The Role of the Mismatch Repair Machinery in Regulating Mitotic and Meiotic Recombination Between Diverged Sequences in Yeast

Wenliang Chen* and Sue Jinks-Robertson*,†

**Graduate Program in Genetics and Molecular Biology and †Department of Biology, Emory University, Atlanta, Georgia 30322*

Manuscript received September 9, 1998
Accepted for publication December 18, 1998

ABSTRACT

Nonidentical recombination substrates recombine less efficiently than do identical substrates in yeast, and much of this inhibition can be attributed to action of the mismatch repair (MMR) machinery. In this study an intron-based inverted repeat assay system has been used to directly compare the rates of mitotic and meiotic recombination between pairs of 350-bp substrates varying from 82% to 100% in sequence identity. The recombination rate data indicate that sequence divergence impacts mitotic and meiotic recombination similarly, although subtle differences are evident. In addition to assessing recombination rates as a function of sequence divergence, the endpoints of mitotic and meiotic recombination events involving 94%-identical substrates were determined by DNA sequencing. The endpoint analysis indicates that the extent of meiotic heteroduplex DNA formed in a MMR-defective strain is 65% longer than that formed in a wild-type strain. These data are consistent with a model in which the MMR machinery interferes with the formation and/or extension of heteroduplex intermediates during recombination.

HOMOLOGOUS recombination involves the formation of heteroduplex DNA in which single strands of DNA derived from different parental duplexes are base-paired. The point at which the duplexes exchange pairing partners is referred to as a Holliday junction, and endonucleolytic cleavage of this junction can either maintain or reverse the linkage of markers that flank the region of heteroduplex DNA. Mismatches present in heteroduplex DNA are corrected by the postreplicative mismatch repair (MMR) machinery and such repair results in the genetic phenomenon of gene conversion. The concerted conversion of a contiguous series of potential mismatches constitutes a gene conversion tract, the length of which can be used as a minimal estimate of the extent of heteroduplex formed during recombination (Ahn and Livingston 1986; Judd and Petes 1988; Symington and Petes 1988; Borts and Haber 1989; Mezard *et al.* 1992; Harris *et al.* 1993; Sweetser *et al.* 1994; Porter *et al.* 1996; Chen and Jinks-Robertson 1998).

Although mismatches often are used to infer the nature of recombination intermediates, sequence divergence has been found uniformly to decrease recombination in bacterial species, yeast, and mammalian cells (for a review, see Modrich and Lahue 1996). Surprisingly, a single mismatch within a region of otherwise perfect identity is sufficient to inhibit transformation in Bacillus (Claverys and Lacks 1986) or mitotic recombination in yeast (Datta *et al.* 1997). In both bacteria and yeast, a log-linear relationship between the rate of recombination and the level of sequence divergence has been observed (Zawadzki *et al.* 1995; Datta *et al.* 1997; Vulic *et al.* 1997). Such a relationship is consistent with the concept of a minimal efficient processing segment (MEPS; Shen and Huang 1986), which is defined as the length of perfect identity needed to efficiently initiate recombination. If one considers recombination substrates as a linear series of overlapping MEPS, then one would predict that the number of MEPS should be a linear function of substrate length and an exponential function of substrate identity.

Much of the limitation imposed on recombination between diverged (homeologous) sequences derives from action of the MMR system. Inactivation of a component(s) of the MMR system usually increases the rate of homeologous recombination, sometimes restoring it to a level comparable to the rate of recombination between identical sequences (Rayssiguier *et al.* 1989; Borts *et al.* 1990; Zahrt *et al.* 1994; de Wind *et al.* 1995; Zawadzki *et al.* 1995; Datta *et al.* 1996, 1997; Hunter *et al.* 1996; Zahrt and Maloy 1997). The biochemistry of the MMR system has been best characterized in *Escherichia coli*, where the primary role of the system is to remove incorrect nucleotides incorporated during DNA synthesis (reviewed in Modrich and Lahue 1996). In *E. coli*, a MutS homodimer binds to the mismatched bases and MutH binds to a nearby hemi-methylated *dam* site, which is present in newly replicated DNA. A MutL homodimer acts as a "molecular matchmaker" to bring together the MutS and MutH proteins, which in turn activates a latent endonuclease activity of MutH. The unmethylated, newly synthesized DNA strand is then

Corresponding author: Sue Jinks-Robertson, Department of Biology, 1510 Clifton Rd., Emory University, Atlanta, GA 30322.
E-mail: jinks@biology.emory.edu

nicked, thus marking it for removal by helicases and exonucleases.

Mismatch recognition systems similar to the *E. coli* MutHLS system have been described in eukaryotes and have attracted much attention because defects have been associated with some forms of human hereditary cancer (reviewed in Kolodner 1996). In yeast, multiple homologs of the *E. coli* MutS (Msh1-6p) and MutL (Pms1p, Mlh1-3p) proteins have been identified, with the major players in nuclear mismatch recognition being Msh2p, Msh3p, Msh6p, Pms1p, and Mlh1p (reviewed in Crouse 1998). Msh2p dimerizes with either Msh3p or Msh6p, and each of these heterodimers recognizes specific types of mismatches. Pms1p likewise forms heterodimers with Mlh1p, and these heterodimers interact with the Msh2p-containing heterodimers in a manner analogous to the bacterial MutS-MutL interaction. Although the yeast Msh2 and Pms1/Mlh1 proteins appear to participate equally in the postreplicative repair of mismatched bases, their antirecombination activities are different. It has been shown in several studies that elimination of Msh2p stimulates recombination between diverged sequences to a greater extent than does elimination of Pms1p (Chambers *et al.* 1996; Datta *et al.* 1996). Methylation is not used as a strand discrimination signal in eukaryotes, so it is not surprising that no eukaryotic MutH homologs have been identified.

The antirecombination activity of the MMR machinery presumably derives from the recognition of mismatches present in heteroduplex recombination intermediates, but how the MMR machinery inhibits recombination once mismatches are detected is not clear. Based on studies in both bacteria (Claverys and Lacks 1986; Zahrt *et al.* 1994; Zahrt and Maloy 1997) and yeast (Alani *et al.* 1994), it has been proposed that mismatch recognition by the MMR machinery might trigger either helicase-driven unwinding of heteroduplex DNA or immediate resolution of the heteroduplex intermediate. According to such models, one might predict that the extent of heteroduplex formation should be greater in the absence of MMR than in its presence. Worth *et al.* (1994) indeed have shown that both the rate and extent of *in vitro* RecA-catalyzed heteroduplex formation are reduced in the presence of purified MutS and MutL proteins. In yeast, Alani *et al.* (1994) have argued that meiotic heteroduplex is longer in MMR-deficient cells than in wild-type cells, and we have found that mitotic gene conversion tracts in a *msh2 msh3* strain are 50% longer than those in a wild-type strain (Chen and Jinks-Robertson 1998).

Homologous recombination in yeast occurs during both mitotic and meiotic cell divisions. In mitotically dividing cells recombination constitutes an important mechanism for repairing broken DNA molecules that arise as a result of random DNA damage. In meiosis, recombination also repairs broken DNA molecules, but the breaks are generated enzymatically at nonrandom sites (Keeney *et al.* 1997). In contrast to the dispensable role recombination plays in yeast mitosis, meiotic recombination serves an essential function by providing a physical link between homologous chromosomes. This link ensures the attachment of homologs to opposite poles of the meiotic spindle and their subsequent disjunction (reviewed in Roeder 1995). Yeast studies have demonstrated that the mitotic (Selva *et al.* 1995, 1997; Datta *et al.* 1996, 1997) and meiotic (Borts *et al.* 1990; Chambers *et al.* 1996; Hunter *et al.* 1996) recombination barriers imposed by sequence divergence (see above) can be partially relieved if one or more of the MMR genes is inactivated. Unfortunately, it has not been possible to directly compare the published mitotic *vs.* meiotic data because of the very different assay systems that have been used. In addition, meiotic studies have not employed the systematic variation in substrate identity that has been used in some mitotic studies (Datta *et al.* 1997).

The relative sequence identity requirements for mitotic *vs.* meiotic recombination is an interesting issue. On the one hand, one might expect the identity requirements of meiotic recombination to be more stringent than those of mitotic recombination in order to ensure that most interactions are allelic interactions between homologs rather than ectopic interactions between dispersed repeats. Ectopic interactions have the potential to generate genome rearrangements, which, if they occur in meiosis, can directly impact gamete viability as well as the fitness of progeny. On the other hand, because recombination is necessary for proper homolog disjunction in meiosis, one might expect meiotic recombination to forego the stringent homology requirements of mitotic recombination in order to guarantee that at least one crossover occurs between each pair of homologs. A fair comparison of mitotic and meiotic sequence identity requirements, as well as the role of the MMR machinery in enforcing these requirements, necessitates the use of the same system to measure the rates of both types of events. In this study an intron-based inverted repeat assay system was used to measure and directly compare the rates of mitotic *vs.* meiotic recombination between pairs of nonidentical substrates. In addition, mitotic and meiotic conversion tract endpoints in wild-type *vs.* MMR-defective strains were determined to ascertain the impact of MMR proteins on the formation of recombination intermediates.

MATERIALS AND METHODS

Media and growth conditions: Yeast strains were grown nonselectively in YEP medium (1% yeast extract, 2% Bacto-peptone; 2.5% agar for plates) supplemented with either 2% glycerol and 2% ethanol (YEPGE) or 2% dextrose (YEPD). Synthetic complete medium (Sherman 1991) supplemented with 2% galactose, 2% glycerol, and 2% ethanol but deficient in histidine (SCGGE-his) was used to select His^+ recombinants. To select Ura^- segregants of Ura^+ strains, 5-fluorooro-

tic acid (5-FOA) was added to minimal synthetic medium supplemented with appropriate nutritional requirements (Boeke *et al.* 1987). For meiotic experiments, diploid strains were pregrown in YEPD overnight followed by sporulation in 2% KOAc supplemented with 10 μg/ml histidine. Both vegetative growth and sporulation were conducted at 30°.

