The *Saccharomyces cerevisiae* Recombination Enhancer Biases Recombination During Interchromosomal Mating-Type Switching but Not in Interchromosomal Homologous Recombination

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ABSTRACT

Haploid Saccharomyces can change mating type through *HO*-endonuclease cleavage of an expressor locus, *MAT*, followed by gene conversion using one of two repository loci, *HML* or *HMR*, as donor. The mating type of a cell dictates which repository locus is used as donor, with **a** cells using *HML* and α cells using *HMR*. This preference is established in part by *RE*, a locus on the left arm of chromosome III that activates the surrounding region, including *HML*, for recombination in **a** cells, an activity suppressed by α2 protein in α cells. We have examined the ability of *RE* to stimulate different forms of interchromosomal recombination. We found that *RE* exerted an effect on interchromosomal mating-type switching and on intrachromosomal homologous recombination but not on interchromosomal homologous recombination. Also, even in the absence of *RE*, *MAT*α still influenced donor preference in interchromosomal mating-type switching, supporting a role of α2 in donor preference independent of *RE*. These results suggest a model in which RE affects competition between productive and nonproductive recombination outcomes. In interchromosome gene conversion, *RE* enhances both productive and nonproductive pathways, whereas in intrachromosomal gene conversion and mating-type switching, *RE* enhances only the productive pathway.

HAPLOID Saccharomyces cells have the remarkable potential to change mating type as often as every generation (reviewed in Haber 1998; Bi and Broach 1999). The mating type of a haploid cell is dictated by the particular allele, **a** or α, present at the mating-type locus, *MAT*, located near the center of chromosome III. Mating-type switching is initiated by a double-strand break at the *MAT* locus, catalyzed by an endonuclease encoded by *HO* (Strathern *et al.* 1982). Switching then occurs by a gene conversion event that replaces the mating information at the *MAT* locus with the opposite mating information present at either of two repository mating loci, *HML* and *HMR*, located at the opposite ends of chromosome III (180 and 90 kb, respectively, away from *MAT*). This results in replacement of one mating-type allele at *MAT* with a copy of the opposite mating-type allele taken from either *HML* or *HMR*.

Mating-type switching follows a precise developmental pattern (Strathern and Herskowitz 1979). This precise choreography results from the intricate pattern of transcriptional regulation of the *HO* gene and from a highly regulated interaction between distant regions of chromosome III. Namely, mothers, but not daughters, transcribe the *HO* gene and do so only during the G_1 phase of the cell cycle. Accordingly, only mothers are capable of switching cell type and the switch occurs prior to DNA replication in the mother (Nasmyth 1993; Long *et al.* 1997; Nasmyth and Jansen 1997). In addition, cell type dictates which donor locus is selected for participation in the gene conversion event. **a** cells predominantly select *HML*, which normally contains α mating information, whereas α cells select *HMR*, which normally contains **a** mating information (Figure 1, bottom). This pattern ensures that most of the switching events result in a change of mating type, rather than a futile replacement of the *MAT* allele with the same sequence (Klar *et al.* 1982).

The fact that cells can select between *HML* and *HMR* loci implies that the two loci possess distinguishable features that are recognized in a cell type-specific manner. Previous studies have shown that this discrimination does not derive from the different alleles resident at the donor loci, from the unique sequences flanking either locus, from any of the DNA sequences distal to either locus on chromosome III, or from any preorganization of the donor and acceptor loci within the nucleus (Weiler and Broach 1992; Simon *et al.* 2002). Sequences flanking *MAT* also do not function in the process of preferential selection. Rather, the left arm of chromosome III exhibits a cell type-dependent difference in ability to participate in recombination. A large (>40 kb) region of the left arm of chromosome III

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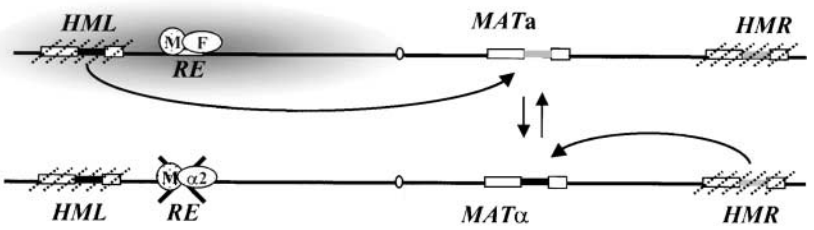

FIGURE 1.—Mating-type switching exhibits cell type-dependent donor preference. A diagram of chromosome III indicating the relative positions of the donor loci, *HML* and *HMR*, the *MAT* locus, and the recombination enhancer, *RE*, is shown. *HML* and *HMR* are transcriptionally silent, indicated by the hatched lines, while *MAT* is transcriptionally active, giving rise to the mating type of the cell. Open rectangles at the three mating loci indicate blocks of homology while the thick line indicates the allele-specific region (α allele is black, **a** allele is gray). In **a** cells (top), Mcm1 (M) and Fkh1 (F), a forkhead transcription factor, occupy *RE* and promote enhanced recombination potential (shaded area) extending over *HML*, rendering it the preferred donor during mating-type switching and resulting in conversion from *MAT***a** to *MAT*α. In α cells (bottom), α2 binds to *RE*, precluding occupation by Fkh1, to suppress enhanced recombination potential, rendering *HMR* the preferred donor through *RE*-independent mechanisms and resulting in conversion from *MAT*α to *MAT***a**.

containing *HML* can undergo intragenic recombination between separated heteroalleles of a test gene at a rate up to 20 times higher in *MAT***a** cells than in *MAT*α cells (Wu and Haber 1995). The *MAT***a** cell-specific enhancement of recombination of the left arm of chromosome III depends on a small (<2 kb) segment ~30 kb from the telomere, 16 kb from *HML* (Wu and Haber 1996). Deletion of this *r*ecombinational *e*nhancer (*RE*) causes *MAT***a** cells to choose *HMR* (the wrong donor) instead of *HML*, without altering the donor preference in *MAT*α cells (*HMR* is preferred as usual; Wu and Haber 1996; Szeto *et al.* 1997). *RE* contains binding sites for the Mcm1 and forkhead (Fkh1 and Fkh2) transcription factors and *FKH1* deletion or certain inactivating mutations of Mcm1 eliminate the ability of *RE* to promote selection of *HML* in *MAT***a** cells (Wu *et al.* 1998; Sun *et al.* 2002).

