Rules of Donor Preference in Saccharomyces Mating-Type Gene Switching Revealed by a Competition Assay Involving Two Types of Recombination

Xiaohua Wu,[1,2] Cherry Wu[1] and James E. Haber

Rosenstiel Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254-9110

Manuscript received March 27, 1997
Accepted for publication June 16, 1997

ABSTRACT

Mating type (*MAT*) switching in *Saccharomyces cerevisiae* is initiated by a double-strand break (DSB) created at *MAT* by HO endonuclease. *MAT*a cells activate the entire left arm of chromosome III; thus *MAT*a preferentially recombines with the silent donor *HML*. In contrast, *MAT*α cells inactivate the left arm, including *HML*, and thus preferentially recombine with *HMR*, 100 kb to the right of *MAT*. We present a novel competition assay, in which the DSB at *MAT* can be repaired either by *MAT* switching or by single-strand annealing (SSA) between two *URA3* genes flanking *MAT*. With preferred donors, *MAT*a or *MAT*α switching occurs 65–70% of the time in competition with SSA. When *HML* is deleted, 40% of *MAT*a cells recombine with the "wrong" donor *HMR*; however, when *HMR* is deleted, only 18% of *MAT*α cells recombine with *HML*. In interchromosomal switching, with donors on chromosome III and *MAT* on chromosome V, *MAT*a retains its strong preference for *HML* and switching is efficient, when the chromosome III recombination enhancer is present. However, *MAT*α donor preference is lost and interchromosomal switching is very inefficient. These experiments demonstrate the utility of using competition between two outcomes to measure the relative efficiency of recombination.

HOMOTHALLIC switching in *Saccharomyces cerevisiae* occurs by site-specific mitotic gene conversion. A DSB at *MAT* promotes the replacement of Ya- or Yα-specific sequences with those copied from one of two unexpressed donor loci, *HMLα* or *HMRa*. One of the most interesting aspects of *MAT* switching is that *MAT*a cells preferentially recombine with *HML*, located 200 kb to the left of *MAT* (Figure 1A), while *MAT*α cells are disposed to select *HMR*, located 100 kb to the right of *MAT* (STRATHERN and HERSKOWITZ 1979; KLAR *et al.* 1982; WEILER and BROACH 1992). The selection of *HML* by *MAT*a involves the activation of the entire left arm of chromosome III for recombination, so that a donor (either *HML* or *HMR*) placed at several different sites along the arm will be used preferentially over another donor situated elsewhere (WU and HABER 1995, 1996). This activation is not specific for mating-type sequences, as *MAT*a cells also exhibit a 25–30-fold higher rate of recombination between *leu2* heteroallelic sequences when one of the *leu2* alleles is inserted in place of *HML* (WU and HABER 1995, 1996). The *cis*-acting sequences required for this activation have been narrowed down to a 700-bp region, located ~19 kb proximal to *HML*. *Trans*-acting elements that activate this recombination enhancer have not yet been identified, except that *MAT*a donor preference is negatively

regulated by expression of the Matα2 protein that represses expression of a-specific genes and that also has a putative binding site within the recombination enhancer (WU and HABER 1996). The *chl1* mutation that affects mitotic stability of all yeast chromosomes also influences *MAT*a donor preference but also affects general chromosome stability (LIRAS *et al.* 1978; GERRING *et al.* 1990; WEILER *et al.* 1995).

*MAT*α donor preference appears to operate by different rules. First, *MAT*α cells are unable to use efficiently a donor inserted at any of several sites located >25 kb to the left of *MAT*, including the centromere-proximal part of the right arm and all of the left arm (KLAR *et al.* 1982; WEILER and BROACH 1992; WU and HABER 1995). Consequently, when *HMR* is deleted, a *MAT*α cell is unable to use *HML* as an efficient alternative donor, and cells die about one-third of the times they attempt to switch because the DSB is not repaired (WU *et al.* 1996). This is quite different from what occurs in *MAT*a cells lacking *HML*, where the "wrong" donor is used sufficiently often that there is no evident cell lethality. Second, *MAT*α cells exhibit only a twofold higher rate of *leu2* heteroallelic recombination when a *leu2* allele is inserted in place of *HMR* (WU and HABER 1995). These results suggest that *MAT*α donor preference involves a different mechanism than *MAT*a.

One problem in assessing the efficiency of a recombination event is that cells may have more than a single opportunity to repair a DSB. Thus, although one-third of *HMLα MAT*α *hmr*Δ cells die after experiencing a DSB, these cells may have attempted to locate and recombine with *HML* many times before the broken chro-
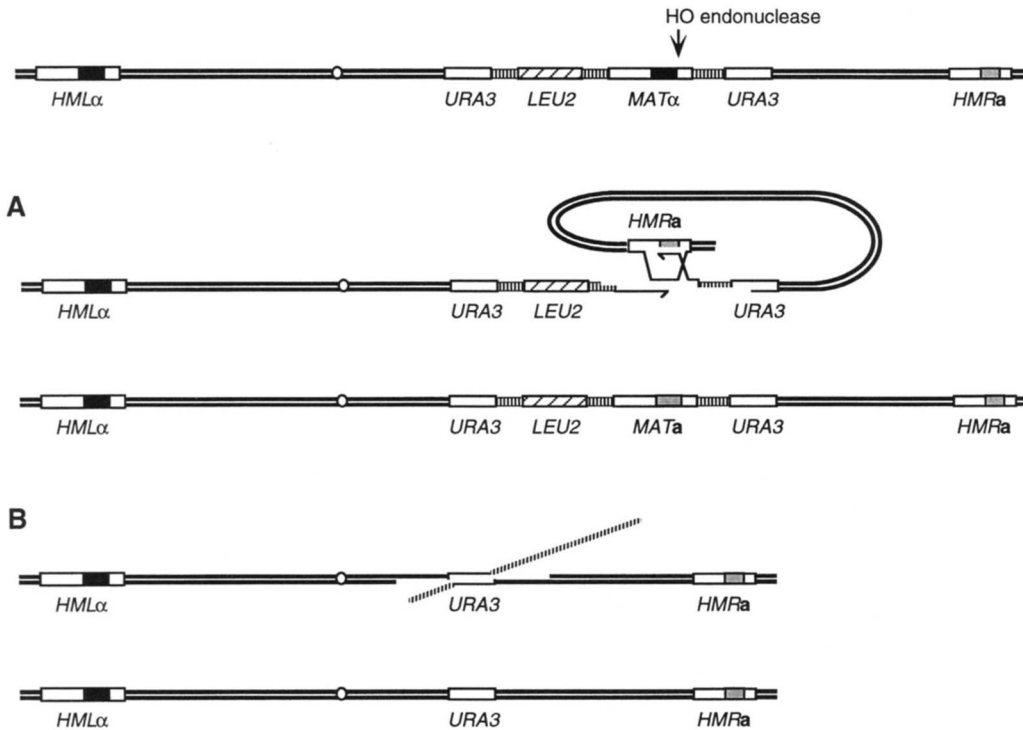

