMR α 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 *HMR α*

gene was cloned as a *HindIII* fragment into a pGEM3Zf(+) vector for further manipulation. The *HMR α -B* allele was created by oligonucleotide-directed mutagenesis (Kolodeij and Young 1991) of C $\rightarrow$ A at position 658 in $\gamma\alpha$ to create a *Bam*HI site. *HMR α -B* was introduced into the chromosome in place of *HMR α* 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 (Boeke et al. 1987). Similar mutagenesis was performed to create *HML α -H* in which position 379 of $\gamma\alpha$ was mutated A $\rightarrow$ T to create a *HindIII* site.

A cassette containing *HMR α* 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 pGEM3Zf(+) 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 *ApaI* site nucleotide 21,974, at a unique *ClaI* site at nucleotide 41,404, at a unique *XhoI* site at nucleotide 67,682 near *HIS4*, and at a unique *XhoI* site at nucleotide 90,286 near *LEU2*. The fragments containing the target sequences flanking *LEU2* and *HMR α* were then transformed into yeast (Rothstein 1983). The *HML α -H* gene, plus an adjacent *URA3* gene, was inserted at the *XhoI* site near *HIS4* (nucleotide 67,682) in a similar fashion.

In some of these constructions, the *XhoI* 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* C₄A₂ 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* heteroallele recombination, *leu2-R* was inserted into a *hml Δ ::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-K* at *LEU2* (91 kb from the left telomere) was accomplished in the same fashion, as was the creation of *hmr Δ ::leu2-R*. Insertions at *MAT* (199 kb) were done by the integration of a *MAT-URA3-(leu2-K)* pBR322 plasmid, as described previously (Lichten et al. 1987), to create a *MAT-URA3-(leu2-K)-MAT* duplication. A *URA3-(leu2-K)* plasmid was integrated into the *ura3-52* locus on chromosome V to create *ura3-52-pBR322-(leu2-K)-URA3* duplications. In strains XW440 and XW421 the same plasmid was inserted into a previously constructed partial deletion of *leu2* to yield *leu2 Δ -pBR322-URA3-(leu2-K)*. PCR-amplified DNA from the regions of 162 kb on chromosome III was cloned into a *URA3-(leu2-R)* pBR322 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 *XhoI-SalI leu2-K* fragment that also carried an *ADE1* insert at the *AflIII* site (W.Y. Leung and J.E. Haber, unpubl.) was inserted at nucleotide 233,174, to create the "233" insertion. The *LEU2* genes in these constructions are all oriented with the 5' $\rightarrow$ 3' direction being left to right on the chromosome in its standard representation (Oliver et al. 1992), except for the *hml Δ ::(leu2-R)* and 249 kb::(*leu2-K*) insertions on chromosome III. Strains XW529, XW530, and XW531 are mei-

otic segregants of a cross between H333 and H334, in which a crossover in the interval containing the duplicated *MAT* loci produces a nonmating (*MAT α -MAT α*) segregant carrying both *MAT α* and *MAT α* .

In strains XW499 and XW500, carrying *hml Δ ::(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 *MAT α* strain XW500 and 1/196 of the *MAT α* 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 $\gamma\alpha$ -specific DNA probe was derived from plasmid pJH315.

PCR amplification of *MAT α* or *MAT α -B* derivatives, after switching from *MAT α* , was accomplished by using primers KK200 (5'-CGACCACTCAAGAAAGA-3') and JK735 (5'-ATGTGAACCGCATGGGCAGT-3') to amplify a 769-bp *MAT α* -specific DNA fragment. *MAT α -B* contains a *Bam*HI site that when cut, yields DNA fragments of 582 and 187 bp. Equivalent analysis was carried out when one donor carried *HML α -H*, to yield *MAT α -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 *MAT α* , *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 *MAT α* mother cells in the first several cell divisions to *MAT α* or *MAT α -B* and their mating with remaining *MAT α* daughter cells. The proportion of switches using *MAT α* or *MAT α -B* was determined from densitometry of Southern blots of DNA cut with *Bam*HI.

In most experiments a *ho MAT α* strain carrying the *URA3*-marked *GAL::HO* plasmid pJH132 (Nickoloff et al. 1989) or the similar *LEU2*-marked plasmid pJH727 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 *MAT α* or *MAT α -B* derivatives were then analyzed by Southern blots or PCR amplification and *Bam*HI cleavage.

Northern blot analysis

Ten-milliliter cultures of cells carrying *hml Δ ::LEU2* and deleted for the normal *leu2* locus were grown in logarithmic phase to a density of 1×10^7 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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