MOLECULAR AND CELLULAR BIOLOGY, Oct. 1995, p. 5607–5617 Vol. 15, No. 10
0270-7306/95/$04.00+0

Replication Slippage between Distant Short Repeats in *Saccharomyces cerevisiae* Depends on the Direction of Replication and the *RAD50* and *RAD52* Genes

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Received 10 April 1995/Returned for modification 25 May 1995/Accepted 6 July 1995

Small direct repeats, which are frequent in all genomes, are a potential source of genome instability. To study the occurrence and genetic control of repeat-associated deletions, we developed a system in the yeast *Saccharomyces cerevisiae* that was based on small direct repeats separated by either random sequences or inverted repeats. Deletions were examined in the *LYS2* gene, using a set of 31- to 156-bp inserts that included inserts with no apparent potential for secondary structure as well as two quasipalindromes. All inserts were flanked by 6- to 9-bp direct repeats of *LYS2* sequence, providing an opportunity for Lys$^+$ reversion via precise excision. Reversions could arise by extended deletions involving either direct repeats or random sequences and by −1- or +2-bp frameshift mutations. The deletion breakpoints were always associated with short (3- to 9-bp) perfect or imperfect direct repeats. Compared with the POL$^+$ strain, deletions between small direct repeats were increased as much as 100-fold, and the spectrum was changed in a temperature-sensitive DNA polymerase δ *pol3-t* mutant, suggesting a role for replication. The type of deletion depended on orientation relative to the origin of replication. On the basis of these results, we propose (i) that extended deletions between small repeats arise by replication slippage and (ii) that the deletions occur primarily in either the leading or lagging strand. The *RAD50* and *RAD52* genes, which are required for the recombinational repair of many kinds of DNA double-strand breaks, appeared to be required also for the production of up to 90% of the deletions arising between separated repeats in the *pol3-t* mutant, suggesting a newly identified role for these genes in genome stability and possibly replication.

Short (4- to 10-bp) direct repeats are abundant throughout the genome, as would be expected for a random distribution of bases (37), and are often found at the ends of deleted nonreiterated sequences in many human genetic diseases (29, 46, 47). These could be an important source of genomic instability, especially if slippage could occur between separated direct repeats during replication. Replication slippage was initially proposed as a mechanism for small deletions and additions in DNA, resulting in frameshift mutations (50; see also the review in reference 44). Deletions and additions in simple repeats (multiply reiterated runs of 2 to 4 bp) are an important source of genetic change and are associated with many human diseases, including various cancers (1, 7, 25, 43, 51). The cancer proneness of humans with a mismatch repair defect has been suggested to be due to an increased likelihood of mutations arising by slippage during the replication of simple repeats (15, 33, 40, 54).

Distantly spaced short direct repeats are also a source of deletion mutations, and these have been proposed to occur primarily through replication slippage, both in *Escherichia coli* (2, 14) and in humans (11). Support for replication slippage as the mechanism for such deletions derives from observations of high levels of deletions when short direct repeats are separated by inverted repeats (IRs) in bacteria and *Saccharomyces cerevisiae*. The IRs are proposed to form stem-like secondary structures during replication, thereby bringing short direct repeats into close proximity, providing greater opportunity for slippage. This stem could inhibit DNA synthesis, which also might provoke slippage. Examples include the IRs of bacterial transposons Tn*10* and Tn*5* (12, 16, 20) and other IRs such as palindromes and quasipalindromes (8, 9, 17, 23, 45). Furthermore, Trinh and Sinden (53) demonstrated that deletion of an IR in *E. coli* which involved short direct repeats depended on the direction of replication. They proposed that deletions occurred primarily during lagging-strand synthesis. We demonstrated that in *S. cerevisiae*, deletions occurred frequently between small repeats within IRs of Tn*5* and that there was polarity in the choice of small repeats (17). This observation along with the involvement of replicative DNA polymerases (17, 18) led us to propose that IR stimulated deletions occurred during lagging-strand synthesis by replication slippage between the small direct repeats.