FIGURE 1.—Repair of an HO endonuclease-induced DSB by gene conversion or by single-strand annealing (SSA). The *MAT* locus was deleted and replaced by *URA3*, after which a pBR322 plasmid containing *MATα*, *LEU2* and *URA3* was integrated at this site to create the duplication shown (top). (A) A DSB created in *MATα* can be repaired by recombination with its preferred donor, *HMRa*. (B) Alternatively, the DSB can be repaired by single-strand annealing, which deletes all sequences between the two *URA3* genes and thus deletes both *LEU2* and *MAT*.

mosome was either degraded or lost during a subsequent mitosis. The actual efficiency might be considerably lower than the final outcome would suggest.

To assess more accurately the efficiency of a particular DNA repair event, it would be useful to provide an alternative, default pathway to repair the DSB if the initial attempts to locate a donor were unsuccessful. One such default pathway is single-strand annealing (SSA) in which a deletion is formed between two homologous sequences flanking a DSB after 5′ to 3′ degradation of the DNA to produce long single-stranded tails that can anneal (Figure 1B). SSA is a very efficient process that competes with gene conversion (FISHMAN-LOBELL *et al.* 1992; LIEFSHITZ *et al.* 1995). For example, in a plasmid carrying two direct repeats of the *Escherichia coli lacZ* gene, one of which carries an HO endonuclease recognition site, a DSB is repaired only ~20% of the time by gene conversion and 80% by SSA. These proportions can be modulated in favor of gene conversion by moving the two flanking regions apart so that it takes more time for the homologous regions to become single-stranded (FISHMAN-LOBELL *et al.* 1992). One might expect that these proportions would also change if the gene conversion donor were less accessible.

We have applied this idea to study the relative efficiencies of different mating type donors to switch *MAT* by providing a competing DNA repair process. This was accomplished by creating a flanking duplication of *URA3* genes, either at the normal *MAT* locus (Figure 1) or on chromosome V. Following HO endonuclease cleavage, the 5′ to 3′ resection of DNA flanking the DSB leads either to a conversion of the *MAT* locus (Figure 1A) or to the deletion of *MAT* and the other se-

quences flanking the two *URA3* genes (Figure 1B). In this way, we could assess the efficiency of switching when the donor and the recipient were in different topological relationships. We find that *MATα* cells recombine with *HMR* as efficiently as *MATa* cells use *HML*, but only when *MATα* and *HMR* are located on the same chromosome.

MATERIALS AND METHODS

Strains and plasmid constructions: All *S. cerevisiae* strains were derived from strain DBY745 (*MATα ade1 leu2-3,112 ura3-52 HMLα HMRa*) that had been modified by mutation or gene replacement also to carry *lys5* and *trp1* (CHATTO *et al.* 1991; WU and HABER 1995; WU *et al.* 1996). The specific modifications of this strain are listed in Table 1 and shown in the figures of this article. Details of their construction are available on request. A deletion of *MAT* was created by a transplacement with a *Hind*III fragment of plasmid pJH526, in which the *MAT* W, X, Y and Z1 regions were replaced with a *URA3* marker. Integration of *MATa* or *MATα* at the *matΔ::URA3* locus or at the *ura3-52* locus was achieved by digestion of pJH67 (*URA3-MATa-pBR322-LEU2*) or pJH270 (*URA3-LEU2-pBR322-MATα*) with *Stu*I, a unique site in *URA3*, and transforming a *matΔ::URA3*, *ura3-52* strain. Integration at *matΔ::URA3* or at *ura3-52* was confirmed by Southern blot analysis. The *HMRα-B* allele was created by site-directed mutagenesis to change 1 bp in *Yα* without changing the amino acid sequence of the α1 gene, creating a *Bam*HI site (WU and HABER 1995). An *HML* deletion was made by replacing the *Xho*I segment of *HMLα* with a cloned *ADE1* gene. An *HMR* deletion extended from a site 57 bp proximal to X border to the site 166 bp distal to the Z1 border, and the deletion was also marked by *ADE1*. Insertion of *HMRa* in the *HML* locus was performed by first replacing the *Xho*I region of a cloned *Bam*HI *HMLα* gene with *URA3* and an adjacent *Nru*I to *Hin*dIII fragment of *HMRa*. This was introduced into chromosome III by transformation of the *Bam*HI fragment and select-

TABLE 1

Strains

Strain	Genotype[a]
CWU3	*ho HMLα matΔ::(URA3,LEU2,MATa,URA3) HMRa pJH283 (GAL::HO, TRP1)*
CWU4	*ho HMLα matΔ::URA3 HMRa ura3-52::(MATa,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU5	*ho HMLα matΔ::(URA3,LEU2,MATα,URA3) HMRa pJH283 (GAL::HO, TRP1)*
CWU6	*ho HMLα matΔ::URA3 HMRa ura3-52::(MATα,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU8	*ho HMLα matΔ::URA3 HMRα-BamHI ura3-52::(MATa,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU12	*ho hmlΔ::(HMRa,URA3) matΔ::URA3 HMRa ura3-52::(MATα,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU15	*ho hmlΔ::(HMRa-BgℓII(X),URA3) matΔ::URA3 HMRa ura3-52::(MATα,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU20[b]	*G1-GAL::HO HMLα matΔ::(URA3,LEU2,MATa,URA3) HMRa*
CWU21[b]	*G1-GAL::HO HMLα matΔ::(URA3,LEU2,MATα,URA3) HMRa*
CWU27[c]	*HMLα ΔREmatΔ::URA3 HMRα-BamHI ura3-52::(MATa,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
CWU65	*ho hmlΔ::HMRa-BgℓII(X) matΔ::(URA3,LEU2,MATα,URA3) HMRa pJH283 (GAL::HO, TRP1)*
CWU66	*ho HMLα matΔ::(URA3,LEU2,MATa,URA3) HMRα-B pJH283 (GAL::HO, TRP1)*
XW600	*hmlΔ::ADE1 matΔ::URA3 HMRα-BamHI ura3-52::(MATa,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
XW601	*hmlΔ::ADE1 matΔ::URA3 HMRa ura3-52::(MATα,LEU2,URA3) pJH283 (GAL::HO, TRP1)*
XW614	*hmlΔ::ADE1 matΔ::(URA3,LEU2,MATα,URA3) HMRa pJH283 (GAL::HO, TRP1)*
XW667	*hmlΔ::(HMRa,URA3) matΔ::(URA3,LEU2,MATα,URA3) hmrΔ::ADE1 pJH283 (GAL::HO, TRP1)*
XW668	*ho hmlΔ::ADE1 matΔ::(URA3,LEU2,MATa,URA3) HMRα-BamHI pJH283 (GAL::HO, TRP1)*
XW669	*HMLα matΔ::(URA3,LEU2,MATa,URA3) hmrΔ::ADE1 pJH283 (GAL::HO, TRP1)*

