Meiotic Recombination Involving Heterozygous Large Insertions in *Saccharomyces cerevisiae*: Formation and Repair of Large, Unpaired DNA Loops

Hutton M. Kearney, David T. Kirkpatrick,[1] Jennifer L. Gerton[2] and Thomas D. Petes

Department of Biology, Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280

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ABSTRACT

Meiotic recombination in *Saccharomyces cerevisiae* involves the formation of heteroduplexes, duplexes containing DNA strands derived from two different homologues. If the two strands of DNA differ by an insertion or deletion, the heteroduplex will contain an unpaired DNA loop. We found that unpaired loops as large as 5.6 kb can be accommodated within a heteroduplex. Repair of these loops involved the nucleotide excision repair (NER) enzymes Rad1p and Rad10p and the mismatch repair (MMR) proteins Msh2p and Msh3p, but not several other NER (Rad2p and Rad14p) and MMR (Msh4p, Msh6p, Mlh1p, Pms1p, Mlh2p, Mlh3p) proteins. Heteroduplexes were also formed with DNA strands derived from alleles containing two different large insertions, creating a large "bubble"; repair of this substrate was dependent on Rad1p. Although meiotic recombination events in yeast are initiated by double-strand DNA breaks (DSBs), we showed that DSBs occurring within heterozygous insertions do not stimulate interhomologue recombination.

In *Saccharomyces cerevisiae*, the exchange of genetic information during meiotic recombination is a highly coordinated process that involves introduction of a double-strand break (DSB) and repair of that break with sequences derived from the homologous chromosome (Roeder 1997; Paques and Haber 1999). The enzyme responsible for DSB formation is the topoisomerase II-related protein Spo11p (Keeney *et al.* 1997). Following DSB formation, the 5′ ends of the break are resected in a process requiring the Rad50p/Mre11p/Xrs2p complex, generating long, single-stranded DNA tails (Alani *et al.* 1990; McKee and Kleckner 1997; Nairz and Klein 1997; Prinz *et al.* 1997). In the modified version of the double-strand break model (Szostak *et al.* 1983; Sun *et al.* 1991), the 3′-ended tails invade the homologous chromosome, creating a heteroduplex DNA molecule (Figure 1). The displaced strand is used as a template for repair of the break. Ligation of the products results in double Holliday junction intermediates (Schwacha and Kleckner 1994, 1995) that can be resolved as crossovers or noncrossovers.

If sequence heterologies are included in the heteroduplex region (either base pair changes or insertion/deletion heterologies), mismatches or unpaired loops will be generated (Figure 1). The repair of these mismatched templates can lead to gene conversion or restoration of Mendelian segregation (Petes *et al.* 1991). Failure to repair the mismatch will result in a *p*ost *m*eiotic *s*egregation (PMS) event. If the heterozygous marker involves an auxotrophic mutation, a PMS event is readily visualized as a sectored spore colony.

One aim of this study is to investigate whether large sequence heterologies can be incorporated into heteroduplexes during meiotic recombination in *S. cerevisiae*. Insertions (often involving transposable elements) and deletions are common in eukaryotic genomes. Gene conversions of large (>5 kb) heterozygous insertions and deletions have been observed (Fink and Styles 1974; Fogel *et al.* 1981; McKnight *et al.* 1981; Pukkila *et al.* 1986; Vincent and Petes 1989), although these conversions have often been attributed to repair of a gapped DNA intermediate rather than repair of a loop within a heteroduplex (Szostak *et al.* 1983; Tran *et al.* 1996). Clikeman *et al.* (2001) recently showed that 2- to 3-kb heterologies can be incorporated into heteroduplexes during mitotic recombination events in yeast. As described below, we find that insertions of up to 5.6 kb can be incorporated into heteroduplex DNA, indicating that meiotic gene conversion of large heterologies can involve DNA loop repair.

A second aim of this study is to identify the proteins involved in the meiotic repair of large, unpaired DNA loops. In a previous study (Kirkpatrick and Petes

Corresponding author: Thomas D. Petes, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.
E-mail: tompetes@email.unc.edu

[1] *Current address:* Department of Genetics, Cell Biology, and Development, University of Minnesota, 250 Biological Sciences Bldg., 1445 Gortner Ave., St. Paul, MN 55108.

[2] *Current address:* Department of Biochemistry and Biophysics, University of California, 513 Parnassus Ave., San Francisco, CA 94143-0448.

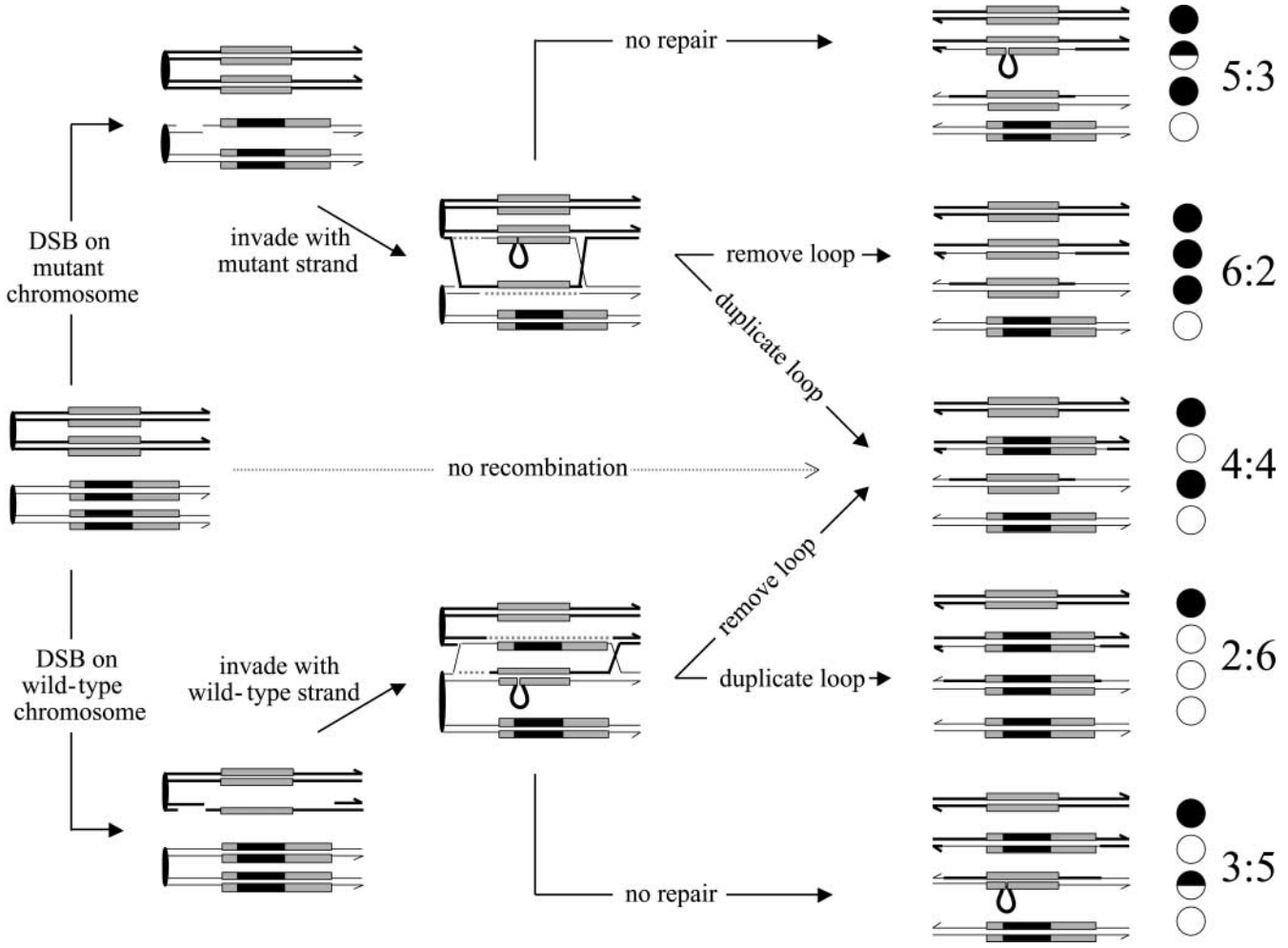

FIGURE 1.—Patterns of aberrant segregation associated with meiotic recombination at *HIS4*. Chromosomes are shown as double-stranded DNA molecules. Sister chromatids are depicted held together at their centromeres (solid ovals). The *HIS4* gene is shown as a shaded rectangle; the solid rectangle within the *HIS4* gene indicates a large insertion. The steps of recombination are derived from the double-strand break repair model (SZOSTAK *et al.* 1983; SUN *et al.* 1991). The top half of the figure shows DSB formation upstream of the mutant allele followed by 5′ to 3′ resection of the broken ends. In the next step, the wild-type chromosome is invaded by a single strand containing the large insertion. Dotted lines represent repair synthesis. Ligation of the free ends in this intermediate would result in the formation of double Holliday junctions. If the recombination intermediate is resolved without repair of the mispaired loop, a 5:3 aberrant tetrad will be produced. The segregation pattern of the spore colonies when replica plated to medium lacking histidine is shown on the far right. Solid circles represent His^+ colonies and open circles represent His^- colonies. The spore containing the heteroduplex with the unrepaired loop is shown as a sectored colony, representing a PMS event. Repair by removal of the loop results in a 6:2 gene conversion. Repair by duplication of the loop results in restoration of 4:4 segregation. The bottom half of the figure shows initiation of recombination upstream of the wild-type *HIS4* allele. The mutant chromosome is invaded by a wild-type strand and the mutant insertion is displaced to form a mispaired loop. Failure to repair the heteroduplex results in a 3:5 PMS tetrad. Repair by removal of the loop results in restoration of 4:4 segregation, while repair by duplication of the loop results in a 2:6 gene conversion tetrad.

1997), we showed that meiotic repair of a small (26-base) DNA loop involved Msh2p, an enzyme involved in mismatch repair (MMR), and Rad1p, an enzyme involved in nucleotide excision repair (NER). In both mitotic and meiotic cells, the MMR enzymes function to repair DNA mismatches generated either by errors made during DNA replication or by heteroduplexes formed between DNA strands derived from nonidentical alleles (CROUSE 1998; HARFE and JINKS-ROBERTSON 2000). In *S. cerevisiae*, base-base mismatches are corrected by a complex containing the MutS homologues, Msh2p and Msh6p, and the MutL homologues, Mlh1p and Pms1p (SIA *et al.* 1997a; KOLODNER and MARSISCHKY 1999). In mitotic cells, DNA loops between 1 and 16 bases are corrected by a complex in which Msh3p is substituted for Msh6p (SIA *et al.* 1997b). Although these two MMR complexes have the major roles in the repair of DNA mismatches, other complexes that include the MutL homologues Mlh2p and Mlh3p are also involved in the repair of very small DNA loops (FLORES-ROZAS and KOLODNER 1998; HARFE and JINKS-ROBERTSON 2000). In addition, the frequency of large (~100 bp) deletions

was increased in strains with *msh3* or *mlh2* mutations (Harfe *et al.* 2000); such deletions could reflect failure to repair large DNA loops formed by DNA polymerase slippage.

In addition to their roles in the repair of DNA mismatches, the MMR enzymes are involved in a number of other cellular processes. First, these enzymes reduce the frequency of recombination between diverged DNA sequences (Selva *et al.* 1995; Datta *et al.* 1996; Hunter *et al.* 1996; Nicholson *et al.* 2000). Second, Msh2p and Msh3p are required for the removal of nonhomologous "tails" of DNA during single-strand annealing recombination events (Saparbaev *et al.* 1996; Sugawara *et al.* 1997). Third, some of the MMR proteins (Msh4p, Msh5p, Mlh1p, and Mlh3p) promote meiotic crossing over in yeast (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Wang *et al.* 1999).

The NER enzymes are required for the efficient repair of a variety of types of DNA damage (reviewed by Prakash *et al.* 1993). Following recognition of a damaged substrate (usually a photoproduct) by Rad14p, the DNA surrounding the lesion is unwound by a helicase to form a small "bubble." The damaged strand is removed by single-strand incisions made both 5′ (Rad1/10p) and 3′ (Rad2p) to the lesion. Repair synthesis of the excised region completes the reaction.

In *S. cerevisiae*, Rad1p and Rad10p are also required in single-strand annealing (Fishman-Lobell and Haber 1992) and the meiotic repair of small DNA loops (Kirkpatrick 1999). Mitotic recombination between inverted repeats containing multiple small heterozygous insertions is inhibited by the Rad1p and Rad10p (Nicholson *et al.* 2000). In *Schizosaccharomyces pombe*, homologues of the *S. cerevisiae* Rad1p, Rad10p, and Rad14p function in short-patch meiotic repair of DNA mismatches (Fleck *et al.* 1999). The *mei9* gene product, homologous to Rad1p, is required for meiotic mismatch repair and crossover resolution in Drosophila (Carpenter 1979, 1982; Sekelsky *et al.* 1995). In the studies described below, we show that the MMR proteins, Msh2p and Msh3p, and the NER proteins, Rad1p and Rad10p, are involved in the meiotic repair of large DNA loops.

MATERIALS AND METHODS

Strains: All haploid yeast strains are derivatives of AS13 or AS4 (Stapleton and Petes 1991). The constructions and genotypes of haploid and diploid strains are described in Tables 1 and 2, respectively. All alterations were introduced by one-step (Rothstein 1983) or two-step (Sherman *et al.* 1982) transplacements. One-step transplacements using PCR-generated DNA fragments were done as described in Wach *et al.* (1994). Primers used to generate PCR cassettes are shown in Table 3. Deletions made with the *kanMX4* or *hygB* cassettes (Wach *et al.* 1994; Goldstein and McCusker 1999) removed most or all of the coding sequence. All constructions were verified by PCR, Southern analysis, or DNA sequencing. To generate diploids homozygous for mutations in *MSH2*, *MSH3*, *MSH6*, *PMS1*, and *MLH1* that have a mutator phenotype, we mated haploids overnight and sporulated the resulting diploids the next day without purification of the diploids. This procedure prevents accumulation of mutations that would reduce spore viability.

