MOLECULAR AND CELLULAR BIOLOGY, Feb. 1997, p. 1027–1036 Vol. 17, No. 2
0270-7306/97/$04.00+0

Altered Replication and Inverted Repeats Induce Mismatch Repair-Independent Recombination between Highly Diverged DNAs in Yeast

HIEP TRAN,[1] NATASHA DEGTYAREVA,[2] DMITRY GORDENIN,[1,2] AND MICHAEL A. RESNICK[1]*

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709,[1] and Department of Genetics, St. Petersburg State University, St. Petersburg 199034, Russia[2]

Received 21 August 1996/Returned for modification 16 October 1996/Accepted 7 November 1996

Replication, DNA organization, and mismatch repair (MMR) can influence recombination. We examined the effects of altered replication due to a mutation in the polymerase δ gene, long inverted repeats (LIRs) in motifs similar to those in higher eukaryotes, and MMR on intrachromosomal recombination between highly diverged (28%) truncated genes in *Saccharomyces cerevisiae*. A combination of altered replication and an LIR increased recombination up to 700-fold, while each alone led to a 3- to 20-fold increase. Homeologous recombination was not altered by *pms1*, *msh2*, and *msh3* mismatch repair mutations. Similar to our previous observations for replication slippage-mediated deletions, there were ≥5-bp identical runs at the recombination breakpoints. We propose that the dramatic increase in recombination results from enhancement of the effects of altered replication by the LIR, leading to recombinationally active initiating structures. Such interactions predict replication-related, MMR-independent genome changes.

Chromosomal recombination has both beneficial and deleterious consequences. During meiosis, recombination is generally considered to be essential to the orderly distribution of chromosomes. In mitotically growing cells of lower organisms, recombination provides for efficient repair of DNA damage, particularly double-strand breaks, through interactions between homologs or sister chromatids. In mammals, somatic recombination is an important component in the development of the immune system.

Recombination could also lead to genome instabilities if it were to occur between diverged DNAs. For example, human chromosomes contain many large DNA repeat sequences such as *Alu*s, LINEs, and pseudogenes (11, 39) in which reciprocal exchange would lead to genome rearrangements and/or deletions. This is presumably prevented by divergence between the repeats.

In all organisms examined, a high level of DNA divergence can reduce recombination substantially. In addition to the level of DNA divergence, there are many factors (see Discussion) that can influence the likelihood of homeologous recombination; these include the mode of initiation, DNA organization, the recombination system examined, and mismatch repair (MMR). For example, conjugational recombination between *Escherichia coli* and *Salmonella typhimurium*, whose DNAs are 16% diverged, is 10^5-fold lower than for intraspecies crosses (34, 44). However, double-strand break-induced intraplasmid recombination between diverged DNAs is reduced less than 10-fold (2). In yeast, a single- or double-strand break can enhance recombination between highly diverged DNAs during transformation (33, 35, 36, 43).

The MMR proteins MutS and MutL in bacteria or their homologs responsible for preventing replication-related mutations in higher organisms are proposed to interact with mismatches in some way to inhibit homeologous recombination, possibly by preventing strand assimilation or strand transfer (37). The role of the MMR system in preventing recombination between highly diverged DNAs appears to depend on other factors that also influence homeologous recombination including mode of initiation (such as DNA damage or type of recombination) and DNA organization. For example, MMR is responsible for most of the reduction in bacterial interspecies recombination (44), but it has little effect on plasmids that contain a large heteroduplex formed between DNAs that are 8% (1, 12) or 16% (57) diverged. For yeast, the effect of MMR on homeologous recombination depends on the system and the level of DNA divergence. The yeast *PMS1* gene, a homolog of the bacterial *mutL* gene, does not appear to affect homeologous recombination in any of the yeast systems (21, 43, 48). Mutations in the *MSH2* and *MSH3* genes of yeast can result in as much as a 40-fold increase in recombination between repeated DNAs that are over 20% diverged; however, the increase is dependent on the organization of the DNAs (48). Mutations in the *PMS1* and *MSH2* genes have no effect on the rate of homeologous recombination between transforming molecules (5) or spontaneous homeologous recombination between plasmid and chromosome (42). Recently, it was shown that mammalian cells defective in *MSH2*, the MutS gene homolog, are much better recipients for gene targeting with related DNA (0.6% base sequence divergence) than are the corresponding wild-type cells. Inactivation of the mouse *MSH2* gene results in mismatch repair deficiency, hyperrecombination, and predisposition to cancer (22). Male mice defective in the DNA repair gene *PMS2* exhibit abnormal chromosome synapsis in meiosis and appear prone to sarcomas and lymphomas (6).

Thus, there are many factors that can influence homeologous recombination, and as a result, they are likely to play an important role in genome stability. Chromosome metabolic activity, such as transcription and DNA replication (for a review, see reference 9), heterochromatin versus euchromatin,

* Corresponding author. Mailing address: Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, 111 Alexander Dr., P.O. Box 12233, Research Triangle Park, NC 27709. Phone: (919) 541-4480. Fax: (919) 541-7593. E-mail: resnick@niehs.nih.gov.

B-DNA versus non-B-DNA (51), and organization, can affect homologous recombination and is also likely to affect recombination between diverged DNAs. For example, altered semiconservative replication in yeast DNA polymerase mutants can increase homologous recombination severalfold in a variety of systems, although the reason remains unclear (3, 28).

Long inverted repeats (LIRs) are an example of the influence of chromosome organization on homologous recombination. An insertion of an LIR into a gene can stimulate interchromosomal heteroallelic recombination as much as 10-fold (28). Previously, LIRs were shown to induce deletions between small surrounding direct repeats in bacteria and yeast (17, 23, 29–31, 47). In transgenic mice, LIRs are mitotically unstable and lead to insertions or deletions (16). Furthermore, inverted repeats have been proposed to induce reciprocal switching of the leading and lagging strands during replication within inverted repeats (8). Based on the ability of LIRs to stimulate deletions between short direct repeats (4 to 9 bp), LIRs have been proposed to form stem-loop structures during replication, thereby blocking replication and leading to replication slippage between repeats flanking the LIRs. Alternatively, the stalled 3′ end interacts with another homologous sequence, resulting in recombination (see Discussion). This view is supported by our observations that yeast mutants defective in semiconservative replication exhibited both enhanced LIR excision and interchromosomal recombination (28, 29).

We have investigated the impact of LIRs and altered replication on both homeologous and homologous intrachromosomal recombination. The altered replication is due to a mutation, *pol3-t*, in the DNA polymerase δ gene (*POL3*), which results in temperature-sensitive growth. Recombination was examined between directly repeated DNAs that were separated by two inverted *URA3* genes (the LIR) or various combinations of directly repeated *URA3* genes or unique DNAs. This motif was chosen because it is common in the DNAs of higher eukaryotes, where large repeat DNAs make up over 10 to 20% of the genome and have been associated with various diseases in humans. Homeologous recombination between directly repeated truncated genes that were 28% diverged was examined.

We have identified new factors that can induce recombination between diverged DNA, dissected the mechanism of replication-slippage and recombination induced by LIRs, and generalized the LIR-induced recombination phenomena. The effect of altered replication or an LIR on homologous recombination extended to recombination between the highly diverged DNAs in that they each increased recombination up to 3- to 20-fold. However, the combination of an LIR and the *pol3-t* mutation had a multiplicative or greater than multiplicative effect on homeologous as well as homologous recombination, resulting in recombination being enhanced up to 700-fold. Mutations in the mismatch repair genes *PMS1*, *MSH2*, and *MSH3* had no effect on recombination between the highly diverged repeats. Based on these results, we suggest that diverged DNA sequences, DNA replication status, and DNA organization—particularly LIRs—can be important factors in genome instability and that the role of mismatch repair depends in part on the mechanism of recombination.