There has been no direct evidence that extended (i.e., more than 1-bp) deletions with breakpoints in small separated direct repeats actually arise by replication slippage when there are no IRs between the repeats. We describe here a system that was developed to study the origin and genetic control of deletions that do not involve IRs or simple repeats. With the sequences studied, we were able to investigate extended deletions of up to 74 bp arising between separated small direct repeats, 1-base frameshift mutations, and the role of replication slippage. Also

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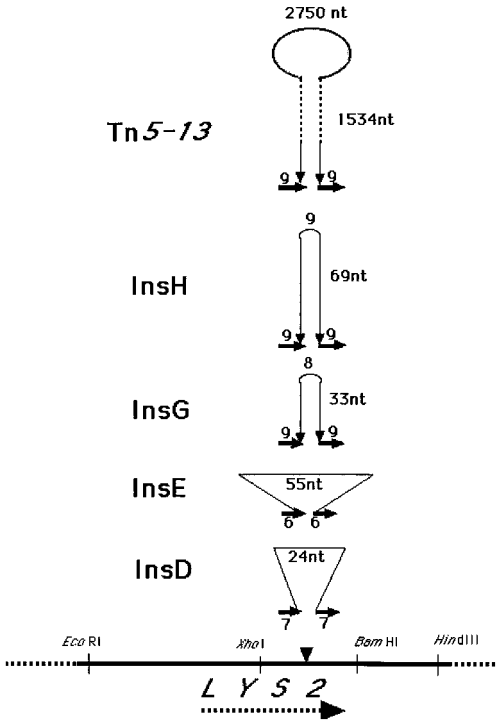


FIG. 1. Inserts in the yeast *LYS2* gene. All inserts are located at the same site as the previously described for Tn*5-13* (17, 20). The site is marked by a filled triangle on the line representing a 4,795-bp *Eco*RI-*Hin*dIII fragment of chromosomal DNA containing *LYS2*. The distances of the other sites from *Eco*RI are as follows: *Xho*I, 3,104 bp; *Bam*HI, 3,486 bp. The horizontal arrow shows the direction of the *LYS2* transcription. For each of the inserts, the lengths of flanking short direct repeats and of inserted regions are shown. For the IR inserts Tn*5-13*, InsG, and InsH, the lengths of the IR and of spacer between the IRs is shown separately. nt, nucleotides.

examined were deletions between repeats separated by 74- and 147-bp quasipalindromes. On the basis of the presence of short direct repeats at the breakpoints of all extended deletions and dependence of the deletion spectrum on orientation relative to the replication origin, replication slippage appears to be the most likely source of deletions. We also show that the *RAD50* and *RAD52* genes, which are required for the recombinational repair of several kinds of DNA double-strand breaks (DSBs), affect deletion rates, suggesting a role for these genes in replication slippage.

MATERIALS AND METHODS

Strains and plasmids. All strains were isogenic and were derived from *pol3-t*-DM (18). All strains were *MAT*α *leu2-2 trp1-Δ1 ura3-X*, and they had various combinations of mutant or wild-type alleles of *pol3*, *RAD50*, and *RAD52* and various inserts (Fig. 1) or a deletion in the *LYS2* gene. The POL$^+$ and *pol3-t* strains with quasipalindrome inserts InsG and InsH and nonpalindrome inserts InsD and InsE, and strain k31-*pol3-t*-DM with deletion DelC in *LYS2*, have been described previously (17). All inserts are at the same site in the chromosomal *LYS2* gene (Fig. 1) as the previously studied insert Tn*5-13* (20). Isogenic *rad50* and *rad52* deletion mutants containing these insertions were derived from POL$^+$ and *pol3-t* strains by using previously described gene transplacement methods and plasmids (18). Plasmids p92, p93, p94, and p95 (Fig. 2) were constructed as follows. First, plasmids p90 and p91 were created to generate the *LYS2*::*InsD* and *LYS2*::*InsE* mutations by gap filling as described below. These plasmids contained a *Hin*dIII-*Pvu*II fragment that included the automously replicating sequence (ARS)-centromere (CEN) cassette (*Cla*I-*Cla*I fragment) and bacterial Amp^r gene from pASZ11 (48), followed by a *Pichia methanolica* chromosomal *Pvu*II-*Pst*I fragment to increase the asymmetry of the fragment (52), the *URA3* gene contained in a *Hin*dIII-*Nsi*I fragment derived from pFL34 (4) (the original *Bgl*II *URA3* fragment was recloned into pFL34 in the opposite orientation), and the *LYS2* gene lacking the *Bam*HI-*Xho*I regions derived from pLL12-BX) (19) in orientations A and B (plasmid p91 and plasmid p90, respectively). The InsD and InsE mutations were transferred to the plasmids p90 and p91 by gap filling (i.e., the plasmids were cut with *Xho*I at the deletion site and transformed into strains having these chromosomal mutations). The Ura$^+$ transformants with putative InsD inserts in the A and B orientations (plasmids p92 and p94, respectively) and with InsE inserts in the A and B orientations (plasmids p93 and p95, respectively) were confirmed by PCR and sequencing of the *Bam*HI-*Xho*I region. The only functional ARS on the plasmids was that contained in the *Cla*I-*Cla*I ARS-CEN cassette; plasmids lacking this cassette did not yield yeast transformants. Thus, we constructed two pairs of CEN plasmids containing *LYS2*::*InsD* or *LYS2*::*InsE* in opposite orientations (Fig. 2) asymmetric with regard to the location of InsD or InsE relative to the position of replication origin. As replication forks in yeast plasmids move with the same speed in both directions from the origin (5), we expect the replication fork to move through the corresponding insert from the side of the *Xho*I site in orientation A and from the side of the *Bam*HI site in orientation B.