Genetic techniques: Standard media and genetic methods were used (Sherman *et al.* 1982). Plates for *kanMX4* and *hygB* selection were YPD + 150 mg/liter geneticin and YPD + 300 mg/liter hygromycin B, respectively. We selected *ura3* strains on medium containing 1 gm/liter 5-fluoroorate (Boeke *et al.* 1984). As in our previous studies (Fan *et al.* 1995), diploid strains were sporulated at 18° on plates containing 1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 2% agar, and supplemented with 6 μg/ml of adenine. Sporulated cells were dissected onto YPD and, following full colony growth at 30°, spore colonies were replica plated to appropriate omission and drug-containing media to score the segregating markers. Spore colonies were then examined for sectored growth patterns by microscopy.

Physical analysis of double-strand breaks: We performed Southern analysis by standard methods (Maniatis *et al.* 1982), using a PCR-generated probe to *HIS4*. Primers used to generate the probe were f: 5′ CCACTTGGAGACCATGTCTTG and r: 5′ CAATGGAACATAGAGCTTGAGTG, resulting in a fragment containing *HIS4* sequences from +690 to +1768. For most of the DSB measurements, strains with the *rad50S* mutation were grown in liquid sporulation media at room temperature as described previously (Nag and Petes 1993). In some experiments, we also isolated and analyzed DNA derived from cells sporulated at 18° on plates. These different sporulation conditions had no effect on the relative levels of DSBs (wild-type *vs.* mutant chromosome) as assayed physically or genetically (data not shown). Levels of DSBs were quantitated using the PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and Image-Quant software.

Statistical analysis: Comparisons were made using the Fisher exact test with two-tailed *P* values or by chi-square analysis (for comparisons involving more than two experimental parameters). Results were considered statistically significant when $P < 0.05$. Instat 1.12 (GraphPad Software) was used for statistical analysis.

RESULTS

Experimental rationale: In our genetic background, heterozygous markers near the 5′ end of *HIS4* have an extraordinarily high rate of non-Mendelian segregation, ~50% of unselected tetrads (Nag *et al.* 1989). This high frequency of aberrant segregation reflects a high level of meiosis-specific DSBs located about 200 bp upstream of the *HIS4* initiating codon (Nag and Petes 1993; Fan *et al.* 1995). Heteroduplexes initiated in the *HIS4* upstream region are efficiently extended through the *HIS4* coding region, a distance of ~2.4 kb (Detloff *et al.* 1992; Porter *et al.* 1993). Heterozygous mutations within the coding sequences lead to mismatches in the heteroduplex; repair of these mismatches results in gene conversion or restoration events, whereas failure to repair the mismatches results in PMS events (Figure 1).

In previous studies, we found that small (26 bp) heterozygous nonpalindromic insertions at position +469 in the *HIS4* coding sequence had high levels of aberrant segregation (26% gene conversion and 4% PMS tetrads;

TABLE 1

Haploid yeast strains

Strain	Relevant genotype[a]	Construction details and/or reference[b]
		AS4-derived haploids
AS4	Wild type	Stapleton and Petes (1991)
HF2	*his4-51 rad50S*	Fan *et al.* (1995)
DNY107	*rad50S*	Nag and Petes (1993)
DNY95	*pms1*-Δ	TST in AS4 with *Bst*XI-cut pJH523 (Kramer *et al.* 1989)
DTK225	*rad1::ura3*	Kirkpatrick and Petes (1997)
DTK240	*rad14::URA3*	Kirkpatrick and Petes (1997)
DTK245	*rad27::HUH*	OST in AS4 with *Bam*HI/*Pvu*II-cut pR2.10 (Sommers *et al.* 1995)
DTK247	*msh4::URA3*	OST in AS4 with *Eco*RI/*Bam*HI-cut p5A (Ross-Macdonald and Roeder 1994)
DTK285	*rad10::URA3*	OST in AS4 with *Sal*I/*Bam*HI-cut pDD37 (Prakash *et al.* 1985)
DTK318	*mlh1::URA3*	OST in AS4 with *Sac*I/*Bam*HI-cut y*mlh1::URA3* (Prolla *et al.* 1994)
DTK339	*hdf1::URA3*	OST in AS4 with *Ssp*1/*Hin*dIII-cut pGEM4Z S-H/URA (Feldmann and Winnacker 1993)
DTK487	*msh3::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *msh3*-Δ and r *msh3*-Δ (pFA6-*kanMX4*)
HMY22	*rad50S*	OST in AS4 with *Eco*RI/*Bam*HI-cut pNKY349 (Cao *et al.* 1990)
HMY50	*his4::U1.1a*	OST in AS4 with 1.1-kb *URA3* fragment in *Sal*I site of *HIS4*; PCR primers f *his4::U1.1* and r *his4::U1.1* (pRS306)
HMY104	*msh2::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *msh2*-Δ and r *msh2*-Δ (pFA6-*kanMX4*)
HMY105	*mlh1::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *mlh1*-Δ and r *mlh1*-Δ (pFA6-*kanMX4*)
HMY106	*pms1::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *pms1*-Δ and r *pms1*-Δ (pFA6-*kanMX4*)
HMY110	*msh3::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *msh3*-Δ and r *msh3*-Δ (pFA6-*kanMX4*)
HMY118	*exo1::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *exo1*-Δ and r *exo1*-Δ (pFA6-*kanMX4*)
HMY123	*rad27::hisG*	5-FOA^r derivative of DTK245
HMY124	*rad10::ura3*	5-FOA^r derivative of DTK285
HMY125	*rad14::ura3*	5-FOA^r derivative of DTK240
HMY134	*msh6::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *msh6*-Δ and r *msh6*-Δ (pFA6-*kanMX4*)
HMY158	*mlh3::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *mlh3*-Δ and r *mlh3*-Δ (pFA6-*kanMX4*)
HMY161	*mlh2::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *mlh2*-Δ and r *mlh2*-Δ (pFA6-*kanMX4*)
HMY166	*rad2::URA3*	OST in AS4 with *Ssp*I-cut pKM55 (from R. Schiestl)
HMY167	*rad2::ura3*	5-FOA^r derivative of HMY166
HMY168	*rad1::URA3 msh3::kanMX4*	OST in HMY110 with *Bam*HI-cut pDG18 (from R. Schiestl)
HMY175	*rad1::ura3 msh3::kanMX4*	5-FOA^r derivative of HMY168
HMY195	*mlh1::hygB his4::U1.1a*	OST in HMY50 with *hygB*; PCR primers f *mlh1*-Δ and r *mlh1*-Δ (pA632)
HMY214	*rad1::hygB his4::U1.1a*	OST in HMY50 with *hygB*; PCR primers f *rad1*-Δ and r *rad1*-Δ (pA632)
HMY221	*msh3::hygB his4::U1.1a*	OST in HMY50 with *hygB;* PCR primers f *msh3*-Δ and r *msh3*-Δ (pA632)
HMY223	*msh4::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *msh4*-Δ and r *msh4*-Δ (pFA6-*kanMX4*)
HMY226	*rad50S rad1::ura3*	OST in DTK225 with *Eco*RI/*Bam*HI-cut pNKY349 (Cao *et al.* 1990)
HMY232	*rad1::URA3 his4-51*	OST in MW30 with *Bam*HI-cut pDG18 (from R. Schiestl)
HMY235	*rad1::ura3 his4-51*	5-FOA^r derivative of HMY232
HMY249	*rad2::hygB his4::U1.1a*	OST in HMY50 with *hygB*; PCR primers f *rad2*-Δ and r *rad2*-Δ (pA632)

(*continued*)

TABLE 1

(Continued)

Strain	Relevant genotype[a]	Construction details and/or reference[b]
JG1	*pol4::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *pol4*-Δ and r *pol4*-Δ (pFA6-*kanMX4*)
JG17	*rev3::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *rev3*-Δ and r *rev3*-Δ (pFA6-*kanMX4*)
JG18	*rad30::kanMX4*	OST in AS4 with *kanMX4*; PCR primers f *rad30*-Δ and r *rad30*-Δ (pFA6-*kanMX4*)
MW30	*his4-51*	White *et al.* (1991)
		AS13-derived haploids
AS13	wild-type	Stapleton and Petes (1991)
DNY24	*his4-lopd*	Nag *et al.* (1989)
DNY25	*his4-lopc*	Nag *et al.* (1989)
DTK221	*rad1::ura3 his4-lopd*	Kirkpatrick and Petes (1997)
DTK237	*rad2::URA3 his4-lopd*	Kirkpatrick and Petes (1997)
DTK238	*rad14::URA3 his4-lopd*	Kirkpatrick and Petes (1997)
DTK244	*rad27::HUH his4-lopd*	OST in DNY24 with *Bam*HI/*Pvu*II-cut pR2.10 (Sommers *et al.* 1995)
DTK243	*msh4::URA3 his4-lopd*	OST in DNY24 with *Eco*RI/*Bam*HI-cut p5A (Ross-Macdonald and Roeder 1994)
DTK248	*pms1*-Δ *his4-lopd*	TST in DNY24 with *BstX*I-cut pJH523 (Kramer *et al.* 1989)
DTK261	*rad10::ura3 his4-lopd*	OST in DNY24 with *Sal*I/*Bam*HI-cut pDD37 (Prakash *et al.* 1985); 5FOA^r derivative
DTK319	*mlh1::URA3 his4-lopd*	OST in DNY24 with *Sac*I/*Bam*HI-cut y*mlh1::URA3* (Prolla *et al.* 1994)
DTK340	*hdf1::URA3 his4-lopd*	OST in DNY24 with *Ssp*1/*Hin*dIII-cut pGEM4Z S-H/URA (Feldmann and Winnacker 1993)
DTK355	α *his4-lopc*	DNY25 with pGAL-HO (Herskowitz and Jensen 1991); 5FOA^r isolate
DTK486	*msh3::kanMX4 his4-lopd*	OST in DNY24 with *kanMX4*; PCR primers f *msh3*-Δ and r *msh3*-Δ (pFA6-*kanMX4*)
DTK489	*msh6::kanMX4 his4-lopd*	OST in DNY24 with *kanMX4;* PCR primers f *msh6*-Δ and r *msh6*-Δ (pFA6-*kanMX4*)
DTK496	*his4-lopd rad1::URA3 msh3::kanMX4*	Spore colony from DTK486 × HMY142 cross
HMY23	*his4-CAG$_{10}$ rad50S*	Moore *et al.* (1999)
HMY35	*rad1::ura3*	OST in DTK221 with *Xho*I-*Bgl*I fragment of *HIS4* to restore wild-type allele
HMY46	*his4::U1.1a rad1::ura3*	OST in HMY35 with 1.1-kb *URA3* fragment in *Sal*I site of *HIS4*; PCR primers f *his4::U1.1* and r *his4::U1.1* (pRS306)
HMY97[c]	*his4::U1.1b*	OST in AS13 with 1.1-kb *URA3* fragment in *Sal*I site of *HIS4*; PCR primers f *his4::U1.1* and r *his4::U1.1* (pRS306)
HMY99	*his4::U1.1a*	OST in AS13 with 1.1-kb *URA3* fragment in *Sal*I site of *HIS4*; PCR primers f *his4::U1.1* and r *his4::U1.1* (pRS306)
HMY101	*msh2::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *msh2*-Δ and r *msh2*-Δ (pFA6-*kanMX4*)
HMY102	*mlh1::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *mlh1*-Δ and r *mlh1*-Δ (pFA6-*kanMX4*)
HMY103	*pms1::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4*; PCR primers f *pms1*-Δ and r *pms1*-Δ (pFA6-*kanMX4*)
HMY111	*msh3::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *msh3*-Δ and r *msh3*-Δ (pFA6-*kanMX4*)
HMY119	*exo1::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4*; PCR primers f *exo1*-Δ and r *exo1*-Δ (pFA6-*kanMX4*)
HMY127	*rad2::ura3 his4-lopd*	5-FOA^r derivative of DTK237
HMY128	*rad27::hisG his4-lopd*	5-FOA^r derivative of DTK244
HMY130	*rad14::ura3 his4-lopd*	5-FOA^r derivative of DTK238
HMY131	α	Spore colony from DTK355 × AS13 cross
HMY132	α *his4::U1.1a*	Spore colony from DTK355 × HMY99 cross
HMY135	*msh6::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4*; PCR primers f *msh6*-Δ and r *msh6*-Δ (pFA6-*kanMX4*)
HMY142	α *rad1::URA3 his4-lopc*	OST in DTK355 with *Bam*HI-cut pDG18 (from R. Schiestl)
HMY145	*his4::U1.1a rad27::hisG*	Spore colony from HMY132 × HMY128

(*continued*)

TABLE 1

(Continued)