MATERIALS AND METHODS

Strains, plasmids, and media. All strains were isogenic and were *MAT*α *leu2-2 trp1-*Δ*1 ura3-*Δ*1 ade2-*Δ derived from *pol3-t* DM strains (28, 29); they had various combinations of mutant or wild-type alleles of *POL*$^+$, *PMS1*, *MSH2*, and *MSH3* and various inserts at the *Xho*I site of the chromosomal *LYS2* gene (see below). The *pol3-t* mutation is due to a G-to-A substitution resulting in an Asp641-to-Asn641 change in the domain VI region (unpublished data). Except for pAM58 (*pms1*::*LEU2* [38]) and *pmsh3*::*LEU2* (*msh3*::*LEU2* [48]), the rest of the plasmids including p203 (*msh2*::*LEU2*) were derived in this study. *E. coli* DH5α was used for plasmid construction. Standard Luria-Bertani and terrific broth (TB) media for bacteria and YPD and synthetic media for yeast were used (50). Medium containing 5-fluoroorotic acid (5-FOA) was used for selection of *URA3* mutations (10).

Constructions. To examine recombination, inserts that contained truncated direct repeats of *ADE* genes, one from the 5′-terminal part and the other from the 3′-terminal part of the gene with a 424-bp overlap, were developed. The first repeat was a 5′ *Pvu*II-*Sal*I fragment (bp 1 to 1930) of the *Pichia methanolica ADE1* gene, including the promoter (1,007 bp) and the first 923 bp after the start codon (56). The second *ADE* repeat was either the 3′-terminal *Hin*dIII-*Bgl*II fragment (bp 1512 to 3036) of *P. methanolica ADE1* (56) or the *Hin*dIII-*Bgl*II fragment of *Saccharomyces cerevisiae ADE2* from plasmid pAZ11 (52). *P. methanolica ADE1* is a functional homolog of *S. cerevisiae ADE2*, and they have a common alignment and a *Hin*dIII site at the same position. These genes encode 5-aminoimidazole ribonucleotide carboxylase. Mutations result in the accumulation of red pigment, and revertants or recombinants give rise to white colonies. The overlapping region of *P. methanolica ADE1* and *S. cerevisiae ADE2* is 28% diverged (see Fig. 2). Following the second *ADE* copy is the *Bgl*II-*Eco*RI fragment of the *TRP1* gene (14) (Fig. 1). Presented in Fig. 1 are the various DNA sequences placed between the two *ADE* repeats. These constructs were inserted in both orientations into the *Xho*I site of the *LYS2* gene on plasmids. The constructions were transferred to the chromosomal *LYS2* gene in the Pol$^+$ DM and *pol3-t* DM strains by using gene disruption procedures (46), resulting in Ura$^+$ Lys$^-$ phenotypes.

Mismatch repair disruption and deletion mutations. Mutations of the *PMS1*, *MSH2*, and *MSH3* genes were made in the Pol$^+$ DM strains containing the above constructs. The *PMS1* gene was disrupted with the *Mlu*I-*Apa*I fragment of *pms1*::*LEU2* from pAM58 (38). The *MSH2* gene was disrupted with the *Sac*I-*Pst*I fragment of *msh2*::*LEU2* from p203. p203 was constructed as follows. The *Xho*I-*Hpa*I *MSH2* gene fragment from pEN11 (4) was cloned into the *Sal*I-*Sma*I sites of the pUC19 polylinker, and then the internal *MSH2 Sal*I-*Sal*I fragment was replaced by the *Sal*I-*Xho*I fragment of *LEU2*. The *Bam*HIII-*Aat*II fragment from *pms3*::*LEU2* (48) was used to disrupt the *MSH3* gene. Disruptions or replacements were verified by PCR with pairs of primers flanking the disrupted regions: for *pms1*::*LEU2*, we used pms1-1 (5′-CTG GAC AAG TTA TCA CCG ACT-3′) and pms1-2 (5′-CCA GCA CCA CAA GTT CAT CAA TG-3′); for *msh2*::*LEU2*, we used msh2-5 (5′-AGG GAA CAT TGA GCA AGT TAA TGA G-3′) and msh2-6 (5′-CAC CAA CCT GTC TGA TGT AAG TAG A 3′); and for *msh3*::*LEU2*, we used msh3-1 (5′-ATT AGA GTA GGC TAC AAG TAC-3′) and msh3-2 (5′-AAC ATA CGT ACC ATC CGC ATC-3′). Amplification was continued for 30 cycles (30 s at 96°C, 1 min at 55°C, and 5 min at 72°C) with TaqPlus polymerase (Stratagene). The anticipated replacement or disruption was confirmed by the identification of the expected PCR fragment. As expected, the *pms1* and *msh2* mutations led to a mutator phenotype resulting in increased frequencies of *can1* mutations (data not shown). The *msh3* deletion had little effect on the appearance of *can1* mutations, as expected. However, a *msh3* deletion mutation in the same strain was previously shown to lead to increased mutation in a homonucleotide run (55).

Genetic and molecular procedures. Insertion of constructions into the *LYS2* gene were verified by Southern blot analysis. The Ade$^+$ recombination rates were determined by fluctuation analysis with 12 to 24 independent cultures as described previously (28) or by using the Leningrad method described in reference 29. Comparable rates were obtained with the two methods. Loss of the intervening *URA3* gene between the *ADE* repeats was selected on 5-FOA medium (10). Genomic DNAs from the Ade$^+$ recombinant were isolated and amplified by PCR with two primers flanking the *ADE1*/*ADE2* overlapping region: ADE1 primer (corresponding to *P. methanolica ADE1*) (5′-CCT ATG ACG GAA GAG GT-3′) and ADE2 primer (corresponding to *S. cerevisiae ADE2*) (5′-GAA CAT TTA GCA TAA TGG-3′). Sequencing of PCR products was done with these primers by using an ABI automated sequencer (Applied Biosystems Inc.).

RESULTS

An LIR stimulates recombination between surrounding homologous and diverged direct repeats. We previously established that an LIR is genetically active in that it is a hot spot for recombination between genes on homologous chromosomes and it can induce illegitimate recombination between short (<10-bp) repeats within or in the vicinity of the LIR (28, 29). We therefore investigated intrachromosomal recombination between direct repeats that were homologous or diverged and examined the consequences of an LIR and other types of inserts (Fig. 1). To study homologous recombination, fragments of the *P. methanolica ADE1* gene were introduced into the *LYS2* gene of *S. cerevisiae* chromosome II. One fragment (*AD*) had the 3′ end deleted, and the other (*DE*) had the 5′