Genetic and molecular procedures. Genetic and molecular procedures were previously described (17–19). Precise or in-frame imprecise deletions of the insertion mutations were selected as Lys$^+$ revertants. Reversion rates were determined by fluctuation analysis using 12 to 24 independent cultures as previously described (17). The 95% confidence intervals for a median rate were determined as described previously (10). Strains were grown at 20, 25, or 30°C, except for the *rad50 pol3-t* and *rad52 pol3-t* strains, which were unable to grow at 30°C. High frequencies of deletions were previously observed in *pol3-t* strains grown at 25°C as well as at 30°C (18). To examine reversion of the InsD and InsE *lys2* alleles residing on the plasmid, plasmids p92, p93, p94, and p95 were transformed into strain k31-*pol3-t*-DM with the nonreverting deletion mutation DelC. The DelC deletion encompasses the insertion site of Tn*5-13* and the InsD and InsE mutations (17) in the chromosomal *LYS2* gene. Thus, Lys$^+$ revertants can arise only by changes in the plasmid alleles. Revertants of the plasmid InsD and InsE were selected at 30°C. The DNA fragments containing revertant sites were amplified by using standard PCR procedures and oligonucleotide primers (BioSynthesis Inc.) 25M (5′-GAGACGCTACCAAGTTCG-3′) and N5 (5′-CG GCTAAGCTCATAACAT-3′), which are complementary to the sequences 185 bp 5′ and 265 bp 3′, respectively, from the ends of the inserts used in this study (17). Amplification was for 35 cycles (1 min at 94°C, 1 min at 50°C, and 2 min at 72°C), using *Taq* polymerase as instructed by the manufacturer (Perkin-Elmer Cetus). The PCR products were sequenced by using the primer (ByoSynthesis Inc.) N1 (5′-AGAATATTTTGTTGAACC-3′) for genomic DNA or G1 (5′-CTCCTACATCCTTGCAGA-3′) for plasmid DNA with an ABI automated sequencer (Applied Biosystems Inc.).

Replication direction. Yeast strain Tn*5*(*URA3*)-*pol3-t*-DM, containing Tn*5* (*URA3*) in the chromosomal *LYS2* gene at the same position as the inserts Tn*5-13*, InsD, InsE, InsH, and InsG in this study (17), was grown in YPD medium to 3×10^7 cells per ml at 30°C, and the chromosomal DNA was isolated as described previously (59). The direction of replication through the chromosomal *LYS2* gene was determined by using two-dimensional neutral/alkaline gel electrophoresis (24; also see the review in reference 6).