Of the three transcriptional regulators encoded by the two *MAT* alleles—**a**1, α1, and α2—only α2 plays a role in donor selection during mating-type interconversion. Cells expressing α2 (typically α cells) select *HMR* as donor while cells lacking α2 (typically **a** cells) select *HML* as donor, independent of other mating-type gene products (Szeto and Broach 1997). α2 functions in transcriptional regulation by interacting with Mcm1 and Tup1/Ssn6 to repress **a**-specific genes in α cells. α2 acts similarly to inactivate *RE* by binding to two α2/Mcm1 sites (named *DPS1* and *DPS2*) located within *RE* to initiate organization of an extended region of highly ordered nucleosomes over the locus (Szeto *et al.* 1997; Weiss and Simpson 1997). The interactions of α2 with *DPS1/2*, Mcm1, and Tup1 are all essential for α2's role in preventing selection of *HML* during interconversion, since mutations of α2 that disrupt its interaction with its DNA target, with Mcm1, or with Tup1 abolish normal donor preference in α cells (but not in **a** cells; Szeto and Broach 1997).

These observations provide a model for the molecular basis of donor preference (Figure 1, bottom). In *MAT***a** cells, binding of Mcm1 to *DPS1* and *DPS2* within *RE* activates the intrachromosomal recombination potential of the left arm of chromosome III by facilitating recruitment of, or acting in conjunction with, Fkh1. Accordingly, *HML* becomes the preferred donor. In *MAT*α cells, binding of α2 adjacent to Mcm1 at *DPS1* and *DPS2* establishes a repressive domain over *RE* and thereby prevents activation of the recombination potential of the left arm. As a result, *HMR* becomes the preferred donor, due to an as yet undefined intrinsic bias for *HMR* as donor (Wu *et al.* 1996). This model provides a heuristic framework for cataloging the roles of all the known players in donor selection. However, the fundamental details of how long-range interactions between donor and acceptor loci occur and how the recombination enhancer affects recombination potential over a large domain remain to be explained.

Here we present results from our studies on the nature of *RE* obtained by examining its ability to stimulate different forms of interchromosomal recombination. In one series of experiments, we examined donor preference during mating-type switching in cells in which the *MAT* locus resided on chromosome II while the donor loci remained on chromosome III. In a second series of experiments we examined the effect of *RE* on interchromosomal homologous recombination between heteroallelic pairs located at various positions on chromosome III homologs. We found that *RE* exerted an effect on interchromosomal mating-type switching and on intrachromosomal homologous recombination but did not do so on interchromosomal homologous recombination. We also found that even in the absence of *RE*, *MAT*α still influenced donor preference in interchromosomal mating-type switching, supporting a role of α2 in donor preference independent of its affect on *RE*. We discuss possible mechanisms that would account for the different effects of *RE* on different types of recombination.

MATERIALS AND METHODS

Plasmid construction: The *lys2::MAT***a***-TRP1* plasmid, B2407, and the *lys2::MAT*α*-LEU2* plasmid, B2422, were created by inserting the genomic *Hin*dIII fragments spanning *MAT***a** and *MAT*α into pRS404 and pRS405, respectively. For *MAT***a**, the resulting plasmid was digested with *Bgl*II and resealed by ligation in the presence of complementary oligonucleotides (5′-GATCAAAGTAAT-3′ and 5′-GATCATTACTTT-3′). This removed the *Bgl*II site without affecting the *MAT***a***1* amino acid sequence. For both plasmids the *LYS2* targeting sequence from pFV8 (Volkert and Broach 1986) was then cloned into the *Xho*I site.

The *MAT* deletion plasmid B2430 was constructed by oligonucleotide mutagenesis of the genomic *Hin*dIII fragment spanning *MAT*α cloned into vector pSELECT, creating a precise deletion of W through Z1 marked with a *Bgl*II site. The *URA3* gene was then inserted at the *Bgl*II site.

Switching the *URA3* marker of pKSW72 (Weiler *et al.* 1995) to *HIS3* created the *HO* deletion plasmid B2428. Plasmid B2574 contains a *ste5*Δ construct. *STE5* was amplified by PCR (5′-GAC GAGCTCTGTATTTGTTCAAGACCG-3′ and 5′-GACGCATG CTTGCTACATCAGCAAC-3′) and cloned into pUC19 at the *Sac*I/*Sph*I sites. The *Xba*I/*Spe*I fragment was then replaced with *HIS3*.

Most of the plasmids used for heteroallelic recombination were obtained from Michael Lichten. Each contains one of two *arg4* mutant alleles (*arg4-nsp* or *arg4-bgl*), the *URA3* marker, and a fragment of yeast chromosome III with a unique restriction site for targeting via circular integration (Wu and Lichten 1994, 1995). Plasmids B2572 and B2573 were created by cloning a 2-kb PCR fragment centromere proximal to *HMR* and inserting it into the *Eco*RI site of plasmids pMJ113 and pMJ-115, respectively (Wu and Lichten 1994). Plasmids pMJ173 and pMJ174 were designed for targeting to *CHA1*, pMJ121 and pMJ-123 to *HIS4*, pMJ483 and pMJ486 to *RIM1*, pMJ113 and pMJ115 to *URA3*, and B2572 and B2573 to *HMR*.

Strain construction: All strains are listed in Table 1. Strains for analysis of mating-type switching were derived from strain Y2201 (Szeto and Broach 1997). Strains Y2901 and Y2902 were created by integrating a plasmid containing *HIS3* adjacent to the Z2 region of *MAT* in strain Y2201. The integration creates a small duplication of Z2 to the first *Hin*dIII site of the *TSM1* gene. Strain Y3149 was obtained by transforming strain Y2201 sequentially with *Bgl*II-digested B2407 (*lys2-MAT***a**-*TRP1*) and *Bgl*II-digested B2422 (*lys2-MAT*α-*LEU2*). Strain Y3149 was then transformed with the *Hin*dIII fragment of plasmid B2430 (which deletes one of the endogenous *MAT* loci from W-Z1) to yield strain Y2934. Strains Y2946 and Y2947 were constructed from strains Y2901 and Y2902, respectively, by a two-step gene replacement using plasmid pLS70 (YIp*URA3-dps1*Δ *dps2*Δ), scoring for the introduction of the *dps1*Δ *dps2*Δ alleles by Southern analysis (Szeto *et al.* 1997). The *re*Δ construct [a *URA3* replacement of *RE* from *Blp*I (chromosome III position 28,961) to *Pme*I (31,213)] was then integrated on the homologous chromosome. Strain Y2948 was created from strain Y2934 in a similar manner, except for using a *HIS3* allele in the *re*Δ construct (B2436). All integrations were confirmed by Southern analysis.

All strains for analysis of heteroallelic recombination were derived from strains Y2811 and Y2812, isogenic derivatives of S288C kindly provided by Mark Rose. *ARG4* was precisely deleted using a targeted deletion cassette marked with *HIS3* (Baudin *et al.* 1993). Integration of the *arg4* recombination cassette at each locus was confirmed by Southern blotting and by segregation analysis. Cassettes were constructed so that all *arg4* alleles were in the same orientation relative to chromosome III. The heteroallelic diploids of different mating types were constructed by mating the *MAT***a** and *MAT*α versions of complementary heteroalleles at the same locus and then deleting *MAT* on either homolog, using plasmid B2438 (*mat*Δ::*LEU2*). *RE* was deleted as above as needed except that the *re*Δ was marked with *TRP1* using plasmid B2434.