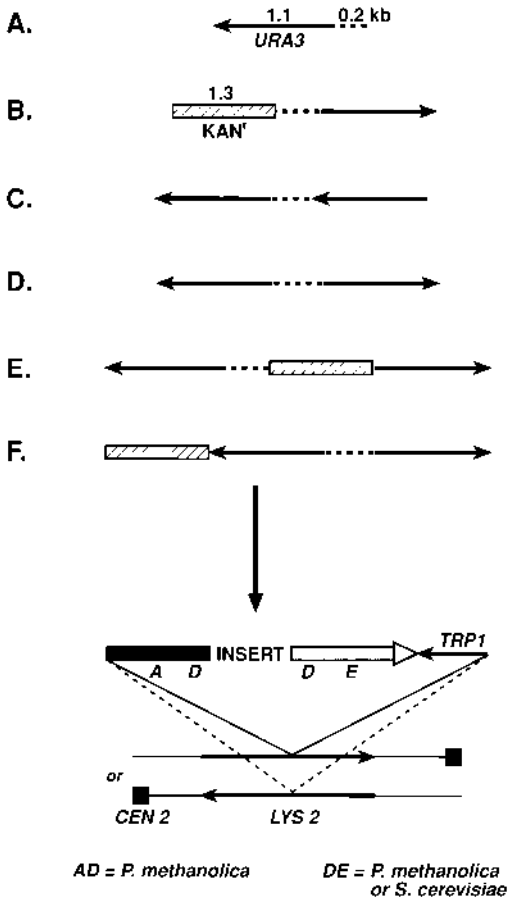

FIG. 1. Constructs used to examine homologous and homeologous recombination between truncated fragments of the *P. methanolica* and *S. cerevisiae ADE1*/*ADE2* genes. The various recombination cassettes (i.e., *AD*-INSERT-*DE*-*TRP1*) were placed in both orientations at the *Xho*I site of the *LYS2* gene on chromosome II. The arrows indicate the direction of transcription of the genes. Recombination in the 424-bp D region results in an Ade^+ phenotype. The INSERT corresponded to the A to F constructs described below: (A) unique DNA, single *URA3* with 0.2-kb spacer (corresponds to the 1.3-kb *Pst*I-*Nsi*I *URA3* fragment from pFL34∗) (54); (B) unique DNA plus large spacer, single *URA3* with 1.5-kb spacer (corresponds to the 1.3-kb *Nsi*I-*Sal*I *URA3* fragment from pFL34∗ plus the 1.3-kb *Sal*I-*Sal*I kanamycin resistance gene from pUC-4K); (C) direct *URA3* repeats (corresponds to the two direct 1.1-kb *Nsi*I-*Bgl*II *URA3* fragments and the 0.2-kb *Bgl*II-*Pst*I spacer from pFL34∗); (D) LIR, inverted *URA3* repeats (corresponds to the two inverted 1.1-kb *Nsi*I-*Bgl*II *URA3* fragments and the 0.2-kb *Bgl*II-*Bam*HIII spacer from pFL34∗); (E) LIR plus large spacer (corresponds to the insertion of a 1.3-kb *Sal*I-*Sal*I kanamycin resistance gene from the pUC-4K at the *Sal*I site of the 0.2-kb spacer of the previous D construct); and (F) LIR plus adjacent spacer (the 1.3-kb *Sal*I-*Sal*I kanamycin resistance gene from pUC-4K was cloned in the *Sal*I site of the *P. methanolica ADE1* fragment).

end deleted. There was an overlapping region of 424 bp so that recombination between the fragments would generate a complete copy of the gene, resulting in complementation of the *ade2* deletion mutation in the *S. cerevisiae* strain.

When the small homologous direct repeats were separated by an LIR, the rate of recombination was considerably enhanced over that when other DNAs were present between the repeats. The rate of recombination between the 424-bp repeats was approximately 16×10^{-5} when they were separated by the *URA3* LIR (Table 1). This was 10-fold higher than the rate when the repeats were separated by direct repeats of comparable size or just a single *URA3* gene. The rates were independent of the direction of insertion of the recombination cassette relative to the *LYS2* gene. Thus, not only does an LIR act as a hot spot for interchromosomal homologous recombination (28), but also it stimulates intrachromosomal recombination.

Since an LIR could stimulate recombination between homologous DNAs, we investigated whether it would also affect recombination between highly diverged DNAs. Homeologous recombination was examined by using similar constructs, except that the *P. methanolica ADE1* 3′ fragment was replaced by its *ADE2* homolog from *S. cerevisiae*. In the region of the 424-bp overlap (as well as the rest of the *P. methanolica ADE1* and *S. cerevisiae ADE2* genes), the sequences are 28% diverged (Fig. 2) (56). *P. methanolica ADE1* is able to complement deletions of *S. cerevisiae ADE2* (56). The truncated gene fragments were separated either by a region containing unique DNA or by direct or inverted 1.1-kb repeats of the *URA3* gene (Fig. 1).

Recombinants were identified by the appearance of Ade^+ colonies on selective medium or loss of the intervening *URA3* marker (by selection on 5-FOA). The gene products from the mosaic *Pichia ADE1*/*Saccharomyces ADE2* gene were functional. Among 161 recombinants that were 5-FOA resistant because they had lost the two intervening *URA3* genes (133 LIRs and 28 direct repeats were examined), all were Ade^+.

Recombination between the diverged repeats was reduced approximately 1,000-fold compared to homologous repeats when separated by unique DNA or DNA containing directly repeated *URA3* genes (Table 2). This is consistent with previous reports of a nearly 200-fold reduction when DNAs are diverged by 25%, although in one study (48) the DNAs were embedded in homologous repeats and in the other study only reciprocal exchange could be detected (21). Since an LIR between the diverged repeats resulted in a three- to sixfold increase in the recombination rate, an LIR can stimulate homeologous as well as homologous recombination. The stimulation is independent of the direction of the cassette relative to the *LYS2* gene in which it is inserted and is independent of the distance between the *URA3* repeats within the LIR when the distance is increased from 200 to 1,500 bp (Table 2). The effect of the LIR is not dependent on its being immediately adjacent to the recombining direct repeats, since insertion of a 1,300-bp spacer sequence did not affect the LIR-stimulated recombination. This suggests that the effect of the LIR on recombination

TABLE 1. Recombination between homologous 424-bp repeats separated by various DNA insertions containing long direct or inverted *URA3* repeats in Pol^+ and *pol3-t* strains grown at 25°C

Strain	Insertion[a]	Orientation[b]	Rate (10^{-5})[c]
Pol+	DIR	A	1.7 (1.5–2.5)
		B	1.9 (1.0–3.2)
	LIR	A	16.4 (14.0–20.7)
		B	16.0 (11.5–18.9)
pol3-t	DIR	A	13.7 (9.4–19.3)
		B	3.7 (2.6–5.5)
	LIR	A	1,270 (729–1,953)
		B	1,094 (811–1,243)

[a] DIR, direct repeat.
[b] For orientation A, the direction of transcription is the same for the *ADE1* promoter in the insert and the *LYS2* gene, for orientation B, the direction of transcription for the *ADE1* promoter in the insert is opposite to that for the *LYS2* gene.
[c] The 95% confidence limit in fluctuation measurement is given in parentheses.

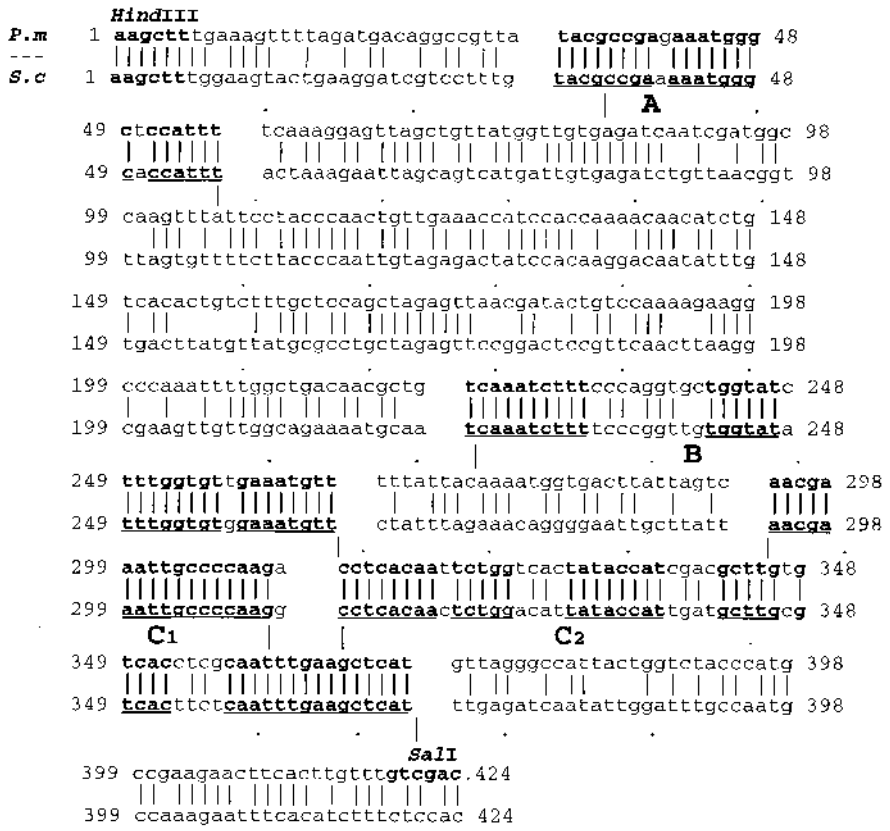

FIG. 2. Alignment of overlapping *P. methanolica ADE1* (*P.m*., upper sequence) and *S. cerevisiae ADE2* (*S.c*., lower sequence) 424-bp fragments. The *P. methanolica ADE1* 5′ fragment extends from the promoter region to the *Sal*I site, while the *S. cerevisiae ADE2* 3′ fragment extends from the *Hin*dIII site to the end of the *ADE2* gene (52, 56). All recombination breakpoints were in the three regions of high identity, A, B, and C (= C1 + C2) (see the text and Table 4). The two primers ADE1 and ADE2, used for amplifying the recombination products, were located outside this 424-bp region. The regions in boldface type correspond to long stretches of identical sequence.

may not simply be due to replication slippage resulting from interaction of the replication complex with the LIR.

