Regulation of Mitotic Homeologous Recombination in Yeast: Functions of Mismatch Repair and Nucleotide Excision Repair Genes

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ABSTRACT

The *Saccharomyces cerevisiae* homologs of the bacterial mismatch repair proteins MutS and MutL correct replication errors and prevent recombination between homeologous (nonidentical) sequences. Previously, we demonstrated that Msh2p, Msh3p, and Pms1p regulate recombination between 91% identical inverted repeats, and here use the same substrates to show that Mlh1p and Msh6p have important antirecombination roles. In addition, substrates containing defined types of mismatches (base-base mismatches; 1-, 4-, or 12-nt insertion/deletion loops; or 18-nt palindromes) were used to examine recognition of these mismatches in mitotic recombination intermediates. Msh2p was required for recognition of all types of mismatches, whereas Msh6p recognized only base-base mismatches and 1-nt insertion/deletion loops. Msh3p was involved in recognition of the palindrome and all loops, but also had an unexpected antirecombination role when the potential heteroduplex contained only base-base mismatches. In contrast to their similar antimutator roles, Pms1p consistently inhibited recombination to a lesser degree than did Msh2p. In addition to the yeast MutS and MutL homologs, the exonuclease Exo1p and the nucleotide excision repair proteins Rad1p and Rad10p were found to have roles in inhibiting recombination between mismatched substrates.

MISMATCH repair (MMR) systems are highly conserved evolutionarily and have important functions in maintaining eukaryotic genome stability (Modrich and Lahue 1996). The MMR proteins not only reduce mutation frequencies by correcting replication errors resulting from nucleotide misincorporation and polymerase slippage, but they also have important antirecombination activities due to their ability to recognize mismatches in recombination intermediates. Eukaryotic MMR systems contain proteins homologous to the well-characterized *Escherichia coli* MMR proteins MutS and MutL (Kolodner 1996; Modrich and Lahue 1996). In *E. coli* MMR, MutS recognizes and binds to mismatches in DNA. MutL interacts with MutS and also with MutH, a protein that recognizes hemi-methylated *dam* sites and thus provides a mechanism for distinguishing between nascent and template strands during DNA replication. Following incision of the nascent strand by MutH, the nicked strand is removed by the combined action of exonucleases and the UvrD helicase, and the resulting gap is filled in by DNA polymerase III (Modrich and Lahue 1996).

In contrast to the single MutS protein in *E. coli*, there are six MutS homologs in yeast (Crouse 1998). Studies of mutation spectra and *in vitro* binding assays indicate that Msh2p is required for repair of all types of mismatches, and that it functions as a heterodimer with either Msh3p or Msh6p. The repair of base-base mismatches appears to be solely dependent on Msh2p/Msh6p and thus is independent of Msh3p (Marsischky *et al.* 1996; Earley and Crouse 1998). On the other hand, *msh3* and *msh2* strains are equally defective in the repair of replication errors that result in loops of four nucleotides or larger, whereas an *msh6* strain has no repair defect for these types of errors (Sia *et al.* 1997). In assays that detect repair of small loops, *msh3* or *msh6* strains exhibit a weak repair defect, whereas *msh3 msh6* double mutants exhibit a very strong, synergistic repair defect equivalent to the repair defect of *msh2* mutants (Johnson *et al.* 1996a; Marsischky *et al.* 1996; Greene and Jinks-Robertson 1997). Heterodimers of either Msh2p/Msh3p or Msh2p/Msh6p thus appear to compete for the repair of small 1- to 2-nucleotide (nt) loops. MutS homologs also have been found to recognize several DNA structures that are intermediates during recombination, including Holliday junctions (Alani *et al.* 1997; Marsischky *et al.* 1999) and branched structures with free 3′ ends (Sugawara *et al.* 1997). Of the three remaining MutS homologs, Msh1p is involved in maintaining the stability of the mitochondrial genome (Reenan and Kolodner 1992), while Msh4p and Msh5p are involved in promoting meiotic interhomolog crossovers (Ross-MacDonald and Roeder 1994; Hollingsworth *et al.* 1995).

In addition to the multiple MutS homologs, there are four MutL homologs in yeast (Crouse 1998). Mlh1p and Pms1p form a heterodimer (Prolla *et al.* 1994a) and are assumed to associate with Msh2p/Msh3p or

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Msh2p/Msh6p heterodimers during nuclear mitotic processes. These MutL homologs are required for repair of replication errors, and disruption of either or both results in the same mutator phenotype as seen in *msh2* strains (Prolla *et al.* 1994b). The MutL homolog Mlh3p interacts with Mlh1p and functions with Msh3p to suppress a portion of frameshift errors (Flores-Rozas and Kolodner 1998; B. Harfe, B. Minesinger and S. Jinks-Robertson, unpublished results). Frameshift spectra analysis indicates that Mlh2p also functions with Msh3p to remove specific types of frameshift intermediates (B. Harfe, B. Minesinger and S. Jinks-Robertson, unpublished results). The helicase(s) and exonucleases involved in yeast MMR have not been characterized fully, although the 5′ to 3′ exonuclease Exo1p has been implicated in mismatch repair (Fiorentini *et al.* 1997; Tishkoff *et al.* 1997), as have the 3′ to 5′ exonuclease activities of DNA polymerases ε and δ (Tran *et al.* 1999).

The MMR machinery has a role not only in removing mutation intermediates; it also recognizes and acts upon mismatches in heteroduplex recombination intermediates derived from parental DNA sequences that are similar but not identical (homeologous sequences). Homeologous substrates recombine much less efficiently than do identical substrates and much of this reduction in recombination is due to the antirecombination activity of the MMR system. It has been shown that disruption of MMR genes is accompanied by increased rates of homeologous recombination in bacteria (Rayssiguier *et al.* 1989; Humbert *et al.* 1995; Abdulkarim and Hughes 1996; Zahrt and Maloy 1997; Majewski and Cohan 1998), yeast (Selva *et al.* 1995; Datta *et al.* 1996; Negritto *et al.* 1997), and mammalian cells (de Wind *et al.* 1995; Ciotta *et al.* 1998). In yeast, the MMR system is exquisitely sensitive to the presence of mismatches in recombination intermediates, as a single base-base mismatch is sufficient to inhibit recombination (Datta *et al.* 1997; Chen and Jinks-Robertson 1999).

In addition to their mismatch recognition role, Msh2p and Msh3p function with the Rad1p/Rad10p endonuclease complex in regulating recombination between direct repeats (Saparbaev *et al.* 1996). Specifically, these proteins have been shown to be important in the removal of nonhomologous ends (Pâques and Haber 1997; Sugawara *et al.* 1997). Recently, repair of certain meiotic recombination intermediates has been shown to involve both MMR proteins and Rad1p in yeast (Kirkpatrick and Petes 1997) or the *RAD1* homolog *mei-9* in Drosophila (Sekelsky *et al.* 1995). In addition, the *Schizosaccharomyces pombe* homologs of Rad1p and Rad10p (Swi10p and Rad16p, respectively) have been found to operate in a Msh2p/Pms1p-independent pathway that removes C-C mispairs (Fleck *et al.* 1999). Although Rad1p and Rad10p clearly have roles in recombination and MMR, they have been best characterized in terms of their role in removal of UV damage via the nucleotide excision repair (NER) pathway. In the yeast NER pathway (Sweder 1994), UV damage is recognized by the Rad14 protein and incisions are made 5′ and 3′ of the damage by the Rad1p/Rad10p and Rad2p endonucleases, respectively. A helicase then removes an oligonucleotide containing the lesion, and DNA polymerase repairs the gap.

In this study we further examine the roles of individual MMR proteins in regulating homeologous recombination in yeast, as well as possible roles of Exo1p and representative NER proteins in this process. Although the antirecombination roles of Msh2p, Msh3p, and Pms1p have been established (Selva *et al.* 1995; Datta *et al.* 1996), the activity of Msh6p or Mlh1p during homeologous recombination has not been reported. In this work we examine the impact of Msh6p and Mlh1p on recombination between 91% identical sequences oriented as inverted repeats. In addition, inverted repeat substrates containing a small number of defined mismatches are used to define the recombination-associated recognition specificities of MutS homologs Msh2p, Msh3p, and Msh6p, and the MutL homolog Pms1p. Finally, the roles of NER proteins Rad1p, Rad2p, Rad10p, and Rad14p and the exonuclease Exo1p in regulating recombination between nonidentical substrates are examined.