Strain	Relevant genotype[a]	Construction details and/or reference[b]
HMY146	*his4::U1.1a rad10::ura3*	Spore colony from HMY132 × DTK261
HMY147	*his4::U1.1a rad14::ura3*	Spore colony from HMY132 × HMY130
HMY153	α *rad1::ura3 his4-lopc*	5-FOA^r derivative of HMY142
HMY154	*his4::U1.1a his4-51*	OST in MW33 with 1.1-kb *URA3* fragment into *Sal*I site of *HIS4*; PCR primers f *his4::U1.1* and r *his4::U1.1* (pRS306)
HMY159	*mlh3::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *mlh3*-Δ and r *mlh3*-Δ (pFA6-*kanMX4*)
HMY162	*mlh2::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *mlh2*-Δ and r *mlh2*-Δ (pFA6-*kanMX4*)
HMY169	α *rad50S*	Spore colony from HMY23 × HMY131 cross
HMY171	*his4::U1.1a rad1::ura3 msh3::kanMX4*	Spore colony from HMY153 × HMY111 cross
HMY172	*his4::U1.1a rad2::ura3*	Spore colony from HMY132 × HMY127 cross
HMY182	*his4-51 his4::U1.1a rad50S*	Spore colony from HMY169 × HMY154 cross
HMY187	*his4::k1.5*	OST in AS13 with *kanMX4* in *Sal*I site of *HIS4*; PCR primers f *his4::k1.5* and r *his4::k1.5* (pFA6-*kanMX4*)
HMY191	*his4::U5.6*	OST in AS13 with 5.6-kb *URA3* fragment into *Sal*I site of *HIS4*; PCR primers f *his4::U5.6* and r *his4::U5.6* (DTK172)
HMY196	*mlh1::hygB his4::k1.5*	OST in HMY187 with *hygB;* PCR primers f *mlh1*-Δ and r *mlh1*-Δ (pA632)
HMY197	*msh3::kanMX4 his4::U5.6*	OST in HMY191 with *kanMX4;* PCR primers f *msh3*-Δ and r *msh3*-Δ (pFA6-*kanMX4*)
HMY202	*rad1::kanMX4 his4::U5.6*	OST in HMY191 with *kanMX4;* PCR primers f *rad1*-Δ and r *rad1*-Δ (pFA6-*kanMX4*)
HMY206	α *rad1::ura3*	Spore colony from HMY131 × HMY35 cross
HMY208	*his4::k1.5 rad50S*	Spore colony from HMY169 × HMY187 cross
HMY209	*his4::U5.6 rad50S*	Spore colony from HMY169 × HMY191 cross
HMY212	*rad1::hygB his4::k1.5*	OST in HMY187 with *hygB;* PCR primers f *rad1*-Δ and r *rad1*-Δ (pA632)
HMY220	*msh3::hygB his4::k1.5*	OST in HMY187 with *hygB;* PCR primers f *msh3*-Δ and r *msh3*-Δ (pA632)
HMY227	*his4::U1.1a rad1::ura3 rad50S*	Spore colony from HMY169 × HMY46 cross
HMY228	*his4::k1.5 rad1::hygB rad50S*	Spore colony from HMY169 × HMY212 cross
HMY233	*his4::k1.5 his4-51*	OST in MW33 with *kanMX4* in *Sal*I site of *HIS4*; PCR primers f *his4::k1.5* and r *his4::k1.5* (pFA6-*kanMX4*)
HMY236	*his4-51 his4::k1.5 rad50S*	Spore colony from HMY169 × HMY233
HMY238	*his4-51 his4::k1.5 rad1::ura3*	Spore colony from HMY206 × HMY233
HMY242	*msh4::kanMX4 his4::U1.1a*	OST in HMY99 with *kanMX4;* PCR primers f *msh4*-Δ and r *msh4*-Δ (pFA6-*kanMX4*)
HMY250	*rad2::hygB his4::k1.5*	OST in HMY187 with *hygB*; PCR primers f *rad2*-Δ and r *rad2*-Δ (pA632)
HMY257	*pol4::hygB his4::U5.6*	OST in HMY191 with *hygB*; PCR primers f *pol4*-Δ and r *pol4*-Δ (pA632)
JG3	*pol4::kanMX4 his4-lopd*	OST in DNY24 with *kanMX4*; PCR primers f *pol4*-Δ and r *pol4*-Δ (pFA6-*kanMX4*)
JG6	*pol4::kanMX4 his4::U1.1b*	OST in HMY97 with *kanMX4*; PCR primers f *pol4*-Δ and r *pol4*-Δ (pFA6-*kanMX4*)
JG7	*rev3::kanMX4 his4::U1.1b*	OST in HMY97 with *kanMX4*; PCR primers f *rev3*-Δ and r *rev3*-Δ (pFA6-*kanMX4*)
JG8	*rad30::kanMX4 his4::U1.1b*	OST in HMY97 with *kanMX4*; PCR primers f *rad30*-Δ and r *rad30*-Δ (pFA6-*kanMX4*)
JG16	*rad50S his4::U1.1b*	OST in 5FOA^r isolate of HMY97 with *Eco*RI/*Bam*HI-cut pNKY349 (Cao *et al.* 1990)
MW33	*his4-51 his4-lop*	White *et al.* (1991)

[a] All strains were derived from the haploid strains AS4 (α *trp1 arg4-17 tyr7-1 ade6 ura3*) or AS13 (*a leu2 ade6 ura3 rme1*) by transformation or by crosses with isogenic derivatives. Only those markers that are different from the progenitor haploids are shown.

[b] Most strains were generated by one-step transplacements (OST) or two-step transplacements (TST). In those constructions in which the one-step transplacement utilized a PCR fragment, the names of the primers are indicated and the template plasmid or yeast strain is shown in parentheses; the references for the template plasmids or strains are: pRS306 (Sikorski and Hieter 1989), pFA6-*kanMX4* (Wach *et al.* 1994), pA632 (Goldstein and McCusker 1999), and DTK172 (Kirkpatrick *et al.* 1998). Ura$^-$ derivatives were selected on 5-fluoroorotic acid medium (Boeke *et al.* 1984).

[c] The *his4::U1.1b* allele differs from the *his4::U1.1a* allele by a T to A polymorphism in the inserted sequence, 74 bp downstream of the *URA3* stop codon.

TABLE 2

Diploid yeast strains

Strain	Relevant homozygous mutations	Cross and/or reference[a]
	Strains heterozygous for *his4-lopd* (26-bp insertion)	
DNY27	Wild type	Nag *et al.* (1989)
DTK223	*msh2::Tn10LUK7-7*	Kirkpatrick and Petes (1997)
DTK230	*rad1::ura3 msh2::Tn10LUK7-7*	Kirkpatrick and Petes (1997)
DTK241	*rad2::URA3*	Kirkpatrick and Petes (1997)
DTK242	*rad14::URA3*	Kirkpatrick and Petes (1997)
DTK246	*rad27::HUH*	DTK245 × DTK244
DTK286	*rad10::URA3*	DTK285 × DTK261
DTK308	*exo1::URA3*	Kirkpatrick *et al.* (2000)
DTK309	*pms1*-Δ	DNY95 × DTK248
DTK320	*mlh1::URA3*	DTK318 × DTK319
DTK327	*msh4::URA3*	DTK247 × DTK243
DTK341	*hdf1::URA3*	DTK339 × DTK340
DTK488	*msh3::kanMX4*	DTK487 × DTK486
DTK494	*msh6::kanMX4*	HMY134 × DTK489
DTK498	*msh5*-Δ	Kirkpatrick *et al.* (2000)
DTK500	*rad1::URA3 msh3::kanMX4*	HMY168 × DTK496
JG20	*pol4::kanMX4*	JG1 × JG3
TP1013	*rad1::URA3*	Kirkpatrick and Petes (1997)
	Strains heterozygous for *his4::U1.1a*	
HMY49	*rad1::ura3*	DTK225 × HMY46
HMY100	Wild type	AS4 × HMY99
HMY107	*msh2::kanMX4*	HMY104 × HMY101
HMY108	*mlh1::kanMX4*	HMY105 × HMY102
HMY109	*pms1::kanMX4*	HMY106 × HMY103
HMY113	*msh3::kanMX4*	HMY110 × HMY111
HMY120	*exo1::kanMX4*	HMY118 × HMY119
HMY136	*msh6::kanMX4*	HMY134 × HMY135
HMY150	*rad27::hisG*	HMY123 × HMY145
HMY151	*rad10::ura3*	HMY124 × HMY146
HMY152	*rad14::ura3*	HMY125 × HMY147
HMY157	*his4-51*	MW30 × HMY154
HMY160	*mlh3::kanMX4*	HMY158 × HMY159
HMY163	*mlh2::kanMX4*	HMY161 × HMY162
HMY176	*rad2::ura3*	HMY167 × HMY172
HMY183	*his4-51 rad50S*	HF2 × HMY182
HMY184	*rad1::ura3 msh3::kanMX4*	HMY175 × HMY171
HMY229	*rad50S rad1::ura3*	HMY226 × HMY227
HMY243	*msh4::kanMX4*	HMY223 × HMY242
	Strains heterozygous for *his4::U1.1b*[b]	
HMY98	Wild type	AS4 × HMY97
JG23	*pol4::kanMX4*	JG1 × JG6
JG24	*rev3::kanMX4*	JG17 × JG7
JG25	*rad30::kanMX4*	JG18 × JG8
JG30	*rad50S*	DNY107 × JG16
	Strains heterozygous for *his4::k1.5*	
HMY190	Wild type	AS4 × HMY187
HMY210	*rad50S*	HMY22 × HMY208
HMY219	*rad1*-Δ	DTK225 × HMY212
HMY230	*rad50S rad1*-Δ	HMY226 × HMY228
HMY234	*his4-51*	MW30 × HMY233
HMY237	*rad50S his4-51*	HF2 × HMY236
HMY239	*his4-51 rad1::ura3*	HMY235 × HMY238

(*continued*)

TABLE 2

(Continued)

Strain	Relevant homozygous mutations	Cross and/or reference[a]
	Strains heterozygous for *his4::U1.1a/his4::k1.5*	
HMY189	Wild type	HMY50 × HMY187
HMY198	*mlh1::hygB*	HMY195 × HMY196
HMY215	*rad1::hygB*	HMY214 × HMY212
HMY222	*msh3::hygB*	HMY221 × HMY220
HMY251	*rad2::hygB*	HMY249 × HMY250
	Strains heterozygous for *his4::U5.6*	
HMY192	Wild type	AS4 × HMY191
HMY199	*msh3::kanMX4*	HMY110 × HMY197
HMY203	*rad1*-Δ	DTK225 × HMY202
HMY211	*rad50S*	HMY22 × HMY209
HMY258	*pol4::hygB*	JG1 × HMY257

[a] Genotypes of the haploids used in the constructions are given in Table 1. In crosses, AS4- and AS13-derived haploids are shown to the left and right of the ×, respectively.

[b] The *his4::U1.1b* allele differs from the *his4::U1.1a* allele by a T to A polymorphism in the insert, 74 bp downstream of the *URA3* stop codon.

Nag *et al.* 1989; Kirkpatrick and Petes 1997). Mutations in the *RAD1* or *MSH2* gene increased the frequency of PMS events and decreased the frequency of gene conversion events, indicating that Rad1p and Msh2p were involved in the repair of 26-base DNA loops (Kirkpatrick and Petes 1997). In this study, we extend our analysis of the gene products required for the efficient repair of the 26-base loop. We also examined the meiotic segregation patterns of larger heterozygous insertions (1.1, 1.5, and 5.6 kb) at the same position in *HIS4*.

Meiotic repair of a 26-base loop: Aberrant segregation patterns of strains heterozygous for a 26-bp insertion in *HIS4* are shown in Table 4. To determine whether various mutations have a significant effect on repair of the 26-base loop, we compared the relative numbers of tetrads with PMS and conversion in the wild-type and mutant strains by Fisher's exact test. By this criterion, in addition to the *rad1* and *msh2* mutations reported previously (Kirkpatrick and Petes 1997), strains homozygous for mutations in *rad10* (DTK286) and *msh3* (DTK488) had a significant repair deficiency (P values of 0.005 and <0.0001, respectively). The *rad1 msh2* and *rad1 msh3* double mutant strains (DTK230 and DTK500, respectively) had PMS frequencies similar to those observed in the single mutant *rad1*, *msh2*, and *msh3* strains, indicating that these gene products are likely to function in a single repair pathway. The requirement for both Rad1p and Rad10p, which function as a heterodimeric structure-specific endonuclease, suggests that this endonuclease activity is required for efficient loop repair; several other NER enzymes, such as Rad2p and Rad14p, however, are not required for loop repair (Kirkpatrick and Petes 1997).

Neither *mlh1* (DTK320) nor *pms1* (DTK309) mutations had a significant effect on repair of the 26-base loop. Similarly, mutations in the MutS homologues Msh6p (DTK494) and Msh5p (DTK498) had no significant effect on loop repair. The *msh4* mutation, however, had a small, but significant effect (P value of 0.005). Since epistasis analysis indicates that Msh4p and Msh5p function in a single pathway in crossover resolution (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995), the differential effects of these two proteins on loop repair is unexpected. Mutations in *EXO1* (DTK308) had a similar subtle defect in repair of the 26-base loop (Kirkpatrick *et al.* 2000). It is possible that Msh4p and Exo1p are involved in a minor pathway of loop repair. Since none of the mutations examined in our study eliminate gene conversion, it is likely that there are pathways of loop repair that are independent of the Rad1p/Rad10p/Msh2p/Msh3p pathway.

The *RAD27* gene encodes the *S. cerevisiae* homologue of the mammalian FEN-1 exo/endonuclease (Johnson *et al.* 1995; Reagan *et al.* 1995). Rad27p is involved in the removal of single-stranded DNA "flaps" and the processing of Okazaki fragments during replication (Harrington and Lieber 1994a,b; Murante *et al.* 1994). Deletion of *RAD27* (DTK246) had no effect on repair of the 26-base loop (Table 4). Finally, we examined the role of the non-homologous end-joining repair pathway in loop repair. A strain homozygous for a deletion of *HDF1/YKU70* (DTK341), a homologue of a component of the mammalian Ku DNA end-binding complex (Feldmann and Winnacker 1993; Troelstra and Jaspers 1994), had no effect on loop repair (Table 4).