Switching assays: Switching assays were performed as described (Weiler *et al.* 1995) with minor modifications. Briefly, diploid strains containing the *mat***a***1*Δ-inc allele at *HMR*, the *mat*α*1*α*2*Δ-inc allele at *HML*, and a genetically marked *MAT* locus were sporulated and then dissected on YEPD plates. After 3 days of growth at 30°, segregants were replica plated to drop-out plates to determine auxotrophies, which identified the initial mating type of the germinated spore as well as other relevant genetic features. Colony PCR to determine the allele at *MAT* was performed using 5′-GACTCTACCCAGATTTGT ATTAGACG-3′ and 5′-GATAAGAACAAAGAATGATGCTAAG AATTGA-3′ as primers. PCR products were run on a 0.7% agarose gel in 1× TAE. Colonies predominantly exhibiting the *mat***a***1* allele were scored as selecting *HMR* as donor, colonies predominantly exhibiting *mat*α*1*α*2* were scored as selecting *HML* as donor, and colonies that failed to exhibit one predominant allele were scored as ambiguous.

Fluctuation test: A slightly modified method of the median was used to determine recombination rates (Lea and Coulson 1949). Cells were plated for YEPD single colonies and grown for 2–3 days at 30°. Individual colonies were recovered in their entirety by coring and the majority of cells were plated on SC-Arg plates to determine the number of arginine prototrophs in the colony. A sample was diluted and plated on YEPD for viable cell count. Between 7 and 11 separate colonies were examined for each rate determination. Triplicate experiments using the same strain on different days yielded values that differed by <30%. For examination of methyl methanesulfonate (MMS)-induced recombination, cells were plated on media containing 0.1% MMS. For examination of gamma ray-induced recombination, the cultures were plated and directly irradiated with a 90 rad/min cesium source for 22 min.

RESULTS

Donor preference is maintained during interchromosomal mating-type switching: Mating-type interconversion normally takes place between donor and recipient loci on the same chromosome. To ask whether donor preference depends on this topology and, more broadly, what types of recombination are influenced by the recombination enhancer, we examined the efficiency of *HML* and *HMR* selection in strains in which the *MAT* locus and the donor loci were located on different chromosomes. This question has been addressed previously, but technical issues limited the reliability of the answer. In fact, two separate but similar experiments yielded two different outcomes (Weiler and Broach 1992; Wu *et al.* 1997).

To examine interchromosomal mating-type switching, we constructed an *HO/HO* diploid strain in which *MAT***a** and *MAT*α were inserted at the *LYS2* loci on the two chromosome II homologs and one chromosome III *MAT* locus was deleted and marked with *URA3*. The strain was further designed so that the initial switching event in spore clones became fixed due to import of the HO-refractory *inc* mutation from either donor locus. The donor locus from which the mating cassette derived in this initial switch could be determined by PCR analysis of the *MAT* locus resident at *lys2* after growth of the spore clone, since the sizes of the mating cassettes derived from the donor loci were different from each other and from the initial allele residing at *MAT*.

To conduct the switching experiment, this strain, Y2934, was sporulated and tetrads were dissected. Marker analysis was performed and spores clones inheriting chromosome III with the *mat*Δ::*URA3* locus were assayed to determine whether the *MAT* locus at *lys2* had undergone a mating-type switch and, if so, which donor provided the mating cassette. Results of this analysis, presented in Figure 2, demonstrated that normal donor

TABLE 1

Strain list

Strain	Genotype
Y2201	*MAT***a**/*MAT*α *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/ his3*Δ*1 trp1-289/trp1-289 HO/HO*
Y2811	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300*
Y2812	*MAT*α *ura3-52 leu2-3,112 ade2-101 his3*Δ*300*
Y2901	*MAT***a***::HIS3/MAT*α *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289 HO/HO*
Y2902	*MAT***a**/*MAT*α*::HIS3 hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289 HO/HO*
Y2934	Δ*mat*(W-Z1)*::URA3/MAT*α *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289 HO/HO lys2::MAT*α*-LEU2/lys2::MAT***a***-TRP1*
Y2935	Δ*mat*(W-Z1)*::URA3/MAT***a** *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289* Δ*ho::HIS3/HO lys2::MAT*α*::LEU2/lys2::MAT***a***::TRP1*
Y2936	Δ*mat*(W-Z1)*::URA3/MAT***a** *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289 HO/HO* Δ*ste5::HIS3/STE5 lys2::MAT*α*::LEU2/lys2::MAT***a***::TRP1*
Y2946	*MAT*α*/MAT***a***::HIS3 hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289* Δ*dps1-*Δ*dps2/*Δ*RE::URA3*
Y2947	*MAT***a**/*MAT*α*::HIS3 hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289* Δ*dps1-*Δ*dps2/*Δ*RE::URA3*
Y2948	Δ*mat*(W-Z1)*::URA3/MAT***a** *hml*α*1*α*2*$_{inc}$/ *hml*α*1*α*2*$_{inc}$ *hmr***a***1*Δ*101*$_{inc}$/ *hmr***a***1*Δ*101*$_{inc}$ *ura3-52/ ura3-52 leu2-3,112/ leu2-3,112 his3*Δ*1/his3*Δ*1 trp1-289/trp1-289 HO/HO lys2::MAT*α*::LEU2/lys2::MAT***a***::TRP1* Δ*dps1-*Δ*dps2/*Δ*RE::HIS3*
Y3100	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 arg4*Δ*::HIS3*
Y3101	*MAT***a** *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 his4::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3102	*MAT*α *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 his4::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3103	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 his4::arg4-bgl::URA3 re*Δ*::TRP1 arg4*Δ*::HIS3*
Y3104	*MAT*α *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 his4::arg4-bgl::URA3 re*Δ*::TRP1 arg4*Δ*::HIS3*
Y3105	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 rim1::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3106	*MAT*α *ura3-52 leu2-3,112 his3*Δ*300 rim1::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3107	*MAT***a** *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3108	*MAT*α *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3109	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 HMR::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3110	*MAT*α *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 HMR::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3111	*MAT***a** *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 rim1::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3112	*MAT*α *ura3-52 leu2-3,112 his3*Δ*300 rim1::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3113	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 MAT::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3114	*MAT*α *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 MAT::arg4-nsp::URA3 CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3115	*MAT***a** *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 ade2-101 CHA1::arg4-nsp::URA3 URA3::arg4-bgl arg4*Δ*::HIS3*
Y3116	*MAT*α *ura3-52 leu2-3,112 trp1*Δ*1 his3*Δ*300 CHA1::arg4-nsp::URA3 URA3::arg4-bgl arg4*Δ*::HIS3*
Y3117	*MAT***a** *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 his4::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3118	*MAT*α *ura3-52 leu2-3,112 his3*Δ*300 ade2-101 his4::arg4-nsp::URA3 HMR::arg4-bgl::URA3 arg4*Δ*::HIS3*
Y3119	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 his4::arg4-nsp::URA3/ his4::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3120	*MAT***a**/*mat*Δ*::LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 his4::arg4-nsp::URA3/ his4::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3121	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 his4::arg4-nsp::URA3/ his4::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3122	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3123	*MAT***a**/*mat*Δ*::LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3124	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3125	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 re*Δ*::TRP1/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*