MATERIALS AND METHODS

Media and growth conditions: All incubations were done at 30°. Nonselective media contained 1% yeast extract and 2% bacto-peptone, as well as 2.5% agar for plates. YEP medium was supplemented with either 4% galactose and 2% glycerol (YEPGG) or 2% dextrose (YEPD) as appropriate. For liquid growth, 0.25 g of adenine was added to each liter after autoclaving. When selection for G418 resistance was required, Geneticin (Sigma, St. Louis) was added to YEPD plates to a final concentration of 0.2%.

Synthetic dextrose (SD) minimal medium contained 0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% dextrose, and 2.5% agar. Ura^- segregants were identified on SD medium supplemented with a complete amino acid mix and 0.1% 5-fluoroorotic acid (5FOA; Boeke *et al.* 1984). For selection of His^+ recombinants, a histidine-deficient amino acid mix was added, and the dextrose in minimal medium was replaced with 2% galactose and 2% glycerol (SG-his medium). SD-Ura plates contained SD medium supplemented with a uracil-deficient amino acid mix.

Plasmid constructions: pAB61 is a *LEU2*-marked integrating plasmid that contains the cβ2/cβ2 inverted repeat (IR) substrates (Figure 1). This plasmid was constructed by ligating the 5.6-kb *Aat*II/*Ngo*MI fragment of pSR406 (Datta *et al.* 1996) to the 2.9-kb *Aat*II/*Ngo*MI vector backbone fragment of pRS305 (Sikorski and Hieter 1989). pAB63 contains the cβ2/cβ7 (91% identical) IR substrates, and was derived from pAB61 by replacing the cβ2 sequence in the 3′ cassette with cβ7 sequence. This was accomplished by ligating the 3.3-kb *Spe*I/*Sca*I fragment of pSR407 (Datta *et al.* 1996) to the 5.1-kb *Spe*I/*Sca*I vector fragment of pAB61.

A 7.4-kb *Xho*I fragment of pAB61 was circularized to form pAB62, which contains only the 5′ portion of the inverted repeat construct. This plasmid was used as the substrate for site-directed mutagenesis with the Chameleon double-stranded

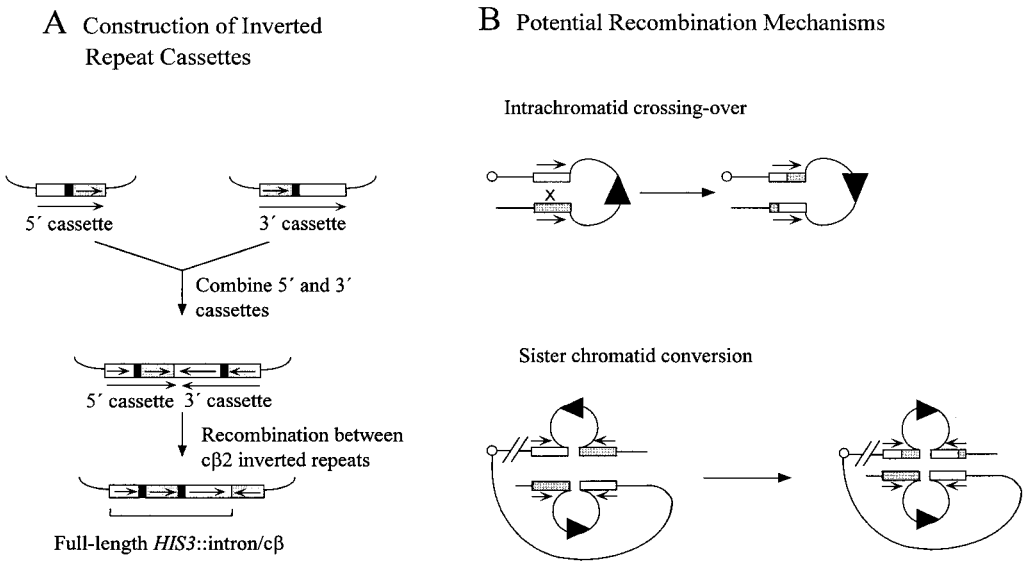

Figure 1.—The IR recombination system. (A) Construction of IR substrates from 5′ and 3′ cassettes (Datta *et al.* 1996). Open boxes represent the *HIS3* selectable marker sequence, solid boxes the intron sequences, and shaded boxes the recombination substrates. Recombination events that reorient the segment between the substrates are identified as His^+ colonies. (B) Recombination between the inverted recombination substrates (open and shaded boxes), by either a sister chromatid conversion process or an intrachromatid crossover (Chen and Jinks-Robertson 1998), flips the 3′ end of a selectable marker gene, represented here as the region with a large arrowhead between the substrates.

site-directed mutagenesis kit (Stratagene, La Jolla, CA), which utilizes a selection primer in addition to one or more mutagenic primers. Site-directed mutagenesis of double-stranded pAB62 was inefficient, so all mutagenesis was done using single-stranded DNA as template.

Two or four mutagenic primers were used to derive each substrate shown in Figure 2. Each primer was designed so that the resulting mutation created and/or destroyed a restriction site, and so that every substrate contained mutations at approximately the same locations (Figure 2). The mutations are described by their coordinate position [position 1 corresponds to position 690 in the published sequence (Sullivan *et al.* 1985)] and the resulting DNA modification. Although insertion of a loop shifts the positions of downstream mutations, coordinate positions are determined based on the assumption that the designated mutation is the only mutation in the substrate. cβ2-ns was created by making the following mutations: 62 C → T (destroys an *Fsp*I site), 157 A → G (creates an *Apa*I site), 231 A → G (creates a *Not*I site), and 281 A → G (creates an *Nco*I site). cβ2-1L, containing four 1-nt additions of G, contains the following mutations: 61 + G (destroys an *Fsp*I site), 151 + G (creates a *Kpn*I site), 231 + G (creates a *Not*I site), and 280 + G (creates a *Nco*I site). To create cβ2-4L, four additions of GATC were made to cβ2: 61 + GATC (creates a *Bam*HI site), 149 + GATC (creates a *Pvu*I site), 231 + GATC (creates a *Pvu*I site), and 277 + GATC (creates a *Bgl*II site). Two 12-bp insertions were introduced into cβ2 to create cβ2-12L: 62 + AAGAGTTCAGGC (destroys an *Fsp*I site) and 231 + AGGTCCTATGAT (destroys an *Eag*I site). The final substrate, cβ2-pal, contains two 18-nt palindromes which can form a hairpin (Nag and Petes 1991): 61 + AGTACTGTACAG TACTCG (destroys an *Fsp*I site and creates a *Bsr*GI site), and 233 + AGTACTGTACAGTACTCG (destroys an *Eag*I site and creates a *Bsr*GI site).

After identifying candidates with the desired combination of restriction sites, both strands of the mutant cβ2 substrate were sequenced. The plasmids that resulted from this process were pAB88 (cβ2-4L), pAB92 (cβ2-ns), pSR534 (cβ2-12L), pSR558 (cβ2-1L), and pSR533 (cβ2-pal). Each of these plasmids was digested with *Spe*I and *Ngo*MI, and the resulting 1.9-kb fragment was inserted into the 6.6-kb *Spe*I/*Ngo*MI vector fragment of pAB61. This replaces the 5′ cβ2 segment of pAB61 with each of the mutagenized cβ2 segments. The resulting