Analysis of strains heterozygous for a 1.1-kb *URA3* insertion: The *his4::U1.1a* allele was constructed by introduction of a 1.1-kb fragment containing the *URA3* gene into the *HIS4* coding sequence at position +469,

TABLE 3

Primers used in strain constructions

Primer name	Sequence (5′–3′)
f *his::U1.1*	GTGAAGTACGTACAGACCGTCCTGACGGTTTATATACCACCCTAGTTGTCcgcttttcaattcaattcat
r *his::U1.1*	ATAGATTTCTTCGAAGAATACACCAACCCTAGACAACGCTCATATTGGTCcagggtaataactgatataa
f *his::k1.5*	GTGAAGTACGTACAGACCGTCCTGACGGTTTATATACCACCCTAGTTGTCcgtacgctgcaggtcgac
r *his::k1.5*	ATAGATTTCTTCGAAGAATACACCAACCCTAGACAACGCTCATATTGGTCatcgatgaattcgagctcg
f *his::U5.6*	GTGAAGTACGTACAGACCGTCCTGACGGTTTATATACCACCCTAGTTGTCattccaggatccaagcgtatgctg
r *his::U5.6*	ATAGATTTCTTCGAAGAATACACCAACCCTAGACAACGCTCATATTGGTCggatccccacctatgggcggg
f *msh2*-Δ	AAAAATCTCTTTATCTGCTGACCTAACATCAAAATCCTCAGATTAAAAGTcgtacgctgcaggtcgac
r *msh2*-Δ	ATCTATATATTATCTATCGATTCTCACTTAAGATGTCGTTGTAATATTAAatcgatgaattcgagctcg
f *msh3*-Δ	GACGGAATATTGCGATCACGTGAATTTTCAATGATAAATAAGCTGGAACAcgtacgctgcaggtcgac
r *msh3*-Δ	ATCATTTATTGTCTGATAATGCTGCATTTAGAACATACGTACCATCCGCAatcgatgaattcgagctcg
f *msh4*-Δ	TAACTAGTTATAGCATTGAAATCTGTAGCTGATCAACGCAAACTATATGCcgtacgctgcaggtcgac
r *msh4*-Δ	TCTGTACAGAAATAATGGATTATAGTTTTAAGCTAAGCGGAAAAGCCAAAatcgatgaattcgagctcg
f *msh6*-Δ	TTTAATTGGAGCAACTAGTTAATTTTGACAAAGCCAATTTGAACTCCAAAcgtacgctgcaggtcgac
r *msh6*-Δ	ACTTTAAAAAAAATAAGTAAAAATCTTACATACATCGTAAATGAAAATACatcgatgaattcgagctcg
f *mlh1*-Δ	ATAGTGATAGTAAATGGAAGGTAAAAATAACATAGACCTATCAATAAGCAcgtacgctgcaggtcgac
r *mlh1*-Δ	CTCAGGAAATAAACAAAAAACTTTGGTATTACAGCCAAAACGTTTTAAAGatcgatgaattcgagctcg
f *mlh2*-Δ	CACATCCCATCATCTCGGTTTGAGGAACAGACGCCTTTTCATAGTTTTGGcgtacgctgcaggtcgac
r *mlh2*-Δ	TCTATTATGAAGTAATCTATTGTGCTGAGTGGTGATAGTGCACCCGATCAatcgatgaattcgagctcg
f *mlh3*-Δ	GCGAGGCTTTCAAGGAAGAATGAACGTGAACTCGTCAACTCAAAAAGAAAcgtacgctgcaggtcgac
r *mlh3*-Δ	CGCAATTTAAAATGCAGGCGACAAACCTTGTTCCAGGATTAAGGTTCTCTatcgatgaattcgagctcg
f *pms1*-Δ	GAACGCGAAAAGAAAAGACGCGTCTCTCTTAATAATCATTATGCGATAAAcgtacgctgcaggtcgac
r *pms1*-Δ	CTCCCTGTATATAATGTATTTGTTAATTATATAATGAATGAATATCAAAGatcgatgaattcgagctcg
f *rad1*-Δ	TAAATGTGTAAAAATAATATTGCACTATCCTGTTGAAAATATCTTTCCAGcgtacgctgcaggtcgac
r *rad1*-Δ	TCGCATTTTATACTGATGTTTTAACAGGGTTCGTTAAATTAAACAATATTatcgatgaattcgagctcg
f *rad2*-Δ	CACGTCATCCATGAAGAAAAGCATTTTCGGGAGAACGCCAAACTTCAGACcgtacgctgcaggtcgac
r *rad2*-Δ	TGCAGAAACAAAGGTAATGTTTATAAATAGTAAATCATACATAAGTATATGTTAatcgatgaattcgagctcg
f *exo1*-Δ	AAAGGAGCTCGAAAAAACTGAAAGGCGTAGAAAGGAATGGGTATCCAAGGTcgtacgctgcaggtcgac
r *exo1*-Δ	CCTCCGATATGAAACGTGCAGTACTTAACTTTTATTTACCTTTATAAACAAATTGGGatcgatgaattcgagctcg
f *pol4*-Δ	CAGTGGTAATAAGTAAAGGATAAACATGCGACCTGTTAGACAAATCGCACcgtacgctgcaggtcgac
r *pol4*-Δ	AAAATTCGCTCCTCTTTTAATGCCTTTATGCAGTTTTTTTTTCCCATTCGatcgatgaattcgagctcg
f *rev3*-Δ	ATTTGAGTCAATACAAAACTACAAGTTGTGGCGAAATAAAATGTTTGGAAggtacgctgcaggtcgac
r *rev3*-Δ	ATAGAAACAAATAACTACTCATCATTTTGCGAGACATATCTGTGTCTAGAatcgatgaattcgagctcg
f *rad30*-Δ	TAGCGCAGGCCTGCTCATTTTTGAACGGCTTTGATAAAACAAGACAAAGCcgtacgctgcaggtcgac
r *rad30*-Δ	ATCAGGACGTTTTAGTTGCTGAAGCCATATAATTGTCTATTTGGAATAGGatcgatgaattcgagctcg

Many of the gene disruptions were made using PCR fragments in which a selectable gene was flanked by sequences of the gene to be disrupted (Wach *et al.* 1994). Sequences homologous to the disruption target gene are shown in uppercase letters; sequences homologous to the selectable gene used to make the disruption are shown in lowercase. f, forward (upstream) primer; r, reverse (downstream) primer. The genes that were used as templates in the PCR reactions are described in Table 1.

the same position as the 26-bp insertion described above. Results from tetrad analysis of strains heterozygous for this insertion are shown in Table 5.

In the wild-type background (HMY100), we observed a high level of gene conversion events (22%) and no PMS events. One interpretation of this result is that heteroduplexes cannot accomodate the 1.1-kb insertion and, consequently, gene conversion occurs through a different pathway (for example, gap repair). Alternatively, heteroduplexes containing the heterozygous insertion are formed, and the resulting loop is repaired with complete efficiency. The second possibility is supported by the observation that the *rad1* strain (HMY49) heterozygous for the 1.1-kb *URA3* insertion had substantial rates of PMS tetrads (Table 5). Because the *URA3* insertion confers the ability to grow on medium lacking uracil, PMS events were visualized as sectored colonies with His^-Ura^+ and His^+Ura^- halves (Figure 2a). The *rad10* mutation had an effect on loop repair similar to that of the *rad1* mutation (comparison of strains HMY49 and HMY151). The other mutations that resulted in PMS events for the 1.1-kb *URA3* insertion were *msh2* (HMY107) and *msh3* (HMY113). The *msh2* and *msh3* mutations had significantly ($P < 0.02$) smaller effects than the *rad1* and *rad10* mutations. Since the efficiency of loop repair for the *URA3* insertion is about the same

TABLE 4

Meiotic segregation patterns of strains heterozygous for *his4-lopd* (26-bp insertion)

Strain name	Relevant genotype[a]	Total tetrads	Ab. seg.[b] (%)	PMS[c] (%)	PMS/Ab.[d] (%)	% of total tetrads						*HIS4-LEU2* distance[f] (cM)
						4:4	6:2	2:6	5:3	3:5	Other[e]	
DNY27[g]	Wild type	359	30	4	12	70	15	11	3	0.3	1	39
TP1013[g]	*rad1*-Δ	471	38	14	37	62	17	6	7	5	2	36
DTK286	*rad10*-Δ	212	34	11	32	66	18	5	6	4	2	33
DTK223[g]	*msh2*-Δ	136	39	15	40	61	13	9	10	3	4	38
DTK488	*msh3*-Δ	295	38	17	45	62	12	9	9	5	3	35*
DTK230[g]	*rad1*-Δ *msh2*-Δ	106	43	24	56	58	9	9	11	10	3	34
DTK500	*rad1*-Δ *msh3*-Δ	136	40	22	55	60	13	5	13	4	5	34*
DTK327	*msh4*-Δ	397	32	9	27	68	11	11	5	3	2	19*
DTK498[h]	*msh5*-Δ	288	24	5	19	76	8	9	3	1	2	24*
DTK494	*msh6*-Δ	216	26	4	16	74	11	11	2	1	1	26
DTK320	*mlh1*-Δ	288	24	5	20	76	10	8	2	2	1	27*
DTK309	*pms1*-Δ	333	29	2	7	72	12	14	2	0	1	28
DTK241[g]	*rad2*-Δ	205	20	2	12	80	11	7	0.5	2	0	21*
DTK242[g]	*rad14*-Δ	228	29	3	9	71	17	8	2	0.4	1	35
DTK308[h]	*exo1*-Δ	292	21	6	30	80	10	4	4	2	1	22*
DTK246	*rad27*-Δ	214	26	4	15	74	11	10	3	0.5	2	27
DTK341	*hdf1*	216	25	3	11	75	12	10	2	1	0.5	27
JG20	*pol4*-Δ	255	28	6	23	72	11	10	3	3	1	33

* Significant ($P < 0.05$) departures from wild type (DNY27) in the numbers of PD:NPD:T asci (contingency chi-square analysis).

[a] Homozygous mutations different from those of the progenitor parental strains.

[b] Percentage of total tetrads with an aberrant segregation pattern (non-4:4).

[c] Percentage of total tetrads with one or more PMS events.

[d] Percentage of aberrant tetrads with one or more PMS events.

[e] Tetrads with two or more PMS events (ab4:4, ab6:2, ab2:6, dev5:3, dev3:5, dev4:4), one PMS event, and one gene conversion (7:1, 1:7), or two gene conversions (8:0 and 0:8).

[f] Calculated by the equation of Perkins (1949).

[g] Recalculated from data of Kirkpatrick and Petes (1997).

[h] Data of Kirkpatrick *et al.* (2000).

in the *rad1 msh3* double mutant (HMY184) and the *rad1* single mutant (HMY49), Rad1p and Msh3p appear to act in a single pathway of loop repair. Mutations in *msh4*, *msh5*, *msh6*, *mlh1*, *pms1*, *mlh2*, *mlh3*, *rad2*, *rad14*, *exo1*, and *rad27* had no effect on the repair of the 1.1-kb loop (Table 5).

Although the *rad27* strain (HMY150) exhibited no defect in repair of the 1.1-kb loop, we observed a significant decrease in the level of aberrant segregants in this background ($P < 0.0001$). This result is specific for the *his4::U1.1a* allele, since we did not observe a similar effect in a *rad27* strain heterozygous for the 26-bp insertion (Table 4) or on other heterozygous point mutations in HMY150 (data not shown). It is possible that the flap endonuclease plays a role in heteroduplex formation between alleles with large insertion/deletion heterologies.

In most models for meiotic recombination (Figure 1), DNA replication is involved in formation of heteroduplexes and in repair of DNA mismatches. *S. cerevisiae* has a number of nonessential DNA polymerases including β (encoded by *POL4*), η (encoded by *RAD30*), and ζ (catalytic subunit encoded by *REV3*; Woodgate 1999). We examined meiotic segregation of the *his4::U1.1b* allele (identical to *his4::U1.1a* except for a single T to A base pair change in the insertion, 74 bp downstream of the *URA3* stop codon) in strains homozygous for null mutations in these three DNA polymerase genes (Table 5). Although the *rad30* and *rev3* mutations had no significant effect, the *pol4* mutation significantly ($P = 0.001$) reduced the frequency of aberrant segregation of *his4::U1.1b*. We also analyzed the effects of the same three DNA polymerase mutations on the frequency of aberrant segregation of a point mutation located in the *HIS4* initiating codon. No significant effect was observed for any of the three mutations (data not shown) and the *pol4* mutation did not significantly affect the frequency of aberrant segregation or the repair of the 26-base loop (Table 4). In addition, DSB formation at the *HIS4* hotspot (monitored in a *rad50S* strain) was unaffected by the *pol4* mutation (data not shown). Although these results suggest that DNA polymerase β may have a role in heteroduplex formation across large heterologies, we found that the *pol4* mutation did not signifi-

TABLE 5

Meiotic segregation patterns of strains heterozygous for *his4-U1.1* (1.1-kb insertion)

Strain name	Relevant genotype	Total tetrads	Ab. seg. (%)	PMS (%)	PMS/Ab. (%)	% of total tetrads						*HIS4-LEU2* distance[a] (cM)
						4:4	6:2	2:6	5:3	3:5	Other	
HMY100	Wild type	349	22	0	0	78	9	13	0	0	0.3	33
HMY49	*rad1*-Δ	362	29	16	55	71	6	7	8	8	0.3	31
HMY151	*rad10*-Δ	164	28	12	41	72	12	4	7	4	1	35
HMY107	*msh2*-Δ	257	26	3	11	74	11	11	2	1	1	30
HMY113	*msh3*-Δ	261	29	7	23	71	11	11	4	2	1	37
HMY184	*rad1*-Δ *msh3*-Δ	282	29	12	43	71	11	6	8	4	1	34
HMY243	*msh4*-Δ	143	19	0	0	81	5	13	0	0	1	16*
HMY136	*msh6*-Δ	207	19	0	0	81	6	13	0	0	0.5	37
HMY108	*mlh1*-Δ	138	21	0	0	79	9	12	0	0	0	24
HMY109	*pms1*-Δ	172	13	0	0	87	4	10	0	0	0	30
HMY163	*mlh2*-Δ	207	19	0	0	81	6	13	0	0	0.5	37
HMY160	*mlh3*-Δ	177	20	0	0	80	6	14	0	0	1	27
HMY176	*rad2*-Δ	144	19	0	0	81	4	15	0	0	0	27
HMY152	*rad14*-Δ	132	15	0	0	85	4	11	0	0	0	34
HMY120	*exo1*-Δ	180	16	0	0	84	5	11	0	0	0	18*
HMY150	*rad27*-Δ	153	8	0	0	92	1	6	0	0	1	18*
HMY98	Wild type[b]	251	23	0	0	77	7	15	0	0	1	37
JG23	*pol4*-Δ[b]	333	12	0	0	88	4	8	0	0	0.3	29
JG24	*rev3*-Δ[b]	172	20	0	0	80	5	15	0	0	1	28
JG25	*rad30*-Δ[b]	167	19	0	0	81	5	13	0	0	0	27

All strains, except for those with the *b* superscript, were heterozygous for the *his4::U1.1a* allele. Headings have the same meanings as in Table 4. *Statistically significant ($P < 0.05$) differences in ratios.

[a] For all strains heterozygous for the *his4::U1.1a* allele, we used contingency chi-square analysis to compare the relative numbers of PD:NPD:T tetrads with those observed in HMY100. The numbers of PD:NPD:T tetrads in strains JG23, JG24, and JG25 were compared to those observed in HMY98; these strains were heterozygous for *his4::U1.1b*.

[b] These strains are heterozygous for the *his4::U1.1b* allele; this allele is described in the text.

cantly reduce the aberrant segregation frequency of a different larger insertion (as described below). Our results are in contrast to those of Leem *et al.* (1994) who found elevated levels of both DSBs and meiotic recombination in *pol4* strains.