E. coli strains used for plasmid manipulations were grown at 37° in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl; 1.5% agar for plates) supplemented with 150 μg/ml ampicillin as appropriate.

Plasmid constructions: All inverted repeat (IR) constructs were contained on a pRS306-based plasmid (Sikorski and Hieter 1989), which has *URA3* as a selectable marker. An outline of the plasmid constructions and the alignments of the IR substrates used in this study are presented in Figures 1 and 2, respectively. Detailed descriptions of the plasmid constructions as well as the IR substrates (with the exception of the cβ2a/cβ2a-4mmA substrates; see below) can be found in Datta *et al.* (1996, 1997). pSR615 contains the cβ2a/cβ2a-4mmA IR substrates, which were derived from pAB96 (obtained from G. F. Crouse). pAB96 contains chicken β tubulin isoform 2 (cβ2a) cDNA sequences in the 5′ recombination cassette, cβ2a-4mmA sequences in the 3′ cassette, and *LEU2* as a selectable marker. cβ2a-4mmA differs from cβ2a by four evenly spaced base substitutions that were introduced by site-directed mutagenesis. pSR615 was constructed by ligating an *Aat*II/*Ngo*MI IR-containing fragment from pAB96 to *Aat*II/*Ngo*MI-digested pRS306, thereby changing the selectable marker from *LEU2* to *URA3*.

Yeast strain constructions: A complete list of strains used in this study is given in Table 1. All diploid strains are isogenic and were constructed by mating derivatives of haploid strain SJR216 with derivatives of either SJR231 or SJR328. A *pms1*Δ derivative of each parental haploid strain was constructed by a standard two-step gene transplacement method (Rothstein 1991) using *Bst*XI-digested pSR211 (Datta *et al.* 1996). A *msh2*Δ*::hisG* derivative of each parental haploid was constructed by one-step gene disruption (Rothstein 1991) using *Aat*II/*Xba*I-digested GC1914 (*msh2*Δ*::hisG-URA3-hisG* plasmid obtained from G. F. Crouse). Ura$^+$ transformants were selected following transformation with either pSR211 or GC1914. Transformants were purified nonselectively on YEPD and then plated on 5-FOA medium to identify Ura$^-$ segregants. The *MSH3* allele of SJR231 *msh2*Δ*::hisG* was converted to *msh3*Δ*::hisG* by one-step gene disruption using *Eco*RI-digested pEN33 (*msh3*Δ*::hisG-URA3-hisG* plasmid; see Datta *et al.* 1996) as described above. All gene disruptions were confirmed by PCR.

Plasmids containing identical or mismatched IR constructs were transformed into the isogenic haploids SJR328, GCY121 (SJR231 *msh2*Δ*::hisG msh3*Δ*::hisG*), GCY128 (SJR231 *pms1*Δ), or SJR626 (SJR328 *pms1*Δ). Plasmids were targeted to the *URA3* locus by digestion with *Stu*I and integration of a single copy of each plasmid was confirmed by Southern analysis.

Measuring mitotic and meiotic recombination rates: Diploid strains were created immediately before rate measurement experiments in order to avoid the accumulation of recessive lethal mutations. Two independent diploids, each derived by mating two independently constructed haploid parents, were used for rate determinations. Diploids were constructed by mixing appropriate haploids on YEPD medium; after 5 hr, the mating mixtures were streaked onto medium selective for diploids. Two-day-old diploid colonies were used directly to inoculate 5 ml of YEPD medium and cultures were grown overnight to a density of $\sim 2 \times 10^8$ cells/ml. Cells were washed with 5 ml of sterile H_2O and resuspended in 1 ml of sterile H_2O. For mitotic rate determinations, aliquots of appropriate dilutions were plated in duplicate on YEPD medium to determine the total viable cell count and on SCGGE-his medium to select for His$^+$ recombinants. Colonies on YEPD and SCGGE-his plates were counted 2 and 5 days, respectively, after plating. Data from 12 or more cultures (6 from each independent diploid) of each strain were used to calculate the mitotic recombination rate by the method of the median (Lea and Coulson 1949).

For meiotic rate determinations, portions of the vegetatively grown cultures used to measure mitotic recombination rates were sporulated at a density of $1–2 \times 10^7$ cells/ml. Random spores were prepared by treating sporulated cultures with β-mercaptoethanol, followed by glusulase treatment to digest the ascal wall and kill vegetative cells (Davidow and Byers 1984). The cultures were then sonicated to disperse the spores, and aliquots of appropriate dilutions were plated nonselectively on YEPD medium and selectively on SCGGE-his medium. Colonies arising on YEPD and SCGGE-his plates were counted 2 and 6 days, respectively, after plating. The meiotic His$^+$ recombination rate for a particular strain was calculated by averaging the rates determined for 6–8 independent cultures (usually half from each independent diploid). All standard deviations were <35% of the rates in wild-type strains and <50% of the rates in the MMR-defective strains.

Generating independent recombinants for mapping conversion tract endpoints: One-ml cultures, each inoculated using a different colony, were grown nonselectively in YEPGE medium to generate mitotic recombinants for molecular analysis. Cells were washed with sterile H_2O, resuspended in 200 μl of sterile H_2O, and 100 μl were plated on SCGGE-his medium to select for His$^+$ recombinants. Only one colony was taken from each culture to ensure that all mitotic recombinants analyzed were of independent origin. Because recombinants generated in meiosis do not divide before selective plating, each meiotic recombinant derived from a given culture can be assumed to be of independent origin. Meiotic recombinants of each strain were, therefore, obtained directly from the SCGGE-his plates used to determine meiotic recombination rates.

Molecular analysis of recombinants: Genomic DNA was extracted by glass bead lysis (Hoffman and Winston 1987) from each recombinant and used as a template for PCR amplification. Recombination products were amplified individually using primers annealing to sequences flanking the recombination substrates (Figure 1). The recombination product located within the intron was amplified using primers HIS3-702F (5′-GTTTCTGGACCATATG) and HIS3-765R (5′-GCACTCAACGATTAG). The recombination product located downstream of the reconstituted *HIS3::intron* gene was amplified using primers HIS3-1751F (5′-GATGGCAAACATGTC) and T3 (5′-TGATGTCGGCGATATAGG). PCR products were purified using Qiaquick Spin Columns (QIAGEN, Chatsworth, CA) and were used directly as templates for DNA sequencing. FAI (5′-ATGGACTAAAGGAGGCT) and T3 were used as sequencing primers for the intron and downstream recombination products, respectively. All sequencing reactions were carried out using ABI Prizm Dye Terminator Cycle Sequencing Ready Reaction Kits and were run on an ABI Prizm 377XL DNA sequencer (PE Applied Biosystems).

RESULTS

The intron-based inverted repeat recombination assay system: The assay system used to examine the effects of sequence divergence on recombination was derived from a galactose-inducible *HIS3* gene containing an artificial intron (*HIS3::intron*). As illustrated in Figure 1,

TABLE 1

Yeast strains

Strain	Genotype and description	Source
SJR216	*MAT***a** *his3Δ200 ura3 ΔNco*	Lab strain
SJR590	SJR216 *msh2Δ::hisG*	This study
SJR588	SJR216 *pms1Δ*	This study
SJR231	*MATα ade2-101$_{oc}$ his3Δ200 ura3-Nhe*	Lab strain
SJR328	SJR231 *lys2ΔRV::hisG leu2-R*	Lab strain
GCY121	SJR231 *msh2Δ msh3Δ::hisG*	Datta *et al.* (1996, 1997)
GCY128	SJR231 *pms1Δ*	Datta *et al.* (1996)
SJR626	SJR328 *pms1Δ*	This study
SJR381	SJR328 with cβ2a/cβ2a 100%-identical substrates (pSR406)	Datta *et al.* (1996, 1997)
SJR484	SJR328 with cβ2a/cβ2a-1mmA substrates (pSR435)	Datta *et al.* (1997)
SJR516	SJR328 with cβ2a/cβ2a-1mmB substrates (pSR449)	Datta *et al.* (1997)
SJR383	SJR328 with cβ2a/cβ2a-3mm substrates (pSR434)	Datta *et al.* (1997)
SJR965	SJR328 with cβ2a/cβ2a-4mmA substrates (pSR613)	This study
SJR485	SJR328 with cβ3a/cβ3a-4mmB substrates (pSR414)	Datta *et al.* (1997)
SJR567	SJR328 with cβ2a/cβ2a-21mm, 94%-identical substrates (pSR424)	Datta *et al.* (1997)
SJR382	SJR328 with cβ2a/cβ7b, 91%-identical substrates (pSR407)	Datta *et al.* (1996, 1997)
SJR487	SJR328 with cβ2b/cβ3b, 85%-identical substrates (pSR421)	Datta *et al.* (1997)
SJR384	SJR328 with cβ3a/cβ7a, 82%-identical substrates (pSR415)	Datta *et al.* (1997)
SJR393	GCY121 with cβ2a/cβ2a 100%-identical substrates (pSR406)	Datta *et al.* (1996, 1997)
SJR488	GCY121 with cβ2a/cβ2a-1mmA substrates (pSR435)	Datta *et al.* (1997)
SJR497	GCY121 with cβ2a/cβ2a-3mm substrates (pSR434)	Datta *et al.* (1997)
SJR490	GCY121 with cβ2a/cβ2a-21mm, 94%-identical substrates (pSR424)	Datta *et al.* (1997)
SJR391	GCY121 with cβ2a/cβ7b, 91%-identical substrates (pSR407)	Datta *et al.* (1996, 1997)
SJR491	GCY121 with cβ2b/cβ3b, 85%-identical substrates (pSR421)	Datta *et al.* (1997)
SJR492	GCY121 with cβ3a/cβ7a, 82%-identical substrates (pSR415)	Datta *et al.* (1997)
SJR385	GCY128 with cβ2a/cβ2a 100%-identical substrates (pSR406)	Datta *et al.* (1996)
SJR524	SJR626 with cβ2a/cβ2a-1mmA substrates (pSR435)	This study
SJR530	GCY128 with cβ2a/cβ2a-3mm substrates (pSR434)	This study
SJR531	GCY128 with cβ2a/cβ2a-21 mm, 94%-identical substrates (pSR424)	This study
SJR386	SJR626 with cβ2a/β7b, 91%-identical substrates (pSR407)	This study
SJR532	GCY128 with cβ2b/cβ3b, 85%-identical substrates (pSR421)	This study
SJR533	GCY128 with cβ3a/cβ7a, 82%-identical substrates (pSR415)	This study