(*continued*)

TABLE 1

(Continued)

Strain	Genotype
Y3126	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 re*Δ*::TRP1/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3127	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 re*Δ*::TRP1/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3128	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 RE/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3129	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 RE/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3130	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 CHA1::arg4-nsp::URA3/CHA1::arg4-bgl::URA3 RE/re*Δ*::TRP1 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3131	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 rim1::arg4-nsp::URA3/rim1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3132	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 rim1::arg4-nsp::URA3/rim1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3133	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 rim1::arg4-nsp::URA3/rim1::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3134	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 TRP1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ade2-101/ADE2 BUD5::arg4-nsp::URA3/BUD5::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3135	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 TRP1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ade2-101/ADE2 BUD5::arg4-nsp::URA3/BUD5::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3136	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 TRP1/trp1*Δ*1 his3*Δ*300/his3*Δ*300 ade2-101/ADE2 BUD5::arg4-nsp::URA3/BUD5::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3137	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 HMR::arg4-nsp::URA3/HMR::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3138	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 HMR::arg4-nsp::URA3/HMR::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3139	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 HMR::arg4-nsp::URA3/HMR::arg4-bgl::URA3 arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3140	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 URA3::arg4-nsp/URA3::arg4-bgl arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3141	*MAT***a**/*mat*Δ::*LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 URA3::arg4-nsp/URA3::arg4-bgl arg4*Δ*::HIS3/arg4*Δ*::HIS3*
Y3142	*mat*Δ*::LEU2/MAT*α *ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1*Δ*1/TRP1 his3*Δ*300/his3*Δ*300 ADE2/ade2-101 URA3::arg4-nsp/URA3::arg4-bgl arg4*Δ*::HIS3/arg4*Δ*::HIS3*

preference was maintained in switching when *MAT* resided on chromosome II and the donor loci on chromosome III. Switching was efficient in that 80–90% of spore clones exhibited predominant replacement of the initial *MAT* allele by a cassette from one of the donor loci. In addition, preference was maintained. A total of 79% of *lys2::MAT***a** spore clones used *HML* as donor while only 12% used *HMR* (comparable to 79% *HML* and 6% *HMR* as donor following intrachromosomal switching in *MAT***a** spore clones from the parental strain Y2201; Figure 2). Similarly, 70% of *lys2::MAT*α cells used *HMR* as donor while only 12% used *HML*. While this is somewhat less efficient than that seen in intrachromosomal switching, likely for reasons discussed below, donor preference is essentially maintained during mating-type switching from donor loci on chromosome III to the expressor locus on chromosome II.

Interchromosomal switching is delayed relative to intrachromosomal switching: We observed that *lys2::MAT*α *mat*Δ spores from strain Y2934 yielded significantly smaller colonies, with a higher percentage of inviable cells, than did *lys2::MAT***a** *mat*Δ spores from the same strain or *MAT*α spores from the parental Y2201 strain (Figure 3). This reduced growth was a consequence of initiation of mating-type interconversion since isogenic spores in which the *HO* gene was inactivated did not yield small colonies or poor viability (Figure 3). While this delay is likely due in part to the persistence of a double-strand break at *MAT* that is not healed in a timely fashion (Weiler and Broach 1992), this explanation cannot be the complete story since *HO lys2::MAT***a** *mat*Δ spore clones do not grow slowly. One explanation for this discrepancy is that *HMR* is less accessible than *HML* in interchromosomal switching, so that the double-strand break is healed by mating-type switching less efficiently in a *MAT*α than in a *MAT***a** background. Another explanation, though, is that the delay in healing the double-strand break at *MAT*α causes a loss of the *MAT*α gene by exonuclease processing and subsequent phenotypic conversion of the cell from α-mating type to **a**-mating type (Laney and Hochstrasser

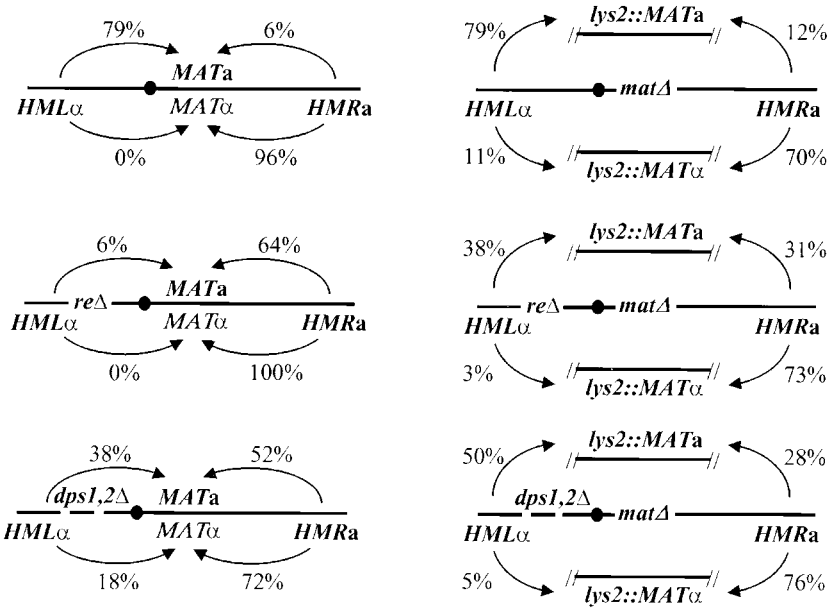

FIGURE 2.—*RE* influences donor preference in both intra- and interchromosomal mating-type switching. Shown are the frequencies of usage of donor alleles in *MAT***a** (above each line) and *MAT*α (below each line) spore clones during *HO*-mediated mating-type switching in six different strains. Strains on the left carry the *MAT* and donor loci at their normal locations on chromosome III while in strains on the right, *MAT* was deleted from chromosome III and inserted at *LYS2* on chromosome II. Strains carry, from top to bottom, wild-type *RE*, a complete deletion of *RE*, or deletion of the two α2/Mcm1 binding sites (*DPS1 DPS2*) within *RE*. Donor preference assays were performed as described in MATERIALS AND METHODS.