Genetic analysis of strains heterozygous for a 1.5-kb *kanMX* insertion or a 5.6-kb *URA3* insertion in *HIS4*: To generalize the results obtained with the 1.1-kb *URA3* insertion, we also examined strains heterozygous for other insertions in *HIS4*. The *his4::k1.5* allele was generated by inserting the 1.5-kb *kanMX4* gene (Wach *et al.* 1994) at position +469 of the *HIS4* coding sequence. Strains with this insertion are His$^-$, but resistant to geneticin. A wild-type strain heterozygous for this insertion (HMY190) had 12% conversion tetrads, but no PMS tetrads (Table 6). In a derivative homozygous for the *rad1* mutation (HMY219), we observed 12% conversion events and 17% PMS events. This result confirms our conclusion that heteroduplexes can be formed that include large heterologies and that the repair of the resulting DNA loop is, at least partly, Rad1p dependent.

We also constructed strains heterozygous for a 5.6-kb insertion of *URA3* at position +469 of the *HIS4* coding sequence (*his4::U5.6*). A wild-type strain heterozygous for this insertion (HMY192) had 13% gene conversion and no PMS tetrads (Table 6). In strains with mutations in either *rad1* (HMY203) or *msh3* (HMY199), however, we observed a small number of PMS tetrads (five tetrads in the two strains). Thus, we conclude that heterologies as large as 5.6 kb can be included within heteroduplexes, although the efficiency of heteroduplex formation with these very large loops is reduced. The *pol4* mutation did not significantly reduce the aberrant segregation frequency of *his4::U5.6* (comparison of HMY192 and HMY258 in Table 6).

DSB formation in strains with heterozygous large insertions: In wild-type strains heterozygous for *his4::U1.1a*, *his4::U1.1b*, *his4::k1.5*, and *his4::U5.6*, we found that 2:6 gene conversion events were more common than 6:2 conversions. One interpretation of such a bias is that the chromosome containing the insertion has fewer DSBs at the upstream *HIS4* hotspot than does the chromosome lacking the insertion (Figure 1). Consequently, we measured DSB formation in *rad50S* derivatives of strains heterozygous for *his4::U1.1b* (JG30) and *his4-k1.5* (HMY210); *rad50S* strains accumulate unprocessed DSBs, simplifying their quantitation (Cao *et al.* 1990).

In addition to the DSB associated with the upstream *HIS4* hotspot (Fan *et al.* 1995), the JG30 strain had a novel minor DSB band that mapped to the 5′ end of

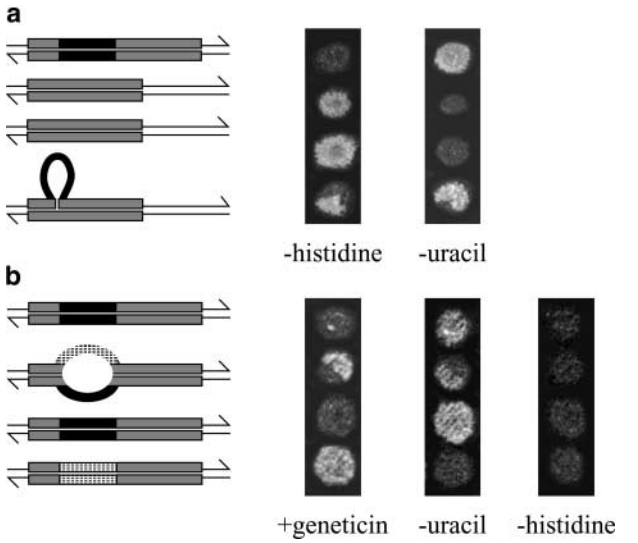

FIGURE 2.—Colony sectoring patterns in strains heterozygous for a single insertion or heterozygous for two different insertions. (a) In a strain heterozygous for *his4::U1.1a* and homozygous for *rad1* or *rad10*, tetrads with PMS events were common. The right side of the figure shows spore colonies derived from HMY49; tetrads were dissected onto a rich growth medium and the resulting spore colonies were replica plated to media lacking histidine or uracil. The left side of the figure depicts the DNA molecules that generate the spore phenotypes (same depictions as in Figure 1). (b) Spore colonies derived from a tetrad of a *rad1* strain (HMY215) that was heterozygous for two different insertions (*his4::U1.1a* and *his4::k1.5*) located at the same position within *HIS4*. Spore colonies were first grown on rich medium and then replica-plated to media lacking histidine or uracil, or containing geneticin. The *URA3* and *kanMX4* insertions are indicated as solid and crosshatched rectangles, respectively.

the *URA3* insert (Figure 3a). The frequency of the insert-associated DSB was about one-third of that associated with the wild-type hotspot. The hotspot-associated DSB on the chromosome with *HIS4* was ~1.3-fold more intense than the upstream DSB on the *his4::U1.1b*-containing chromosome (average of three experiments). The observed conversion bias in HMY98 was 2-fold (Table 5). Thus, the difference in DSB formation on the two homologues accounts for much of the conversion bias observed in HMY98, assuming that DSB formation within the insertion does not contribute to gene conversion (an assumption discussed below).

A very strong DSB was also found within the *kanMX4* insertion (HMY210; Figure 3b). The activity of the upstream *HIS4* hotspot on the mutant chromosome was reduced 3.6-fold when compared to the upstream hotspot on the wild-type chromosome (average of three experiments). This bias in DSB formation accounts for part, although not all, of the 11-fold excess of the 2:6 class seen in the *RAD50* companion strain HMY190 (Table 6). We also found DSBs associated with the insertion of the *his4::U5.6* allele in the *rad50S* strain HMY211 (data not shown).

TABLE 6

Meiotic segregation patterns of strains with larger insertions and bubbles

Strain	*HIS4* alleles	Other homozygous alleles	Total tetrads	Ab. seg. (%)	PMS (%)	PMS/ Ab. (%)	% of total tetrads					*HIS4-LEU2* distance[a] (cM)
							4:4	6:2	2:6	5:3	3:5	Other	
HMY190	*HIS4/his4::k1.5*	Wild type	169	12	0	0	88	1	11	0	0	1	30
HMY219	*HIS4/his4::k1.5*	*rad1-Δ*	175	27	17	63	73	5	5	7	10	1	37
HMY192	*HIS4/his4::U5.6*	Wild type	213	13	0	0	87	2	11	0	0	0	25
HMY203	*HIS4/his4::U5.6*	*rad1-Δ*	472	14	1	6	86	8	5	0.4	0.4	0	28
HMY199	*HIS4/his4::U5.6*	*msh3-Δ*	237	16	0.4	3	84	7	8	0	0	0.4	28*
HMY258	*HIS4/his4::U5.6*	*pol4Δ*	273	10	0	0	90	1	10	0	0	0	25
HMY189	*his4::U1.1a/his4::k1.5*	Wild type	213	6	0	0	94	2	3	0	0	0.5	23
HMY215	*his4::U1.1a/his4::k1.5*	*rad1-Δ*	204	26	19	73	75	5	2	11	7	0.5	20
HMY222	*his4::U1.1a/his4::k1.5*	*msh3-Δ*	138	25	1	3	75	17	8	1	0	0	24
HMY198	*his4::U1.1a/his4::k1.5*	*mlh1-Δ*	137	8	0	0	92	5	3	0	0	0	34
HMY251	*his4::U1.1a/his4::k1.5*	*rad2-Δ*	232	7	0	0	94	4	2	0	0	0.4	36*

This table includes data for *HIS4/his4::k1.5*, *HIS4/his4::U5.6*, and *his4::U1.1a/his4::k1.5* strains. Headings have the same meanings as in Table 4. *Significant ($P < 0.05$) differences.

[a] Statistical comparisons (contingency chi-square analysis) of the relative numbers of PD:NPD:T tetrads were done for HMY190 with HMY219; HMY203, HMY199, and HMY258 with HMY192; and the remainder of the strains with HMY189.

It is likely that the bias in the activities of the upstream hotspots reflects a competitive interaction between the DSBs within the insertions and the upstream DSB. We and others previously found that if two DSB sites are found close together on the same chromosome, the activity of each individual hotspot is reduced when compared to their "solo" activities (Wu and Lichten 1995; Xu and Kleckner 1995; Fan *et al.* 1997). Consistent with this hypothesis, we found that the strong DSB in the *kanMX4* insertion had a larger effect than did the weak DSB in the 1.1-kb *URA3* insertion. In addition, if the insert-associated DSBs do not contribute to gene conversion events at the *HIS4* locus, the effect of the DSB competition would be to generate gene conversion disparity.

Since homology is required to initiate heteroduplex formation, we expected that DSBs within the insertions were unlikely to contribute to gene conversion events at *HIS4* that included the insertions. To test this hypothesis genetically, we constructed strains heterozygous for *his4::U1.1a* (HMY157) or *his4::k1.5* (HMY234) in which the *HIS4* upstream hotspot had been inactivated by mutations of a Rap1p binding site (the *his4-51* allele; Devlin *et al.* 1991; White *et al.* 1991). The results from this analysis are in Table 7.

Deletion of the wild-type hotspot reduced aberrant segregation of *his4::U1.1a* from 22% (HMY100) to 3% (HMY157). The same deletion reduced aberrant segregation of *his4::k1.5* from 13% (HMY190) to 4% (HMY234). In addition, those conversion events that occurred in HMY157 and HMY234 were not biased in favor of the 6:2 class. Such a bias would be expected if DSB formation within the insertion stimulated interhomologue recombination (Figure 1). The deletion of the wild-type hotspot also reduced crossovers between *HIS4* and *LEU2* in HMY157 and HMY234 compared to HMY100 and HMY190, shortening the average map distance from ~32 to 20 cM. In previous studies in which the effect of the hotspot deletion was monitored in the absence of a large insertion at *HIS4*, a similar reduction in crossovers was observed (White *et al.* 1991). We also observed a reduction in aberrant segregation and *HIS4-LEU2* crossovers in strain HMY239, a strain heterozygous for *his4::k1.5* and homozygous for *his4-51* and *rad1* (Table

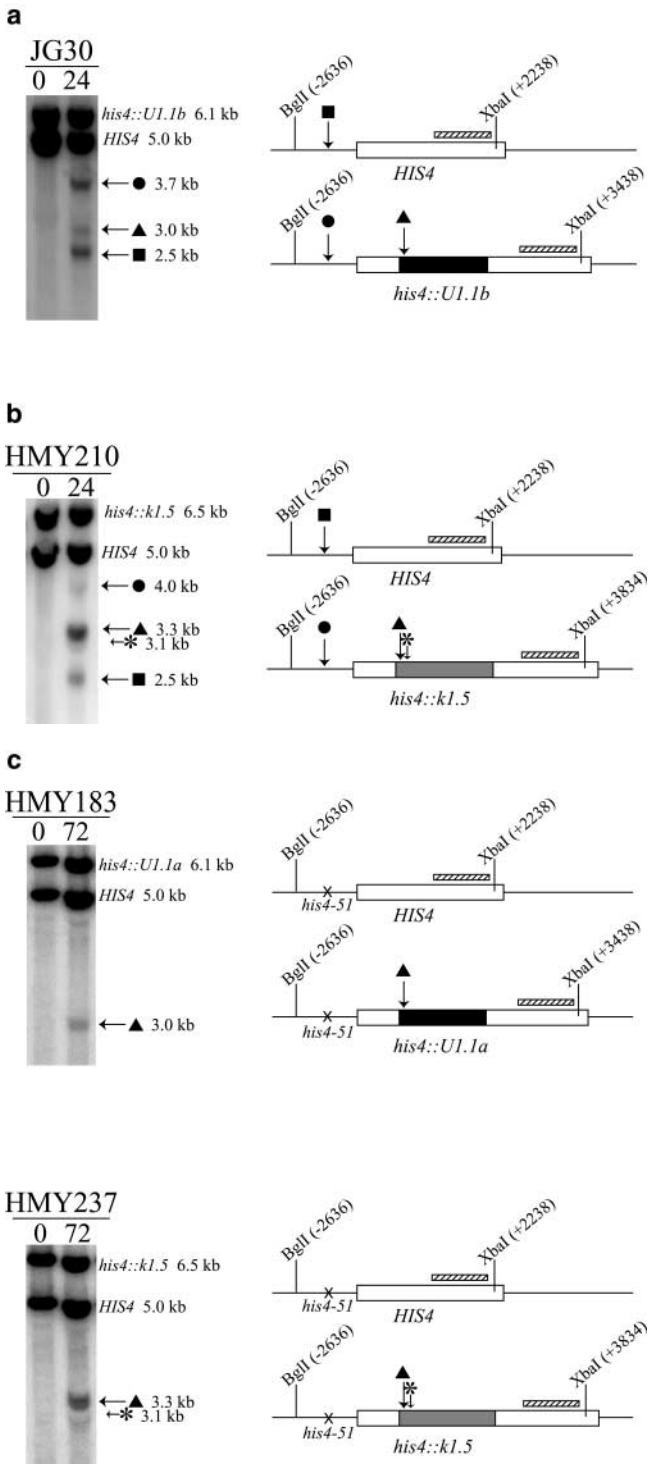

Figure 3.—Double-strand break formation in strains heterozygous for large insertions. Meiotic samples were collected from *rad50S* strains after either 0 and 24 hr of growth in liquid sporulation medium or after 0 and 72 hr on sporulation plates (both methods give equivalent results). DNA was digested with *Bgl*I and *Xba*I and probed with a ^{32}P-labeled *HIS4* fragment (indicated by hatched bar). Parental fragments are labeled at the top of each blot, and DSB-generated bands are indicated with arrows. All positions are given relative to the initiating codon of *HIS4*. (a) Analysis of JG30 (heterozygous for *his4::U1.1b*). DSBs were detected at the upstream *HIS4* hotspot on both the wild-type (solid square) and mutant (solid circle) chromosomes. A third break was also detected (solid triangle) that maps to the 5′ end of the *URA3* insert. (b) Analysis of HMY210 (heterozygous for *his4::k1.5*). DSBs are labeled as described above with the addition of an asterisk to mark a second DSB within the insert. (c) Analysis of strains that are heterozygous for large insertions (HMY183, *his4::U1.1a*; HMY237, *his4::k1.5*) and homozygous for *his4-51* (mutations in the *HIS4* upstream region that eliminate Rap1p binding and the activity of the upstream hotspot). DSBs were still observed in both the 1.1-kb *URA3* insert and the 1.5-kb *kanMX4* insert.