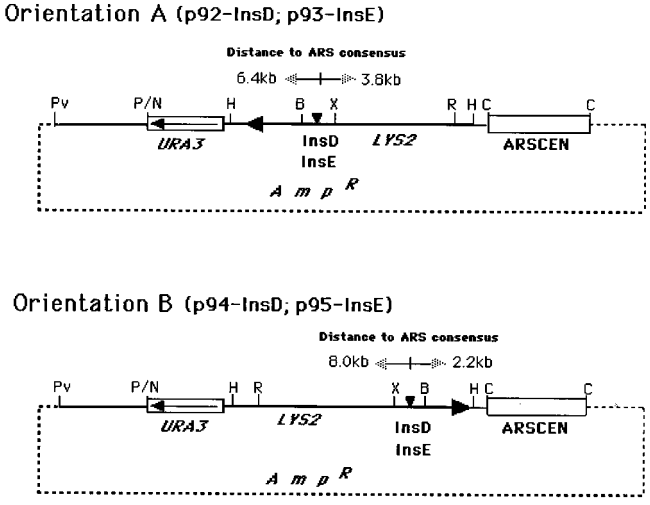


FIG. 2. Plasmids carrying the InsD and InsE inserts. Construction of plasmids with InsD (p92 and p94) and InsE (p93 and p95) in opposite orientations is described in the text. The site where InsD and InsE are located is marked by the filled triangle. Solid arrows show the directions of *LYS2* and *URA3* transcription. Restriction sites: Pv, *Pvu*II; P, *Pst*I; N, *Nsi*I; H, *Hin*dIII; B, *Bam*HI; X, *Xho*I; R, *Eco*RI; C, *Cla*I.

RESULTS

Reversion of small nonpalindromic insertion mutations in POL$^+$ and *pol3* strains. To investigate genetic controls and mechanisms of extended deletion and small (−1-bp) frameshift mutations, we used the previously described InsD (31-bp) and InsE (61-bp) insertion mutations in the chromosomal *LYS2* gene (Fig. 1) (17). These insertions at a common position in *LYS2* are bounded by 7- and 6-bp direct repeats, respectively; the repeats correspond to duplicated *LYS2* sequence. Both inserts shift the reading frame and generate a TGA stop codon adjacent to the 3′ end of the insert (Fig. 3). Reversions can arise by either extended (>1-bp) or small (−1- or +2-bp) deletion or insertion that restores the original *LYS2* reading frame.

Reversion rates were comparable ($\sim 10^{-8}$) for both inserts in POL$^+$ RAD$^+$ strains. (Table 1) over the 20 to 30°C range. As previously reported (17), the reversion rates were 30- to 100-fold higher in the temperature-sensitive polymerase δ *pol3-t* mutant. There was an approximate eightfold increase in mutation rate in the *pol3-t* strain at 30°C over that at 20°C, consistent with a proposed role of this replicative DNA polymerase in mutation production. However, the effect of temperature was not as great as the 50- to 100-fold increase in Tn*5* deletion rates observed in the isogenic background for the same temperature range (17, 18).

The revertants were analyzed by PCR to determine the extent to which reversions arose by deletion (see Materials and Methods). The revertants were classified as complete deletions if they appeared to have lost the entire 31-bp (InsD) or 61-bp (InsE) insert, incomplete if they appeared to have lost only a portion of InsD or InsE, and no detectable loss (NDL) if there was no apparent change in size of the PCR product compared with the original insert. Among 885 revertants of the chromosomal alleles, none yielded a PCR product shorter (in the limits of resolution with PCR) than that obtained with the wild-type *LYS2* gene. The PCR analysis enabled us to detect PCR fragments that were as little as 15 bp shorter than expected for *LYS2* (for example, the e1 class of plasmid revertants described in Fig. 3 gave a PCR product with a mobility clearly different from that for *LYS2*).

The reversion mutations of 245 revertants representing the various groups initially identified by PCR obtained in the strains of five different genotypes were sequenced (Table 2 and Fig. 3). The 18 InsE (class e2) and 86 InsD (classes d1 and d2) revertants identified by PCR as being complete deletions were found to be due to deletions removing the entire InsE sequence between the flanking small repeats. All of the 24 InsE incomplete deletions examined had breakpoints associated with another 5- to 7-bp direct repeat inside or adjacent to InsE. The deletions were either 31 bp (e3 and e4) or 16 bp (e5 and e6). Thus, of the revertants categorized as complete or incomplete deletions, all had breakpoints in small direct repeats.