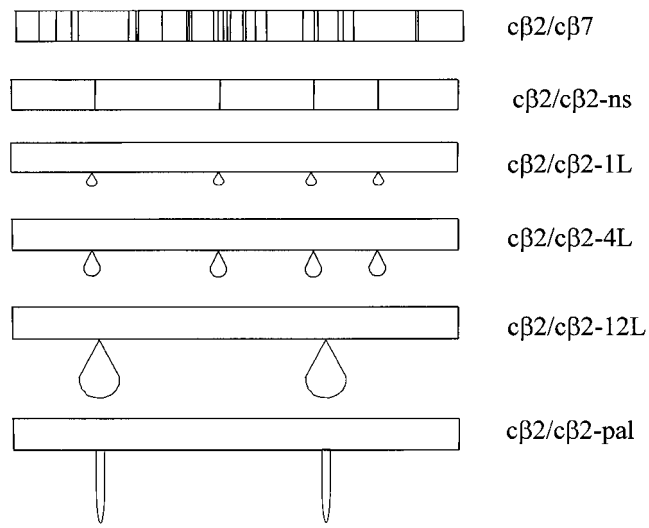

Figure 2.—Recombination substrates. Potential mismatches are represented as either vertical lines for base-base mismatches, or as loops for insertion/deletion mismatches. cβ2/cβ7 was derived from chicken β-tubulin cDNA isoforms 2 and 7; it contains 9% nucleotide substitutions (Datta *et al.* 1996). cβ2/cβ2-ns contains four A to G or C to T nucleotide substitutions; cβ2/cβ2-1L has four 1-nt loops; cβ2/cβ2-4L has four 4-nt loops; cβ2/cβ2-12 contains two 12-nt loops of random sequence; and cβ2/cβ2-pal has two 18-nt palindromic inserts that should form a hairpin (Nag and Petes 1991). Positions of the mismatches are depicted to scale.

plasmids were pAB91 (cβ2/cβ2-4L), pAB96 (cβ2/cβ2-ns), pSR538 (cβ2/cβ2-1L), pSR539 (cβ2/cβ2-12L), and pSR560 (cβ2/cβ2-pal).

p*pms1*Δ was constructed by N. Yu from a plasmid containing a *Bgl*II/*Sal*I *PMS1* fragment in the pIC19R vector (Marsh *et al.* 1984). *PMS1* sequence from the *Mlu*I site to the *Sac*I site was removed and the *hisG-URA3-hisG* cassette (Alani *et al.* 1987) was inserted. pΔ*rad14* contains the *hisG-URA3-KAN-hisG* cassette (Earley and Crouse 1996) in the *Hin*dIII/*Bsr*GI sites of a 2.1-kb *RAD14* PCR product (the primers were 5′-CGGGATCCATAATGGGATACTTCGT-3′ and 5′-GCTCTA GATATAACCAAACAGAA-3′) cloned into the *Pvu*II site of pMTL22 (Chambers *et al.* 1988).

Strain constructions: All strains were derived from SJR328 (*MAT*α *ade2-101 his3*Δ*200 ura3-Nhe lys2*Δ*RV::hisG leu2-R*). The IR cassette plasmids were targeted to the *LEU2* locus on chromosome III by digestion with *Eco*RV, and Leu$^+$ transformants were selected. Southern analysis of candidate strains was done to ensure that only a single copy of the plasmid had integrated at the correct locus.

Following introduction of the IR cassette plasmids into yeast, individual MMR or NER genes were disrupted in one of two ways. The majority of strains were constructed using a one-step disruption plasmid, which was digested with appropriate restriction enzymes and transformed via a lithium acetate protocol into yeast (Ito *et al.* 1983). All transformants were selected on SD-Ura plates. *MSH2* was disrupted by transformation with *Aat*II/*Xba*I-digested pΔ*msh2* (Earley and Crouse 1998), *MSH3* by transformation with *Afl*II/*Msc*I-digested pΔ*msh3* (Earley and Crouse 1998), *MSH4* by transformation with *Eco*RI/*Bam*HI-digested p61 (*msh4*Δ*::URA3*; Ross-MacDonald and Roeder 1994), *MSH5* strains by transformation with *Eco*RI/*Cla*I-digested pNH190-11 (*msh5::URA3*; Hollingsworth *et al.* 1995), *MSH6* by transformation with *Eco*RI/*Sac*I-digested *Msh6*pHUH (Kramer *et al.* 1996), *MLH1* by transformation with *Bam*HI/*Sac*I-digested pmlh1::*URA3* (Prolla *et al.* 1994b), *PMS1* by transformation with *Bam*HI/*Bgl*II-digested p*pms1*Δ, *RAD1* by transformation with *Eco*RI/*Sal*I-digested pR1.6 (Higgins *et al.* 1983), *RAD10* by transformation with *Sal*I/*Bgl*II-digested pMT11-RAD10::URA3 (Weiss and Friedberg 1985), and *RAD14* by transformation with *Pvu*II-digested pΔ*rad14*. In transformations using the *hisG-URA3-hisG* cassette, deletion of the *URA3* gene was selected on 5FOA medium.

The other method of gene disruption involved PCR amplification of the kanamycin resistance gene from plasmid pFA6-kanMX4 (Wach *et al.* 1994) with primers homologous to the relevant gene, followed by transformation and selection for G418-resistant colonies. *RAD2* was disrupted using primers (sequence for the kanamycin resistance gene is in lowercase) 5′-AGGTTCTACACGTCATCCATGAAGAAAAGCATTTTCG GGAGAAcgccagctgaagcttcgtacgc-3′ (Rad2DISF) and 5′-CT GAGATCTTCAAGATGGCGAAAAATAACGTTGCGCGTGT TTGGGgcataggccactagtggatctg-3′ (Rad2DISR). Disruption of the *EXO1* gene was done using primers 5′-TTGGACCACAT TAAAATAAAAGGAGCTCGAAAAAACTGAAAGGcgccagctg aagcttcgtacgc-3′ (Exo1DISF) and 5′-TTTCGACGAGATTT TCATTTG AAAAATATACCTCCGATATGAAACgcataggcca ctagtggatctg-3′ (Exo1DISR). All gene disruptions were confirmed by PCR and/or Southern analysis.

Fluctuation analysis: Individual colonies were inoculated into 5 ml of YEPGG media, and cultures were grown for 2 days. Appropriate dilutions of cells were plated on YEPD or SG-his medium, and plates were incubated for 3 (YEPD) or 4 days (SG-his) prior to counting colonies. For calculation of recombination rates, the median number of His$^+$ colonies per culture was determined based on 12 cultures (6 cultures for each of two isolates). The method of the median (Lea and Coulson 1949) was used to calculate recombination rate (number of recombinants per generation).

RESULTS

The inverted repeat recombination system: The IR system used here was derived from an intron-containing *HIS3* gene and was constructed by combining 5′ and 3′ cassettes containing either identical or nonidentical substrates (Figure 1). The 5′ cassette contained the 5′ end of *HIS3*, the 5′ portion of the intron, and a recombination substrate. The 3′ cassette contained a second recombination substrate (which can be either identical or nonidentical to the substrate in the 5′ cassette), the 3′ portion of the intron, and the 3′ end of the *HIS3* gene. The 5′ and 3′ cassettes were then combined in inverted orientation on a plasmid, and the entire plasmid was integrated into the yeast genome. Recombination between the substrates reorients the 3′ portion of *HIS3*::intron with respect to the 5′ portion, creating a full-length *HIS3*::intron gene that can be identified by growth on selective medium. The recombination events detectable by the inverted repeat system can result from either a sister chromatid conversion process or an intrachromatid crossover (Chen and Jinks-Robertson 1998), as illustrated in Figure 1. It should be noted that this system allows recombination to occur with no functional constraints on the recombination products other than reorientation of the segment between the IR substrates.

The IR constructs were targeted to the *URA3* locus in previous experiments (Datta *et al.* 1996), preventing the use of *URA3* as a selectable marker in subsequent gene disruptions. In the experiments described here, all of the IR constructs were targeted to the *LEU2* locus to avoid this complication. The genome context of the IR construct did not significantly impact the recombination rates (Table 1 and data not shown). In the results that follow, recombination between identical substrates is referred to as "homologous" recombination, while recombination between substrates having one or more potential mismatches is referred to as "homeologous" recombination.

Recombination rates between homologous and homeologous substrates: Previous studies utilized the cβ2/cβ2 100% identical (homologous) and cβ2/cβ7 91% identical (homeologous) substrates to document the antirecombination roles of Msh2p, Msh3p, and Pms1p (Datta *et al.* 1996). We used these same substrates to examine the effects of Msh6p and Mlh1p on recombination between identical *vs.* nonidentical sequences. Recombination rates for wild-type and MMR-defective strains are given in Table 1 and are graphically presented in Figure 3.