Altered replication amplifies the LIR-stimulated homeologous recombination. Altered replication due to mutations in the DNA polymerase δ can stimulate homologous chromosome recombination (28). It also enhances LIR-stimulated illegitimate recombination. We therefore investigated whether a temperature-sensitive mutation, *pol3-t*, in the DNA polymerase δ gene could influence the effect of an LIR on homologous and homeologous recombination.

The *pol3-t* mutation resulted in increased intrachromosomal homologous recombination. When the repeats were separated by directly repeated *URA3* genes, there was a 2- to 10-fold increased recombination rate in the mutant compared to the isogenic Pol$^+$ strain at the 25°C permissive temperature (Table 1). Altered replication also resulted in as much as a 10-fold increase in recombination between the highly diverged DNAs, regardless of whether they were separated by the directly repeated *URA3* genes or unique DNA (Table 2).

The effect of an inverted repeat on recombination was much greater in the *pol3-t* background than in the wild-type background. This is consistent with previous observations on LIR-stimulated replication slippage and interchromosomal recombination (28, 29, 54). In the *pol3-t* mutant, the LIR increased homeologous recombination by 10- to 25-fold over the level observed when the repeats were separated by a direct repeat or unique DNA. As noted above, the relative increase (LIR versus direct repeat) was three- to sixfold in the Pol$^+$ strain. Thus, an inverted repeat in a *pol3-t* background results in an overall 60- to 240-fold increase in homeologous recombination over that in a Pol$^+$ background when the repeats are not separated by an LIR (Table 2). The recombination rates in the *pol3-t* mutant were even higher (nearly 1,000-fold) when rates are measured at the semipermissive temperature of 30°C (Table 2), although they were unaffected in a Pol$^+$ strain (data not shown).

The *pol3-t* mutation leads to similar effects of the LIR on homologous recombination (Table 1). Compared to recombination in the Pol$^+$ strain between repeats not separated by the LIR, the rate was 500- to 700-fold higher when the homologous repeats were separated by an LIR and recombination was examined in the polymerase mutant.

As noted above, the enhancement of the LIR-stimulated recombination by a *pol3-t* mutant is similar to previous observations with LIR-stimulated illegitimate recombination. This suggests that the LIR effect may somehow result from replication slippage between short identical sequences of the truncated *ADE1/2* repeats. If replication slippage in the vicinity of the LIR were responsible for the stimulation (28, 29), separation of the LIR from one of the repeats might be expected to reduce the rate of recombination in at least one of the orientations. A 1,300-bp spacer region of nonhomologous DNA (from the *E. coli* kanamycin resistance gene) was inserted between the *ADE1* fragment and the LIR (Fig. 1F), and the consequences on LIR-stimulated recombination were determined. Both orientations of the cassette were examined to

TABLE 2. Recombination between diverged 424-bp repeats separated by various DNAs

Strain	Insertion[a]	Orientation[b]	Rate (10^{-8})[c]
Pol$^+$	DIR	A	5.3 (1.94–8.34)
		B	4.6 (3.3–8.1)
	LIR	A	28.7 (16.2–63.7)
		B	15.4 (10.7–49.4)
	Single *URA3* + 1.5-kb spacer	A	2.5 (1.3–6.7)
		B	1.7 (1.1–4.8)
	LIR + adjacent 1.3-kb spacer	A	20.5 (5.8–33.5)
		B	16.6 (7.3–31.8)
	LIR + large internal 1.5-kb spacer	A	26.8 (14.8–49.3)
		B	35.6 (21.3–96.9)
pol3-t	DIR	A	53.9 (40.5–80.7)
		B	23.8 (12.2–27.5)
	LIR	A	1,265 (587–1,514)
		B	267 (195–454)
	Single *URA3* + 1.5-kb spacer	A	33.6 (20.8–94.7)
		B	11.0 (7.6–30.6)
	LIR + adjacent 1.3-kb spacer	A	1,187 (849–2,746)
		B	374 (259–529)
	LIR + large internal 1.5-kb spacer	A	263 (221–410)
		B	171 (126–312)
	LIR[d]	A	4,494 (2,928–12,700)[d]
		B	1,309 (1,020–1,979)[d]

[a] See Fig. 1 for a description of inserts. DIR, direct repeat.
[b] For orientation A, the direction of transcription is the same for the *ADE1* promoter in the insert and the *LYS2* gene; for orientation B, the direction of transcription for the *ADE1* promoter in the insert is opposite to that for the *LYS2* gene.
[c] All experiments were done at 25°C except where noted. The rates of recombination in Pol$^+$ strains were comparable at 25 and 30°C. The 95% confidence limit in fluctuation measurement is given in parentheses.
[d] Experiments were carried out at 30°C.

evaluate possible effects of leading- or lagging-strand replication. As shown in Table 2, the spacer did not affect LIR-stimulated homeologous recombination in either orientation of the spacer-containing cassette. Thus, the enhanced recombination does not appear to be explained simply by replication slippage. This conclusion is supported by the observation (see below) that the distribution of recombination breakpoints is comparable for either orientation of the *ADE1*/*ADE2* repeats relative to the direction of replication.

Recombination breakpoints occur in regions of greater identity. Because of sequence differences between diverged DNAs, it is possible to analyze the recombination breakpoints, or junctions. We therefore examined recombinants from the Pol$^+$ and *pol3-t* strains for both orientations of the cassette relative to the direction of replication. The DNA of the Ade$^+$ recombinants (first selected as 5-FOA-resistant clones) was PCR amplified from total genomic DNA with primers to the *P. methanolica ADE1* (5′-CCT ATG ACG GAA GAG GT-3′) and *S. cerevisiae ADE2* (5′-GAA CAT TTA GCA TAA TGG -3′) regions and subsequently sequenced.

There appears to be a requirement for a small region of identity for recombination to occur. In the 424-bp region of overlap, 100 bases are in sequences of less than 5-base identity and yet there were few breakpoints in such regions. Over 95% of the 133 breakpoints examined occurred in identical sequences (homology blocks) of at least 5 bp (Table 3). Similar to other reports for homeologous recombination in yeast, all recombination junctions are at regions of identical sequences.

There was no difference in the size distribution of homology blocks at the breakpoints between the Pol$^+$ and the *pol3-t* strains (Table 3), and there were no recombinants that had multiple breakpoints. For the LIR constructs (Fig. 1D), the recombination breakpoints occurred in regions A, B, and C (= C1 + C2) (Fig. 2), which had greater than 85% homology over a stretch of ≥24 bases (Table 4). While the length of A plus B is comparable to that of C, nearly 80% of the recombinants occurred in the latter region. The distribution of breakpoints among regions A, B, and C did not appear to differ with orientation. (The replication fork moves in the same direction as *LYS2* transcription [54].) However, there did appear to be an effect of orientation for the C1 component of C, which is characterized by a run of 17 identical bases. Overall, these results suggest that recombination induced by an LIR is not simply due to slippage events as proposed for deletion of an LIR.

TABLE 3. Distribution of recombination breakpoints by length of homology blocks

Strain	Total no. of breakpoints	% Distribution of breakpoints			
		0–4[a]	5	6–10	>10
Pol$^+$	67	1.5	15	34	50
pol3-t	66	3	12	44	41

[a] Number of identical bases at breakpoints.

LIR-stimulated homeologous recombination is not influenced by MMR. Recombination between highly diverged DNAs has been proposed to lead to multiple mismatches, which might be subject to MMR (44), thereby reducing the incidence of recombination. There are many examples supporting this proposal (24, 34, 41, 49, 59), as well as the contrary view that MMR may play only a small role in preventing homeologous recombination in bacteria and in yeast (1, 2, 5, 32, 42, 43). Furthermore, given the suggested role for replication in LIR-stimulated genetic events and the observation that MMR is associated with correction of replication-associated mutations, it is possible that MMR is involved in LIR-stimulated recombination.