[a] All strains are isogenic derivatives of strain DBY745 of genotype: *HMLα MATα HMRa ura3-52 leu2-3,112 ade1 ho* but also carrying *trp1* created by gene replacement.
[b] G1-GAL::HO is a galactose-inducible HO gene expressed only in the G1 phase of the cell cycle (MOORE and HABER 1996)
[c] ΔRE is a 1.7-kb deletion of the recombination enhancer for *MAT* donor preference (nt 29,139–30,877) marked by *ADE1* (WU and HABER 1996).

ing Ura+ transformants. The *hmlΔ::HMRa-BgℓII(X)* allele was created by end-filling of the *BgℓII* site of *HMRa* and subsequent religation to destroy the *BgℓII* site. All gene replacements and modifications were confirmed by Southern blot and PCR analysis.

Analysis of HO-induced recombination: A galactose-inducible *HO* gene was carried on plasmid pFH800 (NICKOLOFF *et al.* 1989), marked by *TRP1*. All media specified below are described by SHERMAN *et al.* (1986). Induction of *HO* gene was performed as follows. Yeast cells were cultured overnight in Trp dropout medium with glucose and then diluted in YEP-lactate medium so that the cell concentration would reach 1–5 × 10⁶/ml the next day. Twenty-four hours after changing to YEP-lactate medium, galactose (final concentration 2%) was added for 1.5 hr after which cells were diluted and spread on YEPD plates. Subsequently cells were replica-plated to synthetic complete plates lacking either uracil, leucine or tryptophan. Only cells retaining the *TRP1*-marked pFH800 plasmid were subsequently analyzed. Mating tests were carried out by cross-streaking patches with *his3* or *ade5* cells of each mating type and scoring for the formation of wild-type diploids.

The switching of the *MAT* locus flanked by *URA3* genes was monitored both genetically and by Southern blots. A conversion of the *MAT* locus from either *HML* or *HMR* resulted in cells that retained the duplication and the intervening *LEU2* gene (Figure 1A). Failure to complete gene conversion led to single-strand annealing and the loss of both the *MAT* and *LEU2* genes (Figure 1B). When more than one donor could be used to switch cells to the opposite mating type, their proportional use could be ascertained by identifying restriction site polymorphisms in the Ya or Yα regions of the two donors that were introduced at *MAT*. For *MATα* cells, *HMRa-BgℓII(X)* was inserted in place of *HML* and could be distinguished from the *BgℓII*-containing sequences donated by *HMRa*. *MATa* cells could switch either from *HMLα* or from *HMRα-B*, containing a *BamHI* site (WU *et al.* 1996). DNA was digested with *Hind*III plus either *BgℓII* or *BamHI*, and the

DNA was probed with a 700-bp segment distal to *MAT* (WHITE and HABER 1990).

Statistical analysis: A contingency G-test (SOKOL and ROHLF 1969) was used in a version written in Hypercard for the Macintosh computer by E. LOUIS (personal communication).

RESULTS

Intrachromosomal donor selection is similar in *MATa* and *MATα* cells: To construct strains to analyze the competition between *MAT* switching and a competing SSA process, the normal *MAT* locus was first deleted and replaced with a *URA3* gene. A pBR322 plasmid carrying *URA3, LEU2* and either *MATa* or *MATα* was inserted by targeted integration into the *URA3* locus to produce a *URA3-LEU2*-pBR322 *MAT-URA3* duplication (Figure 1A). HO endonuclease was induced for 1.5 hr from a galactose-inducible *GAL::HO* gene carried on the centromeric *TRP1* plasmid pFH800, and then cells were plated on YEPD to allow single cells to grow into colonies in the absence of further galactose induction. There was no detectable loss of viability after HO induction. As shown in Figure 2, A and B, and in Table 2, ~65% of the HO-induced events led to a successful switch of mating type (retaining *LEU2*), while ~35% yielded the Leu⁻ Mat⁻ phenotype expected after SSA. The results for *MATa* (66%) and *MATα* (65%) were nearly identical. Thus, although *MAT* switching is essentially 100% efficient when there are no nearby flanking homologous sequences to accomplish SSA, this assay
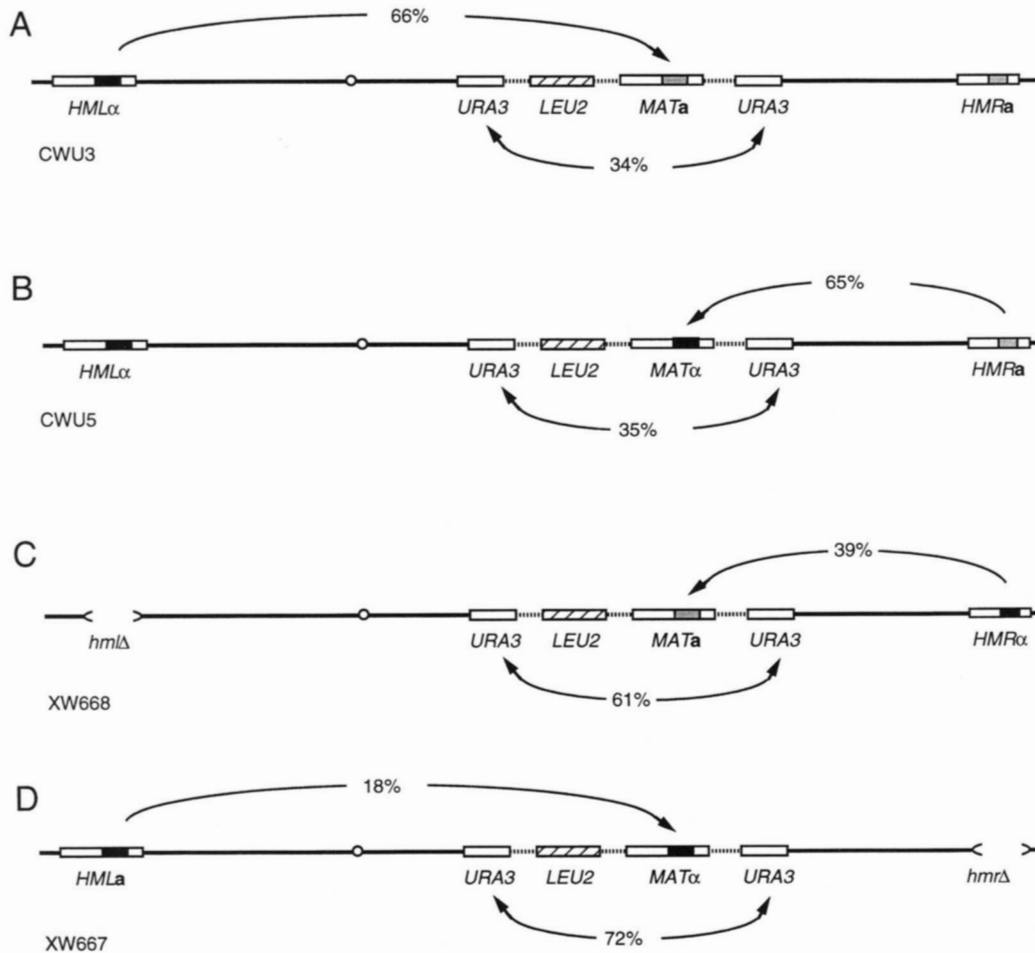
FIGURE 2.—Competition between gene conversion and SSA for intrachromosomal recombination. The percent of HO-induced events involving gene conversion using the preferred donors for *MAT*a (A) and *MAT*α (B) are shown (→) above the illustrated chromosome III. Repair by SSA is shown below the chromosome. Similar measurements are shown for *MAT*a (C) and *MAT*α (D) using the wrong donors, *HMR*α or *HML*a. Data are taken from Table 2.