For the cβ2/cβ2 homologous substrates, strains with deficiencies in either Msh2p or Msh3p had a 2-fold increase in recombination rate relative to wild-type, *msh6*, *pms1*, or *mlh1* strains. This increase is consistent with results in other studies using the IR recombination system (Datta *et al.* 1996). To account for effects unrelated

TABLE 1

Recombination rates for Cβ2/Cβ2 (100% identical) or Cβ2/Cβ7 (91% identical) substrates in wild-type and mismatch-repair-deficient strains

Recombination substrates	Strain	Genotype	Rate of His^+ recombinants $\times 10^{-6}$	Homeologous rate normalized to homologous rate in strain of same genotype	Mutant homeologous rate relative to wild-type homeologous rate[a]
Cβ2/Cβ2	GCY313	Wild type	1.5		
	GCY416,GCY417	*msh2*Δ	3.0		
	GCY421,SJR785	*msh3*Δ	2.7		
	GCY413,SJR788	*msh6*Δ	1.1		
	GCY420	*msh3*Δ *msh6*Δ	3.3		
	GCY418	*msh2*Δ *msh3*Δ	3.4		
	GCY422	*pms1*Δ	1.2		
	GCY414,GCY415	*mlh1*Δ	1.5		
	SJR1229-1,SJR1229-2	*pms1*Δ *mlh1*Δ	1.5		
Cβ2/Cβ7	GCY314	Wild type	0.046	0.031	1.0
	GCY400,GCY401	*msh2*Δ	1.9	0.63	20
	GCY402	*msh3*Δ	0.26	0.096	3.1
	GCY403,GCY404	*msh6*Δ	0.22	0.20	6.5
	GCY405,GCY406	*msh3*Δ *msh6*Δ	1.9	0.58	19
	GCY407,GCY408	*msh2*Δ *msh3*Δ	1.8	0.53	17
	GCY409,GCY410	*pms1*Δ	0.37	0.32	11
	GCY411,GCY412	*mlh1*Δ	0.35	0.23	7.5
	SJR1228-1,SJR1228-2	*pms1*Δ *mlh1*Δ	0.23	0.15	4.9

[a] For all calculated relative recombination rates, the normalized homeologous rates in the previous column were used, thus correcting for effects unrelated to the nonidentities present in the substrates.

to sequence divergence between substrates, recombination rates between homeologous cβ2/cβ7 substrates were normalized to those obtained with the cβ2/cβ2 substrates in strains of the same genotype (see the fifth column of Table 1). The normalized rates were used to assess the specific effects of repair defects on homeologous recombination rates. In a wild-type background, the rate of homeologous recombination was reduced 33-fold relative to the rate of homologous recombination (homeologous/homologous rate = 0.03). Eliminating Msh2p, Msh3p and Msh6p, or Msh2p and Msh3p elevated homeologous recombination ~20-fold. In *msh3* and *msh6* strains, the homeologous recombination rates were elevated 3- and 7-fold, respectively, relative to the rate in the wild-type strain. Finally, elimination of Pms1p or Mlh1p resulted in an 11- or 8-fold elevation in the homeologous recombination rate, respectively. A *pms1 mlh1* double mutant strain showed a 5-fold increase in the homeologous recombination rate, which was similar to the increase observed in the single mutants.

Recombination substrates containing defined types of mismatches: Although heteroduplex recombination intermediates formed between the cβ2/cβ7 substrates should contain only single base-base mismatches, recombination rates were elevated for this substrate in both *msh3* and *msh6* strains. This was surprising because only the Msh2p/Msh6p heterodimer is thought to bind base-base mismatches. To further examine the effects of MMR genes on specific types of mismatches, site-directed mutagenesis was used to create substrates containing evenly spaced mutations (Figure 2). These substrates, when recombining with the original cβ2 recombination substrate, can form either base-base mismatches (cβ2/cβ2-ns), 1-nt loops (cβ2/cβ2-1L), or 4-nt loops (cβ2/cβ2-4L) in the heteroduplex recombination intermediate. In addition to recognizing base-base mismatches and small insertion/deletion mismatches, the MMR machinery can recognize large loops (Umar *et al.* 1994; Kirkpatrick and Petes 1997) but not palindromes (Nag and Petes 1991). To examine the effect of these structures on recombination rates, substrates were made that should contain either 12-nt loops (cβ2/cβ2-12L) or 18-nt palindromes (cβ2/cβ2-pal) in heteroduplex recombination intermediates. Recombination rates between the defined mismatch substrates were measured in wild-type and various MMR-deficient and NER-deficient strains. These data are given in Table 2 and are graphically presented in Figure 4. In Figure 4 and in the description of the results that follows, it should be noted that rates for the homeologous substrates were normalized to those for the homologous control substrates for the strain of the same genotype. The normalization was done to eliminate recombination effects that are unrelated to the nonidentities present in the substrates.

Effects of defined mismatches on recombination

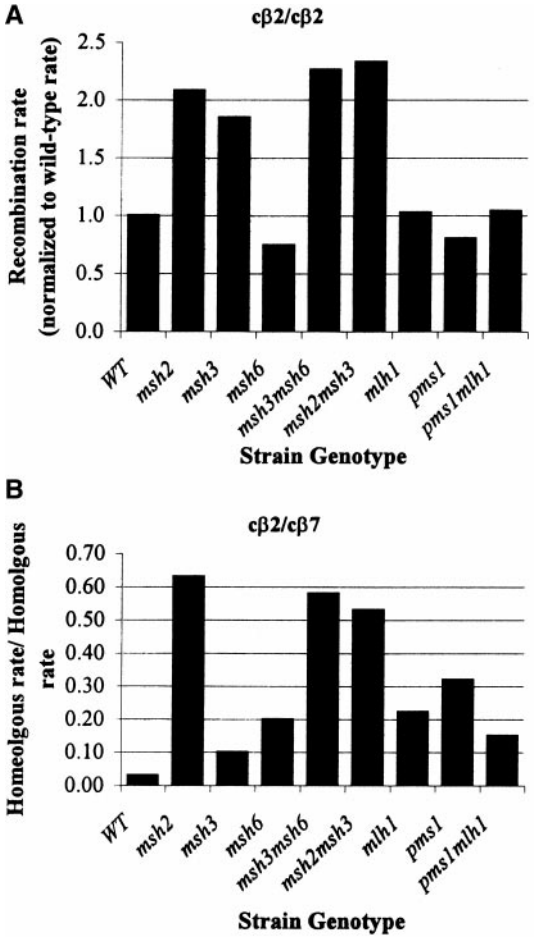

Figure 3.—Recombination rates between homologous and homeologous substrates in wild-type and MMR-defective strains. (A) Recombination rates of MMR-defective strains containing the cβ2/cβ2 recombination substrates were normalized to the wild-type rate. (B) Recombination rates of the homeologous cβ2/cβ7 substrates were normalized to those obtained with the homologous cβ2/cβ2 control substrates in strains with the same genotype.

rates in wild-type and repair deficient backgrounds: Relative to the homologous control substrates, the greatest inhibition of recombination (a 22-fold decrease; homeologous/homologous = 0.045) was obtained with the cβ2/cβ2-ns substrates. This reduction was almost as large as that observed with cβ2/cβ7 91% identical substrates (a 33-fold decrease). Substrates potentially forming 1-nt loops (cβ2/cβ2-1L) or 4-nt loops (cβ2/cβ2-4L) exhibited 9- and 13-fold decreases, respectively, in recombination relative to the 100% control substrates. The cβ2/cβ2, cβ2/cβ2-ns, cβ2/cβ2-1L, and cβ2/cβ2-4L had very similar recombination rates in *msh2* strains, and these rates were the same as those obtained with the homologous control substrates. Thus, the antirecombination activity due to the MMR system is completely Msh2p dependent and mismatch specific. That is, the mismatch repair system is responsible for essentially all of the mismatch-associated inhibition of recombination, and four base-base mismatches have a more inhibitory effect on recombination than do four 1-nt insertion/deletions or four 4-nt insertion/deletions. Relative to the homologous substrates, the potential 12-nt loops and 18-nt palindromes reduced recombination only 3.8- and 2.4-fold, respectively. Some inhibition remained in the *msh2* strains, suggesting that these structures inhibit recombination in both MMR-dependent and MMR-independent manners.