replacement of the 3′ or 5′ half of *HIS3::intron* with a 350-bp recombination substrate created a 5′ or 3′ recombination cassette, respectively. All recombination substrates were derived from chicken β-tubulin cDNA (cβ) sequences and substrate pairs varied in identity from 82% to 100% (Figure 2). Juxtaposition of a 5′ and 3′ cassette in reverse orientation creates an IR construct with the recombination substrates flanking the 3′ half of the *HIS3::intron* gene. Recombination between the substrates via either intrachromatid crossover or sister chromatid conversion flips the intervening *HIS3::intron* sequences (the "invertible segment"), thus reconstituting a full-length *HIS3* gene with a complete intron containing one of the two recombination products. The other recombination product is located distal to the intact *HIS3::intron* gene. Because the recombinant cβ sequences within the *HIS3::intron* gene are spliced out of the primary transcript and do not impact the gene product, there are no functional constraints on either the recombination substrates or the recombination products. It should be noted that neither intrachromatid gene conversion nor sister chromatid crossover produces His^+ recombinants. Intrachromatid gene conversion does not reorient the 3′ *HIS3::intron* segment and so recombinants are His^-; a sister chromatid crossover produces acentric and dicentric chromosomes and hence inviable His^+ progeny.

We previously used the intron-based assay system to demonstrate that mitotic recombination between diverged sequences in yeast is regulated in large part by the MMR machinery (Datta *et al.* 1996, 1997). These studies, however, provided no information concerning the antirecombination role of the MMR machinery in meiotic recombination. To specifically address this issue and to directly compare the identity requirements of mitotic *vs.* meiotic recombination, *MAT*α strains containing IR constructs were mated with appropriate *MAT***a** haploid strains to create wild-type, *MSH2*-defec-

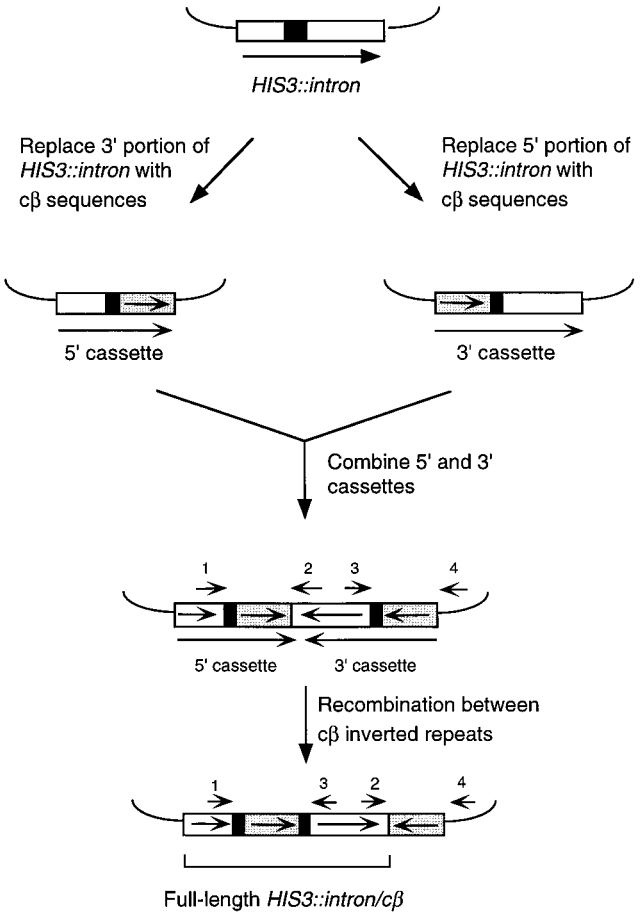

Figure 1.—Construction of IR substrates. The *pGAL-HIS3::intron* construct contained on plasmid pSR266 is shown at the top. Open boxes correspond to *HIS3* sequences, solid boxes to artificial intron sequences, and gray boxes to cβ sequences; boxes are not to scale. The positions of the oligonucleotides used as PCR primers are indicated by arrows numbered as follows: 1, HIS3-702F; 2, HIS3-1751F; 3, HIS3-765R; 4, T3. The recombinant cβ segment within the intron was amplified using primers 1 and 3; the recombinant segment downstream of the reconstituted *HIS3::intron* gene was amplified using primers 2 and 4.

tive (*msh2*Δ/*msh2*Δ *MSH3*/*msh3*Δ), or *PMS1*-defective (*pms1*Δ/*pms1*Δ) diploid strains. The *MSH2*-defective and *PMS1*-defective diploid strains will hereafter be referred to as *msh2*Δ and *pms1*Δ strains, respectively. It should be noted that all diploids were heterozygous for the IR plasmid, thus precluding the production of His^+ recombinants by interchromosomal interactions.

Recombination rates between identical and mismatch-containing substrates: Mitotic and meiotic recombination rates were inferred in each strain by measuring the rates of His^+ prototroph formation by fluctuation analysis and random spore analysis, respectively, and these rates are presented in Table 2. Recombination rates between 100% identical cβ2a sequences were measured in the wild-type, *msh2*Δ, and *pms1*Δ strains, and these rates were used as a normalization standard when assessing the effects of mismatches on recombination in the presence or absence of MMR proteins. Mitotically, the cβ2a/cβ2a 100% substrates recombined at a rate of $\sim 1 \times 10^{-6}$ in the wild-type, *msh2*Δ, and *pms1*Δ strains. This similarity in recombination rates was unexpected because we consistently have found a two- to threefold elevation in recombination rates between identical sequences in *msh2*Δ strains relative to MMR-competent strains (Datta *et al.* 1997; A. Bayliss, M. Hendrix, G. F. Crouse and S. Jinks-Robertson, unpublished results). Although we do not understand the reason for this discrepancy, we speculate that it could reflect either a haploid/diploid effect (all previous studies were done with haploid strains) or strain background differences (one of the haploid parents used in this study is the same as that used in other studies, but the other is unrelated).

The rate of meiotic recombination between the cβ2a/cβ2a 100% substrates was ~100-fold greater than the corresponding mitotic rate in the wild-type, *msh2*Δ, and *pms1*Δ strains. It should be noted, however, that the induction of meiotic recombination is usually assessed using recombination frequencies rather than rates. Whereas the meiotic recombination frequency is equal to meiotic rate (all events occur in a single generation), the mitotic frequency is generally higher than mitotic rate because of the random occurrence of mitotic recombination over several generations. With our substrates, the 100-fold difference between mitotic and meiotic recombination rates translates to approximately a 10-fold difference in mitotic *vs.* meiotic recombination frequencies. This is considerably less than the several hundred-fold meiotic induction generally observed with allelic sequences, and may be related to the inherent bias for intrachromosomal events in mitosis (Fabre *et al.* 1984; Kadyk and Hartwell 1992; Jinks-Robertson *et al.* 1993) *vs.* interchromosomal events in meiosis (Haber *et al.* 1984; Game *et al.* 1989; Schwacha and Kleckner 1997). It can be estimated that ~10% of "meiotic" events involving the IR substrates are likely of mitotic origin, but we do not believe that this 10% significantly impacts the meiotic data.

We reported previously that a single mismatch within the 350-bp IR substrates was sufficient to reduce mitotic recombination 4-fold in a wild-type haploid strain, and this reduction was entirely dependent on the MMR machinery (Datta *et al.* 1997). Mitotic recombination rates in diploid strains were measured for two pairs of substrates whose sequences differ by a single nucleotide: 1mmA and 1mmB cβ2a sequences differing at positions 884 (A884G) and 883 (G883C), respectively. Although the 1mmA and 1mmB substrates differ in the types of mismatches that potentially can be formed in heteroduplex recombination intermediates (A/C or G/T mismatch for 1mmA; G/G or C/C mismatch for 1mmB), their mitotic recombination rates in a wild-type background were decreased to similar extents relative to the 100% control rate (6.0-fold for 1mmA and 4.4-fold for