reveals that *MAT* gene conversion is in active competition with SSA.

The introduction of the competing recombination system does not alter donor preference. This is illustrated by using strain CWU66, which is identical to CWU3 except that it carries two α-containing donors, *HML*α and *HMR*α-B, which are distinguishable by molecular analysis because of the presence of a *Bam*HI site in *HMR*α-B (WU and HABER 1995). As with strain CWU3, 70% (55/79) of the HO-induced recombination events were gene conversions, switching *MAT*a either to *MAT*α or *MAT*α-B. This proportion is very similar to that found with CWU3 (66%), where the only donor of Yα sequences was *HML*α. Twenty α-mating colonies were subjected to Southern blot analysis to determine if they had used *HML*α or *HMR*α-B as the donor. Donor preference was similar to what has been previously observed when there was no competing SSA event, as all 20 (100%) used *HML* as the donor. A similar experiment was carried out with strain CWU65, identical to CWU5 except that it carried *hml*Δ*::HMR*a-*Bgl*II(X) instead of *HML*α. After HO induction, 69% switched mating type (47/68) while the rest experienced deletions between the flanking *URA3* genes. Among 20 individual a-mating derivatives, 19 were *MAT*a and only one was *MAT*a-*Bgl*II(X). Thus donor

preference was not altered by surrounding the *MAT* locus with *URA3*, *LEU2* and pBR322 sequences. We note also that *MAT* conversion and SSA accounted for all the observed events. When homologous recombination is prevented, HO-induced DSBs can be inefficiently repaired by several nonhomologous end-joining repair pathways, at frequencies of ~1 in 10^3 cells (MOORE and HABER 1996). No such events were observed when *MAT* conversion and SSA could both occur.

Locating the "wrong" donor in *MAT*a and *MAT*α cells: Does the proportion of switching and SSA change when cells are confronted only with the wrong donor? In the case of *MAT*a, strain XW668 was constructed (Figure 2C) in which *HML* was deleted and *HMR*α-B was inserted in place of *HMR*a. When HO was induced, the proportion of successful switches fell from 66 to 39%. Thus, although the wrong donor can be used efficiently enough so that *MAT*a cells do not die when only *HMR* is available, its effectiveness in switching is notably lower than for *HML*. The competition assay supports the idea that it is inherently more difficult for *MAT*a to use an unactivated donor than an activated one.

A *MAT*α strain deleted for the normal *HMR* locus but carrying *HMR*a inserted in the place of *HML* (*hml*Δ*::HMR*a), strain XW667, was similarly constructed

TABLE 2

Competition between *MAT* switching and deletions

Strain	*MAT*[a]	Donors[b]		*MAT* switching	SSA
CWU3	*MAT*a (III)	**HML**α	*HMR*a	71/108 (0.66)	37/108 (0.34)
CWU5	*MAT*α (III)	*HML*α	**HMR**a	80/123 (0.65)	43/123 (0.35)
CWU66	*MAT*a (III)	**HML**α	*HMR*α-B	55/79 (0.70)	24/79 (0.30)
CWU65	*MAT*α (III)	*hml*Δ::**HMRa-*Bgl*II(X)**	*HMR*a	47/68 (0.69)	21/68 (0.31)
XW668	*MAT*a (III)	*hml*Δ	*HMR*α-B	32/82 (0.39)	50/82 (0.61)
XW614	*MAT*α (III)	*hml*Δ	**HMR**a	58/97 (0.60)	39/97 (0.40)
XW669	*MAT*a (III)	**HML**α	*hmr*Δ	78/105 (0.74)	27/105 (0.26)
XW667	*MAT*α (III)	*hml*Δ::**HMRa**	*hmr*Δ	19/106 (0.18)	87/106 (0.92)
XW601	*MAT*a (V)	*hml*Δ	*HMR*α-B	6/91 (0.07)	85/91 (0.93)
XW600	*MAT*α (V)	*hml*Δ	**HMR**a	20/243 (0.08)	223/243 (0.92)
CWU4	*MAT*a (V)	**HML**α	*HMR*a	39/122 (0.32)	83/122 (0.68)
CWU6	*MAT*α (V)	*HML*α	**HMR**a	6/133 (0.05)	127/133 (0.95)
CWU8	*MAT*a (V)	**HML**α	*HMR*α-B	41/115 (0.36)	74/115 (0.64)
CWU12	*MAT*α (V)	*hml*Δ::**HMRa**	*HMR*a	61/263 (0.23)	202/263 (0.77)
CWU15	*MAT*α (V)	*hml*Δ::**HMRa-*Bgl*II(X)**	*HMR*a	17/96 (0.18)	79/96 (0.82)
CWU27	*MAT*a (V)	**HML**α	*HMR*α-B	12/124 (0.10)	112/124 (0.90)
		RE deleted			
CWU20	*MAT*a (III) G1-GAL::HO	**HML**α	*HMR*a	122/136 (0.90)	14/136 (0.10)
CWU21	*MAT*α (III) G1-GAL::HO	*HML*α	**HMR**a	74/82 (0.90)	8/82 (0.10)

SSA, Single-strand annealing.