The presence of evenly spaced nucleotide substitutions (cβ2/cβ2-ns substrates) in a wild-type background reduced recombination to a level that was only 5% of the recombination rate seen with the cβ2/cβ2 homologous substrates. Relative to the wild-type strain, an *msh6* strain showed a 14-fold elevation in homeologous recombination rate, indicating that most of the antirecombination effect is Msh6p dependent. Surprisingly, an *msh3* mutant showed an 8-fold increase in the homeologous/homologous ratio obtained in wild-type cells, indicating a significant role of Msh3p in antirecombination. This was unexpected, as Msh3p is not thought to be involved in recognition of base-base mismatches. As expected, homeologous recombination rates did not increase upon disruption of *MSH4* or *MSH5*.

Elimination of Pms1p resulted in an 8-fold increase in recombination between the cβ2/cβ2-ns substrates. There was also a measurable effect of Exo1p deficiency, with an observed 2.7-fold increase in recombination. Surprisingly, *rad1* and *rad10* strains had 6.4- and 8.4-fold increases in homeologous recombination relative to a wild-type strain, respectively. Similar observations have been made in *rad1* strains when substrates contained 1 or 6% base-base mismatches (J. McDougal and S. Jinks-Robertson, unpublished results). To test whether these recombination rate increases were due to the NER pathway or were specific to the Rad1p/Rad10p complex, disruptions of *RAD2* and *RAD14* were made. No increase in homeologous recombination was seen in *rad2* or *rad14* strains.

In a wild-type background, the recombination rate for the cβ2/cβ2-1L substrates was only 11% of the recombination rate between the cβ2/cβ2 control substrates. Both Msh3p and Msh6p had roles in the suppression of recombination between substrates containing single nucleotide insertion/deletion mismatches, as evidenced by the 3.4- and 2.0-fold recombination increases in *msh3* and *msh6* strains, relative to the wild-type strain, respectively. In a *pms1* strain, homeologous recombination was elevated 4.3-fold, again indicating that Pms1p has less antirecombination activity than does Msh2p (*msh2* strains had a 9.7-fold elevation in recombination). In *rad1* strains, the homeologous recombination rate was elevated 2.9-fold.

In wild-type strains, recombination between cβ2/cβ2-4L substrates was reduced to 8% of the control homologous recombination. Recombination rates between the cβ2/cβ2-4L substrates in *msh2* or *msh3* strains were ele-

TABLE 2

Recombination rates for substrates containing specific mismatches in wild-type and MMR- or NER-deficient strains

Recombination substrates	Strain	Genotype	Rate of His^+ recombinants $\times 10^{-6}$	Homeologous rate normalized to homologous rate in strain of same genotype	Mutant homeologous rate relative to wild-type homeologous rate
Cβ2/Cβ2	GCY313	Wild type	1.5		
	GCY416,GCY417	*msh2*Δ	3.0		
	GCY421,SJR785	*msh3*Δ	2.7		
	SJR786 (2 isolates)	*msh4*Δ	0.97		
	SJR787 (2 isolates)	*msh5*Δ	0.98		
	GCY413,SJR788	*msh6*Δ	1.1		
	GCY420	*msh3*Δ *msh6*Δ	3.3		
	GCY418	*msh2*Δ *msh3*Δ	3.4		
	GCY422	*pms1*Δ	1.2		
	GCY414,GCY415	*mlh1*Δ	1.5		
	GCY788,GCY789	*exo1*Δ	2.9		
	GCY703,GCY709	*rad1*Δ	2.1		
	GCY778,GCY779	*rad10*Δ	1.9		
	GCY804,GCY805	*rad2*Δ	2.1		
	GCY767,GCY768	*rad14*Δ	1.6		
Cβ2/Cβ2-ns	GCY562,GCY615	Wild type	0.067	0.045	1.0
	GCY713,GCY714	*msh2*Δ	3.0	1.0	22
	GCY569,GCY616	*msh3*Δ	0.97	0.36	8.0
	GCY633,GCY634	*msh4*Δ	0.058	0.060	1.3
	GCY637,GCY638	*msh5*Δ	0.064	0.065	1.5
	GCY593,GCY594	*msh6*Δ	0.68	0.62	14
	GCY648,GCY647	*pms1*Δ	0.43	0.36	8.0
	GCY762,GCY763	*exo1*Δ	0.35	0.12	2.7
	GCY721,GCY722	*rad1*Δ	0.60	0.29	6.4
	GCY747,GCY748	*rad10*Δ	0.71	0.37	8.4
	GCY752,GCY753	*rad2*Δ	0.088	0.042	0.93
	GCY764,GCY765	*rad14*Δ	0.066	0.041	0.92
Cβ2/Cβ2-1L	SJR767 (2 isolates)	Wild type	0.17	0.11	1.0
	SJR774 (2 isolates)	*msh2*Δ	3.2	1.1	9.7
	SJR775 (2 isolates)	*msh3*Δ	1.0	0.37	3.4
	SJR776 (2 isolates)	*msh4*Δ	0.11	0.11	1.0
	SJR777 (2 isolates)	*msh5*Δ	0.10	0.10	0.93
	SJR778 (2 isolates)	*msh6*Δ	0.24	0.22	2.0
	GCY649,GCY650	*pms1*Δ	0.57	0.48	4.3
	GCY824,GCY825	*exo1*Δ	0.54	0.19	1.7
	GCY723,GCY727	*rad1*Δ	0.68	0.32	2.9

(*continued*)

vated ~10-fold, making them comparable to recombination rates between the cβ2/cβ2 control substrates in these genetic backgrounds. This suggests that all antirecombination activity is due to action of the Msh2p/Msh3p complex. In agreement with this, elimination of Msh6p (leaving the Msh2p/Msh3p heterodimer active) had no impact on recombination between homeologous substrates. The homeologous recombination rates of *pms1* and *rad1* strains were elevated 3.1- and 4.2-fold, respectively.

The recombination rate between the cβ2/cβ2-12L substrates in a wild-type genetic background was 26% of recombination rate for the cβ2/cβ2 control substrates and was elevated 2.1-fold in an *msh2* mutant. The increase in homeologous recombination in an *msh3* strain was similar to the increase observed in an *msh2* strain, indicating that all mismatch-associated antirecombination activity is derived from the Msh2p/Msh3p complex. Neither *msh4*, *msh5*, *msh6*, *pms1*, nor *exo1* strains showed a significant increase in homeologous recombination. In *rad1* and *rad10* strains, homeologous recombination was elevated 2.4- and 2.6-fold, respectively, which is similar to the increases seen in *msh2* and *msh3* strains. As with the cβ2/cβ2-ns substrates, no increase in homeologous recombination was seen in *rad2* or *rad14* strains.

In wild-type cells, the cβ2/cβ2-pal substrates recombined at a rate that was 42% of the recombination rate

TABLE 2

(Continued)

Recombination substrates	Strain	Genotype	Rate of His^+ recombinants $\times 10^{-6}$	Homeologous rate normalized to homologous rate in strain of same genotype	Mutant homeologous rate relative to wild-type homeologous rate[a]
Cβ2/Cβ2-4L	GCY560,GCY559	Wild type	0.12	0.080	1.0
	GCY582,GCY583	*msh2*Δ	2.5	0.83	10
	GCY566,GCY567	*msh3*Δ	2.6	0.96	12
	GCY631,GCY632	*msh4*Δ	0.11	0.11	1.4
	GCY635,GCY636	*msh5*Δ	0.099	0.10	1.3
	GCY591,GCY592	*msh6*Δ	0.088	0.080	1.0
	GCY596,GCY595	*pms1*Δ	0.30	0.25	3.1
	GCY810,GCY811	*exo1*Δ	0.39	0.13	1.7
	GCY681,GCY717	*rad1*Δ	0.70	0.33	4.2
Cβ2/Cβ2-12L	SJR768 (2 isolates)	Wild type	0.39	0.26	1.0
	SJR779 (2 isolates)	*msh2*Δ	1.6	0.53	2.1
	SJR780 (2 isolates)	*msh3*Δ	1.6	0.59	2.3
	SJR781 (2 isolates)	*msh4*Δ	0.21	0.22	0.83
	SJR782 (2 isolates)	*msh5*Δ	0.22	0.22	0.86
	SJR783 (2 isolates)	*msh6*Δ	0.21	0.19	0.73
	GCY651,GCY652	*pms1*Δ	0.25	0.21	0.80
	GCY786,GCY787	*exo1*Δ	0.73	0.25	0.97
	GCY682,GCY720	*rad1*Δ	1.3	0.62	2.4
	GCY781,GCY782	*rad10*Δ	1.3	0.68	2.6
	GCY803	*rad2*Δ	0.49	0.23	0.90
	GCY780	*rad14*Δ	0.31	0.19	0.75
Cβ2/Cβ2-pal	SJR849 (2 isolates)	Wild type	0.63	0.42	1.0
	SJR850 (2 isolates)	*msh2*Δ	2.1	0.70	1.7
	SJR851 (2 isolates)	*msh3*Δ	1.8	0.67	1.6
	SJR852 (2 isolates)	*msh4*Δ	0.30	0.31	0.74
	SJR853 (2 isolates)	*msh5*Δ	0.23	0.23	0.56
	SJR854 (2 isolates)	*msh6*Δ	0.24	0.22	0.52
	GCY653,GCY654	*pms1*Δ	0.43	0.36	0.85
	GCY683,GCY684	*rad1*Δ	1.7	0.81	1.9