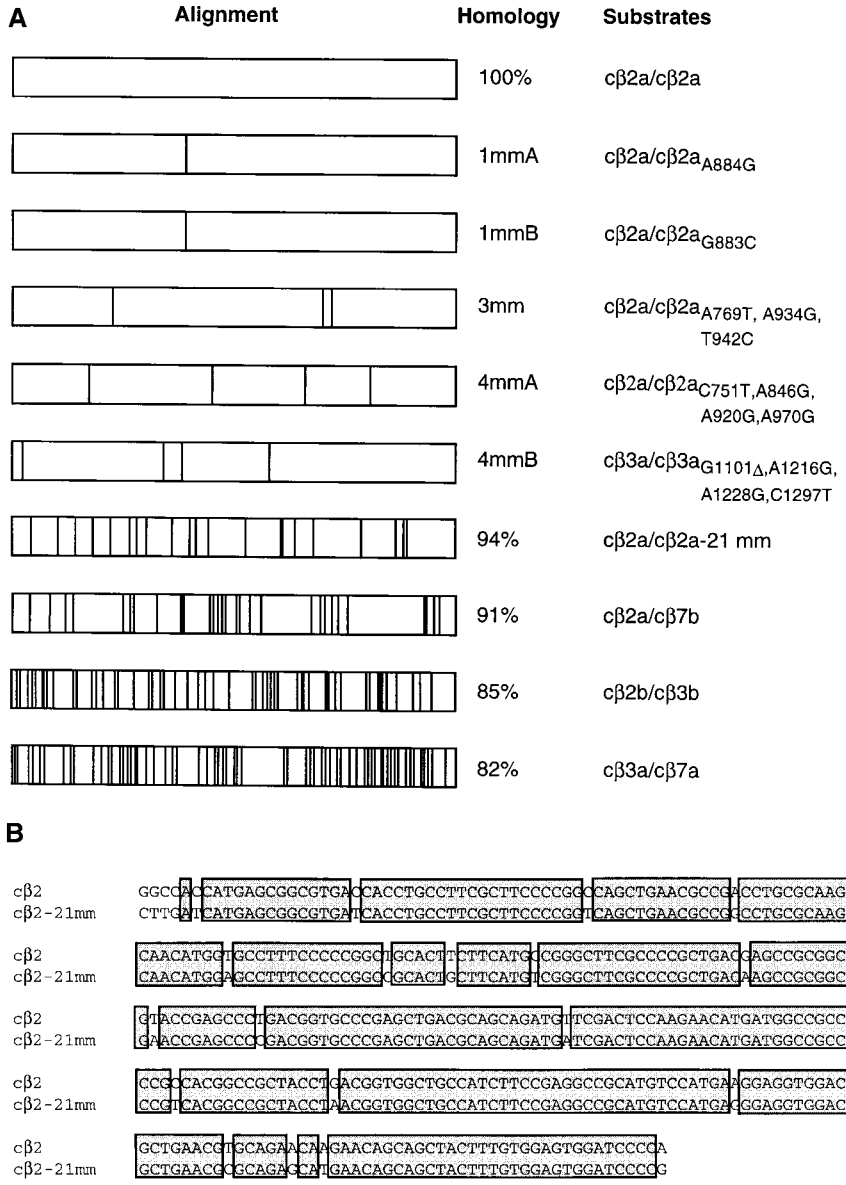

Figure 2.—Alignment of recombination substrates derived from cβ cDNA sequences. (A) cβ sequences were numbered as in the GenBank files (see Datta *et al.* 1997 for details). For substrates containing one to four mismatches, the nature and position of the mismatches are indicated as a subscript. Each potential mismatch (mm) between a given pair of sequences is indicated by a vertical line. All sequences are ~350 bp. (B) Alignment of cβ2/cβ2-21 mm sequences. Sequences of perfect identities are boxed and shaded in gray. Although we refer to positions within the aligned sequences using the numbering system shown, it should be noted that the cβ2a/cβ2a-21mm homology does not actually begin until position 7.

1mmB). In contrast to the approximately 5-fold effect observed mitotically, the mismatches in the 1mmA and 1mmB substrates reduced meiotic recombination only 2-fold and 1.8-fold, respectively, relative to the 100% control substrates. This indicates that a single potential mismatch has less impact on meiotic than on mitotic recombination rates. Elimination of *MSH2* restored the mitotic and meiotic recombination rates of 1mmA to those of the 100% control substrates. Although deletion of the *PMS1* gene also increased the mitotic recombination rate between the 1mmA substrates, the *pms1*Δ rate for the mismatched substrates was lower than the 100% control rate. Meiotic recombination between the 1mmA substrates was not impacted by elimination of Pms1p.

To examine the effects of multiple mismatches on mitotic and meiotic recombination, substrates containing three or more mismatches were introduced into the wild-type and MMR-defective diploid strains. As observed for the 1mm substrates, three mismatches reduced mitotic recombination rates more than meiotic rates (6.7-fold *vs.* 3.5-fold) in a wild-type background, and both reductions were completely dependent on the MMR machinery. When the recombination substrates differed by four or more nucleotides, however, meiotic recombination rates in a wild-type background were reduced just as much as the mitotic rates (Table 2). Although elimination of Msh2p or Pms1p increased both mitotic and meiotic recombination rates, the rates for the 94%, 91%, 85%, and 82% identical substrates were not equivalent to those observed with the 100% control substrates. The MMR-independent decrease in recombination rates with these lower levels of sequence identity presumably reflects an inability of the recombination machinery to efficiently initiate recombination between these sequences (Datta *et al.* 1997). In contrast to the similar inhibitory effects of sequence divergence

TABLE 2

Rates of His$^+$ recombinants

Genotype	Sequence identity	Mitotic recombination		Meiotic recombination	
		Rate ($\times 10^{-8}$)	Fold ↑↓	Rate ($\times 10^{-6}$)	Fold ↑↓
Wild type	100%	120	1×	92	1×
	1 mmA	20	↓6.0×	46	↓2.0×
	1 mmB	27	↓4.4×	52	↓1.8×
	3mm	18	↓6.7×	26	↓3.5×
	4mmA	5.3	↓23×	4.5	↓20×
	4mmB	8.3	↓14×	5.7	↓16×
	94%	0.86	↓140×	0.76	↓120×
	91%	0.93	↓130×	0.83	↓110×
	85%	0.17	↓710×	0.13	↓710×
	82%	0.089	↓1300×	0.059	↓1600×
*msh2*Δ	100%	110	1×	91	1×
	1mmA	98	↓1.1×	130	↑1.4×
	3mm	110	1×	94	1×
	94%	62	↓1.8×	29	↓3.1×
	91%	38	↓2.9×	16	↓5.7×
	85%	9.8	↓11×	3.1	↓29×
	82%	3.5	↓31×	0.57	↓160×
*pms1*Δ	100%	100	1×	210	1×
	1mmA	40	↓2.5×	84	↓2.5×
	3mm	55	↓1.8×	170	↓1.2×
	94%	14	↓7.1×	13	↓16×
	91%	11	↓9.1×	12	↓18×
	85%	2.1	↓48×	2.0	↓110×
	82%	1.1	↓91×	0.31	↓680×

on mitotic and meiotic recombination rates between the 94%, 91%, 85%, and 82% identical substrates in MMR-competent strains, it should be noted that, in MMR-defective cells, meiotic recombination between these substrates was consistently impacted more by a decrease in sequence identity than was mitotic recombination. Mitotic recombination rates between the 85% or 82% identical substrates, for example, were reduced an average of 16-fold in the *msh2*Δ strains and 63-fold in the *pms1*Δ strains. The corresponding meiotic recombination rates between the 85% or 82% identical substrates were reduced an average of 50-fold in the *msh2*Δ strains and 180-fold in the *pms1*Δ strains relative to the 100% control substrates.

Mitotic and meiotic gene conversion tract endpoints in wild-type and *msh2*Δ strains: One approach to addressing the mechanism of the MMR-associated inhibition of recombination is to determine whether the formation of recombination intermediates is altered by the MMR machinery. Because gene conversion tracts are generally assumed to be a direct reflection of the heteroduplex DNA intermediate formed during recombination, 94% identical substrates were used to map the endpoints of conversion tracts in mitotic and meiotic His$^+$ recombinants derived from both wild-type and *msh2*Δ diploid strains. Conversion tract endpoints were determined by individually sequencing the recombinant cβ segments flanking the invertible *HIS3::intron* segment.

As illustrated in Figure 2, the cβ2a and cβ2a-21mm sequences differ at 21 positions, thereby dividing the substrates into 21 intervals of perfect identity (two mismatches are adjacent to each other). A given mismatch was considered to have been converted if the corresponding nucleotides in the recombinant cβ2a sequences were identical. A gene conversion tract encompasses a series of contiguous mismatches and has endpoints in two discrete intervals. An endpoint was assigned to a given interval (intervals are identified by the positions of the flanking mismatches) if the mismatch defining one side of the interval was converted but the mismatch defining the other side was not. If one assumes that gene conversion tracts start and end at random, then the number of endpoints contained in a given identity interval should be directly proportional to the length of that interval. Interval 318–350, for example, contains 31 bp of perfect identity, which constitutes ~10% of the 322 bp of perfect identity shared between the cβ2a/cβ2a-21mm substrates. One would predict, therefore, that this interval should contain ~10% of all conversion tract endpoints. The experimentally determined conversion tract endpoint distributions were compared to the expected distribution by subtracting the percentage of expected endpoints in

TABLE 3

Distribution of conversion tract endpoints

Identity interval (nt)	Expected endpoints (%)	WT mitotic endpoints		WT meiotic endpoints		*msh2*Δ mitotic endpoints		*msh2*Δ meiotic endpoints	
		No.	%	No.	%	No.	%	No.	%
1 (6–21)	4	4	7	2	3	5	4	2	3
2 (21–43)	7	2	3	1	2	8	7	6	10
3 (43–57)	4	1	2	3	5	2	2	3	5
4 (57–70)	4	2	3	2	3	2	2	1	2
5 (70–84)	4	2	3	1	2	6	5	3	5
6 (84–99)	4	4	7	1	2	2	2	2	3
7 (99–105)	2	0	0	0	0	2	2	3	5
8 (105–113)	2	1	2	1	2	5	4	0	0
9 (113–133)	6	1	2	2	3	6	5	2	3
10 (133–147)	4	2	3	1	2	7	6	1	2
11 (147–152)	1	0	0	0	0	1	1	0	0
12 (152–162)	3	2	3	1	2	1	1	2	3
13 (162–191)	9	2	3	4	7	5	4	6	10
14 (191–219)	8	7	12	5	8	11	10	8	13
15 (219–229)	2	1	2	0	0	1	1	1	2
16 (229–244)	4	0	0	0	0	7	6	6	10
17 (244–282)	11	5	8	7	12	12	11	9	15
18 (282–309)	8	10	17	8	13	12	11	2	3
19 (309–315)	2	2	3	1	2	0	0	0	0
20 (315–318)	1	0	0	0	0	0	0	0	0
21 (318–350)	10	12	20	20	33	19	17	3	5
Total	100	60	100	60	100	114	100	60	100

each identity interval from the percentage of observed endpoints. This yields positive and negative percentages which, when plotted, indicate an excess or deficit of endpoints, respectively.