We examined the consequences of mutations in the MMR genes *PMS1*, *MSH2*, and *MSH3* on recombination between the *ADE1-ADE2* gene pair (*PMS1* is a homolog of *E. coli mutL*, and *MSH2* and *MSH3* are homologs of *E. coli mutS*.) The Ade$^-$ repeats were separated by direct or inverted copies of the *URA3* genes or a single *URA3* gene plus 1,500 bp of bacterial DNA (Fig. 1B). As described above, the presence of an

TABLE 4. Distribution of recombination breakpoints shown as preference for breakpoints in regions of greater identity[a]

Strain	Orientation[b]	Total no. of breakpoints	No. of breakpoints[c]			
			A (2/24)	B (6/43)	C1 (0/17)	C2 (8/59)
Pol$^+$	A	30	5	2	11	12
	B	30	1	0	13	16
pol3-t	A	27	1	5	11	9
	B	26	2	3	1	16

[a] Regions A, B, C1, and C2 are shown in Fig. 2. The distribution is only for LIR constructs.
[b] For orientation A, the direction of transcription is the same for the *ADE1* promoter in the insert and the *LYS2* gene; for orientation B, the direction of transcription for the *ADE1* promoter in the insert is opposite to that for the *LYS2* gene.
[c] Numbers in parentheses show the number of mismatches per total number of nucleotides in the sequences of high homology (Fig. 2).

TABLE 5. Effect of mismatch repair genes *PMS1*, *MSH2*, and *MSH3* in Pol$^+$ strains on recombination between diverged DNAs separated by direct or inverted repeats or unique sequence

Genotype	Inserts[a]	Ade$^+$ recombination rate (10^{-8}) for[b]:	
		Orientation A	Orientation B
Mmr$^+$	DIR	2.8 ± 0.3	3.6 ± 0.2
pms1		1.9 ± 0.1	2.1 ± 0.5
msh2		4.3 ± 1.0	2.7 ± 0.7
msh3		2.4 ± 0.9	1.0 ± 0.3
Mmr$^+$	LIR	33 ± 5	33 ± 9
pms1		35 ± 9	14 ± 2
msh2		27 ± 10	16 ± 2
msh3		38 ± 3	25 ± 11
Mmr$^+$	Unique[c]	4.6 ± 0.1	1.7 ± 0.3
pms1		5.1 ± 2.0	2.0 ± 0.2
msh2		1.7 ± 0.1	0.8 ± 0.2
msh3		1.5 ± 0.3	0.7 ± 0.1

[a] DIR, direct repeat.
[b] Rates were measured by the Leningrad method with a 121-pin replicator as described previously (29). For orientation A, the direction of transcription is the same for the *ADE1* promoter in the insert and the *LYS2* gene; for orientation B, the direction of transcription for the *ADE1* promoter in the insert is opposite to that for the *LYS2* gene.
[c] Unique DNA inserts as described in Fig. 1B.

LIR enhances homeologous recombination nearly 10-fold in a Pol$^+$ strain. The absence of the *PMS1*, *MSH2*, or *MSH3* gene product did not affect the rates of homeologous recombination (Table 5) between the direct repeats regardless of the orientation of the repeats relative to the direction of replication or whether the region between them contained unique DNA, a direct *URA3* repeat, or an LIR.

DISCUSSION

Recombination between diverged DNA sequences could be a major contributor to genome instability, especially in higher eukaryotes, where diverged repeats are common. Such recombination could lead to genomic rearrangement such as translocations, deletions, or inversions. The prevention of recombination between highly diverged DNAs may be important not only for genomic stability but also for prevention of interspecies exchange of genetic information (34).

Many factors could influence recombination between highly diverged DNAs. These include initiation, association between the recombining DNAs, strand transfer and resolution, and MMR. As demonstrated with various systems in yeast, the contributions of the various factors can depend on the organization of the recombining DNAs. We have shown that replication defects and DNA organization similar to that in the human genome can induce intragenic recombination between highly diverged DNAs as well as homologous DNAs in comparable ways. They also induce ectopic recombination (in preparation).

Stimulation of homeologous recombination by altered replication. The altered replication in the *pol3-t* mutant resulted in as much as a 12-fold increase in homeologous recombination with a comparable or possibly smaller effect on homologous recombination. The LIR also enhanced homeologous recombination in the Pol$^+$ strains up to sixfold. However, the effects of the combination of the Polδ mutation and the LIR were not additive but, instead, resulted in at least a multiplicative increase in the rate of homeologous recombination. For example, there was an approximately 5- to 10-fold (B and A orientations, respectively) increase in recombination for the direct repeats in the *pol3-t* versus the Pol$^+$ strain, while there was an approximately 4- to 6-fold increase in recombination in the Pol$^+$ strain when the repeats were separated by an LIR compared to when they were separated by a direct repeat. If the effects were multiplicative, the expected increase would be 20- to 60-fold, which is in the same range as the observed 60- to 240-fold increase for orientations B and A. The effect was also at least multiplicative for homologous repeats. These results are similar to our previous report (28) for LIR-stimulated homologous interchromosomal recombination, where there was also a synergistic stimulation of allelic recombination by the *pol3-t* mutation and an LIR (which was the bacterial transposon Tn*5*). The high level of homologous recombination detected in the present study suggests that intermediates can be isolated and examined directly.

To address possible mechanisms of stimulated recombination, we characterized the breakpoints among the homeologous recombinants. Over 95% occurred in regions of at least 5-bp identity (Table 3), although one-quarter of the repeat sequence DNAs was in stretches of ≤4-bp identity. This bias is consistent with our previous observations of mutations arising by replication slippage in wild-type and *pol3-t* mutants, as well as LIR-stimulated deletions in a *pol3-t* mutant (28, 29, 54). Most events occur between repeats of at least 5-bp identity (Table 3). We suggest that this common feature may reflect a minimal sequence requirement for the replication apparatus to continue replication from a nascent strand at a new slipped position.

Based on results in yeast (28, 29, 31, 47) and bacteria (18, 20, 23), the LIR has been proposed to be genetically active via an interaction between the replicating apparatus and the inverted repeats of the LIR. The LIRs could increase replication slippage type mutations several hundred-fold in yeast (29–31, 47). Recently, Canceill and Ehrlich (13) demonstrated that while an LIR can inhibit in vitro replication by the *E. coli* Pol3 holoenzyme, slippage could occur between small direct repeats at the base of the LIRs depending on replication conditions. There are several common features of deletion mutations resulting from illegitimate recombination via replication slippage and homology-driven recombination between highly diverged or homologous DNAs. First, there is a combined effect of replication defects and inverted repeats. Second, the breakpoints are of comparable size. Finally, while they were not examined in the present experiments, the *RAD52* pathway genes which are required for much of mitotic recombination are also involved in replication slippage deletions in yeast (29, 54).

Based on the combination of effects of LIRs on several genetic end points that can be related to replication slippage, we propose that the multiplicative interaction with a defect in replication is due to an enhancement of the consequences of replication blockage on recombination. The observation that homeologous recombination and homologous recombination are similarly stimulated suggests a common mechanism. These results are consistent with stimulation of allelic recombination between homologous chromosomes by an LIR and altered replication (28). However, because of DNA divergence, it is possible to investigate the recombination junctions.