Consistent with the PCR results, sequencing revealed that the NDL reversion category was due to small additions and deletions inside or in the vicinity of the original insert. Among 44 NDL revertants of the InsD and InsE mutations in the POL$^+$ (POL$^+$ RAD$^+$ and POL$^+$ *rad52*) strains, 41 were due to 1-bp deletions and only 3 appeared to be due to 2-bp additions. Of nine independent InsD NDL revertants isolated from the *pol3-t* strains (*pol3-t* RAD$^+$, *pol3-t rad50*, and *pol3-t rad52*), all arose by 1-bp deletions. However, for InsE, many (21 of 64) of the NDL revertants in the *pol3-t* strains arose by 7-bp deletions between small repeats, and the rest were due to 1-bp deletions. Most of the 1-bp deletions occurred in runs of two or more identical bases.

In summary, complete and incomplete deletions can be reliably identified by PCR. In the POL$^+$ strain, the NDL class contained almost exclusively 1-bp deletions, whereas in the *pol3* strains, it also contained 7-bp deletions. Results of PCR and sequence analyses indicated that reversions in the POL$^+$ strains were due primarily to complete deletions and 1-bp deletions for InsD and to 1-bp deletions for InsE. There was a large increase in reversions for both these inserts in the *pol3-t* RAD$^+$ strains, and these were primarily due to either complete or incomplete deletions involving small repeats separated by as much as 61 bp. All extended deletions appeared to be through mechanisms involving direct repeats, regardless of the genetic background. We note that there is no evidence for extended deletions with breakpoints in random DNA sequences.

Deletions of InsD and InsE in the *pol3-t* mutant can involve nonidentical repeats. Among the chromosomal InsD and InsE revertants, 10 types of deletions were identified (Table 2 and Fig. 3). The breakpoints were in identical repeats or repeats that differed by one base. For imperfect pairs of repeats, it was possible to identify the breakpoint relative to the position of the nonidentical bases (for example, the repeats involved in the d2 deletion; Fig. 3A). In this and similar cases, breakpoints were to the right of the nonidentical base (a or g) and therefore outside the stretch of seven identical bases (CTGCAGG) (BOR [breakpoint outside repeats]). For perfect repeats, the breakpoints were apparently in the stretch of seven precisely matched bases (BIR [breakpoints in repeats]; for example, d1 in Fig. 3A). While the positions of d1 and d2 breakpoints differ, the deletions are of the same size.

There were four sets of deletions in which one deletion was BIR and the other(s) was BOR (d1-d2, e3-e4, e5-e6, and e7-e8-e9) (Fig. 3). Among InsD revertants in the *pol3-t* RAD$^+$ strain, approximately half (18 of 39) of the deletions were of the BOR type, while there were no BOR deletions in the POL$^+$ RAD$^+$ strain (0 of 18). (All revertants grew well on lysineless medium, excluding the possibility of revertant selection due to growth differences.) For the InsE revertants, deletions were observed only in the *pol3-t* strain. Of 13 revertants that were due to 31-bp deletions, all were in the BOR (e4) category. (Since there were no apparent differences in the BIR/BOR ratio between the sets of revertants obtained at different temperatures, the revertants originating at 20, 25, and 30°C were combined for these analyses.)

There may be an effect of size of repeats or number of matched bases on the observed incidence of BOR or BIR deletions. Although the d1-d2 and e3-e4 pairs of repeats are separated by the same distance (31 bp) and share identical sequence in a common downstream repeat (Fig. 3), the BIR/BOR ratios in the *pol3-t* RAD$^+$ strain were quite different: 21/18 for the d1-d2 pairs and 0/13 for the e3-e4 pairs. There are seven identical bases (or 9 bp with one mismatch) in the d1-d2 pair, while the e3-e4 pair has only five identical bases (or 7 bp with one mismatch). Consistent with this finding, the BIR/BOR ratio was 1/7 for deletions associated with the e7-e8 repeats. This pair of repeats share three identical bases (or 5 bp with one mismatch) (Fig. 3 and Table 2).