[a] For all calculated relative recombination rates, the normalized homeologous rates in the previous column were used, thus correcting for effects unrelated to the nonidentities present in the substrates.

for the cβ2/cβ2 control substrates. Although elimination of Msh2p, Msh3p, or Rad1p elevated the recombination rate similarly, the increase did not correspond to full restoration of homeologous recombination to levels seen with the control homologous substrates. Elimination of Msh4p, Msh5p, Msh6p, or Pms1p did not increase homeologous recombination.

Epistatic relationships between repair genes in regulating homeologous recombination: To gain a better understanding of the implications of intermediate effects of repair proteins on homeologous recombination (such as was seen in *pms1* strains) and to determine if individual proteins act in the same or different antirecombination pathways, double mutant strains were constructed. The nucleotide substitution substrate was chosen for the double mutant studies because it showed the largest range of recombination rates in the single mutant studies. The homologous and homeologous recombination rates of double mutant strains are presented in Table 3 along with recombination rates of relevant single mutant strains.

The *msh3 msh6* double mutant had a recombination rate identical to that of the *msh2* mutant, as expected based on previous studies (Johnson *et al.* 1996a; Marsischky *et al.* 1996; Greene and Jinks-Robertson 1997). We found that *pms1 msh2* and *rad1 msh2* double mutants had recombination rates similar to the *msh2* strain; no further combinations of double mutants containing *msh2* were examined. However, recombination rates between the homeologous cβ2/cβ2-ns and homologous cβ2/cβ2 substrates were examined in strains containing every possible double mutant combination of *msh3*, *msh6*, *pms1*, *rad1*, and *exo1*.

Some of the double mutant strains exhibited a homeologous recombination rate similar to the highest rate of recombination in the relevant single mutant strains. This was true of the *pms1 msh6*, *exo1 msh3*, and *rad1 msh3* strains and suggests that the relevant proteins

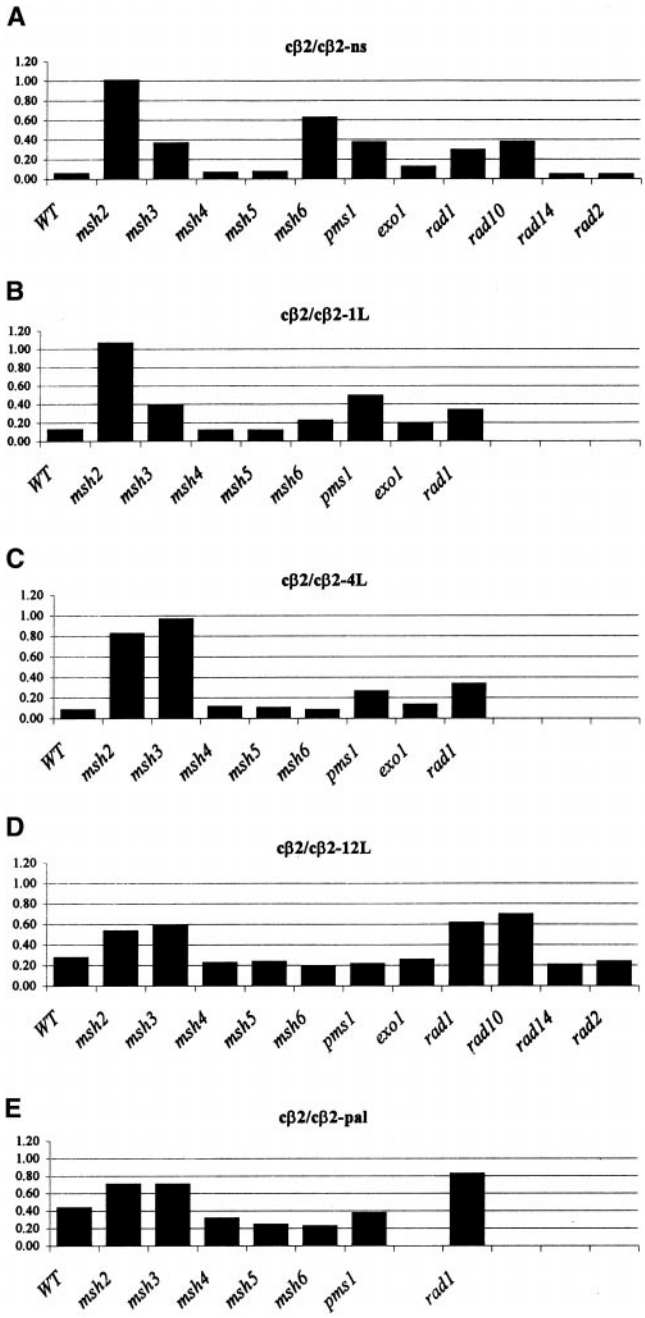

Figure 4.—Recombination rates for MMR-deficient and NER-deficient strains between substrates containing (A) four base-base mismatches, (B) four 1-nt loops, (C) four 4-nt loops, (D) two 12-nt loops, or (E) two 18-nt palindromes. Homeologous recombination rates are shown normalized to the rates obtained with homologous control substrates in strains of the same genotype.

act in the same pathway. In the *pms1 msh3* and *pms1 exo1* double mutants, the effects of the mutations on homeologous recombination rates appeared to be additive, suggesting that these proteins may act in separate pathways. In the *exo1 msh6*, *rad1 msh6*, *exo1 rad1*, and *rad1 pms1* double mutants, the effects of the mutations were greater than additive when compared to the single mutants. For the *exo1 msh6* strain, the apparently greater than additive effect disappeared when the recombination rate was normalized to the *exo1* rate for the homologous control substrate, leaving an effect similar to that seen in an *msh6* strain. To determine whether the synergism in the other double mutants was due to a nonspecific effect on homologous recombination or a specific effect on homeologous recombination, we examined recombination between the homologous cβ2/cβ2 substrates in the double mutant strains. The importance of normalizing the homeologous rates to the homologous rates obtained in a double mutant strain of the same genotype was evident with these double mutants; the homologous rates in the double mutants were greater than the rates in either of the relevant single mutants. Following this normalization, the increases in homeologous recombination in the *rad1 exo1* and *rad1 pms1* mutants appeared additive, while the increase in the *rad1 msh6* double was similar to that observed in the *msh6* single mutant.

To correlate the effects of repair defects on homeologous recombination with the effects of repair defects on general mutation processes, rates of forward mutation to canavanine resistance were determined for *msh2*, *msh3*, *msh6*, *pms1*, *exo1*, and *rad1* strains. The rates obtained were similar to previously published rates. Double mutant *msh3 msh6*, *exo1 msh3*, *exo1 msh6*, *exo1 rad1*, *rad1 msh3*, *rad1 msh6*, and *rad1 pms1* strains also were examined for forward mutation rate at *CAN1*. With the exception of the *exo1 rad1* strain (which showed a slight elevation in mutation rate over either single mutant), all double mutants examined had a mutation rate approximately equivalent to the highest mutation rate observed in the relevant single mutants (data not shown).