A model for altered replication-stimulated recombination. The results obtained in studies of replication slippage between small repeats (54), LIR and/or replication-stimulated recombination (28, 29; see above), and recombination between highly diverged DNAs (42, 43) are consistent with previously developed models. In the replication model for the generation of

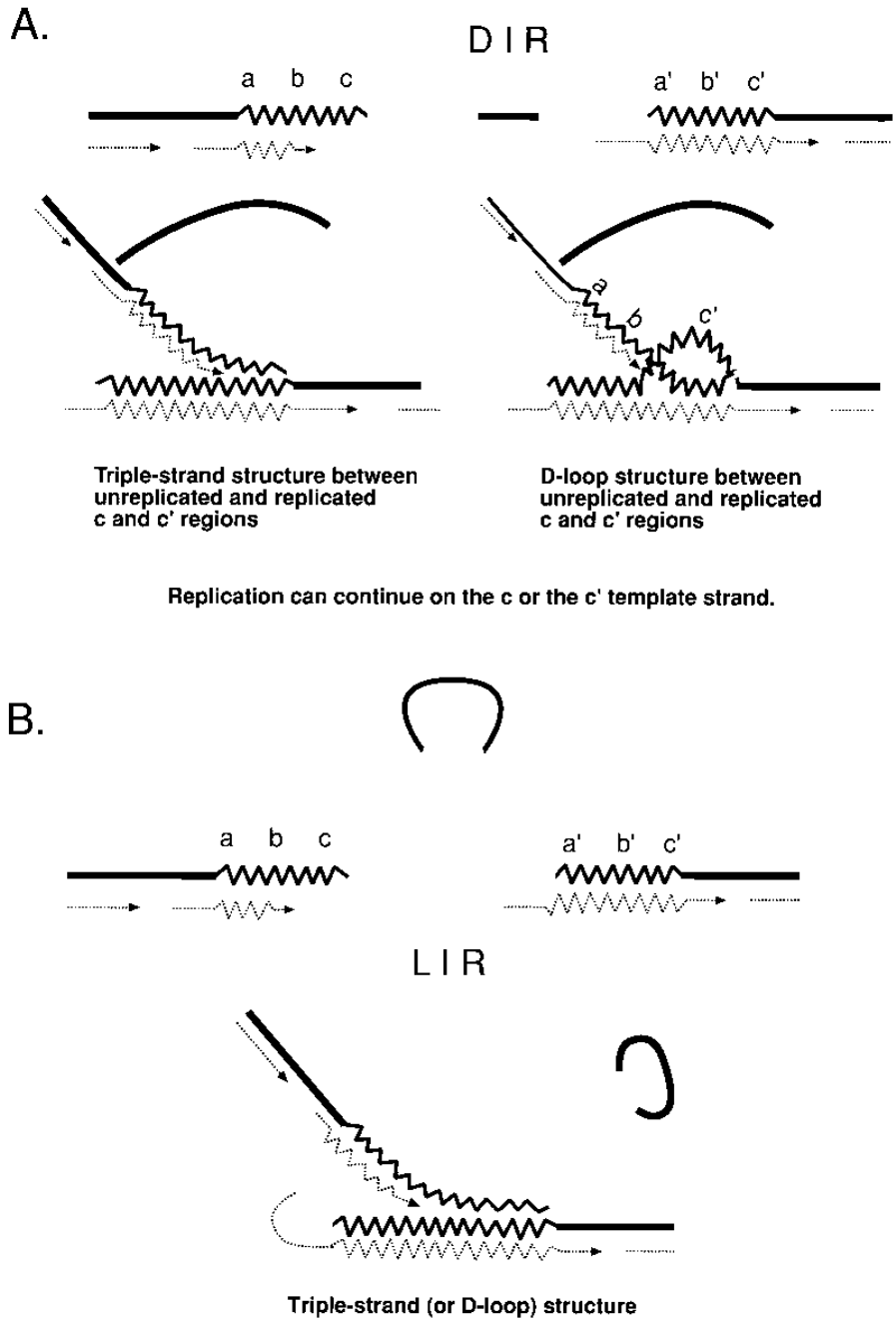

FIG. 3. Model for replication-driven recombination between partially and fully replicated repeats separated by a direct repeat (DIR) (A) or an LIR (B). As described in Discussion, a replication defect and/or LIR is proposed to lead to single-stranded regions during replication, and these could lead to recombinational interactions between direct repeats. We and others had previously proposed (28, 29, 47) that during replication there can be internal pairing of an LIR undergoing replication and that this could result in a blockage to replication. For an LIR (gray arrows in panel B), the blockage might also lead to degradation of the nascent strand (dotted line). The resulting single-stranded and double-stranded repeats (wavy lines) could undergo a recombinational interaction so that replication could continue on the original template strand, or there could be strand switching at a small region of identity (i.e., ≥5 bases). Once there is strand switching and replication continues, the DNA would not have mismatches; therefore, MMR would not be expected to have an effect. An alternative version of this model is that the nascent 3′ end in one of the repeats can invade the other duplicated repeat at a small region of identify and that replication could continue.

deletions and recombination, (i) alterations in lagging-strand replication (i.e., in *pol3* or *pol1* mutants) can lead to slippage between small identical repeats (29, 47, 54), (ii) slippage is enhanced by an LIR as a result of delaying replication further (28), and (iii) stalled replication can be resolved via slippage or recombination. Our results cannot be explained simply by replication slippage. First of all, the *ADE1*/*ADE2* recombination breakpoints in this work do not depend on the direction of replication (Table 4), in contrast to observations for LIR-induced deletions resulting from replication slippage (28, 54). Second, the breakpoints of LIR-induced deletions are always located close to the base of the LIR (28, 29, 31, 47). We have shown that insertion of a 1.3-kb spacer between the LIR and one of the repeats (Fig. 1F) does not reduce recombination, regardless of cassette orientation (Table 2).

We suggest a model for homeologous recombination (Fig. 3)

that accounts for interrupted replication, as suggested for replication slippage induced by an LIR (28), and that is driven by DNA synthesis. (The model incorporates features of the model proposed by Porter et al. [42] and Priebe et al. [43] and is based in part on the original one-ended model for double-strand break repair [45].) We had proposed that (i) while there may be association between diverged molecules, a heteroduplex would be unstable; (ii) a duplex could be invaded by a 3′ single-stranded end of diverged DNA at a small region of identity (i.e., corresponding to the ≥5-bp junctions) (42, 43; see above); and (iii) the resulting recombinational intermediate would be stabilized by the subsequent replication that would lead to homoduplex DNA. There would be little mismatched DNA on which MMR could act; therefore, this model also explained why MMR was not found to affect homeologous recombination whereas it did influence homologous recombination events (42, 43).

We propose that a pause in replication, as could occur in a replication-defective mutant, can lead to a partially replicated single-stranded region. This enables recombinational interactions to occur between a partially replicated repeat and a repeat that has been duplicated. The LIR would increase the likelihood of blockage and might act to initiate recombination. For example, the blockage in replication might also lead to some degradation of the nascent 3′ strand so as to result in one of the repeats becoming single stranded. (Degradation of the 3′ strand has been established for a double-strand break in yeast [26], and this possibly also occurs at a site of replication blockage.) The recombinational interaction could arise by displacement of the end of the nascent 3′ strand to the duplicated region, such as was proposed for synthesis past a lesion in bacteriophage T4 (27). Alternatively, it could arise through an interaction of the unreplicated single strand of the repeat with the duplicated repeat, as diagrammed in Fig. 3, possibly through a *recA* mediated D-loop structure (in combination with a topoisomerase) or a triple-strand structure (15, 19). The net result would be that the nascent replicating strand in one repeat would be placed at a corresponding position in the fully replicated repeat. This would increase the likelihood of strand switching, which could lead to newly replicated homoduplex DNA. However, we cannot rule out an alternative model in which replication arrest induced by an LIR could lead to a double-strand break that is subsequently repaired through a single strand-annealing mechanism (26). However, there is no information concerning single-strand annealing between highly diverged DNAs.

Based on results in this and our previous studies of replication slippage and of homeologous recombination, we further propose that there is a minimum requirement of approximately 5-bp identity for the replication switch. This type of replication-driven recombination between diverged DNAs might not be subject to MMR, as noted above for double-strand break initiated events (43). The reduced incidence of recombination for homeologous versus homologous repeats simply would reflect the decreased likelihood of a recombinational interaction between the single-stranded and double-stranded regions of the two repeats. We assume that all other factors, such as the likelihood of replication blockage and size of the single-stranded region, are independent of whether the recombining DNAs are homologous or diverged.