TABLE 7

Meiotic segregation patterns of strains homozygous for the *his4-51* allele and isogenic controls

Strain	*HIS4* alleles	Other relevant alleles	Total tetrads	Ab. seg. (%)	PMS (%)	PMS/ Ab. (%)	% of total tetrads					*HIS4-LEU2* distance[a] (cM)
							4:4	6:2	2:6	5:3	3:5	
HMY100	*HIS4/his4::U1.1a*		349	22	0	0	78	9	13	0	0	33
HMY157	*HIS4/his4::U1.1a* *his4-51/his4-51*		156	3	0	0	97	1	2	0	0	17*
HMY190	*HIS4/his4::k1.5*		169	12	0	0	88	1	11	0	0	30
HMY234	*HIS4/his4::k1.5* *his4-51/his4-51*		186	4	0	0	96	2	3	0	0	20
HMY219	*HIS4/his4::k1.5*	*rad1*-Δ/*rad1*-Δ	175	27	17	63	73	5	5	7	10	37
HMY239	*HIS4/his4::k1.5* *his4-51/his4-51*	*rad1*-Δ/*rad1*-Δ	142	9	6	69	91	1	2	3	4	16*

The *his4-51* mutation results in loss of the binding site for the Rap1p transcription factor; this loss eliminates the upstream *HIS4* DSB (White *et al.* 1991). Headings have the same meanings as in Table 4. *Significant ($P < 0.05$) differences.

[a] Statistical comparisons (contingency chi-square analysis) of the relative numbers of PD:NPD:T tetrads were done for HMY100 with HYM157, for HMY190 with HMY234, and for HMY219 with HMY239.

7). This reduction was less than that observed for HMY234, presumably reflecting the recovery of PMS events that would have been repaired as restoration events (Figure 1) in the *RAD1* strain.

To be sure that strains without the upstream *HIS4* hotspot had insertion-associated DSBs, we examined DSB formation in *rad50S* derivatives of HMY157 and HMY234 (HMY183 and HMY237, respectively). Since DSBs were observed (Figure 3c), we conclude that DSBs that occur within heterozygous insertions do not effectively stimulate gene conversion between homologous chromosomes. It is likely that such DSBs are repaired by genetically silent sister-strand recombination.

Since the disparity of gene conversion in strains heterozygous for insertion-generated mutations is reduced in *rad1* strains, we also analyzed DSB formation in *rad50S* derivatives of the *rad1* strains, HMY49 (HMY229) and HMY219 (HMY230), heterozygous for the *his4::U1.1a* and *his4::k1.5* insertions, respectively. The patterns of DSBs in these *rad1* strains were identical to those seen in the *RAD1* strains (data not shown). Therefore, we conclude that Rad1p does not influence patterns of DSB formation.

Ectopic recombination between the *his4::U* insertions and the *ura3* locus on chromosome V: Although it is likely (as discussed above) that most DSBs within the insertions are repaired by sister-strand interactions, we also observed tetrads that had ectopic gene conversion events between the *his4::U1.1a* or *his4::U5.6* alleles on chromosome III and the mutant *ura3* gene on chromosome V. Two types of spore colonies are indicative of ectopic recombination events: class 1, in which part or all of the colony is His^- Ura^-, and class 2, in which part or all of the colony is His^+ Ura^+. There were a total of 12 class 1 spore colonies in strains that were heterozygous for *his4::U1.1a* and the frequency of tetrads with such colonies was 1.4% or less in all strains; there was only a single class 2 tetrad in these strains. Strains heterozygous for the *his4::U5.6* allele had a higher frequency of ectopic events with an average frequency of 3.8% class 1 tetrads and 1.5% class 2 tetrads.

In all class 1 spore colonies examined (12 of 12), the *his4* gene retained the *URA3* insertion. The simplest explanation of this event is that mutant information derived from the chromosome V *ura3* gene was donated to the wild-type gene located in *HIS4* on chromosome III. In confirmation of this explanation, in 12 of 12 His^- Ura^- spore colonies examined, we showed that the mutant *ura3* insertion in *his4* had the same nonsense mutation (G to T change at position 721) as the mutant genes on chromosome V. In class 2 spore colonies, the *HIS4* gene lacked an insertion. The simplest explanation for such colonies is that ectopic conversion between one of the *his4::U* alleles and one of the mutant chromosome V *ura3* genes resulted in a wild-type *URA3* gene on one copy of chromosome V. Cosegregation of this allele with the *HIS4* allele lacking an insertion would produce a His^+ Ura^+ spore colony or sector. In summary, there was a fairly high frequency of meiotic ectopic recombination events in strains with *his4::U* alleles, as expected from previous studies (Jinks-Robertson and Petes 1985; Lichten *et al.* 1987). Since these ectopic events do not directly contribute to aberrant segregation at the *HIS4* locus, they were not included in the data shown in Tables 5 and 6.

Formation and repair of bubble structures: Diploid strains were constructed in which each homologue contained an insertion at the same position in *HIS4*, but the insertions had different sequences (*his4::U1.1a* and *his4::k1.5*). Heteroduplexes that include these insertions would be expected to form a large "open" bubble (Figure 2b). Spore colonies diagnostic for such a heteroduplex would be expected to be His^-, but have Gen^R Ura^- and Gen^S Ura^+ sectors (Figure 2b).

In the wild-type background (HMY189), we observed only 6% aberrant tetrads, and all were gene conversion events (Table 6). Deletion of *RAD1* (HMY215) resulted in a dramatic increase in aberrant segregants (26%) with the recovery of many PMS tetrads (73% of the aberrant tetrads). The *msh3* deletion (HMY222) also elevated aberrant segregation frequencies, but in contrast to the results obtained with HMY215, very few PMS events were recovered (25% aberrant with 5% PMS/Ab; Table 6). Possible explanations for this difference will be discussed further below.

It has been reported previously that strains with mutations in *MLH1* and *PMS1* have elevated frequencies of PMS events involving the mating type locus (Wang *et al.* 1999). PMS events involving this locus would be expected to reflect formation of a bubble of ~700 bp. In our genetic background, the *mlh1* mutation had no effect on bubble repair (HMY198, Table 6). It is possible that the difference in our results and those of the previous study reflect size or sequence differences in the insertions. Alternatively, the colonies sectored at the mating type locus might reflect an Mlh1p-Pms1p-dependent increase in mating type switch as a consequence of mitotic gene conversion (Chen and Jinks-Robertson 1999).

DISCUSSION

The main conclusions from this study are: (1) heteroduplexes formed during meiotic recombination can include large (5.6 kb) insertions; (2) heteroduplexes can be formed between alleles that include two different large insertions; (3) the efficient repair of DNA loops formed during meiotic recombination requires Rad1p, Rad10p, Msh2p, and Msh3p; (4) gene conversion events that involve large insertions usually duplicate, rather than delete, the insertions; and (5) DSBs within insertions do not stimulate recombination between homologues.

Accommodation of heterologies within heteroduplexes: The finding that meiotic heteroduplexes can include regions of heterology as large as 5.6 kb is somewhat surprising. There are three related mechanisms that could result in heteroduplex formation through a large heterology during meiotic recombination. First, there could be extensive degradation of the broken DNA ends, followed by invasion of the resulting single-stranded DNA into the other homologue (Figure 1). The inclusion of the heterology in the heteroduplex requires either a single strand to migrate through the inserted sequence in a homoduplex, or the ability of the single strand to invade the homoduplex simultaneously on both sides of the insertion. It should be noted, however, that the ability of *Escherichia coli* strand exchange proteins to bypass 1-kb heterologies *in vitro* is limited, even in the presence of the RuvAB complex (Iype *et al.* 1994; Morel *et al.* 1994; Adams and West 1996). In the second model, the resection resulting in heteroduplex formation is limited to the DNA upstream of the insertion; the resulting Holliday junction is translated through the heterology by branch migration. The genetic evidence indicating that branch migration *in vivo* in yeast is limited or nonexistent (Fogel *et al.* 1981; Petes *et al.* 1991) argues against this model. In addition, formation of a heteroduplex that includes a bubble structure (Figure 2b) is unlikely to occur by branch migration. In our favored model, heteroduplex formation is driven by DNA replication rather than by branch migration (Figure 4). This model is similar to synthesis-dependent strand annealing models of recombination (Paques and Haber 1999), except that there is no requirement for migration of a D-loop.

In many previous yeast studies (reviewed in Petes *et al.* 1991), efficient meiotic conversions of large heterozygous insertions have been observed. Our results demonstrate that at least some of these conversion events are likely to reflect Rad1p/Rad10p/Msh2p/Msh3p-dependent repair of large DNA loops within heteroduplexes. We cannot rule out the alternative possibility that conversion in the presence of these proteins occurs through gap repair rather than the repair of a DNA loop in a heteroduplex. The observation of large (2 kb) DNA loops in heteroduplexes generated during mitotic recombination in *RAD1* strains (Clikeman *et al.* 2001), however, argues against this interpretation.

Proteins required for DNA loop repair: We examined the effects of many different mutations on the efficiency of repair of the 26-base and 1.1-kb DNA loops (Tables 4 and 5). Repair of both types of loops involved the Rad1p, Rad10p, Msh2p, and Msh3p. These same four proteins are required for the removal of nonhomologous ends during certain types of mitotic recombination events (Paques and Haber 1999). We found that the requirement for the four proteins was approximately the same in the repair of the 26-base loop, but the requirement for Msh2p and Msh3p for the repair of the 1.1-kb loop was reduced. Similarly, all four proteins are required to approximately the same extent for single-strand annealing events when the interacting repeats are short (~200 bp), but the requirement for Msh2p and Msh3p is substantially reduced when the repeats are long (1 kb; Sugawara *et al.* 1997). One interpretation of our results is that the Msh2p and Msh3p stabilize the interactions of Rad1p and Rad10p with the substrate, and this stabilization is more important with the smaller region of single-stranded DNA represented by the 26-base loop.

The observed effect of mutating *RAD1* or *RAD10* in strains heterozygous for *his4-lopd* or *his4::U1.1a* was an increase in the frequency of both 5:3 and 3:5 PMS tetrads and a reduction in the frequency of 2:6, but not 6:2, gene conversion tetrads (Tables 4 and 5; Kirkpatrick and Petes 1997). The reductions in the frequencies of 2:6 tetrads relative to wild type were statistically significant for the *rad1* strains TP1013 and HMY49 (*P*

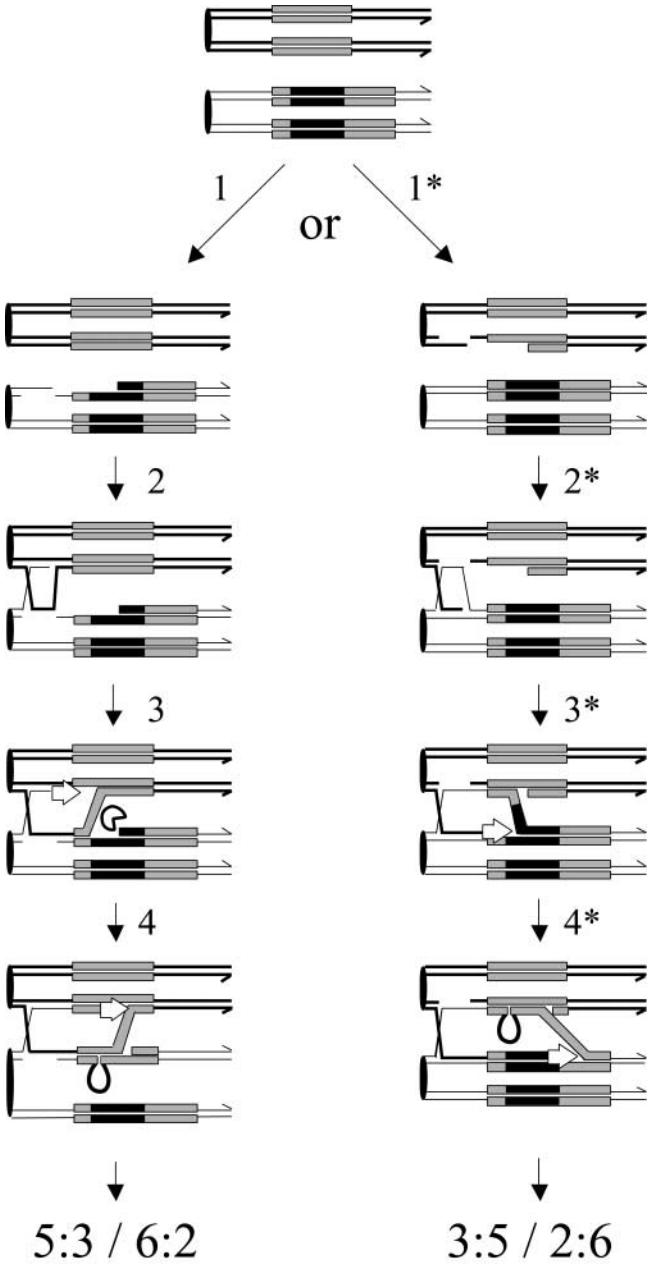

FIGURE 4.—Polymerase-driven heteroduplex formation involving large insertions. (1) The DSB is formed at the upstream hotspot on the mutant chromosome and is resected through part, but not all of the insertion. (2) The "left" arm invades the intact duplex and creates a D-loop. (3) Polymerase (indicated by an arrow) drives expansion of the D-loop through the insertion. Note that continued degradation of the "right" arm of the recipient chromosome is required to expose complementary regions of the DNA. (4) Continued synthesis will result in the formation of a mispaired loop in the recombination intermediate. Further processing of this intermediate (Figure 1) will result in a 5:3 or 6:2 tetrad. (1*) The DSB is formed on the wild-type chromosome and is resected. Note that less resection of the DSB is required in the absence of the large insertion. (2*) The left arm invades the intact duplex and creates a D-loop. (3*) Polymerase drives expansion of the D-loop and duplicates the large insertion. (4*) Continued synthesis will result in the formation of a mispaired loop in the recombination intermediate. Further processing of this intermediate (Figure 1) can result in a 3:5 or 2:6 tetrad.

values of 0.02 and 0.01, respectively) and the *rad10* strains DTK286 and HMY151 (*P* values of 0.02 and 0.003, respectively).