DISCUSSION

The inverted repeat assay system: The IR assay system selects for reorientation of the segment of DNA between homologous or homeologous recombination substrates. Such reorientation can occur either via a sister chromatid conversion mechanism or an intrachromatid crossover mechanism (Figure 1). DNA sequence analysis of recombination products (Chen and Jinks-Robertson 1998) indicates that recombination between inverted repeats in this system occurs predominantly via a sister chromatid conversion process, thereby creating a recombination intermediate that may, at least transiently, contain a large mismatched region. The increased homologous recombination observed in the *rad1* and *rad10* strains, and perhaps the *msh2* and *msh3* strains as well, may be due to a role of these proteins in the removal of such large heterologies. The normalization of homeologous recombination rates to the homologous rates should ameliorate effects that are unrelated to the homeology. In designing the homeologous substrates containing defined types of mismatches, care was

TABLE 3

Recombination rates for the cβ2/cβ2-ns substrates in double mutant strains

Recombination substrates	Strain	Genotype	Rate of His^+ recombinants $\times 10^{-6}$	Homeologous rate normalized to homologous rate in strain of same genotype	Mutant homeologous rate relative to wild-type homeologous rate[a]
Cβ2/Cβ2	GCY313	Wild type	1.5		
	GCY416,GCY417	*msh2*Δ	3.0		
	GCY421,SJR785	*msh3*Δ	2.7		
	GCY413,SJR788	*msh6*Δ	1.1		
	GCY422	*pms1*Δ	1.2		
	GCY703,GCY709	*rad1*Δ	2.1		
	GCY788,GCY789	*exo1*Δ	2.9		
	GCY418	*msh3*Δ *msh6*Δ	3.3		
	SJR1230-1,SJR1230-2	*pms1*Δ *msh3*Δ	2.8		
	SJR1233-1,SJR1233-2	*exo1*Δ *rad1*Δ	3.9		
	SJR1232-1,SJR1232-2	*rad1*Δ *msh6*Δ	3.0		
	SJR1231-1,SJR1231-2	*rad1*Δ *pms1*Δ	2.7		
Cβ2/Cβ2-ns	GCY562,GCY615	Wild type	0.067	0.045	1.0
	GCY713,GCY714	*msh2*Δ	3.0	1.0	22
	GCY569,GCY616	*msh3*Δ	0.97	0.36	8.0
	GCY593,GCY594	*msh6*Δ	0.68	0.62	14
	GCY647,GCY648	*pms1*Δ	0.43	0.36	8.0
	GCY721,GCY722	*rad1*Δ	0.60	0.29	6.3
	GCY762,GCY763	*exo1*Δ	0.35	0.12	2.7
	GCY888,GCY889	*rad1*Δ *msh2*Δ	3.7		
	GCY741,GCY756	*pms1*Δ *msh2*Δ	2.8		
	GCY834	*msh3*Δ *msh6*Δ	3.2	0.97	22
	GCY733,GCY742	*pms1*Δ *msh3*Δ	1.6	0.57	13
	GCY736,GCY737	*pms1*Δ *msh6*Δ	0.82		
	GCY797,GCY798	*pms1*Δ *exo1*Δ	0.89		
	GCY859,GCY860	*exo1*Δ *msh3*Δ	1.0		
	GCY819,GCY820	*exo1*Δ *msh6*Δ	1.8		
	GCY890,GCY891	*exo1*Δ *rad1*Δ	2.2	0.56	13
	GCY863,GCY864	*rad1*Δ *msh3*Δ	0.67		
	GCY872,GCY887	*rad1*Δ *msh6*Δ	1.8	0.60	13
	GCY807,GCY814	*rad1*Δ *pms1*Δ	2.1	0.78	17

[a] For all calculated relative recombination rates, the normalized homeologous rates in the previous column were used, thus correcting for effects unrelated to the nonidentities present in the substrates.

taken to space them evenly across the recombination substrates and to introduce all types of mismatches at the same relative positions. Thus, the substrates differed only in the type of mismatch they contain and not in their basic architecture. Even so, we cannot eliminate the possibility that the introduced mismatches may differentially influence either the way that recombination initiates or the mechanism of recombination followed, and thus may alter the observed recombination rates in unforeseen ways.

Impact of defined mismatches on recombination in mismatch repair proficient strains: Four nucleotide substitutions in the 350-bp cβ2 substrates (cβ2/cβ2-ns) reduced recombination rates ~20-fold, which is comparable to the decrease seen with the cβ2/cβ7 91% identical substrates, which contain 29 nucleotide substitutions. Insertion/deletion loops of 1 or 4 nt in the substrates (cβ2/cβ2-1L and cβ2/cβ2-4L, respectively) caused a 10-fold reduction in recombination rates and so had a smaller impact on recombination than did nucleotide substitutions. The larger loops (cβ2/cβ2-12L) and palindromes (cβ2/cβ2-pal) were the least efficient at blocking recombination, causing only a 2–4-fold reduction in recombination rates. Although one could attribute the relatively small effects of the larger loops and palindromes to the difference in the number of potential mismatches (four nucleotide substitutions or small loops *vs.* two large loops or palindromes), previous work indicates that the first mismatch has the largest impact on recombination (Datta and Jinks-Robertson 1995). Further evidence that the difference is not merely due to the number of mismatches is the finding that recombination rates in wild-type strains bearing the cβ2/cβ7 91% identical substrates were very similar to rates in wild-type strains with the cβ2/cβ2-ns substrates. We suggest that large loops and palindromes are not

recognized as efficiently by the mismatch repair machinery as other types of mismatches when they occur in mitotic recombination intermediates.

Antirecombination roles of the yeast MutS homologs: For all substrates examined, the *msh2* mutants exhibited the largest increase in homeologous recombination rates relative to the wild-type strains. For substrates containing defined types of small mismatches (cβ2/cβ2-ns, cβ2/cβ2-1L, cβ2/cβ2-4L), the *msh2* strains had recombination rates equivalent to the rate of homologous recombination in an *msh2* strain. With the cβ2/cβ2-12L and cβ2/cβ2-pal substrates, the recombination rates in *msh2* strains were lower than homologous recombination in *msh2* strains, indicating that large loops and palindromes in recombination intermediates interfere with recombination in an MMR-independent manner. The high density of base-base mismatches in the cβ2/cβ7 substrates also interfered with recombination in an MMR-independent manner. For the cβ2/cβ7 and cβ2/cβ2-ns substrates, *msh3 msh6* double mutant strains had recombination rates similar to *msh2* strains, which is consistent with mutation data; other substrates were not examined in the *msh3 msh6* double mutants.

The Msh2p/Msh3p complex is generally considered to only recognize extrahelical loops corresponding to insertion/deletion mismatches, whereas the Msh2p/Msh6p complex recognizes base-base mismatches as well as small loops (Crouse 1998). Thus, one would expect recombination rates between the cβ2/cβ7 91% substrates and between the cβ2/cβ2-ns substrates to be similarly elevated in *msh2* or *msh6* strains and to be unaffected in an *msh3* strain. Instead, similar increases in recombination rates were observed for *msh3* and *msh6* strains, with each strain having a lower homeologous recombination rate than the corresponding *msh2* strain. Although the clustered point mutations in the cβ2/cβ7 substrates might create distortions in heteroduplex recombination intermediates that could be recognized by Msh3p, the base substitutions in the cβ2/cβ2-ns substrates are well separated. Thus, these data suggest an unsuspected role for Msh3p in the recognition of base-base mismatches in recombination intermediates. Although yeast mutation rate studies have indicated no role of Msh3p in repair of base-base mismatches (Marsischky *et al.* 1996; Earley and Crouse 1998), the low-affinity binding of the Msh2p/Msh3p complex to base mispairs *in vitro* (Habraken *et al.* 1996) and the residual repair of some base-base mismatches during transformation of plasmid heteroduplex DNA constructs into *msh6* strains (Lühr *et al.* 1998) suggest that Msh3p may be involved in the repair of some types of base-base mismatches. Also, it has been observed that transfer of the chromosome containing hMSH3 into human tumor-derived cells lacking both hMSH3 and hMSH6 restores some repair of base-base mismatches (Umar *et al.* 1998). Thus, a role for Msh3p in recognition of base-base mismatches in recombination intermediates, while surprising based on results of mutation studies, is not inconsistent with other observations. As an alternative to a role in participating in the recognition of base-base mismatches, Msh3p might have a structural role within a protein complex that inhibits recombination at a step subsequent to the initial mismatch recognition.