[a] The *MAT* (III) locus is located within a duplication of *URA3* genes at the site of the normal *MAT* locus on chromosome III, as illustrated in Figure 1. The *MAT* (V) locus is inserted between *ura3-52* and *URA3*, as shown in Figure 3. In all cases except CWU20 and CWU21, a galactose-inducible HO gene, expressed at all stages of the cell cycle, was induced for 1.5 hr, and then cells were plated and grown into colonies. Identification of colonies that had switched mating type and those that had undergone a deletion event (becoming Leu2⁻) was determined. CWU20 and CWU21 carry a galactose-inducible HO gene expressed only in the G1 stage of the cell cycle. These strains were treated in a similar fashion. Cells that neither switched nor underwent a deletion are not shown.

[b] The donors that could be used to switch *MAT* to the opposite mating type are shown in boldface for each experiment. Note that one of the two donors may be used preferentially. RE is the *cis*-acting recombination enhancer on chromosome III (WU and HABER 1996).

(Figure 2D). It has been previously shown that replacing *HML* with *HMR* sequences does not perturb donor preference rules (WEILER and BROACH 1992; WU and HABER 1995; WU *et al.* 1996). This strain switched only 18% of the time compared with 65% when the donor was at *HMR*. The diminished ability of *MAT*α, compared with *MAT*a, to use the wrong donor (0.18 *vs.* 0.39) is apparently sufficient to account for the lethality seen in *MAT*α *HML*α *hmr*Δ cells, where one-third of the cells die when they attempt to use the unfavored donor. There is no such lethality in a *MAT*a *hml*Δ *HMR*α strain.

The use of HMR is different in *MAT*a and *MAT*α strains: Most of the regulation of donor preference appears to be exerted by regulating the accessibility of *HML*, being unusually "hot" in *MAT*a strains and unusually "cold" in *MAT*α. Nevertheless, there may be

some enhancement of *HMR* in *MAT*α strains. This was suggested by the 2.5-fold higher rate of spontaneous recombination in *MAT*α *vs.* *MAT*a when a *leu2-R* allele was inserted in place of *HMR* and a *leu2-K* allele was inserted near *MAT*, though this is much less than the 30-fold difference when a similar measurement was conducted with *leu2-R* in place of *HML* (WU and HABER 1995). This conclusion is supported by data presented here, comparing the use of *HMR*a to switch *MAT*α in strain XW614 (60%) *vs.* the selection of *HMR*α to switch *MAT*a in strain XW668 (39%).

Efficiency of donors in interchromosomal switching: Mating type switching can occur interchromosomally. To learn whether *HML* and *HMR* are equally efficient in interchromosomal interactions, we constructed a set of strains in which the *MAT* locus on chromosome III was deleted and a pBR322-*URA3-LEU2* plasmid car-
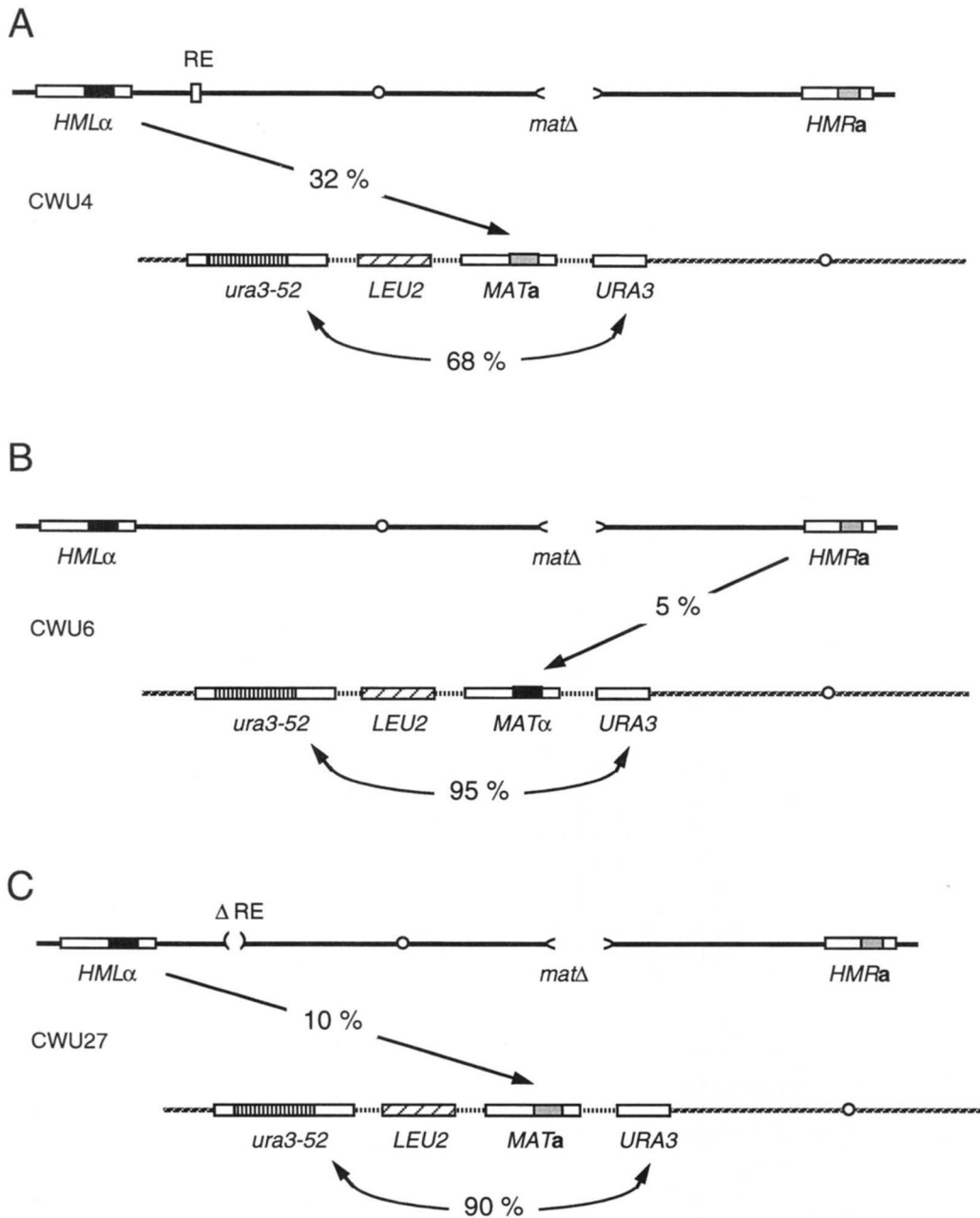
FIGURE 3.—Competition between gene conversion and SSA for interchromosomal recombination, in which *URA3-LEU2-MAT*-pBR322-*URA3* is present on chromosome V and gene conversion donors are present on chromosome III (see MATERIALS AND METHODS). The percentages of HO-induced events involving gene conversion using the preferred donors for *MAT*a (A) and *MAT*α (B) are shown (→) between the two illustrated chromosomes. Repair by SSA is shown below chromosome V. The effect of the recombination enhancer (RE) on interchromosomal *MAT*a switching is shown in (C). Data are taken from Table 2.