Gene conversion tracts were determined for 30 mitotic His^+ recombinants generated in the wild-type strain. Twenty-eight of the recombinants had continuous conversion tracts with all mismatches converted in the same direction. The other two recombinants had no detectable conversion events and, therefore, were assumed to have both endpoints in the same interval. The expected and observed distributions of conversion tract endpoints are presented in Table 3 and are compared graphically in Figure 3A. Several of the 3′ intervals (*e.g.*, intervals 318–350 and 282–309) have a notable excess of endpoints, indicating that the ends of the substrates proximal to the invertable *HIS3::intron* segment may be preferred sites for initiating and/or resolving recombination intermediates.

To directly compare mitotic and meiotic conversion tracts, conversion tracts were determined for 33 meiotic His^+ recombinants isolated from the same wild-type strain. Of these conversion tracts, 27 were continuous and asymmetric and 3 had no mismatches converted. The remaining 3 recombinants contained either interrupted conversion tracts or continuous but bidirectional (symmetric) tracts. Because of their complexity, these latter tracts were not included in the determination of endpoint distributions or in calculations of conversion tract lengths (see below). The distribution of the meiotic conversion tract endpoints is presented in Table 3 and graphically compared to the expected distribution in Figure 3B. Strikingly, the most 3′ interval, interval 318–350, contained 33% of all endpoints whereas only 10% were predicted to be in this interval on the basis of its length. Most of the remaining identity intervals exhibited a mild deficit of endpoints. A comparison of the distributions in Figure 3, A and B, indicates that the moderate mitotic clustering of endpoints at the 3′ end of the substrates is exaggerated in the corresponding meiotic recombinants.

In the absence of a functional MMR system, the mismatches present in heteroduplex molecules formed during recombination should be segregated at the next round of DNA replication, thus producing the equivalent of a gene conversion tract. Sixty-three mitotic His^+ recombinants derived from the *msh2*Δ strain were sequenced to estimate the extent of heteroduplex formation. Fifty recombinants had continuous asymmetric conversion tracts, 6 had no mismatches converted, and 7 had complex conversion events. This class distribution is not statistically different from that of the mitotic conversion tracts in wild-type cells ($P > 0.1$ by χ^2 contingency test). The distribution of conversion tract endpoints is presented in Table 3 and graphically compared to the expected distribution in Figure 3C. In contrast

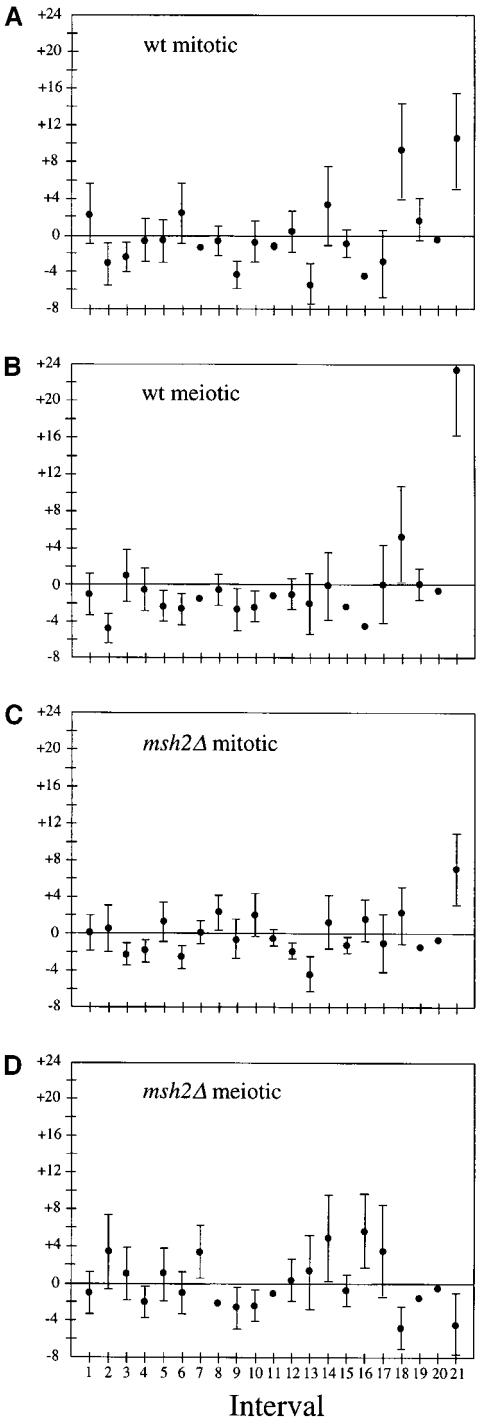

Figure 3.—Conversion tract endpoint distributions. The percentages of endpoints expected in each interval (see Table 3 for the interval numbers) were subtracted from the observed percentages, and the residual values were plotted (solid circles). Positive or negative deviations of observed from expected percentages indicate an excess or deficit of endpoints, respectively. Vertical bars correspond to standard deviations (SDs), each of which was approximated by taking the square root of the number of endpoints observed in a given interval. The SDs thus obtained were converted to percentages, and these percentages were added to and subtracted from the corresponding percentage deviations. The SD for interval 21 in B is off the *y*-axis scale.

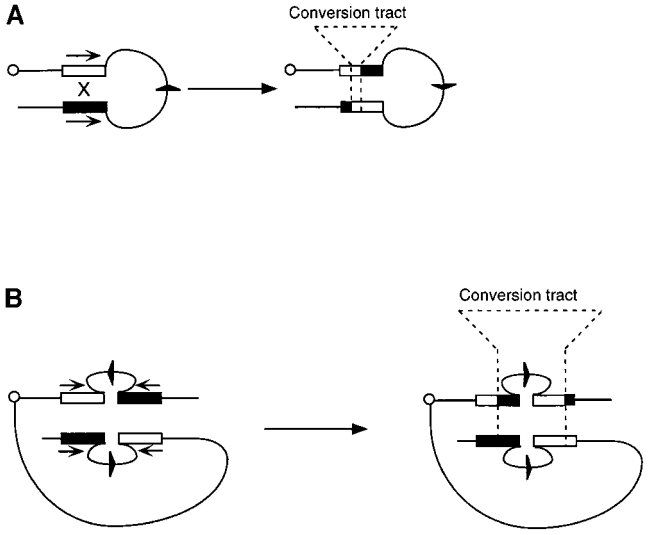

Figure 4.—Models of IR recombination. Inversion of a segment of DNA between inverted repeats can occur by either (A) intrachromatid crossover or (B) sister chromatid gene conversion. The open and solid boxes correspond to the IR substrates that flank the invertible *HIS3::intron* segment, which is represented by a loop with an arrow indicating orientation. Open circles denote centromeres. It should be noted that if the internally paired chromatid in A is straightened, it is identical to the top chromatid in B in terms of the recombination endpoints. Thus, although the conversion tract in A would be calculated to be quite short, a conversion tract with identical endpoints would be relatively long if one assumes the model in B.

to the weak 3′ clustering of mitotic conversion tract endpoints evident in wild-type cells (Figure 3A), endpoints appeared to be more or less randomly distributed in the MMR-defective cells.

Thirty-three meiotic His^+ recombinants derived from the *msh2*Δ strain also were analyzed. Twenty-six recombinants had continuous asymmetric conversion tracts, 4 had no mismatches converted, and 3 had complex tracts. This class distribution is the same as that of the meiotic recombinants isolated from a wild-type strain ($P > 0.9$ by χ^2 contingency test). The meiotic conversion tract endpoint distribution is presented in Table 3 and is compared graphically to the expected distribution in Figure 3D. In contrast to the very striking clustering of meiotic endpoints in interval 318–350 in wild-type cells (Figure 3B), there was no evident clustering of meiotic endpoints in MMR-defective cells.

Mitotic and meiotic conversion tract lengths in wild-type and *msh2*Δ strains: In the assay system used here, reorientation of the *HIS3::intron* segment between the recombination substrates is required in order for a recombinant to be His^+ (Figure 1). Reorientation involves interactions between the flanking cβ segments and can occur either by intrachromatid crossover or by sister chromatid conversion. As illustrated in Figure 4, an intrachromatid crossover requires pairing of two substrates on the same DNA molecule, whereas sister chro-

TABLE 4

Conversion tract parameters

	Wild type[a]		*msh2*Δ[b]		
	Mean	95% CI	Mean	95% CI	*P* Value
Mitotic conversion tracts					
Minimal length	258	194, 321	290	243, 338	0.43
Maximal length	303	243, 363	335	289, 380	0.42
Average length	280	218, 342	312	266, 359	0.42
Mismatches converted	15.1	11.3, 18.9	16.7	13.9, 19.5	0.50
Meiotic conversion tracts					
Minimal length	204	152, 256	339	288, 390	0.0006
Maximal length	255	206, 305	382	334, 430	0.0006
Average length	230	179, 281	361	312, 410	0.0006
Mismatches converted	11.9	8.8, 15.0	19.3	16.0, 22.6	0.002

The mean value and the 95% confidence interval (CI) are presented for each parameter. A Student's *t*-test was performed for each parameter to compare the mean value of the wild-type group to that of the *msh2*Δ group; the *P* value is reported. *n* denotes the number of conversion tracts included in each analysis. The minimal, maximal, and average conversion tract lengths, as well as the number of mismatches converted, were determined according to the sister chromatid conversion model. The minimal extent of a given conversion tract was calculated as the sum of conversion tracts on each side of the invertible *HIS3::intron* segment; these tracts were individually calculated by subtracting from 350 bp the position of the most-distal mismatch converted with respect to the invertible segment. The maximal length of the same conversion tract was determined by adding to the minimal length the lengths of the identity intervals that flanked the minimal tract on either side. The average length of a given conversion tract was calculated as the mean of minimal and maximal lengths. The number of mismatches converted was manually counted for each recombinant according to the sister chromatid conversion model.