For the 1-nt loop substrates (cβ2/cβ2-1L), both Msh3p and Msh6p exhibited antirecombination activity, which is consistent with their overlapping *in vivo* roles in repair of loop-containing mutational intermediates (Johnson *et al.* 1996a; Marsischky *et al.* 1996; Greene and Jinks-Robertson 1997; Sia *et al.* 1997; Harfe and Jinks-Robertson 1999). For the substrates containing the larger 4-nt loops (cβ2/cβ2-4L), disruption of either *MSH2* or *MSH3* increased the recombination rate to the homologous level, while disruption of *MSH6* had no detectable effect. This indicates that Msh2p/Msh3p is solely responsible for blockage of recombination between these substrates, and that Msh2p/Msh6p is not capable of recognizing a 4-nt loop in recombination intermediates. When the substrates contained a potential 12-nt loop or an 18-nt palindrome, we observed a similar pattern; *msh2* and *msh3* strains showed equivalent increases in recombination, whereas a *msh6* strain showed no increase. This pattern of recognition of loops that are 4 nt or larger during recombination is consistent with observations made regarding microsatellite instability, where repeats 4 bp or larger were destabilized equally in *msh2* and *msh3* strains, but not at all in *msh6* strains (Sia *et al.* 1997).

MSH4 and *MSH5* were disrupted in strains containing the defined mismatch substrates, and in no case did we observe associated increases in recombination rates. This demonstrates that Msh4p and Msh5p have no role in blocking mitotic homeologous recombination, which is consistent with a meiotic-specific function of these proteins (Ross-MacDonald and Roeder 1994; Hollingsworth *et al.* 1995). We note, however, a small decrease in recombination in *msh4* and *msh5* strains when the recombination intermediate potentially contains palindromes.

Antirecombination roles of the yeast MutL homologs: In previous studies, the elevation of homeologous recombination in *pms1* strains was consistently less than that in *msh2* strains (Datta *et al.* 1996; Chen and Jinks-Robertson 1999). This could have been due to redundancy of the MutL homologs or to a MutL-independent antirecombination activity of yeast MutS homologs. The *pms1* (Datta *et al.* 1996) and *mlh1* (this article) strains show similar increases in homeologous recombination rates, consistent with the idea that they function as a heterodimer. Based on the *pms1 mlh1* double mutant results, we suggest that some MutS-dependent blockage of homeologous recombination occurs in the absence of Pms1p and Mlh1p. This is contrary to the apparently complete dependence of MutS homologs on Pms1p/Mlh1p for repair of mutational intermediates (Crouse

1998). It is also possible that the remaining two MutL homologs in yeast (Mlh2p and Mlh3p) may play a more prominent role in antirecombination than they do in mutation avoidance.

Antirecombination roles of endonucleases and exonucleases: Rad1p and Rad10p form a heterodimeric endonuclease (Bardwell *et al.* 1992; Siede *et al.* 1993) that functions in NER and in recombination (Davies *et al.* 1995) to recognize and cleave 5′ of the junction of double- and single-stranded DNA. Rad1p and Rad10p, along with Msh2p and Msh3p, are involved in removal of nonhomologous single-stranded tails during double-strand break repair (Pâques and Haber 1997; Sugawara *et al.* 1997), although there is also a minor *RAD1*- and *MSH2*-independent pathway for removal of nonhomologous tails (Colaiácovo *et al.* 1999). Surprisingly, disruption of *RAD1* increased the recombination rates between all of the substrates containing defined mismatches. When the heteroduplex formed during recombination potentially contained nucleotide substitutions or small loops (1 or 4 nt), *rad1* strains showed a substantial increase in recombination, but this increase was less than the increase seen in *msh2* strains. For large loops and palindromes, a Rad1p deficiency was equivalent to a deficiency in Msh2p or Msh3p. For both nucleotide substitution and 12-nt loop substrates, recombination rates in *rad1* strains were similar to those in *rad10* strains, indicating that Rad1p and Rad10p are acting as a heterodimer in regulating recombination, as has been observed for other processes in both recombination and repair. Although Rad1p has been implicated in the removal of large loops in recombination (Kirkpatrick and Petes 1997) and mutation (Harfe and Jinks-Robertson 1999) intermediates, this is the first report of the Rad1p/Rad10p endonuclease being involved in recognition or processing of base-base mismatches or small loops. This function of the Rad1p/Rad10p complex may be related to its endonuclease activity or may be simply structural, resulting from its association with Msh2p/Msh3p. Whether Rad1p/Rad10p (or Exo1p, see below) has a structural or enzymatic role could be determined by using mutant proteins that are structurally normal but have no nucleolytic activity. The complete lack of any increase in homeologous recombination when the *RAD2* or *RAD14* gene was disrupted suggests that Rad1p/Rad10p is acting outside of its role in the nucleotide excision repair pathway.

Exo1p is a 5′ → 3′ exonuclease (Huang and Symington 1993) that has been shown to associate with Msh2p (Tishkoff *et al.* 1997). Strains deficient in Exo1p showed small (twofold) increases in recombination when the heteroduplex intermediate potentially contains base-base mismatches or small loops. This effect could be due to exonucleolytic processing of mismatch-containing recombination intermediates, or could result from a structural role of Exo1p in MMR complexes. Elimination of other exonucleases in combination with Exo1p might reveal synergistic interactions with regard to homeologous recombination, which would indicate a role for exonuclease activity in antirecombination.

Roles of mismatch repair and nucleotide excision repair proteins in recombination: The mismatches formed during recombination between the cβ2/cβ2-ns, cβ2/cβ2-1L, and cβ2/cβ2-4L substrates are structurally similar to mismatches formed as a consequence of replication errors. Because Rad1p has not been implicated in the repair of these types of mismatches in replication intermediates, the increases in recombination rates between these substrates in *rad1* strains were surprising. Double mutant studies indicated additive recombination effects upon elimination of Rad1p and Pms1p, of Rad1p and Exo1p, or of Pms1p and Exo1p. This genetic behavior suggests the involvement of multiple distinct pathways or complexes in the regulation of homeologous recombination. In addition to the unexpected role of Rad1p in regulating homeologous recombination, we found that Msh3p has an antirecombination role when the recombination substrates contain potential base-base mismatches. This is in stark contrast to the apparent inability of Msh3p to remove replication errors resulting in base-base mismatches (Crouse 1998).

The model of mismatch repair in which a Mlh1p/Pms1p heterodimer pairs with either a Msh2p/Msh6p or a Msh2p/Msh3p heterodimer to effect repair does not fully explain the results of the recombination studies reported here, again indicating that antirecombination is more complex than the repair of replication errors. *Msh6 pms1* double mutants showed no increase in recombination over *msh6* levels, which indicates that Pms1p does not have a role independent of Msh6p. In contrast, the rate of recombination in a *pms1 msh3* strain was increased relative to the *msh3* and *pms1* single mutants, indicating that these two genes may work in separate pathways. It is possible that Pms1p is coordinating the recognition of the base-base mismatch by the Msh2p/Msh6p heterodimer and that Msh3p is primarily involved in some separate step, perhaps in complex with Rad1p or Exo1p but not Pms1p. The observation that *pms1*, *mlh1*, and *pms1 mlh1* strains had lower recombination rates than *msh2* strains is also inadequately explained by the model of MMR derived from mutational studies. As noted previously, DNA sequence analysis of recombination products suggests that most recombination between IR substrates occurs between sister chromatids (Chen and Jinks-Robertson 1998; Figure 1B). Sister chromatid recombination can occur only during the G2 phase of the cell cycle, after chromosomes have replicated, while mutational intermediates arise and presumably are repaired during the S phase of the cell cycle. It is known that *MSH2*, *MSH6*, and *PMS1* are cell-cycle-regulated in *Saccharomyces cerevisiae*, being most highly expressed during S phase, whereas *MSH3* and *MLH1* are constitutively expressed (Kramer *et al.* 1996). Thus, the protein complexes formed during S phase

may differ from protein complexes formed during G2 phase. For example, MMR proteins are known to associate with replication proteins such as PCNA (Johnson *et al.* 1996b; Umar *et al.* 1996), but this association may not occur during G2. The potential cell-cycle differences in complex composition as well as the documented association of MMR and NER proteins (Bertrand *et al.* 1998) could account for the unexpected interactions between MMR and NER proteins observed with the IR recombination assay.

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