rying either *MAT*a or *MAT*α was integrated into the *ura3-52* locus on chromosome V (Figure 3). When HO was induced in strain CWU4, the *ura3-52—LEU2—MAT*a—*URA3* duplication was deleted 68% of the time, while 32% successfully converted *MAT*a to *MAT*α.

A related strain (CWU8), but containing *HML*α and *HMR*α-B on chromosome III, was constructed. The efficiency of successful switching was approximately the same (36%) as when only *HML*α could donate. It was also possible to ask if the preference for *HML*α over *HMR*α-B that is seen when *MAT*a is on chromosome III is maintained when *MAT*a is on chromosome V. When *MAT*a switches to *MAT*α-B, there is a diagnostic *Bam*HI site present that is lacking when *MAT* uses *HML*α as the donor. We analyzed 30 independent

switching events by Southern blot analysis and found that 29 of them had used *HML*α; thus preference is maintained when *MAT* is on a different chromosome from its donors.

Interchromosomal *MAT*α donor preference was analyzed in a similar fashion, using strain CWU6 in which the *MAT* locus was deleted and in which *MAT*α was introduced on chromosome V by the integration of a *URA3-LEU2-MAT*-pBR322 plasmid at the *ura3-52* locus (Figure 3B). Unexpectedly, 95% of the HO-induced events led to deletions. This is statistically significantly different from the results obtained with *MAT*a ($P < 0.01$). Thus although *MAT*α uses *HMR* as efficiently as *MAT*a selects *HML*, when the recombining partners are on the same chromosome, the use of the two donors is

very different during interchromosomal interactions. *MATα* cannot locate *HMR* efficiently when the donor is on a different chromosome.

We also examined the case where *MATα* on chromosome V could use either *HMR*a or a copy of *HMR*a integrated in place of *HML* (CWU12). Here *MATα* switched to *MAT*a 23% of the time, suggesting that the wrong donor, *hmlΔ::HMR*a, was at least as efficient in *trans* as the normally preferred donor, *HMR*a. To verify that the donor at the left end of chromosome III was being used as often as the normally preferred *HMR*a, we created a *Bgl*II(X) mutation in *hmlΔ::HMR*a, so that we could distinguish whether *MATα* on chromosome V switched from *HMR*a or from *hmlΔ::HMR*a-*Bgl*II(X) (strain CWU15). Here, ~18% of the HO-induced cells switched mating type. Among 36 switches analyzed by Southern blot, 18 used *HMR*a and 18 used *hmlΔ::HMR*a-*Bgl*II(X). Thus, donor preference is lost when *MATα* is located on a different chromosome.

Finally we compared the use of *HMR* in interchromosomal switching when *HML* was deleted and where *MATα* (strain XW600) or *MAT*a (XW601) was inserted on chromosome V. The use of *HMR*a to switch *MATα* (7%) was indistinguishable from the ability of *HMR*α-B to convert *MAT*a (8%). Thus, the constraint on interchromosomal switching using *HMR* is not mating-type regulated.

One possibility that we have not excluded is that the interchromosomal events involving *HMR* are frequently accompanied by crossing-over, which would produce a lethal combination of a dicentric and an acentric reciprocal recombinant chromosomes, while events involving *HML* would simply produce translocations. We think this is unlikely. It has been shown that intrachromosomal switches are rarely accompanied by crossing-over (HABER *et al.* 1980; KLAR and STRATHERN 1984). It is possible, though, that interchromosomal recombination could be less constrained. Thus, we analyzed switches between *HMLα* (III) and *MAT*a (V) in strain CWU4, where a crossing-over would be viable, but lead to a reciprocal translocation. Such translocations can readily be detected by Southern blot analysis. Using this test, we showed that none of 20 *MAT*a (V) conversions to *MATα* were accompanied by crossing-over. Thus, it seems unlikely that the low success of *HMR* in recombining with *MATα* on chromosome V can be attributed to frequent (lethal) crossovers accompanying switching.

Deletion of the *MAT*a recombination enhancer reduces the use of *HML* in interchromosomal recombination: Recently we demonstrated that *MAT*a donor preference depends on a 700-bp *cis*-acting, orientation-independent recombination enhancer (RE) on chromosome III that affects recombination along the entire left arm of that chromosome. Deletion of this site causes a *MAT*a cell to recombine with *HMR* 85–90% of the time instead of 10–15% when the enhancer is present. We wished to know if the deletion would also interfere

with recombination in *trans* in the same fashion. Accordingly, we deleted RE as part of a 1.7-kb deletion replaced by the *ADE1* locus (WU and HABER 1996) in strain CWU27. As shown in Figure 3C, the absence of this sequence dropped the efficiency of *HMLα* to gene convert *MAT*a on chromosome V from 32 to 10%. Thus, interchromosomal switching is strongly influenced by the RE, as is intrachromosomal recombination (WU and HABER 1996).