Effect of mismatch repair on homeologous recombination. We have shown that while recombination between highly diverged DNA repeats was reduced over 400-fold compared to homologous recombination, the reduction was not affected by the *PMS1*, *MHL1*, or *MSH2* components of the yeast MMR system. The stimulation by the LIR was also MMR insensitive. The model described above and our previous results have provided explanations why homeologous recombination might not be subject to MMR. The factors determining when MMR can prevent such recombination appear to include initiating events, the type of recombination, the level of divergence, and, as we have now shown, the DNA organization and altered replication.

The MMR system is important for genome integrity because of its role in preventing mutations. It is responsible for repairing many of the mistakes generated by polymerases during chromosomal replication. In yeast, mutations of the *E. coli* homologs *PMS1*, *MHL1*, and *MSH2* can lead to several-hundredfold enhancements in the frequency of alterations in simple dinucleotide repeats (53). Mutations in human MMR genes can lead to carcinogenesis due to accumulation of mutations (37). The proteins of the MMR systems from *E. coli* to humans can recognize and bind a mismatch and process it (4, 25, 40).

The MMR systems also play a role in several types of homeologous recombination. In bacteria, interspecies recombination and recombination between highly diverged DNA were increased several orders of magnitude in *mutS* or *mutL* strains (44). Recently, it was shown that mammalian cells defective in the *E. coli* MutS homolog were a much better recipient for gene targeting with related DNA (0.6% divergence) than was the corresponding wild-type cell line (22).

Based on results in yeast, it appears that the DNA organization and level of divergence are important (discussed in reference 42) in determining if the MMR system can act as a barrier to homeologous recombination. A defect in the yeast MMR genes *MSH2* and *MSH3*, but not *PMS1*, resulted in as much as 100-fold enhanced mitotic crossing over between diverged DNA sequences (91% identity), and there was up to a 10-fold effect for sequences that were much more diverged (77%) (21). (These results also argue against the view that the lack of an MMR effect in our experiments is simply due to too many mismatches.) Selva et al. (48) examined recombination between short homeologous DNAs (25% diverged) flanked by long identical sequences; recombination was probably initiated in the region of homology. They found that for one of the two orientations examined, recombination was increased (up to 43-fold) by mutations in *MSH2* and *MSH3* but not the *PMS1*. Alani et al. (5) demonstrated that mutations in *MSH2* had no effect on homeologous recombination between copenetrating molecules during transformation. Porter et al. (42) also showed that the MMR system was not involved in mitotic recombination between 15% diverged sequences that are located on a chromosome and a plasmid.

Thus, the likelihood that MMR will have an effect on recombination between highly diverged DNAs appears to depend on a variety of factors. Possibly, it has its greatest effect if it can act to prevent strand exchange, as suggested by the in vitro results of Worth et al. (58). They have shown that bacterial MutS can inhibit strand transfer between diverged DNAs and that MutL could exacerbate that inhibition. Alani et al. (5) suggested that in yeast, Msh2P blocks branch migration when mismatched bases are encountered. Thus, the effect of MMR may occur prior to or during the formation of a multiply mismatched heteroduplex. If so, the replication-initiated recombination described in the present study and modeled in Fig. 3 may be insensitive to MMR. Recently, we showed that heteroduplex DNA formed between 18%-diverged DNA sequences can efficiently transform Mmr^+ *E. coli*, suggesting that the multiply mismatched recombinational intermediates are not inactivated by the MMR system (57).

Implications. There are many repeats in the genome of most higher eukaryotes. Possibly, small palindromes or large inverted identical or related repeats (such as *Alu*s) could stimulate recombination between frequent surrounding repeats that are highly diverged even when they are not immediately adjacent to the inverted repeats (see, for example, results with an adjacent spacer and LIR). It will be interesting to examine the ability of small palindromes of the size common to the human genome to induce recombination between highly diverged DNAs. Possibly, other cellular factors or external agents such as replication inhibitors could also stimulate such recombination. It will also be interesting to determine if LIRs, which are known to stimulate replication slippage in bacteria, can lead to recombination between diverged or even homologous DNAs and whether there is a role for MMR. Since bacterial Tn*5* LIRs are frequently deleted during conjugation (7), suggesting that they form stem-like structures in the single-stranded DNA during transfer, they might help to overcome the barrier to recombination in bacterial interspecies crosses (44). Along with this, the role of MMR should be investigated, since the impact of MMR on homeologous recombination appears to depend on the route of recombination in bacteria (1, 2, 32, 44) as well as yeast (5, 21, 42, 43).

ACKNOWLEDGMENTS

We are grateful to W. Taylor, M. Roberts, and E. Golovanova for assistance in experiments; to G. Crouse and R. Lahue for plasmid p*msh3*::*LEU2* and R. Kolodner for plasmid pEN11; and to A. Waldman and L. Worth for discussion and critical comments on the manuscript.

Support was provided by the Russian Foundation for Basic Research (grant 96-04-48996 to D.A.G.) and by international research grant 75195-545401 from the Howard Hughes Medical Institute (to D.A.G. and M.A.R.). Partial support was provided by interagency agreement DE-A105-94ER61940 from the Department of Energy.