As illustrated in Figure 1, mispaired loops can be repaired either by loop removal or duplication of the loop. Repair of 5:3-type heteroduplexes by removal of the loop will result in a 6:2 tetrad. Duplication of the loop will result in restoration of 4:4 segregation. Alternatively, repair of a 3:5-type heteroduplex by removal of the loop will result in restoration of 4:4 segregation, while duplication of the loop will result in a 2:6 gene conversion. A strain deficient in the ability to repair mispaired loops by duplication would generate fewer 2:6 conversions (with a concomitant increase in the production of 3:5 PMS tetrads), without an effect on the 6:2 conversion class. One would still observe 5:3 PMS tetrads, however, due to the inability to complete restoration repair from this intermediate. Thus, the reduction of the 2:6 conversion class in *rad1* and *rad10* strains suggests that the Rad1p/Rad10p endonuclease is involved in loop repair events that require cleavage of the strand opposite the loop (KIRKPATRICK and PETES 1997).

As shown in Figure 5a, Rad1p/Rad10p is a junction-specific endonuclease that recognizes single- to double-strand transitions (BARDWELL *et al.* 1994). The human homologues of Rad1p/10p (XPF/ERCC1) incise duplex DNA 2–8 nucleotides 5′ of a junction with single-stranded DNA (MATSUNAGA *et al.* 1996; DE LAAT *et al.* 1998a,b). In one study, the polar binding of RPA to the single-stranded substrate affected the incision activity of XPF/ERCC1 (DE LAAT *et al.* 1998b). As shown in Figure 5b, RPA bound to 3′-protruding single-stranded arms inhibits incision by XPF/ERCC1, whereas binding of RPA to 5′-protruding single-stranded arms greatly stimulates incision. On the basis of these observations, we suggest that binding of RPA on the large DNA loops preferentially directs Rad1p/Rad10p cleavage to the strand opposite the DNA loop. The repair events subsequent to this cleavage would result in duplication of the insertion (Figure 5c).

Several additional points concerning this model are relevant. First, there must be a second, as yet unidentified, repair system that results in deletion, rather than duplication, of the insertion. Second, since *in vitro*, XPF/ERCC1 (in the presence of RPA) cleaves 30-base loop substrates on both strands (MATSUNAGA *et al.* 1996; T. MATSUNAGA and A. SANCAR, personal communication), it is possible that Msh2p/Msh3p contribute to the specificity of strand cleavage. Third, CLIKEMAN *et al.* (2001) found that DNA loop repair in mitotic yeast cells required both Msh2p and Pms1p. This result suggests that meiotic and mitotic repair of loops may have different genetic requirements. Alternatively, since the substrates examined by Clikeman *et al.* contained both point mutations and insertions, it is possible that their results reflect an interaction between two different repair systems. Fourth, the model is consistent with obser-

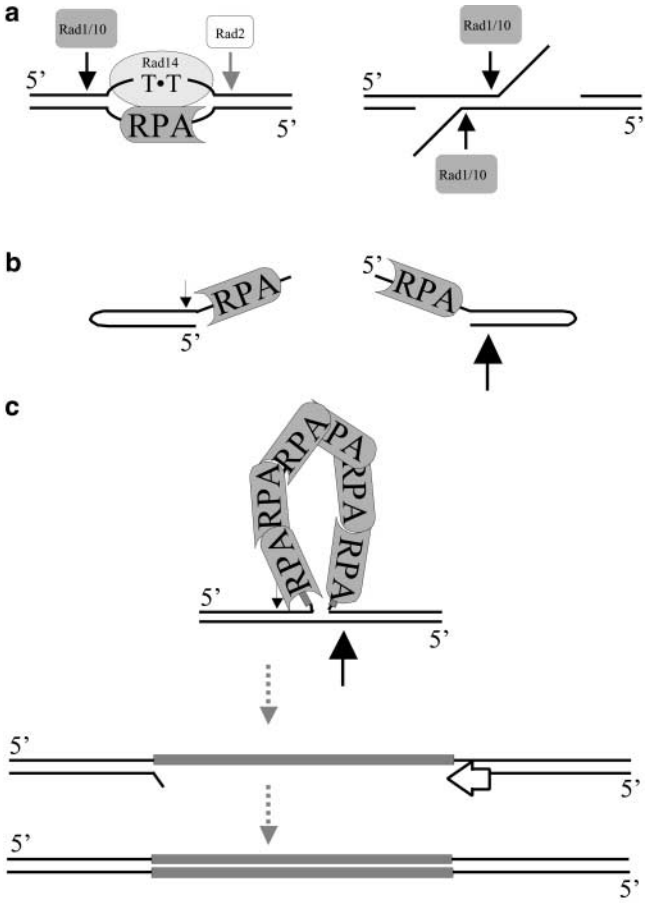

Figure 5.—The enzymatic activity of the Rad1p/10p endonuclease. (a) The Rad1p/Rad10 (XPF/ERCC1) endonuclease makes single-strand nicks at single-stranded to double-stranded transitions in DNA. The incision is always made in the duplex DNA, 5′ to the single-stranded region (Park *et al.* 1995; Matsunaga *et al.* 1996; de Laat *et al.* 1998a). This activity is consistent with the known roles for Rad1p/Rad10p in nucleotide excision repair (shown on the left) and single-strand annealing (shown on the right). (b) The binding of RPA to single-stranded tails influences XPF/ERCC1 cleavage. When the 3′-binding side of RPA faces the cleavage site, XPF/ERCC1 is stimulated, but when the 5′-binding side of RPA faces the cleavage site, cleavage is inhibited (de Laat *et al.* 1998b). (c) Our genetic results suggest that RPA (and, perhaps, Msh2p/Msh3p) might direct Rad1p/10p cleavage in loop repair. We show a large single-stranded loop bound with RPA. The two potential incision sites 5′ to the single- to double-strand junction are indicated by arrows. We suggest that RPA stimulates cleavage across from the loop while inhibiting cleavage on the same strand as the loop. This incision would then allow subsequent polymerase activity to duplicate the insertion.

vation that the efficiency of targeted integration of transforming DNA is greatly reduced by mutations in *ERCC1*, the mammalian equivalent of *RAD10* (L. Niedernhofer and R. Kanaar, personal communication). If integration of transforming DNA involves a heteroduplex intermediate (such as that shown in Figure 5c), the Rad1p/Rad10p endonuclease would be required for the cleavage event necessary to integrate the insertion.

We previously showed that loops composed of palindromic sequences frequently escape meiotic repair (Nag *et al.* 1989; Nag and Petes 1991; Moore *et al.* 1999). Since the Rad1p/Rad10p endonuclease requires a single- to double-strand transition for cleavage, it is likely that these hairpin-forming loops do not present an appropriate substrate for repair. This conclusion is consistent with the observations that the efficiency of incision in stem-loop substrates (Figure 5c) increases with the size of the loop and that no incisions are observed for loops eight bases or smaller (De Laat *et al.* 1998a). In preliminary studies, we find no effect of *rad1* on the repair of a heterozygous palindromic insertion (H. M. Kearney and T. D. Petes, unpublished data).

The roles of Rad1p and Msh3p in the repair of bubble substrates are different from their roles in the repair of DNA loops. Although mutations in either *RAD1* or *MSH3* result in a fourfold elevation in the frequency of gene conversion (comparison of HMY215 and HMY222 with HMY189, Table 6), the *rad1*, but not the *msh3*, mutation leads to substantially elevated PMS frequencies. One interpretation of this result is that Rad1p and Msh3p reduce the formation of the bubble substrate. Since crossovers between *HIS4* and *LEU2* are not substantially affected by these mutations (Table 6), this reduction probably does not involve DSB formation, but a subsequent step. In addition to its role in preventing formation of the bubble substrate, Rad1p, but not Msh3p, is significantly involved in its repair. Although we favor this interpretation, we cannot exclude other possibilities. For example, the Rad1p and Msh3p may be involved in directing repair events to restorations rather than gene conversions.

Disparity of gene conversion in wild-type strains: Gene conversion events involving heterozygous point mutations usually show no disparity (equal frequencies of 6:2 and 2:6) or subtle disparities (Fogel *et al.* 1981; Nagylaki and Petes 1982). In contrast, conversion events involving either large insertions or deletions often (although not always) show disparity in favor of the conversion events that duplicate the insertion or result in loss of the deletion (Fink and Styles 1974; Fogel *et al.* 1981; McKnight *et al.* 1981; Pukkila *et al.* 1986; Vincent and Petes 1989). In our study, we also observed that conversion events tend to duplicate, rather than delete, the insertion. We suggest that this disparity reflects two factors. First, if the insertion contains a site susceptible to DSB formation, then competition for adjacent DSB formation (Wu and Lichten 1995; Xu and Kleckner 1995; Fan *et al.* 1997) reduces DSB formation at the normal *HIS4* upstream site (Figure 3b). Since only DSB formation at the normal upstream region results in gene conversion involving the homologues (as discussed below), this effect results in disparity.

We suggest that a second factor in generating disparity is the relative efficiency of heteroduplex formation across the insertion. As shown in Figure 4, recombina-

tion events initiated on the chromosome with large (1.1–5.6 kb) insertions require extensive degradation (>1.1–5.6 kb) of one of the strands of the recipient chromosome in order to allow DNA loop formation. Events initiated on the chromosome with the wild-type allele require less degradation, although more extensive DNA synthesis. If strand degradation is limited, then one would expect a bias in favor of 2:6 conversions. This expectation assumes that heteroduplex formation events that are initiated, but not completed, can nonetheless give rise to viable spore products. In summary, the degree of conversion bias observed for heterozygous insertions is likely to reflect the strength of DSB formation within the insertion, the size of the insertion, and the position of the insertion relative to the initiating DSB.

***HIS4-LEU2* crossovers in wild-type and mutant strains:** A number of the mutations examined in our study significantly affected crossovers in the *HIS4-LEU2* interval (Tables 4–7). Crossover distances were determined by measuring the number of PD:NPD:T tetrads (Perkins 1949). The relative numbers of these tetrads is a complicated function of the frequency of initiating recombination intermediates, the resolution of these intermediates as crossovers or noncrossovers, and the ratio of various classes of double crossovers. In previous studies, it has been shown that mutations in *MLH1*, *MSH4*, *MSH5*, and *EXO1* reduce crossovers (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Hunter and Borts 1997; Khazanehdari and Borts 2000; Kirkpatrick *et al.* 2000). These same mutations significantly affected crossovers in strains heterozygous for *his4-lopd* (Table 4). In addition, the *msh3* and *rad2* mutations altered the distribution of the PD, NPD, and T tetrads. The effect of *msh3* was not completely straightforward. The *msh3* strains had a reduction in the relative number of NPD tetrads (presumably representing four-strand double crossovers), but an increase in the relative number of T tetrads (presumably representing single crossovers and three-strand double crossovers; data not shown). The *rad2* mutation reduced the relative numbers of both NPD and T tetrads. Since the level of aberrant segregation at *HIS4* was also reduced in the *rad2* strain (Table 4), the reduction in crossovers might reflect an effect of *rad2* on the initiation of recombination.

In strains heterozygous for *his4::U1.1a*, we also observed significant reductions in crossovers in *msh4* and *exo1* mutant strains (Table 5). In addition, the *rad27* mutation significantly reduced both crossovers and aberrant segregation. As suggested above, the endonuclease encoded by *RAD27* may be involved in heteroduplex formation involving large heterozygous insertions or deletions.

DSBs formed within heterozygous insertions stimulate ectopic recombination, but not recombination between homologues: We found that DSBs were efficiently formed within the *his4::U1.1* and *his4::k1.5* insertions. These breaks, however, did not contribute to recombination between homologues, although the breaks in the *URA3* insertions stimulated ectopic recombination with the *ura3* genes on chromosome V. The DNA ends formed by DSBs within the insertion contain nonhomologous DNA sequences that presumably block interactions with the homologous chromosome. If the nonhomologous sequences were efficiently removed from both strands, as expected if conversion could result from DNA gap repair, it is likely that these DSBs would stimulate interaction with the homologues. Thus, our results argue that gap repair in yeast occurs rarely. It is likely that DSBs formed within the insertion, if not used for ectopic recombination events, are repaired by sister-strand interactions. One argument for this type of repair is that we find no loss in spore viability in strains heterozygous for the *his4::k1.5* insertion, despite the DSBs that occur in the *kanMX4* insertion (which shares no homology with the yeast genome).

Conclusions: Heteroduplexes can be formed during meiotic recombination in *S. cerevisiae* through very large heterologies. The resulting DNA loops are repaired by a process requiring Rad1p, Rad10p, Msh2p, and Msh3p. This mechanism duplicates, rather than removes, large loops. DSBs that occur within heterozygous insertions do not efficiently initiate interhomologue exchange.

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LITERATURE CITED

Adams, D. E., and S. C. West, 1996 Bypass of DNA heterologies during RuvAB-mediated three- and four-strand branch migration. J. Mol. Biol. **263:** 582–596.

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Bardwell, A. J., L. Bardwell, A. E. Tomkinson and E. C. Friedberg, 1994 Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. Science **265:** 2082–2085.

Boeke, J. D., F. Lacroute and G. Fink, 1984 A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast. Mol. Gen. Genet. **197:** 345–346.

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae.* Cell **61:** 1089–1101.

Carpenter, A. T., 1979 Recombination nodules and synaptonemal complex in recombination-defective females of *Drosophila melanogaster.* Chromosoma **75:** 259–292.

Carpenter, A. T., 1982 Mismatch repair, gene conversion, and crossing-over in two recombination-defective mutants of *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **79:** 5961–5965.

Chen, W., and S. Jinks-Robertson, 1999 The role of the mismatch repair machinery in regulating mitotic and meiotic recombination between diverged sequences in yeast. Genetics **151:** 1299–1313.

Clikeman, J. A., S. L. Wheeler and J. A. Nickoloff, 2001 Efficient incorporation of large (>2 kb) heterologies into heteroduplex

DNA: *PMS1/MSH2*-dependent and -independent large loop mismatch repair in yeast. Genetics **157:** 1481–1491.