[a] $n = 30$ for mitotic and meiotic conversion tracts.

[b] $n = 57$ for mitotic conversion tracts; $n = 30$ for meiotic conversion tracts.

matid conversion involves pairing of two pairs of substrates, one on either side of the invertible segment.

Given the same conversion tract endpoints, the calculation of conversion tract length is very different depending on whether a recombination event occurs through the intrachromatid crossover pathway *vs.* the sister chromatid conversion pathway (see Figure 4). Although intrachromatid crossover and sister chromatid conversion are genetically indistinguishable, we have argued previously that most of the His^+ recombinants selected by our inverted repeat system arise via the sister chromatid conversion pathway (Chen and Jinks-Robertson 1998). Using the experimentally determined conversion tract endpoints for each mitotic or meiotic recombinant, the corresponding conversion tract length was calculated according to the sister chromatid conversion model. As for the conversion tract endpoint analysis, only recombinants with continuous conversion tracts or no conversion tracts were included in the calculation of conversion tract lengths. It should be noted that a "no conversion" event corresponds to a sister chromatid conversion in which the endpoints on either side of the *HIS3::intron* invertible segment are in the same interval (*e.g.*, an event that initiates in interval 191–219 on one side of the invertible segment, proceeds through the invertible segment, and ends in interval 191–219 on the other side of the invertible segment).

Table 4 presents the mean values of the minimal, maximal, and average conversion tract lengths, as well as the average number of mismatches converted. The mitotic gene conversion tracts in the wild-type diploid strain averaged 275 bp, which agrees very well with the 280-bp average length reported in haploid cells (Chen and Jinks-Robertson 1998). In *msh2*Δ diploid cells, the average mitotic tract length was 312 bp, which is slightly, but not significantly, longer than the tract length in wild-type diploid cells. This is in contrast to the statistically significant lengthening of tracts (from 275 bp to 385 bp) observed previously by us in a *msh2*Δ *msh3*Δ haploid strain (Chen and Jinks-Robertson 1998). One possible reason for the tract length difference in the *msh2*Δ diploid cells *vs.* the *msh2*Δ *msh3*Δ haploid cells is that Msh3p might be involved specifically in regulating conversion tract length independently of Msh2p. We tested this possibility by analyzing conversion tracts in a *msh2*Δ haploid strain isogenic to the previously used *msh2*Δ *msh3*Δ haploid (data not shown). The average conversion tract length in the *msh2*Δ haploid was 349 bp, which is significantly different from the 275 bp length in wild-type haploid cells ($P < 0.05$ by Student's *t*-test) but not significantly different from the 385-bp average length in the *msh2*Δ *msh3*Δ haploid cells ($P = 0.33$ by Student's *t*-test). We thus are left with the speculation that the discrepancy between our haploid and diploid results

may reflect a haploid/diploid difference in recombination or could reflect the fact that the haploid and diploid strains are not isogenic.

The average length of meiotic conversion tracts was 204 bp in the wild-type diploid strain *vs.* 339 bp in the *msh2*Δ diploid strain. This 65% difference in meiotic tract lengths is statistically significant and suggests that the MMR machinery regulates the extent of heteroduplex formation during meiosis. Although mitotic tracts were longer than meiotic tracts in wild-type cells (280 bp *vs.* 230 bp) and meiotic tracts were longer than mitotic tracts in *msh2*Δ cells (361 bp *vs.* 312 bp), neither of these differences is statistically significant.

DISCUSSION

An intron-based IR assay system was used to examine mitotic and meiotic recombination rates between identical and mismatched sequences in both MMR-competent and MMR-defective yeast strains. Recombination rates were measured in isogenic wild-type, *msh2*Δ, and *pms1*Δ diploid strains, thus allowing a direct comparison of the impact of sequence divergence on mitotic *vs.* meiotic recombination events. In addition, recombination products derived from 94%-identical substrates were sequenced to estimate the extent of meiotic *vs.* mitotic heteroduplex formation in wild-type and *msh2*Δ strains. It should be noted that the substrates in all strains were present on only one copy of chromosome *V*, which limits detectable recombinants to intrachromosomal events and precludes the production of recombinants via recombination between homologs.

Recombination rates between mismatch-containing substrates in wild-type cells: The rates of mitotic and meiotic recombination were measured between cβ substrates varying in identity from 82% to 100% (see Figure 2 for substrate alignments). Recombination rates are given in Table 2, and all recombination rates obtained with a strain of a given genotype (wild-type, *msh2*Δ, or *pms1*Δ) were normalized to the rate obtained with 100%-identical cβ2a control substrates. These normalized data are presented graphically in Figure 5 to more easily compare and contrast mitotic and meiotic recombination rates in the three strain backgrounds used. In a wild-type strain, the presence of one or three mismatches in the recombination substrates reduced mitotic recombination more than meiotic recombination (see inset in Figure 5). Although the mitotic *vs.* meiotic differences are subtle, they suggest that mismatches in meiotic heteroduplex intermediates are either recognized less efficiently than those in mitotic intermediates, or that once recognized, meiotic mismatches have a less negative impact on the overall recombination process. Alternatively, one could hypothesize that meiotic heteroduplex is shorter than mitotic heteroduplex and, therefore, less likely to include the mismatch(es) that trigger the MMR-associated antirecombination activity. The conversion

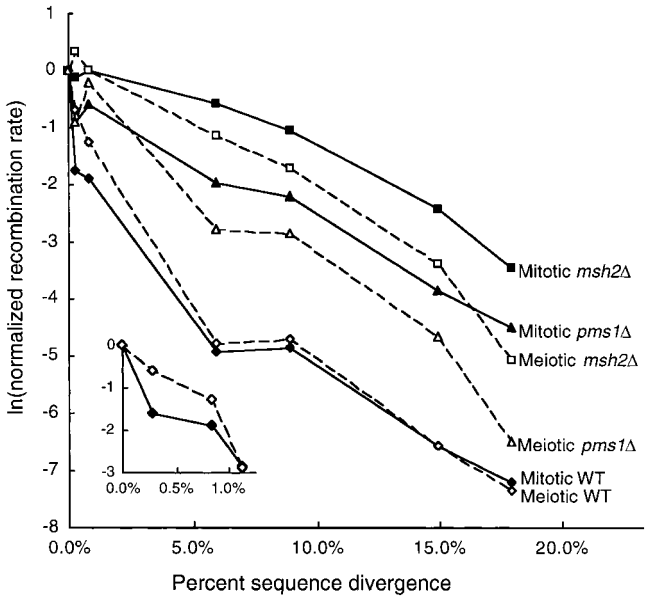

Figure 5.—Relationship between recombination rates and sequence divergence. The natural logarithms of the normalized recombination rates in Table 2 are plotted as a function of the percentage of sequence divergence. The mitotic or meiotic normalized rates for each strain genotype were obtained by dividing each rate by the rate obtained with the 100% identical control substrates.

tract length analysis indicates, however, that similar extents of heteroduplex are formed in mitosis and meiosis. With four or more mismatches, the normalized levels of mitotic and meiotic recombination rates for a given level of substrate identity were indistinguishable in a wild-type background. If one assumes that mismatches have a cumulative negative effect on recombination, then the probability of escaping the antirecombination activity of the MMR machinery will become essentially zero at some level of divergence, and the inclusion of additional mismatches will not further impact recombination. We suggest that the probability that a given mismatch triggers the antirecombination activity of MMR proteins is less in meiosis than in mitosis, but that the cumulative effect of multiple mismatches in both cases is to eventually trigger antirecombination with the same efficiency. The net result would be less inhibition of meiotic recombination than mitotic recombination by one or a few mismatches, but similar levels of inhibition by higher levels of sequence divergence.

The differential effect of a very low level of sequence divergence on the overall efficiency of mitotic *vs.* meiotic recombination in wild-type cells makes biological sense if one considers the relative roles of recombination in mitosis *vs.* meiosis. Recombination is responsible for the repair of double-strand breaks (DSBs) generated by random DNA damage in mitosis and by nonrandom enzymatic cleavage in meiosis. Although mitotic ectopic recombination between nonhomologous chromosomes occurs at about the same level as that between allelic

sequences on homologous chromosomes, intrachromosomal interactions (both sister chromatid and intrachromatid) are much more efficient than interchromosomal interactions in mitosis (Fabre *et al.* 1984; Lichten and Haber 1989; Kadyk and Hartwell 1992; Jinks-Robertson *et al.* 1993). It has been argued that recombination between sister chromatids should be favored over interchromosomal interactions to ensure that mitotic DNA repair occurs with very high fidelity (sister chromatids are identical) and also to lower the occurrence of deleterious chromosome rearrangements. We suggest that the very stringent identity requirements imposed by the yeast MMR machinery in mitosis would discourage nonsister interactions and thereby further strengthen the natural bias for sister chromatid interactions. In meiosis, the bias for recombination between homologs *vs.* intrachromosomal interactions is reversed (Haber *et al.* 1984; Game *et al.* 1989; Schwacha and Kleckner 1997). This reversal of the bias may be related to formation of the synaptonemal complex between homologs, which could serve either to encourage allelic interactions, or to discourage both intrachromosomal interactions and interactions between nonhomologous chromosomes. Because meiotic recombination generally provides an essential physical link between homologous chromosomes that ensures their proper disjunction, maintaining a high efficiency of meiotic recombination in the presence of subtle sequence polymorphisms between homologs is crucial. We suggest that the relaxed identity requirements observed here with very small amounts of sequence divergence may aid in promoting the requisite nonsister interactions in meiosis. Alternatively, the differential effect of low levels of sequence divergence could reflect a switch from mismatch-triggered heteroduplex rejection in mitosis to mismatch-triggered heteroduplex repair in meiosis.