2003). This would permit mating between these phenotypic "**a**" cells carrying a break at *MAT* and adjacent *MAT*α cells, which, being in the daughter lineage, would not have initiated mating-type switching. This diploid cell would also suffer from double-strand-break-induced arrest, either from the inherited unrepaired break or from newly initiated *HO* cutting of the *MAT*α locus. Such phenotypic conversion and mating cannot occur in a *lys2::MAT***a** cell. To address this possible explanation, we deleted *STE5* from the strain. *STE5* encodes a scaffold protein involved in the pheromone response pathway and is required for mating. As evident in Figure 3, *lys2::MAT*α spore clones deleted for *STE5* showed normal viability and no decrease in colony size. Further, *ste5 lys2::MAT*α cells exhibit normal donor preference, with 82% of such spore clones selecting *HMR* as donor

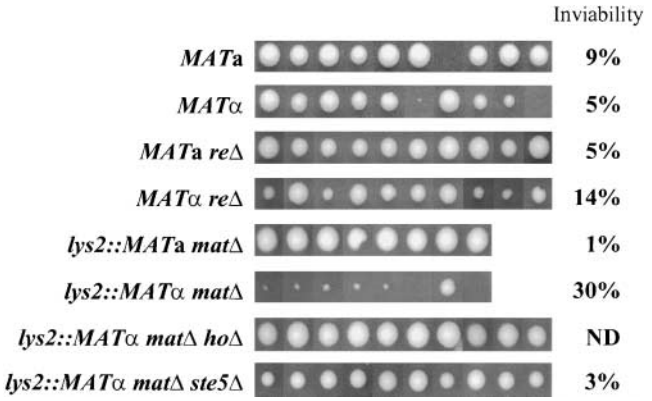

FIGURE 3.—Interchromosomal switching in *MAT*α strains causes cell inviability. Shown are photographs of spore clones with the relevant genotypes indicated to the left taken after 3 days of growth on YEPD at 30°. The percentage of dissected spores of each genotype that failed to yield colonies is indicated to the right.

(data not shown). Thus, the decrease in colony size and cell viability seen in spores inheriting a *lys2::MAT*α allele depends both on persistence of the double-strand break at *MAT* and on the ability of cells to mate. Similar results were observed for **a** cells attempting to switch in a strain deleted for HML (WU *et al.* 1996).

The recombination enhancer influences donor preference during interchromosomal recombination: Previous studies identified a region on chromosome III required for activation of *HML* as donor in *MAT***a** cells and responsible for suppressing this activation in *MAT*α cells. Deletion of this locus, *RE*, does not affect donor selection in *MAT*α cells but does result in inappropriate selection of *HMR* in *MAT***a** cells. To enhance our understanding of the function of *RE*, we asked what effect *RE* mutations would have on donor locus selection when *MAT* was located on another chromosome. Control strains with *MAT* at the wild-type location were also constructed for comparison. As seen previously and in Figure 2, in a strain in which *MAT* resides at its normal location, deletion of *RE* results in a reversal of donor preference in *MAT***a** cells, such that *HMR* rather than *HML* becomes the preferred donor. Consistent with previous reports (WU and HABER 1996; SZETO *et al.* 1997), *MAT*α cell preference for *HMR* remained unchanged by deletion of *RE*. *RE* contains two Mcm1/α2p binding sites. In strains in which these two sites are precisely deleted, then selection of donor in *MAT***a** cells became random and selection of donor in *MAT*α cells was unchanged, consistent with previous observations.

In contrast with the above results, deletion of *RE* in a strain with *MAT* resident on chromosome II resulted in essentially random selection of donor loci in a *MAT***a** background, although as with intrachromosomal recombination deletion of *RE* did not affect donor preference in a *MAT*α background. Similar but less dramatic results

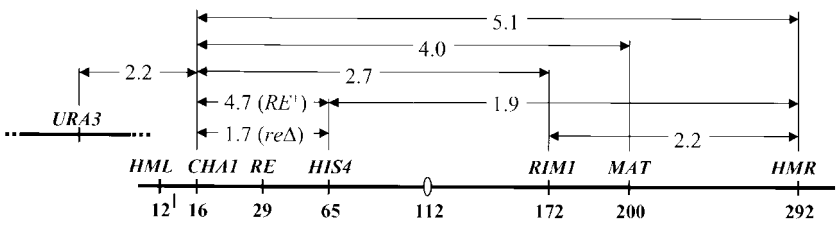

FIGURE 4.—*RE* promotes cell type-specific bias in the rate of intrachromosomal homologous recombination. Recombination rates between heteroalleles of ARG4 inserted at the indicated sites on chromosome III were determined for isogenic **a** and α haploid strains. Shown are the ratios of the rate obtained in the **a** strain *vs.* that in the α strain. The absolute rates are presented in Table 2.

are observed with deletion of only the Mcm1/α2p binding sites in *RE*, consistent with the fact that these elements play critical but not absolutely essential roles in *RE* function (SUN *et al.* 2002). The fact that *MAT***a** and *MAT*α cells exhibit a difference in donor preference during the interchromosomal mating-type switch in the *re*Δ background suggests that α2p exerts an influence on donor preference through some mechanism independent of *RE*. This observation is discussed below.

The recombination enhancer affects intrachromosomal but not interchromosomal gene conversion: We used a reporter system developed by Michael Lichten to examine the effects of the recombination enhancer on intra- and interchromosomal gene conversion (WU and LICHTEN 1995; BORDE *et al.* 1999). The system consists of two *arg4* genes, each carrying a distinct restriction site fill-in mutation, inserted at two separate sites on chromosome III (for intrachromosomal conversion) or at equivalent sites on the two chromosome III homologs in diploids (for interchromosomal conversion). Since the reversion rate of either mutation is quite low ($<10^{-6}$/cell/generation) and since the strain is deleted for *ARG4* at its normal location, appearance of Arg^+ prototrophs signals a recombination event between the two alleles. For interchromosomal recombination these recombination events could be gene conversion of one or the other allele or reciprocal recombination with a crossover site between the two alleles. For intrachromosomal recombination only gene conversion events are recorded, since the two *arg4* alleles are in direct orientation on the chromosome and reciprocal recombination would lead to excision of intervening sequences, generally a lethal event in the haploid background. The rates of recombination between the two alleles in a strain were determined by the method of the median (LEA and COULSON 1949).

WU and HABER (1995) previously reported a mating-type-dependent bias in intrachromosomal gene conversion between *leu2* heteroalleles inserted at various sites on chromosome III, with a significant increase in conversion rates in **a** *vs.* α cells if one of the alleles resided within 20 kb of *RE* on the left arm of chromosome III. We observed similar but less dramatic effects with the *arg4* heteroalleles. As evident in Figure 4 and Table 2, recombination between an *arg4* allele resident at *CHA1* (adjacent to *HML*) and an allele located at any other site on chromosome III was fivefold higher in a *MAT***a** background than in a *MAT*α background. This bias was reduced to less than twofold in a strain for which the *RE* was deleted. Recombination between heteroalleles at other sites also showed a bias in *MAT***a** *vs. MAT*α but not as marked. Thus, this work confirms the previous observation that *RE* promotes enhanced recombination over the left arm of chromosome III in **a** cells relative to α cells.