Deletions of InsD and InsE depend on orientation with respect to replication origin. Deletions involving nonidentical repeats provided a unique opportunity to address both mechanisms of replication slippage and the role of replication direction. If slippage occurs during semiconservative replication, then the deletion spectrum might be expected to change when the replication direction is reversed (i.e., the leading and lagging strands are switched). The *LYS2* gene containing either InsE or InsD was therefore inserted into a plasmid in opposite

A

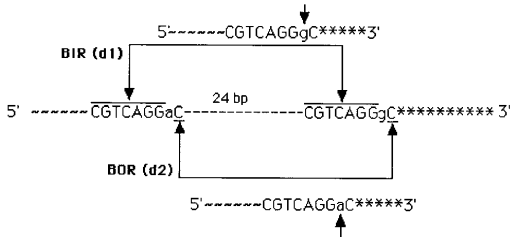
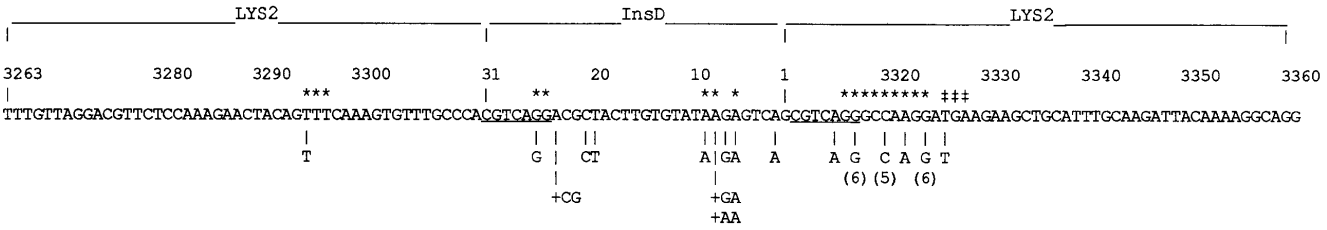


FIG. 3. Sequences of the InsD and InsE revertants. (A) Possible locations of the breakpoints for d1 (BIR) and d2 (BOR) deletions. Deletion borders are shown by vertical arrows. Horizontal lines show possible regions where the deletion breakpoints could be located. (B) InsD revertants. (C) InsE revertants. Nucleotide numbers in the nontranscribed strand of *LYS2* are according to reference 38. For InsD and InsE, representing the terminal part of Tn*5-13* (17), nucleotide numbers are according to reference 3. ‡‡‡, position of the TGA stop codon in the *LYS2* reading frame generated by inserts InsD and InsE. *, runs ≥2 of identical single bases or dinucleotide repeats in the sites where 1-bp deletions or 2-bp additions occurred. Deleted or added nucleotides of the 1-bp deletions and 2-bp additions are indicated beneath the sequences of InsD and InsE. If more than one mutation was obtained in a particular site, the number of mutations obtained independently is shown in parentheses. Short direct repeats flanking InsD and InsE and deleted regions of extended deletions are underlined. Deleted nucleotides of the extended deletions are shown by lowercase letters. For BOR deletions, the mismatches that could form during slippage between nonidentical repeats are shown.

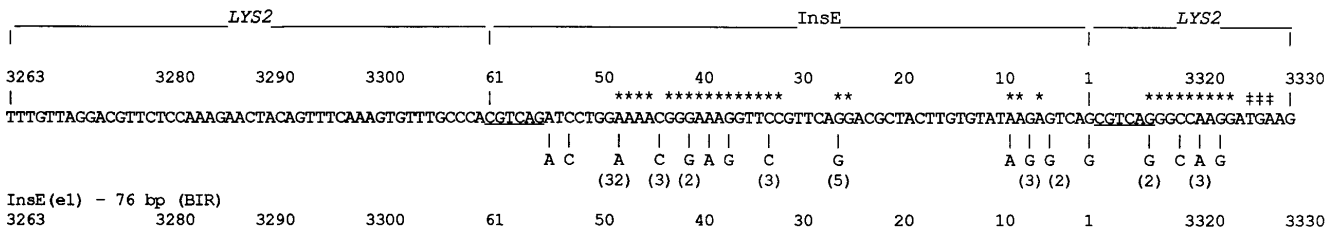
B



InsD (d-1) -31 bp (precise, BIR)
3263 3280 3290 3300 31 20 10 1 3320 3330 3340 3350 3360
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGGacgctacttgtgtataagagtcagcgtcaggGCCAAGGATGAAGAAGCTGCATTTGCAAGATTACAAAAGGCAGG