Recombination rates between mismatch-containing substrates in MMR-defective cells: The effect of sequence divergence on mitotic *vs.* meiotic recombination was examined in two different types of MMR-defective diploid strains: *msh2*Δ and *pms1*Δ. Whereas strains deleted for *MSH2* (the MutS homolog essential for all mismatch repair) should have no mismatch binding activity, *in vitro* studies indicate that mismatch recognition can occur in the absence of the MutL homologs Pms1p and Mlh1p (Habraken *et al.* 1997). To delineate the antirecombination role of the MMR machinery (factors other than the MMR machinery may affect recombination in a mismatch-dependent manner), an "MMR index" was calculated for each pair of substrates. The MMR index is defined here as the ratio of normalized recombination rate measured in the *msh2*Δ or *pms1*Δ strain to that measured in the wild-type, MMR-competent strain. As shown in Figure 6, both the *msh2*Δ and the *pms1*Δ mitotic and meiotic MMR indices increased initially with increasing sequence divergence, demonstrating that the MMR machinery actively inhibits recombination in a mismatch-dependent manner. Although mismatches had a cumulative negative effect on recombination in the presence of the MMR machinery, the increases in the MMR indices eventually plateau. As noted above, this behavior suggests that after a critical number of mismatches has been sensed by the MMR machinery, the probability of escaping the antirecombination activity of the MMR proteins is zero and the presence of additional mismatches is inconsequential. Although the plateau is not evident with substrates having <94% identity (21 mismatches), we have no substrates in the 4-to-21 mismatch range so the exact point(s) where the plateau occurs is not clear. It should be noted that these observations are very similar to those reported previously by Datta *et al.* (1997).

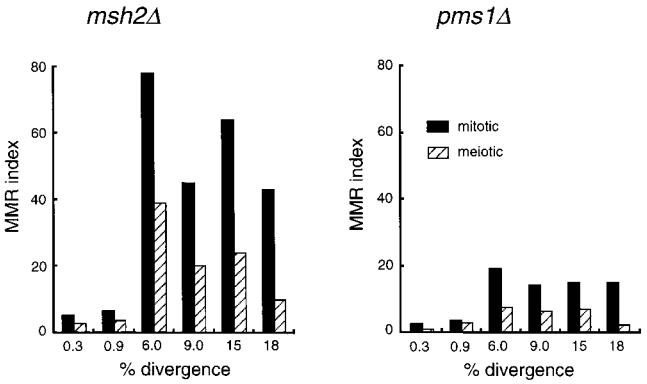

Figure 6.—MMR indices. The MMR index for a given level of substrate identity in a MMR-defective strain (either *msh2*Δ or *pms1*Δ) was calculated by dividing the normalized recombination rate for the mutant strain by that obtained for the wild-type, MMR-competent strain. The normalized recombination rates are the rates obtained in a given genetic background divided by the rate for the 100% control substrates in the same genetic background.

The mitotic MMR index is greater than the meiotic index for every pair of substrates examined in both the *msh2*Δ and the *pms1*Δ strains. This observation suggests that the maximal antirecombination activity of the yeast MMR machinery is more efficient in mitosis than in meiosis. It may be, for example, that, regardless of the number of mismatches present, 5% of all meiotic heteroduplexes "escape" the antirecombination activity of the MMR machinery, whereas only 1–2% of all mitotic heteroduplexes escape this activity. Alternatively, a larger percentage of meiotic recombinants may be produced through a mechanism that does not involve extensive heteroduplex formation. Gap repair, for example, does not involve extensive heteroduplex formation. Another example of such a mechanism has been proposed by Resnick and colleagues (Porter *et al.* 1996) and involves the production of recombination intermediates by replicative extension of an invading 3′ end (thus generating true homoduplex) rather than by continued assimilation of the (mismatched) invading strand.

We previously reported that *msh2*Δ strains exhibit higher levels of mitotic recombination between diverged substrates than do isogenic *pms1*Δ strains (Datta *et al.* 1996). The data summarized in Figures 5 and 6 confirm the mitotic observation and demonstrate that meiotic recombination behaves similarly. The normalized values of mitotic and meiotic recombination between diverged substrates were uniformly higher in a *msh2*Δ background than in a *pms1*Δ background (Figure 5) and the MMR indices were consistently larger for the *msh2*Δ strains than for the *pms1*Δ strains (Figure 6). Thus, although the yeast MutL homolog Pms1p is apparently indispensable for the repair of DNA replication errors (reviewed in Crouse 1998), it is not absolutely required for the antirecombination activity of the MMR machinery in either mitosis or meiosis. This is not to imply that the yeast MutL homologs have no antirecombination role, but rather that some inhibition of recombination occurs in their absence. We suggest that mismatch binding by MutS homologs in the absence of MutL homologs may be sufficient to impede recombination, whereas such binding is apparently unable to trigger repair of DNA replication errors. The specific requirement of the MutL homologs for the repair of replication errors may be related to their interactions with proliferating cell nuclear antigen (PCNA; Johnson *et al.* 1996; Umar *et al.* 1996), which have been speculated to link MMR directly to the process of DNA replication.

For both the *msh2*Δ and *pms1*Δ strains, meiotic recombination was more negatively impacted by sequence divergence than was mitotic recombination (Figure 5). The slightly steeper slopes for the plots of the meiotic *vs.* mitotic data suggest that the MEPS for meiotic recombination may be longer than that for mitotic recombination. That is, a greater length of perfect identity may be required to successfully initiate meiotic recombination than to initiate mitotic recombination. Once initiation occurs, however, mismatches that become incorporated into a heteroduplex intermediate would become potential targets of the MMR-associated antirecombination activity.

Gene conversion tracts: Recombination products derived using the 94%-identical substrates in both wild-type and *msh2*Δ strains were sequenced to determine the endpoints and estimate the lengths of mitotic and meiotic conversion tracts (Figure 3 and Table 4, respectively). It is assumed in analyses of this sort that conversion tracts are accurate representations of the extent of heteroduplex formed in MMR-competent cells. We acknowledge the possibility, however, that a conversion tract border may not always correspond to the extent of the underlying heteroduplex intermediate, but rather may reflect the border of mismatch correction. The most notable feature of conversion tracts in the wild-type strain is that intervals close to the invertible *HIS3::intron* segment showed an excess of endpoints, while the more distal intervals exhibited a deficit of endpoints. A similar, but more pronounced, pattern of endpoint distribution was observed previously using one of the wild-type haploid parents of the diploid used in this study (Chen and Jinks-Robertson 1998). In the earlier study, we systematically addressed possible explanations for the endpoint distribution by altering the substrates in defined ways. Most notably, reversal of the orientations of both substrates with respect to the invertible *HIS3::intron* segment reversed the distribution of endpoints so that intervals proximal (and formally distal) to the invertible *HIS3::intron* segment still contained an excess of endpoints. We concluded that the proximity of an interval to the *HIS3::intron* segment was the primary determinant of the proportion of endpoints likely to be located in that interval, regardless of the length or sequence of the interval. Because the same intron-based system was used in this study, we suggest that the distributions of mitotic and meiotic conversion tract endpoints in a wild-type strain (Figure 3, A and B, respectively) similarly reflect the relative proximity of intervals to the invertible *HIS3::intron* segment. In contrast to the results obtained with an MMR-competent strain, the distributions of mitotic and meiotic conversion tracts in a *msh2*Δ strain lacked an obvious clustering of endpoints (compare Figure 3A to 3C, and 3B to 3D). A similar observation was made by us using both a haploid *msh2 msh3* strain and a haploid *pms1* strain (Chen and Jinks-Robertson 1998).

We argued previously (Chen and Jinks-Robertson 1998) that a sister chromatid conversion model could more readily account for the observed conversion tract endpoint distributions than could an intrachromatid crossover model (Figure 4). According to the sister chromatid conversion model, each recombination event that successfully reorients the invertible *HIS3::intron* segment must initiate within substrates on one side of the invertible segment, extend through the invertible segment, and resolve within the substrates on the other side of the invertible segment. The endpoint clustering evident in a wild-type strain thus reflects a bidirectional gene conversion gradient that extends in both directions from the selected site of conversion (the invertible segment). Using the assumption of sister chromatid conversion, the lengths of mitotic and meiotic gene conversion tracts were calculated for events occurring in both wild-type and *msh2*Δ strains (Table 4). As predicted by the endpoint distributions, these analyses indicated that mitotic and meiotic tracts were longer in a *msh2*Δ strain than in a wild-type strain, although the length difference was only significant for meiotic tracts.

The conversion tract data strongly implicate the MMR machinery in determining the distribution of conversion tract endpoints in recombination events involving diverged sequences. We suggest that these data are relevant to the antirecombination activity of MMR proteins and indicate that recombination intermediates are tar-

geted by the MMR machinery. The recombination products observed in a wild-type strain presumably are those that escaped the antirecombination activity of the MMR machinery, but nevertheless may provide useful insight into the mechanism of antirecombination. We suggest that the gradient of conversion tract endpoints observed in wild-type cells reflects increasing obstruction of recombination by the MMR machinery as the heteroduplex formation progresses and more and more mismatches are incorporated. One possibility is that mismatches are recognized by the MMR machinery during or immediately after the formation of heteroduplex DNA. Such recognition might block further extension of the heteroduplex intermediate and thereby trigger helicase-driven unwinding of the intermediate.