For interchromosomal recombination we created diploid strains with the two *arg4* alleles inserted at homologous sites at various positions along the chromosome III homolog (Figure 5A). We then created phenotypic **a** or α versions of this diploid by deleting one of the *MAT* loci from the strain and examined recombination rates in the resulting isogenic **a**/α, **a**, and α diploid strains. Measurements of spontaneous recombination rates at various sites along the chromosome (Figure 5B) confirmed previous observations that mitotic recombination is higher in **a**/α strains relative to isogenic **a** or α strains (ESPOSITO and WAGSTAFF 1981). Further, we found that recombination rates at all sites were equiva-

TABLE 2

Rates of intrachromosomal recombination

Loci		MT	*RE*	Rate[a]	**a**/α[b]
CHA1	*HIS4*	**a**	+	9.8×10^{-5}	4.7
		α	+	2.1×10^{-5}	
		a	Δ	1.8×10^{-5}	1.7
		α	Δ	1.1×10^{-5}	
CHA1	*RIM1*	**a**	+	0.69×10^{-5}	2.5
		α	+	0.28×10^{-5}	
CHA1	*MAT*	**a**	+	0.90×10^{-5}	4.0
		α	+	0.22×10^{-5}	
CHA1	*HMR*	**a**	+	1.8×10^{-5}	5.1
		α	+	0.37×10^{-5}	
CHA1	*URA3*	**a**	+	0.24×10^{-5}	2.0
		α	+	0.12×10^{-5}	
HIS4	*HMR*	**a**	+	0.21×10^{-5}	1.9
		α	+	0.11×10^{-5}	
RIM1	*HMR*	**a**	+	0.80×10^{-5}	2.2
		α	+	0.37×10^{-5}	

MT, mating type.

[a] Rates of Arg^+ prototrophs in strains carrying *arg4* heteroalleles inserted at the indicated loci on chromosome III were determined in isogenic **a** and α strains as described in MATERIALS AND METHODS. Values are in prototrophs per cell per generation.

[b] Ratio of the rate observed in the **a** strain *vs.* that in the α strain.

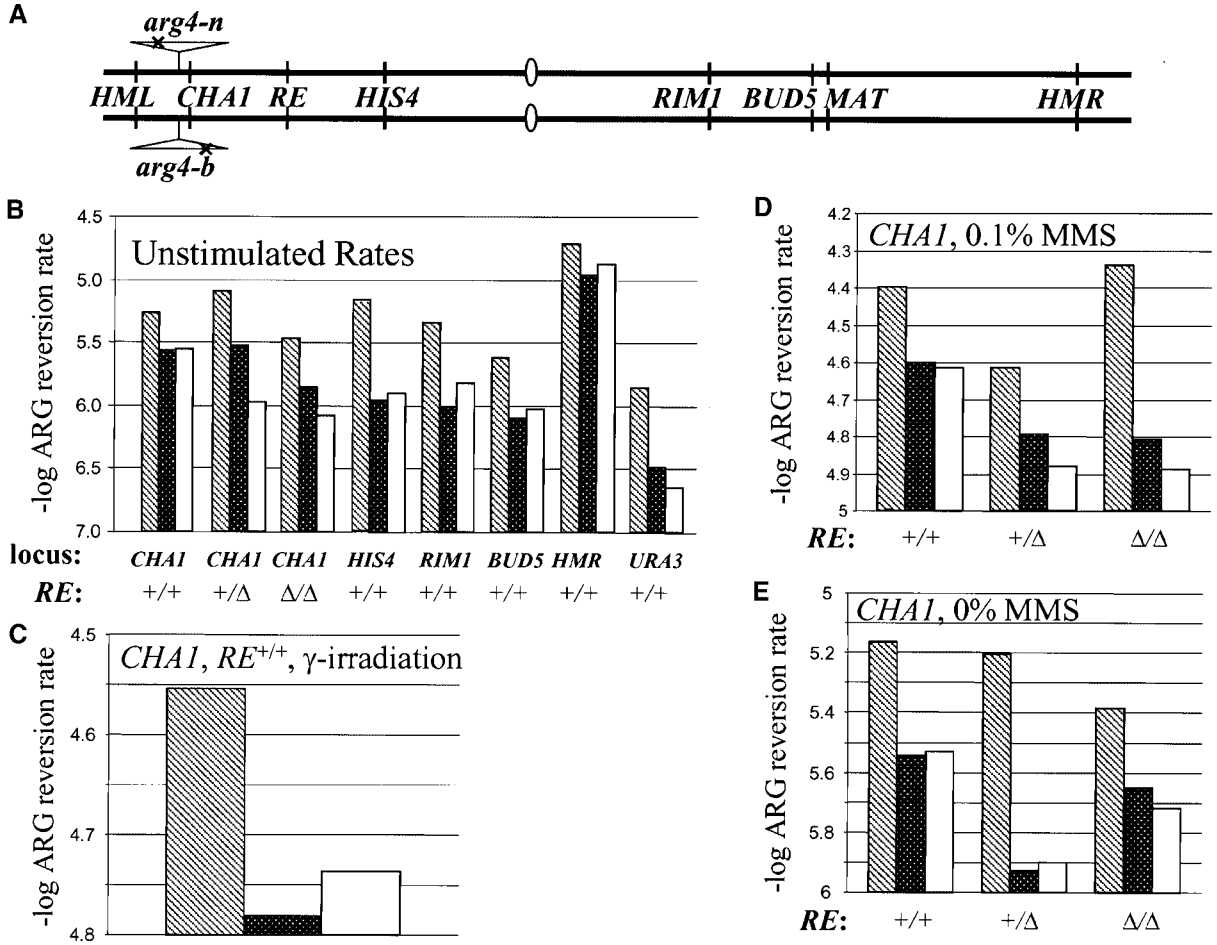

FIGURE 5.—*RE* does not affect interchromosomal homologous recombination. (A) Configuration of chromosome III in diploids used to measure recombination rates between heteroalleles of *arg4* inserted at various homologous sites along the chromosome. Arg^+ reversion rates are shown for isogenic **a**/α (hatched bars), **a** (stippled black bars), and α (open bars) diploid strains containing *arg4* heteroalleles inserted at the indicated locus and carrying the indicated *RE* genotype in unstimulated cells (B), in cells treated with 2000 rads γ-irradiation (C), or in cells either treated with 0.1% MMS (D) or plated without MMS treatment (E).

lent in **a** *vs.* α strains, indicating that the bias for enhanced recombination in **a** cells during intramolecular recombination is not recapitulated during intermolecular recombination. Finally, while deletion of *RE* caused a small (approximately threefold) decrease in recombination rates for alleles inserted at the *CHA1* locus, such deletions did not bestow an **a** *vs.* α recombination bias (Figure 5, B and E). Thus, *RE* does not confer an apparent cell type-specific recombination bias to intermolecular recombination.