InsD (d-2) -31 bp (BOR; mismatch A:C)
3263 3280 3290 3300 31 20 10 1 3320 3330 3340 3350 3360
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGGACgctacttgtgtataagagtcagcgtcagggcCAAGGATGAAGAAGCTGCATTTGCAAGATTACAAAAGGCAGG

C



InsE(e1) - 76 bp (BIR)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGttctccaaagaactacagtttcaaagtgtttgcccacgtcagatcctggaaaacgggaaaggttccgttcaggacgCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

InsE(e2) -61 bp (precise; BIR)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGatcctggaaaacgggaaaggttccgttcaggacgctacttgtgtataagagtcagcgtcagGGCCAAGGATGAAG

InsE(e3) -31 bp (BIR)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGATCCTGGAAAACGGGAAAGGTTCCGTTCAGGacgctacttgtgtataagagtcagcgtcaggGCCAAGGATGAAG

InsE(e4) -31 bp (BOR; mismatch A:C)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGATCCTGGAAAACGGGAAAGGTTCCGTTCAGGACgctacttgtgtataagagtcagcgtcagggcCAAGGATGAAG

InsE(e5) -16 bp (BIR)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGaactacagtttcaaagTGTTTGCCCACGTCAGATCCTGGAAAACGGGAAAGGTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

InsE(e6) -16 bp (BOR; mismatch A:A)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGaactacagtttcaaagtGTTTGCCCACGTCAGATCCTGGAAAACGGGAAAGGTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

InsE(e7) -7 bp (BIR)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGATCCTGGAAAcgggaaaGGTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

InsE(e8) -7 bp (BOR; mismatch C:C)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGATCCTGGAAAcgggaaaggTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

InsE(e9) -7 bp (BOR; mismatch +G)
3263 3280 3290 3300 61 50 40 30 20 10 1 3320 3330
TTTGTTAGGACGTTCTCCAAAGAACTACAGTTTCAAAGTGTTTGCCCACGTCAGATCCTggaaaacGGGAAAGGTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAG

TABLE 1. Reversion of the nonpalindromic insertion mutations InsD (31 bp) and InsE (61 bp)

Genotype	Temp (°C)	Rate of reversion (10^{-8})[a]						
		InsD			InsE			
		Total rate	Complete deletions	NDL	Total rate	Complete deletions	Incomplete deletions	NDL
POL$^+$ RAD$^+$	20	0.3 (0.2–0.4[b])	0.10 (10[c])	0.20[b] (23[c])	0.4 (0.3–0.5)	0[b] (0)	0[b] (0[c])	0.40[b] (20)
	25[d]	**0.7 (0.5–0.8)**	**0.22 (8)**	**0.48 (17)**	**0.4 (0.3–0.5)**	**0.02 (1)**	**0 (0)**	**0.38 (18)**
	30	0.6 (0.5–0.7)	0.33 (12)	0.27 (10)	0.5 (0.4–0.6)	0 (0)	0 (0)	0.50 (15)
POL$^+$ *rad50*	**25**	**0.6 (0.4–0.8)**	**0.03 (1)**	**0.57 (18)**	**0.7 (0.5–0.9)**	**0 (0)**	**0 (0)**	**0.70 (20)**
	30	0.6 (0.4–0.8)	0 (0)	0.60 (9)	0.8 (0.6–1.0)	0 (0)	0 (0)	0.80 (7)
POL$^+$ *rad52*	**25**	**4.1 (2.7–6.1)**	**0 (0)**	**4.1 (10)**	**2.0 (1.1–3.3)**	**0 (0)**	**0 (0)**	**2.0 (16)**
	30	5.9 (5.0–6.8)	0 (0)	5.9 (20)	3.3 (2.9–3.7)	0 (0)	0 (0)	3.3 (20)
pol3 RAD$^+$	20	10 (7.2–13)	9.8 (48)	0.2 (1)	8.7 (5.8–11)	4.7 (28)	1.5 (9)	2.5 (15)
	25	**3.4 (27–71)**	**34.0 (60)**	**0 (0)**	**14 (4.7–28)**	**9.2 (88)**	**4.2 (40)**	**0.6 (6)**
	30	77 (18–210)	69.0 (26)	8.0 (3)	72 (40–85)	32 (17)	27 (14)	13 (7)
pol3 rad50	20	7.8 (1.4–13)	6.2 (24)	1.6 (6)	4.5 (2.7–7.9)	0.55 (4)	0.68 (5)	3.3 (24)
	25	**11 (5.1–19)**	**10.3 (29)**	**0.37 (1)**	**4.0 (2.3–16)**	**1.4 (14)**	**0.7 (7)**	**1.9 (19)**
pol3 rad52	20	6.1 (2.9–11)	3.3 (13)	2.8 (11)	11 (7.2–15)	0.5 (2)	0.8 (3)	9.7 (36)
	25	**15 (6.9–27)**	**11.0 (22)**	**4.0 (8)**	**14 (7.9–34)**	**1.1 (3)**	**2.2 (6)**	**10.7 (31)**