Cell cycle dependency on the competition between *MAT* switching and SSA: The competition between *MAT* gene conversion and SSA is somewhat different in cells where HO cleavage is confined to the G1 stage of the cell cycle. This was shown by constructing strains in which the HO gene is galactose-inducible but only in the G1 phase of the cell cycle (NASMYTH *et al.* 1987; MOORE and HABER 1996). The proportion of *MAT*a cells that switched to *MATα* was ~90% (strain CWU20, Table 2), and the proportion of *MATα* cells switching to *MAT*a was 90% (strain CWU21, Table 2). The difference between these results and those obtained for cells induced at all cell cycle stages could have several explanations, as discussed below.

DISCUSSION

The use of a competing recombination process has allowed us to assess in a new way the efficiency of *MAT* gene conversion with different donors in various locations. In theory it should be possible to show that a good donor recombines more rapidly with *MAT* than a poor one, but in practice it has been difficult to make the process sufficiently synchronous to see such differences, even though it is been possible to show that various mutants slow down the appearance of gene conversion by 30–60 min (IVANOV *et al.* 1994). Instead, we use the competition between gene conversion and SSA to estimate the kinetics of gene conversions. We assume that the default SSA process, involving a given pair of flanking homologous sequences at a given distance, proceeds with the same kinetics whether or not a competing donor for gene conversion is efficient or inefficient (or even present). We have recently shown that the completion of SSA for two constructs at different chromosomal locations, each similar in size to that used here, takes ~1 hr (HABER and LEUNG 1996). However, it should be noted that cells may become committed to the completion of gene conversion (by strand invasion and the initiation of new DNA synthesis) or SSA (by complementary base pairing) more rapidly than the time of physical appearance of the final product (WHITE and HABER 1990; FISHMAN-LOBELL *et al.* 1992).

One striking result of this approach is that *MAT* switching is not as efficient, relative to SSA as one might have anticipated. In logarithmically growing cells, *MAT* switching accounted for only 65–70% of the outcomes. This suggests that frequently the 5′ to 3′ resection of

the ends of the DSB [which we have estimated to proceed 1–2 nt/sec (FISHMAN-LOBELL et al. 1992)] continues far outside of the regions of homology shared by MAT and its donors until the flanking URA3 regions can interact. The 65% success of MAT gene conversions is somewhat better than the 33% gene conversion vs. SSA that we observed between direct LacZ sequences on a plasmid, when the distance between the direct repeat was about the same as the URA3—MAT—URA3 sequences used in this study (FISHMAN-LOBELL et al. 1992). Based on earlier results from this laboratory, we were initially surprised that deletion formation was so successful, as we had found that 5' to 3' exonuclease digestion was extensive distal to the cut, but very limited on the proximal side, though there was degradation in both directions when donors were deleted (WHITE and HABER 1990). More recently, however, we have found that the degradation of regions proximal to MAT appears to occur similarly to that which occurs at distal regions, even when donors are present (N. SUGAWARA, A. HOLMES and J. E. HABER, unpublished results). We have not resolved this difference. Possibly the degradation of the Y region involves excising both strands so that it appears there is no 5' to 3' degradation, while resection of sequences more centromere proximal to the DSB may then involve only 5' to 3' degradation. The fact that we observe a significant fraction of deletions argues that such degradation occurs. Moreover, although we and others have presented several lines of evidence that support the idea that deletion formation between homologous regions flanking the DSB normally occurs predominantly by SSA (FISHMAN-LOBELL et al. 1992; SUGAWARA and HABER 1992; LIEFSCHITZ et al. 1995; HABER and LEUNG 1996), it is possible that other types of recombination, initiated from single-stranded regions distal to the cut, are responsible for the deletions we see (PRADO and AGUILERA 1995). In any case, it is clear that deletions, involving regions of DNA initially several kilobases from the regions of homology shared by MAT and its donors, occur 35% of the time in competition with gene conversion.

Efficiency of donors during intrachromosomal MAT switching: Our results substantiate and extend our previous conclusions that MATa cells activate HML for recombination when the RE sequence is present, but in the absence of the RE, MATa cells behave like MATα cells and discourage the use of HML (WU and HABER 1995, 1996; WU et al. 1996). These experiments also enrich our understanding of the ways that MATa and MATα cells use the wrong donor. In our previous studies, we showed that MATa cells could use HMR well enough to ensure that essentially all DSBs were repaired; however, the assay we have applied here shows that the use of HMR is only half as efficient as HML. This difference is less than the four- to fivefold preference of HML over HMR and suggests that there are additional factors that influence the search for homol-

ogy. The competition assay also supports our previous conclusion, based on the frequent inviability of MATα cells using only HML, that HML is somehow excluded in MATα cells (or in MATa cells in which the RE is deleted) (WU and HABER 1996).

We also have found evidence of a minor, but statistically significant ($P < 0.01$), activation of HMR in MATα cells in strains XW614 (60%) and XW668 (39%). This conclusion is supported by previous studies using leu2 recombination when one allele is located in place of HMR, where MATα cells recombined about twice as often as MATa (WU and HABER 1995). The basis for this small difference in HMR usage is not known.

There also appears to be some cell cycle effect on donor preference. The higher proportion of gene conversions when HO is induced only in the G1 stage of the cell cycle could be explained in several ways. There may be cell cycle-dependent differences in the rate of 5' to 3' resection to produce the long single strands needed for SSA. Alternatively, there may be cell cycle regulation of the expression of gene products required for gene conversion that are not needed for SSA. We have previously shown that the genetic requirements of these two processes are not identical (IVANOV et al. 1996), and we have also previously shown that other DNA repair processes are cell cycle dependent (MOORE and HABER 1996). Finally, there may be additional gene products that act in G1 (which is the only time when the normal HO gene is expressed) to facilitate the interaction of donors with MAT.

Constraints on interchromosomal gene conversion: In this article, we show that there is a fundamental difference in the two donor interactions for interchromosomal gene conversion. HML can work efficiently in trans, only about twofold less efficiently than when it acts in cis under similar competitive circumstances. In contrast, although HMR converts MATα about as efficiently as HML recombines with MATa intrachromosomally, HMR is very inefficient when recombining interchromosomally. The interchromosomal constraint on the use of HMR is also seen when chromosome V carries MATa.

The basis of the constraint on interchromosomal interactions of HMR is not known. It is possible that neither the behavior of HML in MATa cells (with or without the RE) nor the behavior of HMR in MATα cells is representative of general recombination between ectopic sites. Previous studies of heteroallelic leu2 recombination in mitotic cells suggested that interchromosomal sites were ~5–10-fold less efficiently used than intrachromosomal sites (LICHTEN and HABER 1989). It will be necessary to place donors in several locations on different chromosomes to learn if both of the normal mating-type donors have evolved special features of accessibility.