One significant difference between mating-type switching and mitotic intermolecular recombination is that initiation of recombination is rate limiting in mitotic recombination whereas the initiating double-strand break occurs in almost all sensitive cells during mating-type interconversion. To address whether a cell type bias would be revealed in cells in which initiation of mitotic recombination were enhanced, we treated strains carrying *arg4* heteroalleles inserted at *CHA1* with MMS or γ-irradiation. Both of these treatments induce double-strand breaks and enhance the rate of mitotic recombination. As noted in Figure 5, C and D, both treatments increased recombination rates ~10-fold. However, in neither case did such treatment reveal a bias in recombination between **a** and α cell types. Also, while a small reduction in recombination rates was still observed in strains deleted for *RE*, no cell type bias was manifest. Thus, even when initiation of intermolecular mitotic recombination is enhanced by induction of double-strand breaks, *RE* does not confer a cell type-specific effect on recombination.

DISCUSSION

Donor preference is maintained during interchromosomal switching: The results presented in this report show that donor preference is maintained even when donor and recipient loci reside on different chromosomes, indicating that donor selection does not depend on interactions *in cis*. These results confirm previous work from this lab using a different assay in which the behavior of certain switching events could be only in-

ferred (Weiler and Broach 1992). This also confirms work suggesting that the relative three-dimensional organization of donor and acceptor loci on chromosome III does not play a significant role in donor locus selection (Simon *et al.* 2002). Rather, our results suggest that donor preference results from cell type differences in the intrinsic ability of the donor loci to participate in gene conversion following HO-initiated cleavage of *MAT*: in **a** cells *HML* is more "accessible" than *HMR* while in α cells the opposite is true.

Our results differ from those of Wu *et al.* (1997), who have previously reported that interchromosomal switching results in the decreased ability of *MAT*α resident on chromosome V to utilize either donor locus following HO cleavage. In addition, when these cells did switch, they selected donor loci randomly. There are several possible explanations for this discrepancy. A trivial possibility is that the difference in location of the *MAT* locus (chromosome II *vs.* chromosome V), perhaps reflecting different relative positions of donor and acceptor loci within the nuclear landscape, affected donor preference. A second possible explanation lies in the different assays. In the experiments by Wu *et al.*, the *MAT* locus was flanked by *URA3* alleles. Repair of the HO-induced double-strand break was thus a competition between gene conversion using the donor loci and recombination between the flanking *URA3* alleles via the single-strand annealing pathway. If the cells could have repaired the double-strand break only by conversion with the donor loci, donor preference might have been maintained. Finally, the switching experiments conducted by Wu *et al.* were performed with a galactose-induced *HO* gene. Thus, switching events were initiated in all stages of the cell cycle, not just G_1. This might suggest that the bias in donor preference is operative only during switching in G_1. In any event, the positive results from our experiments indicate that donor preference can be maintained during intermolecular switching.

One noteworthy phenotype associated with interchromosomal switching was decreased *lys2::MAT*α spore colony size and viability. This phenotype is similar to that observed in intrachromosomal switching in *MAT*α strains deleted for its preferred donor *HMR* (Wu *et al.* 1996). In both cases the phenotype is reversed by inactivation of either *HO* or the mating response pathway. Thus, the cause of the phenotype likely results from a delay in the repair of the double-strand break, the subsequent phenotypic conversion from α to **a** of the delayed cell, and, finally, mating of cells harboring the break with neighboring unswitched cells. That a similar small phenotype does not arise in *MAT***a** cells is likely not due to the fact that switching occurs more efficiently in **a** cells than in α cells but rather that phenotypic conversion and mating cannot occur in **a** cells delayed in switching. In fact, our previous data on interchromosomal switching suggest that the delay occurred equally in *MAT***a** and *MAT*α cells (Weiler and Broach 1992).

MAT*α influences donor switching independently of its affect on *RE*:** Cell type dictates donor preference strictly through MATα2 (Szeto and Broach 1997). In the absence of α2, *RE* activates *HML* for recombination and in the presence of α2 or the absence of *RE* this enhancement of *HML* is abrogated. Repression of *RE*-mediated activation occurs through two binding sites for α2 situated within *RE*. Data from studies presented in this report indicate that α2 also affects donor preference independently of not only these binding sites but also *RE* itself. In particular, interchromosomal switching in *MATa** cells in a strain deleted for *RE* exhibits random donor selection whereas switching in *MAT*α *re*Δ strains exhibits a strong bias for *HMR*. Therefore, α2 either suppresses selection of *HML* or enhances selection of *HMR* during intermolecular switching by a mechanism that does not involve *RE*. Wu *et al.* (1996) previously showed that *HMR* inserted at any site in the left half of chromosome III was selected inefficiently in *MAT*α cells. This domain is larger than that influenced by *RE* and thus their results are consistent with an *RE*-independent mechanism for suppression of donor selection by α2 of loci located on the left arm of chromosome III. Notably, we find that intramolecular homologous recombination over chromosome III was generally lower in α cells than in **a** cells, a phenomenon that might be related to the *RE*-independent donor bias observed. The mechanism for this *RE*-independent α2 effect could involve some as yet undefined activation of *HMR* or repression of *HML* as donor in *MAT*α cells. However, since the effect is seen in intermolecular switching, we can conclude that the position of *HML* or *HMR* relative to *MAT* on chromosome III does not contribute to this process.

***RE* affects intra- but not interchromosomal homologous recombination:** Our results confirmed that intramolecular gene conversion on chromosome III is enhanced in **a** cells *vs.* α cells when one of the participating alleles is resident on the left arm of the chromosome. The effects we observed are not as dramatic as those found previously (Wu and Haber 1995), which may reflect a difference in the reporter alleles used for the analysis. Nonetheless, these observations confirm that the recombination enhancer affects homologous recombination and not just mating-type interconversion. Thus, the fact that the recombination enhancer does not confer a similar cell type bias in interchromosomal homologous recombination is surprising and even more so given that it confers such a cell type bias in interchromosomal mating-type switching. One possibility is that the competition for gene conversion leading to prototropy is with sister chromatid repair of initiating lesions, which would not lead to prototrophy (Figure 6). In interchromosomal recombination, the enhancer would activate the sister to an equivalent extent as the homolog, so that the rate of recombination might be increased but most of the events would be with the sister chromatid. In intrachromosomal allele recombination at heter-