REFERENCES

1. **Abastado, J.-P., B. Cami, T. H. Dinh, J. Igolen, and P. Kourilsky.** 1984. Processing of complex heteroduplexes in *Escherichia coli* and *Cos*-1 monkey cells. Proc. Natl. Acad. Sci. USA **81:**5792–5796.
2. **Abastado, J.-P., S. Darche, F. Godeau, B. Cami, and P. Kourilsky.** 1987. Intramolecular recombination between partially homologous sequences in *Escherichia coli* and *Xenopus laevis* oocytes. Proc. Natl. Acad. Sci. USA **84:** 6496–6500.
3. **Aguilera, A., and H. L. Klein.** 1988. Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. Genetics **119:**779–790.
4. **Alani, E., N.-W. Chi, and R. D. Kolodner.** 1995. The *Saccharomyces cerevisiae* Msh2 protein specifically binds to duplex oligonucleotides containing mismatched DNA base pairs and insertions. Genes Dev. **9:**234–247.
5. **Alani, E., R. A. G. Reenan, and R. D. Kolodner.** 1994. Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. Genetics **173:**19–39.
6. **Baker, S. M., C. E. Bronner, L. Zang, A. W. Plug, M. Robatzek, G. Warren, E. A. Elliott, J. Yu, T. Ashley, N. Arnheim, R. A. Flavell, and R. M. Liskay.** 1995. Male mice defective in the DNA mismatch repair gene *PMS2* exhibit abnormal chromosome synapsis in meiosis. Cell **82:**309–319.
7. **Berg, D. E., C. Egner, and J. B. Lowe.** 1983. Mechanism of F factor-enhanced excision of Tn5. Gene **22:**1–7.
8. **Bi, X., and L. F. Liu.** 1996. DNA rearrangement mediated by inverted repeats. Proc. Natl. Acad. Sci. USA **93:**819–823.
9. **Bierne, H., and B. Michel.** 1994. When replication folk stops. Mol. Microbiol. **13:**17–23.
10. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.
11. **Brosius, J., and S. J. Gould.** 1992. On "genomenclature": a comprehensive (and respectful) taxonomy for pseudogenes and other "junk DNA". Proc. Natl. Acad. Sci. USA **89:**10706–10710.
12. **Cami, B., P. Chambon, and P. Kourilsky.** 1984. Correction of complex heteroduplexes made of mouse *H-2* gene sequences in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **81:**503–507.
13. **Canceill, D., and S. D. Ehrlich.** 1996. Copy-choice recombination mediated by DNA polymerase III holoenzyme from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **93:**6647–6652.
14. **Celniker, S. E., K. Sweder, F. Srienc, J. E. Bailey, and J. L. Campbell.** 1984. Deletion mutation affecting autonomously replicating sequence *ARS1* of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**2455–2466.
15. **Chiu, S. K., B. J. Rao, R. M. Story, and C. M. Radding.** 1993. Interactions of three strands in joints made by RecA protein. Biochemistry **32:**13146–13155.
16. **Collick, A., J. Drew, J. Penberth, P. Bois, J. Luckett, F. Scaerou, A. Jeffreys, and W. Reik.** 1996. Instability of long inverted repeats within mouse transgenes. EMBO J. **15:**1163–1171.
17. **Collins, J.** 1980. The instability of palindromic DNA. Cold Spring Harbor Symp. Quant. Biol. **45:**409–416.
18. **Collins, J., G. Volckaert, and P. Nevers.** 1982. Precise and nearly precise excision of the symmetrical inverted repeats of Tn5: common features of *recA*-independent deletion events in *E. coli*. Gene **19:**139–146.
19. **Cunningham, R. P., A. M. Wu, T. Shibata, C. DasGupta, and C. M. Radding.** 1981. Homologous pairing and topological linkage of DNA molecules by combined action of *E. coli* RecA protein and topoisomerase I. Cell **24:**213–223.
20. **d'Alencon, E., M. Petranovic, B. Michel, P. Noirot, A. Aucouturier, M. Uzest, and S. D. Ehrlich.** 1994. Copy-choice illegitimate DNA recombination revisited. EMBO J. **13:**2725–2734.
21. **Datta, A., A. Adouda, L. New, G. 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.
22. **de Wind, N., M. Dekker, A. Berns, M. Radman, and H. te Riele.** 1995. Inactivation of the mouse *MSH2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell **82:**321–330.
23. **Ehrlich, D. S.** 1989. Illegitimate recombination in bacteria, p. 799–832. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
24. **Feinstein, S. I., and J. A. Low.** 1986. Hyper-recombining recipient strains in bacterial conjugation. Genetics **113:**13–33.
25. **Fishel, R., A. Ewel, A. Lee, M. K. Lescoe, and J. Griffith.** 1994. Binding of mismatched microsatellite DNA sequences by the human Msh2 protein. Science **266:**1403–1405.
26. **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.
27. **Formosa, T., and B. Alberts.** 1986. DNA synthesis dependent on genetic recombination: characterization of a reaction catalyzed by purified bacteriophage T4 proteins. Cell **47:**793–806.
28. **Gordenin, D. A., K. S. Lobachov, N. P. Degtyareva, A. L. Malkova, E. Perkins, and M. A. Resnick.** 1993. Inverted DNA repeats: a source of eucaryotic genomic instability. Mol. Cell. Biol. **13:**5315–5322.
29. **Gordenin, D. A., A. L. Malkova, A. Peterzen, V. N. Kulikov, Y. I. Pavlov, E. Perkins, and M. A. Resnick.** 1992. Transposon Tn5 excision in yeast: the influence of DNA polymerases alpha, delta, epsilon and repair genes. Proc. Natl. Acad. Sci. USA **89:**3785–3789.
30. **Gordenin, D. A., Y. Y. Proscyavichus, A. L. Malkova, M. V. Trofimova, and A. Peterzen.** 1991. Yeast mutants with increased transposon Tn5 excision. Yeast **7:**37–50.
31. **Henderson, S. T., and T. D. Petes.** 1993. Instability of a plasmid borne inverted repeat in *Saccharomyces cerevisiae*. Genetics **133:**57–62.
32. **Humbert, O., M. Prudhomme, R. Hakenbeck, C. G. Dowson, and J. P. Claverys.** 1995. Homeologous recombination and mismatch repair during transformation in *Streptococcus pneumoniae*: saturation of the Hex mismatch repair system. Proc. Natl. Acad. Sci. USA **92:**9052–9056.
33. **Larionov, V., N. Kouprina, M. Eldarov, E. Perkins, G. Porter, and M. A. Resnick.** 1994. Transformation-associated recombination between diverged and homologous DNA repeats is induced by strand breaks. Yeast **10:**93–104.
34. **Matic, I., C. Rayssiguier, and M. Radman.** 1995. Interspecies gene exchange in bacteria: the role of SOS and mismatch repair systems in evolution of species. Cell **80:**507–515.
35. **Mezard, C., and A. Nicolas.** 1994. Homologous, homeologous, and illegitimate repair of double-strand breaks during transformation of a wild-type strain and a *rad52* mutant strain of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**1278–1292.
36. **Mezard, C., D. Pompon, and A. Nicolas.** 1992. Recombination between similar but not identical DNA sequences during yeast transformation occurs within short stretches of identity. Cell **70:**659–670.
37. **Modrich, P., and R. Lahue.** 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:**101–133.
38. **Morrison, A., A. L. Johnston, L. H. Johnston, and A. Sugino.** 1993. Pathway correcting DNA replication errors in *S. cerevisiae*. EMBO J. **12:**1467–1473.
39. **Nowak, R.** 1994. Mining treasures from "junk DNA." Science **263:**608–610.
40. **Parker, B. O., and M. G. Marinus.** 1992. Repair of DNA heteroduplexes containing small heterologous sequences in *E. coli*. Proc. Natl. Acad. Sci. USA **89:**1730–1734.
41. **Petit, M. A., J. Dimpfl, M. Radman, and H. Echols.** 1991. Control of large chromosomal duplications in *Escherichia coli* by the mismatch repair system. Genetics **129:**327–332.

42. **Porter, G., J. Westmoreland, S. Priebe, and M. A. Resnick.** 1996. Homologous and homeologous intermolecular gene conversion are not differentially affected by mutations in the DNA damage or the mismatch repair genes *RAD1*, *RAD50*, *RAD51*, *RAD52*, *RAD54*, *PMS1* and *MSH2*. Genetics **143:** 755–767.
43. **Priebe, S. D., J. Westmoreland, T. Nilsson-Tillgren, and M. A. Resnick.** 1994. Induction of recombination between homologous and diverged DNAs by double-strand gaps and breaks and role of mismatch repair. Mol. Cell. Biol. **14:**4802–4814.
44. **Rayssiguier, C., D. S. Thaler, and M. Radman.** 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature **342:**396–401.
45. **Resnick, M. A.** 1976. The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59:**97–106.
46. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–210.
47. **Ruskin, B., and G. R. Fink.** 1993. Mutations in *POL1* increase the mitotic instability of tandem inverted repeats in *Saccharomyces cerevisiae*. Genetics **133:**43–56.
48. **Selva, E. M., L. New, G. F. Crouse, and R. Lahue.** 1995. Mismatch correction acts as a barrier to homeologous recombination in *S. cerevisiae*. Genetics **139:**1175–1188.
49. **Shen, P., and H. V. Huang.** 1989. Effect of base pair mismatches on recombination via the RecBCD pathway. Mol. Gen. Genet. **218:**358–360.
50. **Sherman, F., G. F. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Sinden, R. R.** 1994. DNA structure and function. Academic Press, Inc., San Diego, Calif.
52. **Stotz, A., and P. Linder.** 1990. The *ADE2* gene from *Saccharomyces cerevisiae*: sequence and new vectors. Gene **95:**91–98.
53. **Strand, M., T. A. Prolla, R. M. Liskay, and T. D. Petes.** 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature **365:**274–276.
54. **Tran, H. T., N. P. Degtyareva, N. N. Koloteva, A. Sugino, H. Masumoto, D. A. Gordenin, and M. A. Resnick.** 1995. Replication slippage between distant short repeats in *Saccharomyces cerevisiae* depends on the direction of replication and the *RAD50* and *RAD52* genes. Mol. Cell. Biol. **15:**5607–5617.
55. **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.
56. **Tran, H. T., V. N. Kulikov, V. N. Noskov, G. I. Sizonenko, Y. O. Chernoff, and Y. I. Pavlov.** 1993. The 5′-aminoimidazole ribonucleotide-carboxylase structural gene of methylotrophic yeast *Pichia methanolica*: cloning, sequencing and homology analysis. Yeast **9:**1251–1258.
57. **Westmoreland, J., G. Porter, M. Radman, and M. A. Resnick.** Highly mismatched molecules resembling recombination intermediates efficiently transform mismatch repair proficient *E. coli*. Genetics, in press.
58. **Worth, L., S. Clark, M. Radman, and P. Modrich.** 1994. Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. Proc. Natl. Acad. Sci. USA **91:**3238–3241.
59. **Zahrt, T. C., G. C. Mora, and S. Maloy.** 1994. Inactivation of mismatch repair overcomes the barrier to transduction between *Salmonella typhimurium* and *Salmonella typhi*. J. Bacteriol. **176:**1527–1529.