Crouse, G. F., 1998 Mismatch repair systems in *S. cerevisiae*, pp. 411–448 in *DNA Damage and Repair: DNA repair in prokaryotes and lower eukaryotes*, Vol. 1, edited by J. A. Nickoloff and M. F. Hoekstra. Humana, 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.

de Laat, W. L., E. Appeldoorn, N. G. J. Jaspers and J. H. J. Hoeijmakers, 1998a DNA structural elements required for ERCC1-XPF endonuclease activity. J. Biol. Chem. **273:** 7835–7842.

de Laat, W. L., E. Appeldoorn, K. Sugasawa, E. Weterings, N. G. Jaspers *et al.*, 1998b DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. Genes Dev. **12:** 2598–2609.

Detloff, P., M. A. White and T. D. Petes, 1992 Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. Genetics **132:** 113–123.

Devlin, C., K. Tice-Baldwin, D. Shore and K. T. Arndt, 1991 RAP1 is required for BAS1/BAS2- and GCN4-dependent transcription of the yeast *HIS4* gene. Mol. Cell. Biol. **11:** 3642–3651.

Fan, Q., F. Xu and T. D. Petes, 1995 Meiosis-specific double-strand DNA breaks at the *HIS4* recombination hot spot in the yeast *Saccharomyces cerevisiae*: control in *cis* and *trans*. Mol. Cell. Biol. **15:** 1679–1688.

Fan, Q. Q., F. Xu, M. A. White and T. D. Petes, 1997 Competition between adjacent meiotic recombination hotspots in the yeast *Saccharomyces cerevisiae*. Genetics **145:** 661–670.

Feldmann, H., and E. L. Winnacker, 1993 A putative homologue of the human autoantigen Ku from *Saccharomyces cerevisiae*. J. Biol. Chem. **268:** 12895–12900.

Fink, G. R., and C. A. Styles, 1974 Gene conversion of deletions in the *HIS4* region of yeast. Genetics **77:** 231–244.

Fishman-Lobell, J., and J. E. Haber, 1992 Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. Science **258:** 480–484.

Fleck, O., E. Lehmann, P. Schar and J. Kohli, 1999 Involvement of nucleotide-excision repair in *msh2 pms1*-independent mismatch repair. Nat. Genet. **21:** 314–317.

Flores-Rozas, H., and R. D. Kolodner, 1998 The *Saccharomyces cerevisiae MLH3* gene functions in *MSH3*-dependent suppression of frameshift mutations. Proc. Natl. Acad. Sci. USA **95:** 12404–12409.

Fogel, S., R. K. Mortimer and K. Lusnak, 1981 Mechanisms of meiotic gene conversion, or "wandering on a foreign strand," pp. 289–339 in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:** 1541–1553.

Harfe, B. D., and S. Jinks-Robertson, 2000 DNA mismatch repair and genetic instability. Annu. Rev. Genet. **34:** 359–399.

Harfe, B. D., B. K. Minesinger and S. Jinks-Robertson, 2000 Discrete *in vivo* roles for the MutL homologs Mlh2p and Mlh3p in the removal of frameshift intermediates in budding yeast. Curr. Biol. **10:** 145–148.

Harrington, J. J., and M. R. Lieber, 1994a The characterization of a mammalian DNA structure-specific endonuclease. EMBO J. **13:** 1235–1246.

Harrington, J. J., and M. R. Lieber, 1994b Functional domains within FEN-1 and RAD2 define a family of structure-specific endonucleases: implications for nucleotide excision repair. Genes Dev. **8:** 1344–1355.

Herskowitz, I., and R. E. Jensen, 1991 Putting the *HO* gene to work: practical uses for mating-type switching, pp. 132–146 in *Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego.

Hollingsworth, N. M., L. Ponte and C. Halsey, 1995 *MSH5*, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. **9:** 1728–1739.

Hunter, N., and R. H. Borts, 1997 Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. Genes Dev. **11:** 1573–1582.

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.

Iype, L. E., E. A. Wood, R. B. Inman and M. M. Cox, 1994 RuvA and RuvB proteins facilitate the bypass of heterologous DNA insertions during RecA protein-mediated DNA strand exchange. J. Biol. Chem. **269:** 24967–24978.

Jinks-Robertson, S., and T. D. Petes, 1985 High-frequency meiotic gene conversion between repeated genes on non-homologous chromosomes in yeast. Proc. Natl. Acad. Sci. USA **82:** 3350–3354.

Johnson, R. E., G. K. Kovvali, L. Prakash and S. Prakash, 1995 Requirement of the yeast *RTH1* 5′ to 3′ exonuclease for the stability of simple repetitive DNA. Science **269:** 238–240.

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.

Khazanehdari, K. A., and R. H. Borts, 2000 *EXO1* and *MSH4* differentially affect crossing-over and segregation. Chromosoma **109:** 94–102.

Kirkpatrick, D. T., 1999 Roles of the DNA mismatch repair and nucleotide excision repair proteins during meiosis. Cell. Mol. Life Sci. **55:** 437–449.

Kirkpatrick, D. T., and T. D. Petes, 1997 Repair of DNA loops involves DNA-mismatch and nucleotide-excision repair proteins. Nature **387:** 929–931.

Kirkpatrick, D. T., M. Dominska and T. D. Petes, 1998 Conversion-type and restoration-type repair of DNA mismatches formed during meiotic recombination in *Saccharomyces cerevisiae*. Genetics **149:** 1693–1705.

Kirkpatrick, D. T., J. R. Ferguson, T. D. Petes and L. S. Symington, 2000 Decreased meiotic intergenic recombination and increased meiosis I nondisjunction in *exo1* mutants of *Saccharomyces cerevisiae*. Genetics **156:** 1549–1557.

Kolodner, R. D., and G. T. Marsischky, 1999 Eukaryotic DNA mismatch repair. Curr. Opin. Genet. Dev. **9:** 89–96.

Kramer, W., B. Kramer, M. S. Williamson and S. Fogel, 1989 Cloning and nucleotide sequence of DNA mismatch repair gene *PMS1* from *Saccharomyces cerevisiae*: homology of *PMS1* to procaryotic MutL and HexB. J. Bacteriol. **171:** 5339–5346.

Leem, S. H., P. A. Ropp and A. Sugino, 1994 The yeast *Saccharomyces cerevisiae* DNA polymerase IV: possible involvement in double strand break DNA repair. Nucleic Acids Res. **22:** 3011–3017.

Lichten, M., R. H. Borts and J. E. Haber, 1987 Meiotic gene conversion and crossing-over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics **115:** 233–246.

Maniatis, T., E. F. Fritsch and J. Sambrook, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Matsunaga, T., C.-H. Park, T. Bessho, D. Mu and A. Sancar, 1996 Replication protein A confers structure-specific endonucleasse activities to the XPF-ERCC1 and XPG subunits of human DNA repair excision nuclease. J. Biol. Chem. **271:** 11047–11050.

McKee, A. H., and N. Kleckner, 1997 A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene *SAE2*. Genetics **146:** 797–816.

McKnight, G. L., T. S. Cardillo and F. Sherman, 1981 An extensive deletion causing overproduction of yeast iso-2-cytochrome c. Cell **25:** 409–419.

Moore, H., P. W. Greenwell, C. P. Liu, N. Arnheim and T. D. Petes, 1999 Triplet repeats form secondary structures that escape DNA repair in yeast. Proc. Natl. Acad. Sci. USA **96:** 1504–1509.

Morel, P., A. Stasiak, S. D. Ehrlich and E. Cassuto, 1994 Effect of length and location of heterologous sequences on RecA-mediated strand exchange. J. Biol. Chem. **269:** 19830–19835.

Murante, R. S., L. Huang, J. J. Turchi and R. A. Bambara, 1994 The calf 5′- to 3′-exonuclease is also an endonuclease with both activities dependent on primers annealed upstream of the point of cleavage. J. Biol. Chem. **269:** 1191–1196.

Nag, D. K., and T. D. Petes, 1991 Seven-base-pair inverted repeats

in DNA form stable hairpins *in vivo* in *Saccharomyces cerevisiae*. Genetics **129:** 669–673.

Nag, D. K., and T. D. Petes, 1993 Physical detection of heteroduplexes during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 2324–2331.

Nag, D. K., M. A. White and T. D. Petes, 1989 Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature **340:** 318–320.

Nagylaki, T., and T. D. Petes, 1982 Intrachromosomal gene conversion and the maintenance of sequence homogeneity among repeated genes. Genetics **100:** 315–337.

Nairz, K., and F. Klein, 1997 *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. **11:** 2272–2290.

Nicholson, A., M. Hendrix, S. Jinks-Robertson and G. F. Crouse, 2000 Regulation of mitotic homeologous recombination in yeast. Functions of mismatch repair and nucleotide excision repair genes. Genetics **154:** 133–146.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Park, C. H., T. Bessho, T. Matsunaga and A. Sancar, 1995 Purification and characterization of the XPF-ERCC1 complex of human DNA repair excision nuclease. J. Biol. Chem. **270:** 22657–22660.

Perkins, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

Petes, T. D., R. E. Malone and L. S. Symington, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis, and Energetics*, edited by J. Broach, E. Jones and J. Pringle. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Porter, S. E., M. A. White and T. D. Petes, 1993 Genetic evidence that the meiotic recombination hotspot at the *HIS4* locus of *Saccharomyces cerevisiae* does not represent a site for a symmetrically processed double-strand break. Genetics **134:** 5–19.

Prakash, L., D. Dumais, R. Polakowska, G. Perozzi and S. Prakash, 1985 Molecular cloning of the *RAD10* gene of *Saccharomyces cerevisiae*. Gene **34:** 55–61.

Prakash, S., P. Sung and L. Prakash, 1993 DNA repair genes and proteins of *Saccharomyces cerevisiae*. Annu. Rev. Genet. **27:** 33–70.

Prinz, S., A. Amon and F. Klein, 1997 Isolation of *COM1*, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. Genetics **146:** 781–795.

Prolla, T. A., D. M. Christie and R. M. Liskay, 1994 Dual requirement in yeast DNA mismatch repair for *MLH1* and *PMS1*, two homologs of the bacterial MutL gene. Mol. Cell. Biol. **14:** 407–415.

Pukkila, P. J., M. D. Stephens, D. M. Binninger and B. Errede, 1986 Frequency and directionality of gene conversion events involving the *CYC7–H3* mutation in *Saccharomyces cerevisiae*. Genetics **114:** 347–361.

Reagan, M. S., C. Pittenger, W. Siede and E. C. Friedberg, 1995 Characterization of a mutant strain of *Saccharomyces cerevisiae* with a deletion of the *RAD27* gene, a structural homolog of the *RAD2* nucleotide excision repair gene. J. Bacteriol. **177:** 364–371.

Roeder, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev. **11:** 2600–2621.

Ross-Macdonald, P., and G. S. Roeder, 1994 Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell **79:** 1069–1080.

Rothstein, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

Saparbaev, M., L. Prakash and S. Prakash, 1996 Requirement of mismatch repair genes *MSH2* and *MSH3* in the *RAD1–RAD10* pathway of mitotic recombination in *Saccharomyces cerevisiae*. Genetics **142:** 727–736.

Schwacha, A., and N. Kleckner, 1994 Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell **76:** 51–63.

Schwacha, A., and N. Kleckner, 1995 Identification of double Holliday junctions as intermediates in meiotic recombination. Cell **83:** 783–791.

Sekelsky, J. J., K. S. McKim, G. M. Chin and R. S. Hawley, 1995 The Drosophila meiotic recombination gene *mei-9* encodes a homologue of the yeast excision repair protein Rad1. Genetics **141:** 619–627.

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.

Sherman, F., G. R. Fink and J. B. Hicks, 1982 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sia, E. A., S. Jinks-Robertson and T. D. Petes, 1997a Genetic control of microsatellite stability. Mutat. Res. **383:** 61–70.

Sia, E. A., R. J. Kokoska, M. Dominska, P. Greenwell and T. D. Petes, 1997b Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes. Mol. Cell. Biol. **17:** 2851–2858.

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.

Sommers, C. H., E. J. Miller, B. Dujon, S. Prakash and L. Prakash, 1995 Conditional lethality of null mutations in *RTH1* that encodes the yeast counterpart of a mammalian 5′- to 3′-exonuclease required for lagging strand DNA synthesis in reconstituted systems. J. Biol. Chem. **270:** 4193–4196.

Stapleton, A., and T. D. Petes, 1991 The Tn3 beta-lactamase gene acts as a hotspot for meiotic recombination in yeast. Genetics **127:** 39–51.

Sugawara, N., F. Paques, M. Colaiacovo and J. E. Haber, 1997 Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. Proc. Natl. Acad. Sci. USA **94:** 9214–9219.

Sun, H., D. Treco and J. W. Szostak, 1991 Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **64:** 1155–1161.

Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein and F. W. Stahl, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

Tran, H. T., D. A. Gordenin and M. A. Resnick, 1996 The prevention of repeat-associated deletions in *Saccharomyces cerevisiae* by mismatch repair depends on size and origin of deletions. Genetics **143:** 1579–1587.

Troelstra, C., and N. G. Jaspers, 1994 Recombination and repair. Ku starts at the end. Curr. Biol. **4:** 1149–1151.

Vincent, A., and T. D. Petes, 1989 Mitotic and meiotic gene conversion of Ty elements and other insertions in *Saccharomyces cerevisiae*. Genetics **122:** 759–772.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Wang, T. F., N. Kleckner and N. Hunter, 1999 Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc. Natl. Acad. Sci. USA **96:** 13914–13919.

White, M. A., M. Wierdl, P. Detloff and T. D. Petes, 1991 DNA-binding protein RAP1 stimulates meiotic recombination at the *HIS4* locus in yeast. Proc. Natl. Acad. Sci. USA **88:** 9755–9759.

Woodgate, R., 1999 A plethora of lesion-replicating DNA polymerases. Genes Dev. **13:** 2191–2195.

Wu, T. C., and M. Lichten, 1995 Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics **140:** 55–66.

Xu, L., and N. Kleckner, 1995 Sequence non-specific double-strand breaks and interhomolog interactions prior to double-strand break formation at a meiotic recombination hot spot in yeast. EMBO J. **16:** 5115–5128.

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