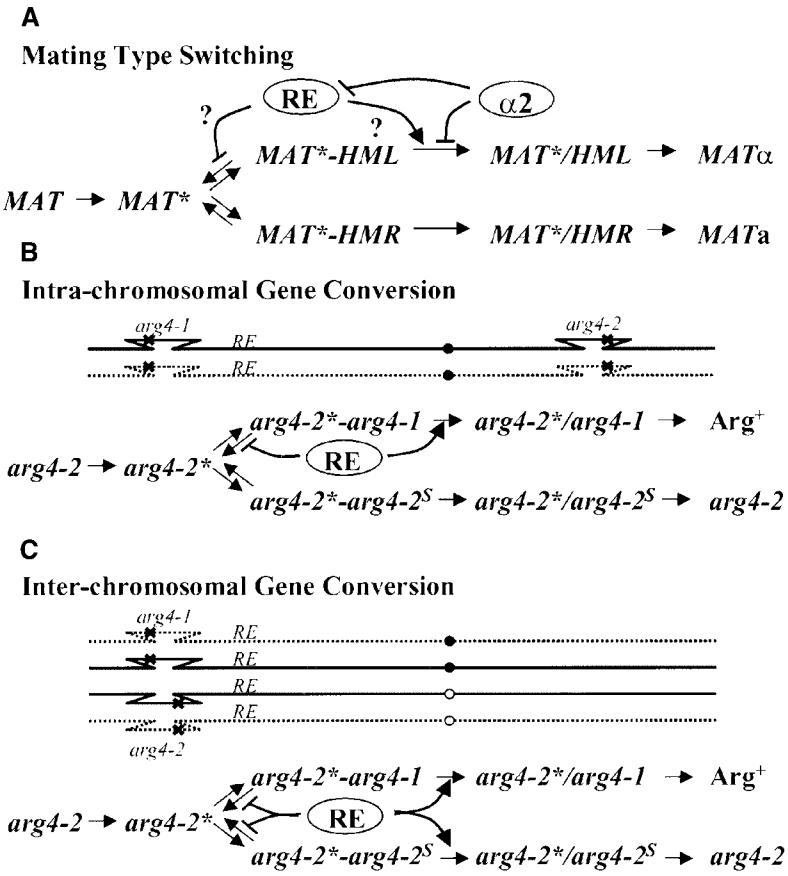

FIGURE 6.—RE biases selection of competing donors for double-strand-break repair. Proposed effect of RE on donor selection in mating-type interconversion (A), intramolecular homologous recombination (B), and interchromosomal homologous recombination (C) is shown. In all cases, recombination is initiated by formation of a recombinogenic lesion (*) at a participating locus, followed by rapid and reversible formation of ternary complexes with alternative templates for repair [*HML* or *HMR* in the case of mating-type interconversion and the *arg4* heteroallele or the *arg4* sister allele (^{S}) in the case of homologous recombination]. Transition to the closed intermediate (*e.g.*, *MAT**/*HML*) represents a relatively irreversible commitment to recombination, which fixes the final outcome. We propose that *RE* acts by stimulating the conversion to the closed intermediate or by slowing the dissolution of the ternary complex. α2 inhibits *RE* and can also inhibit recombination progression by an *RE*-independent mechanism. (A and B) *RE* acts on only one branch of the alternative pathways and thus affects the overall outcome of the process. (C) *RE* acts on both branches and does not affect the overall outcome.

ologous sites, events initiated outside the recombination domain would not have an enhanced interaction with the sister chromatid but rather with the heteroallele lying within the activated recombination domain. This possibility would suggest that interchromosomal recombination restricted to G_1 might exhibit a cell type bias that is not evident for recombination at other stages of the cell cycle.

The mechanism of recombination enhancement: While our results do not yet provide a molecular mechanism for the function of *RE*, they can be appreciated in the context of a model positing competition between different sites as a template for repairing a broken DNA molecule, with *RE* biasing that competition (Figure 6). For mating-type interconversion, the HO-cleaved *MAT* locus likely samples both *HML* and *HMR* repeatedly through a Rad52-dependent homology search that is rapid compared to commitment to recombination. This initial three-strand synapse can either decompose back to the initial broken *MAT* DNA and the intact donor locus or convert to a stable recombination intermediate consisting, for instance, of a D-loop at the donor loci formed from invasion of a single-stranded region from the resected double-strand break at *MAT*. In the absence of *RE* and α2, this conversion to a stable intermediate would be essentially equivalent for both donor locus complexes and thus the relative conversion to *MAT***a** or *MAT*α would depend on the relative abundance of the three-strand synapses formed with *HML vs. HMR*. When *MAT* is located on a different chromosome than *HML* and *HMR*, the relative concentration of the two complexes should be essentially equivalent, and thus the yield of *MAT***a** and *MAT*α would be expected to be equal, which is what we observe. When *MAT* resides on the same chromosome as the donor loci, the proportion of *HML* and *HMR* complexes would depend on the frequency of colliding with one *vs.* the other, making the physically closer locus the predominant donor. Since *HMR* is closer on the chromosome and in the three-dimensional space of the nucleus (SIMON *et al.* 2002), it becomes the default preferred donor.

In this context we would anticipate that *RE* would function to stabilize the interaction of HML and MAT, either by retarding the decomposition of the ternary complex (essentially rendering *HML* and the surrounding DNA more "sticky") or by accelerating the formation of the stable recombination intermediate. For instance, *RE* could promote an increase in the localized concentration of specific helicases, which would increase the probability of strand invasion following initial synapsis. This would bias selection in favor of *HML*. As noted previously, α2 inhibits *RE* and, as we have observed in this report, α2 also inhibits selection of *HML* by an *RE*-independent function that could act at the same step or at a different step from that affected by *RE*. Thus, in α cells, *HMR* becomes the preferred donor.

This same mechanism could account for the effects of *RE* on intramolecular *vs.* intermolecular gene conversion. Recent evidence suggests that most recombination intermediates arise as a consequence of replication (Tercero *et al.* 2003), so that repair of the recombinogenic lesion occurs in the presence of a sister chromatid. Thus, for heteroalleles located on the same chromosome, repair of a recombinogenic lesion would involve a competition between the heteroallele on the same chromosome, potentially yielding a prototroph, and the identical allele on the sister chromatid, which would not yield a prototroph. As shown in Figure 6B, the former event but not the latter event could be influenced by *RE*, by the same mechanism involved in donor preference. This would result in a cell type-dependent change in the rate of prototroph formation, as is observed. In the case of heteroalleles at the same site on homologous chromosomes (Figure 6C), the initiating lesion again could be repaired by interaction with either the heteroallele on the homolog chromatid or the identical allele on the sister chromatid. In this case, *RE* would affect both reactions, with the consequence that the balance between prototroph-generating and non-prototroph-generating pathways would be unchanged. As observed, *RE* would not affect the rate of prototroph formation. Thus, this biased competition model can account for all the effects reported here and previously. Further data, though, will be required to pinpoint the precise step affected by *RE*.

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