Other applications of the competition assay: The strategy of using a competing recombination event to

measure the efficiency of gene conversion was necessary to make an accurate assessment of recombination efficiency. In the absence of a competitive event, there is nothing to prevent cells from simply delaying their progression through the cell cycle until the DSB has been repaired. This may account for the distinct difference in our results from those previously presented by WEILER and BROACH (1992), who concluded that *MAT* donor preference was maintained when donors on chromosome III were asked to switch a *MAT* locus on chromosome V, but where there was no possibility of single-strand annealing.

We believe that using a second, competing process is a strategy that has other important applications in the study of recombination. For example, this approach provides a good way to assess the effect of DNA sequence divergence on the efficiency of DSB-induced recombination. Previously, we used a related strategy to evaluate the relative efficiency of SSA of small flanking regions of homology, by providing a larger, more distant sequence that would surely succeed in recombining if the first sequence were too small (SUGAWARA and HABER 1992). We have recently extended that work to show that a divergence of 3% in a 205-bp region is sufficient to cause a >20-fold reduction in its ability to recombine successfully, in competition with a large, more distal region. However, when the additional homologous locus was removed, so that cells either recombined using the 205-bp region or else died, the use of this small diverged region was only threefold less than the fully homologous sequence, presumably because the cell made a number of attempts to recombine while delaying progression of the cell through the cell cycle (SUGAWARA *et al.* 1997). A similar strategy could be used to examine, in a systematic way, the accessibility of regions along a chromosome.

LITERATURE CITED

CHATTO, B. B., F. SHERMAN, D. A. AZUBALIS, T. A. FJELLSTEDT, D. MEHNERT *et al.*, 1991 Selection of *lys2* mutants of the yeast *Saccharomyces cerevisiae* by the utilization of α-aminoadipate. Genetics **93:** 51–65.

FISHMAN-LOBELL, J., N. RUDIN and J. E. HABER, 1992 Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. Mol. Cell. Biol. **12:** 1292–1303.

GERRING, S. L., F. SPENCER and P. HIETER, 1990 The *CHL1* (*CTF1*) gene product of *Saccharomyces cerevisiae* is important for chromosome transmission and normal cell cycle progression in G2/M. EMBO J. **9:** 4347–4358.

HABER, J. E., and W.-Y. LEUNG, 1996 Lack of chromosome territoriality in yeast: promiscuous rejoining of broken chromosome ends. Proc. Natl. Acad. Sci. USA **93:** 13949–13954.

HABER, J. E., D. T. ROGERS and J. H. MCCUSKER, 1980 Homothallic conversions of yeast mating-type genes occur by intrachromosomal recombination. Cell **22:** 277–289.

IVANOV, E. L., N. SUGAWARA, C. I. WHITE, F. FABRE and J. E. HABER, 1994 Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 3414–3425.

IVANOV, E. L., N. SUGAWARA, J. FISHMAN-LOBELL and J. E. HABER, 1996 Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics **142:** 693–704.

KLAR, A. J., and J. N. STRATHERN, 1984 Resolution of recombination intermediates generated during yeast mating type switching. Nature **310:** 744–748.

KLAR, A. J., J. B. HICKS and J. N. STRATHERN, 1982 Directionality of yeast mating-type interconversion. Cell **28:** 551–561.

LICHTEN, M., and J. E. HABER, 1989 Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. Genetics **115:** 261–268.

LIEFSHITZ, B., A. PARKETT, R. MAYA and M. KUPIEC, 1995 The role of DNA repair genes in recombination between repeated sequences in yeast. Genetics **140:** 1199–1211.

LIRAS, P., J. MCCUSKER, S. MASCIOLI and J. E. HABER, 1978 Characterization of a mutation in yeast causing nonrandom chromosome loss during mitosis. Genetics **88:** 651–671.

MOORE, J. K., and J. E. HABER, 1996 Genetic and cell cycle control of two different pathways of nonhomologous end-joining repair of a double-strand break in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 2164–2173.

NASMYTH, K., B. STILLMAN and D. KIPLING, 1987 Both positive and negative regulators of HO transcription are required for mother cell specific mating type switching in yeast. Cell **48:** 579–587.

NICKOLOFF, J. A., J. D. SINGER, M. F. HOEKSTRA and F. HEFFRON, 1989 Double-strand breaks stimulate alternative mechanisms of recombination repair. J. Mol. Biol. **207:** 527–541.

PRADO, F., and A. AGUILERA, 1995 Role of reciprocal exchange, one-ended invasion crossover and single- strand annealing on inverted and direct repeat recombination in yeast: different requirements for the *RAD1*, *RAD10*, and *RAD52* genes. Genetics **139:** 109–123.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SOKOL, R. R., and F. J. ROHLF, 1969 *Biometrics.* Freeman, San Francisco.

STRATHERN, J. N., and I. HERSKOWITZ, 1979 Asymmetry and directionality in production of new cell types during clonal growth: the switching pattern of homothallic yeast. Cell **17:** 371–381.

SUGAWARA, N., and J. E. HABER, 1992 Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol Cell Biol **12:** 563–575.

SUGAWARA, N., F. PÂQUES, M. CÓLAIÁCOVO and J. E. HABER, 1997 Role of *Saccharomyces cerevisiae* mismatch repair genes Msh2 and Msh3 in double-strand break-induced recombination. Proc. Natl. Acad. Sci. USA (in press).

WEILER, K. S., and J. R. BROACH, 1992 Donor locus selection during *Saccharomyces cerevisiae* mating type interconversion responds to distant regulatory signals. Genetics **132:** 929–942.

WEILER, K. S., L. SZETO and J. R. BROACH, 1995 Mutations affecting donor preference during mating type interconversion in *Saccharomyces cerevisiae*. Genetics **139:** 1495–1510.

WHITE, C. I., and J. E. HABER, 1990 Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. **9:** 663–674.

WU, X., and J. E. HABER, 1995 *MAT*a donor preference in yeast mating-type switching: activation of a large chromosomal region for recombination. Genes Dev. **9:** 1922–1932.

WU, X., and J. E. HABER, 1996 A 700 bp *cis*-acting region controls mating-type dependent recombination along the entire left arm of yeast chromosome III. Cell **87:** 277–285.

WU, X., J. K. MOORE and J. E. HABER, 1996 Mechanism of *MAT*α donor preference during mating-type switching of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 657–668.