Conclusions: In MMR-competent cells, mitotic recombination was impacted more than meiotic recombination by a low level of sequence divergence, whereas both types of recombination were similarly impacted by higher levels of divergence. The differential effects of low levels of divergence on recombination were MMR-dependent and might possibly serve to strengthen the biases for mitotic intrachromosomal interactions *vs.* meiotic interchromosomal interactions. In MMR-defective cells, meiotic recombination was impacted more by sequence divergence than was mitotic recombination, suggesting that the MEPS may be slightly longer for meiosis than for mitosis. Recombination rates between nonidentical sequences were consistently higher in *msh2*Δ strains than in *pms1*Δ strains, indicating that the yeast MutS homologs can exert antirecombination activity in the absence of the MutL homolog Pms1p. For both mitotic and meiotic recombination events, low levels of sequence divergence impeded recombination predominantly via action of the MMR machinery whereas higher levels of sequence divergence impeded recombination via action of the MMR machinery plus an additional MMR-independent process. This latter process may reflect a requirement for a minimal length of perfect homology (the MEPS) to successfully initiate a recombination event.

This work was supported by National Institutes of Health grant GM-38464 (S.J.-R.). W.C. was supported in part by the Graduate Division of Biological and Biomedical Sciences and by a National Institutes of Health Medical Scientist Training grant (Emory University, Atlanta, GA).

LITERATURE CITED

Ahn, B.-Y., and D. M. Livingston, 1986 Mitotic gene conversion lengths, coconversion patterns, and the incidence of reciprocal recombination in a *Saccharomyces cerevisiae* plasmid system. Mol. Cell. Biol. **6:** 3685–3693.

Alani, E., R. A. G. Reenan and R. D. Kolodner, 1994 Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. Genetics **137:** 19–39.

Boeke, J. D., J. Trueheart, G. Natsoulis and G. R. Fink, 1987 5-fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175.

Borts, R. H., and J. E. Haber, 1989 Length and distribution of meiotic gene conversion tracts and crossovers in *Saccharomyces cerevisiae*. Genetics **123:** 69–80.

Borts, R. H., W.-Y. Leung, W. Kramer, B. Kramer, M. Williamson *et al.*, 1990 Mismatch repair-induced meiotic recombination requires *PMS1* gene product. Genetics **124:** 573–584.

Chambers, S., N. Hunter, E. J. Louis and R. H. Borts, 1996 The mismatch repair system reduces meiotic homeologous recombination and stimulates recombination-dependent chromosome loss. Mol. Cell. Biol. **16:** 6110–6120.

Chen, W., and S. Jinks-Robertson, 1998 Mismatch repair proteins regulate heteroduplex formation during mitotic recombination in yeast. Mol. Cell. Biol. **18:** 6525–6537.

Claverys, J.-P., and S. A. Lacks, 1986 Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. Microbiol. Rev. **50:** 133–165.

Crouse, G. F., 1998 Mismatch repair systems in *Saccharomyces cerevisiae*, pp. 411–448 in *DNA Damage and Repair, Volume 1: DNA Repair in Prokaryotes and Lower Eukaryotes*, edited by J. A. Nickoloff and M. F. Hoekstra. Humana Press, Totowa, NJ.

Datta, A., A. Adjiri, L. New, G. F. Crouse and S. Jinks-Robertson, 1996 Mitotic crossovers between diverged sequences are regulated by mismatch repair proteins in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 1085–1093.

Datta, A., M. Hendrix, M. Lipsitch and S. Jinks-Robertson, 1997 Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc. Natl. Acad. Sci. USA **94:** 9757–9762.

Davidow, L. S., and B. Byers, 1984 Enhanced gene conversion and postmeiotic segregation in pachytene-arrested *Saccharomyces cerevisiae*. Genetics **106:** 165–183.

De Wind, N., M. Dekker, A. Berns, M. Radman and H. Te Riele, 1995 Inactivation of the mouse *Msh2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell **82:** 321–330.

Fabre, F., A. Boulet and H. Roman, 1984 Gene conversion at different points in the mitotic cycle of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **195:** 139–143.

Game, J. C., K. C. Sitney, V. E. Cook and R. K. Mortimer, 1989 Use of a ring chromosome and pulsed-field gels to study interhomolog recombination, double-strand DNA breaks and sister-chromatid exchange in yeast. Genetics **123:** 695–713.

Haber, J. E., P. C. Thorburn and D. Rogers, 1984 Meiotic and mitotic behavior of dicentric chromosomes in *Saccharomyces cerevisiae*. Genetics **106:** 185–205.

Habraken, Y., P. Sung, L. Prakash and S. Prakash, 1997 Enhancement of MSH2-MSH3-mediated mismatch recognition by the yeast MLH1-PMS1 complex. Curr. Biol. **7:** 790–793.

Harris, S., K. S. Rudnicki and J. E. Haber, 1993 Gene conversions and crossing over during homologous and homeologous ectopic recombination in *Saccharomyces cerevisiae*. Genetics **135:** 5–16.

Hoffman, C. S., and F. Winston, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:** 267–272.

Hunter, N., S. R. Chambers, E. J. Louis and R. H. Borts, 1996 The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. EMBO J. **15:** 1726–1733.

Jinks-Robertson, S., M. Michelitch and S. Ramcharan, 1993 Substrate length requirements for efficient mitotic recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 3937–3950.

Johnson, R. E., G. K. Kivvali, S. N. Guzder, N. S. Amin, C. Holm *et al.*, 1996 Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J. Biol. Chem. **271:** 27987–27990.

Judd, S. R., and T. D. Petes, 1988 Physical lengths of meiotic and mitotic gene conversion tracts in *Saccharomyces cerevisiae*. Genetics **118:** 401–410.

Kadyk, L. C., and L. H. Hartwell, 1992 Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics **132:** 387–402.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kolodner, R., 1996 Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev. **10:** 1433–1442.

Lea, D. E., and C. A. Coulson, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

Lichten, M., and J. E. Haber, 1989 Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. Genetics **123:** 261–268.

Mezard, C., D. Pompon and A. Nicolas, 1992 Recombination between similar but not identical DNA sequences during yeast transformation occurs within short stretches of identity. Cell **70:** 659–670.

Modrich, P., and R. Lahue, 1996 Mismatch repair in replication fidelity, genetic recombination and cancer biology. Annu. Rev. Biochem. **65:** 101–133.

Porter, G., J. Westmoreland, S. Priebe and M. A. Resnick, 1996 Homologous and homeologous intermolecular gene conversion are not differentially affected by mutations in the DNA damage or mismatch repair genes *RAD1, RAD50, RAD51, RAD52, RAD54, PMS1* and *MSH2*. Genetics **143:** 755–767.

Rayssiguier, C., D. S. Thaler and M. Radman, 1989 The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature **342:** 396–401.

Roeder, G. S., 1995 Sex and the single cell: meiosis in yeast. Proc. Natl. Acad. Sci. USA **92:** 10450–10456.

Rothstein, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:** 281–301.

Schwacha, A., and N. Kleckner, 1997 Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell **90:** 1123–1135.

Selva, E. M., L. New, G. F. Crouse and R. S. Lahue, 1995 Mismatch correction acts as a barrier to homeologous recombination in *Saccharomyces cerevisiae*. Genetics **139:** 1175–1188.

Selva, E. M., A. B. Maderazo and R. S. Lahue, 1997 Differential effects of the mismatch repair genes *MSH2* and *MSH3* on homeologous recombination in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **257:** 71–82.

Shen, P., and H. V. Huang, 1986 Homologous recombination in *Escherichia coli*: dependence on substrate length and homology. Genetics **112:** 441–457.

Sherman, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 3–20.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Sweetser, D. B., H. Hough, J. Wheldon, M. Arbuckle and J. A. Nickoloff, 1994 Fine-resolution mapping of spontaneous and double-strand break-induced gene conversion tracts in *Saccharomyces cerevisiae* reveals reversible mitotic conversion polarity. Mol. Cell. Biol. **14:** 3863–3875.

Symington, L. S., and T. D. Petes, 1988 Expansions and contractions of the genetic map relative to the physical map of yeast chromosome III. Mol. Cell. Biol. **8:** 595–604.

Umar, A., A. B. Buermeyer, J. A. Simon, D. C. Thomas, A. B. Clark *et al.*, 1996 Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell **87:** 65–73.

Vulic, M., F. Dionisio, F. Taddei and M. Radman, 1997 Molecular keys to speciation: DNA polymorphism and the control of genetic exchange in enterobacteria. Proc. Natl. Acad. Sci. USA **94:** 9763–9767.

Worth, L., Jr., S. Clark, M. Radman and P. Modrich, 1994 Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. Proc. Natl. Acad. Sci. USA **91:** 3238–3241.

Zahrt, T. C., and S. Maloy, 1997 Barriers to recombination between closely related bacteria: MutS and RecBCD inhibit recombination between *Salmonella typhimurium* and *Salmonella typhi*. Proc. Natl. Acad. Sci. USA **94:** 9786–9791.

Zahrt, T. C., G. C. Mora and S. Maloy, 1994 Inactivation of mismatch repair overcomes the barrier to transduction between *Salmonella typhimurium* and *Salmonella typhi*. J. Bacteriol. **176:** 1527–1529.

Zawadzki, P., M. S. Roberts and F. M. Cohan, 1995 The log-linear relationship between sexual isolation and sequence divergence in Bacillus transformation is robust. Genetics **140:** 917–932.

Communicating editor: M. Lichten