[a] The types of events leading to a reversion were classified on the basis of the structure of revertant *LYS2* alleles, as determined by PCR (see text), as complete, incomplete, or NDL. The rate for each type of event (R_e) was calculated as follows: $R_e = R_t \cdot (N_e/N_t)$, where R_t is the total rate of reversions, R_e is the rate of a particular event, N_t is the total number of revertants analyzed by PCR, and N_e is the number of revertants with a particular structure.
[b] 95% confidence intervals for the median value of the rates of InsD and InsE reversion, determined by fluctuation analysis (see Materials and Methods).
[c] Number of revertants.
[d] The results obtained at 25°C are in boldface, since this was the only temperature at which every strain was tested.

orientations, A and B (Fig. 2), near the origin of replication (see Materials and Methods and Table 3, footnote *b*). The *Eco*RI site was nearest the plasmid replication origin in the A orientation (Fig. 2). Using two-dimensional electrophoresis analysis (see Materials and Methods), we determined the direction of replication fork movement through the *LYS2* gene in the chromosome. The replication fork moves in a direction going from the *Eco*RI site toward the *Hin*dIII site (i.e., in the same direction as *LYS2* transcription; Fig. 1) (data not shown), resulting in the same direction of replication across the chromosomal *LYS2* gene as for orientation A in the plasmids.

The Lys$^+$ plasmid revertants were isolated at 30°C in a *pol3-t* mutant. The reversion rates ($\sim 80 \times 10^{-8}$) for both orientations of InsE in the plasmid were comparable to those for InsE when it was in the chromosomal *LYS2* gene. The Lys$^+$ reversion rates for the two orientations of InsD in the plasmid were similar; however, they were four- to sevenfold greater than for InsD in the chromosome (data not shown).

All of the InsD revertants (54 for orientation A and 48 for orientation B) were complete deletions, as judged from PCR analysis. Many of the deletion revertants were sequenced to determine the breakpoints (Table 3). Among 15 of the InsD deletions that arose for the A orientation, 10 were in the d1 class (BIR) and 5 were d2 (BOR). When the orientation was reversed, only the BIR category (d1) was retained among 23 deletions sequenced, whereas the BOR (d2) category disappeared. d1/d2 ratios were significantly different ($P < 0.01$) for the two orientations of InsD. The ratio obtained with orienta-

TABLE 2. Revertants of the InsD (31-bp) and InsE (61-bp) inserts in the chromosomal *LYS2* gene[a]

Genotype	InsD				InsE								
	Complete deletions, −31 bp		NDL		Deletions					NDL			
					Complete, −61 bp, BIR e2	Incomplete							
						−31 bp		−16 bp		−7 bp			−1 bp
	BIR d1	BOR d2	−1 bp	+2 bp		BIR e3	BOR e4	BIR e5	BOR e6	BIR e7	BOR e8	BIR e9	
POL$^+$ RAD$^+$	18	0	16	3	NA[b]	NA	NA	NA	NA	0	0	0	20
POL$^+$ *rad52*	NA	NA	3	0	NA	NA	NA	NA	NA	0	0	0	2
pol3 RAD$^+$	21	18	1	0	14	0	13	0	0	1	8	3	13
pol3 rad50	9	5	4	0	3	0	6	1	1	0	2	1	14
pol3 rad52	10	5	4	0	1	1	2	0	0	0	6	0	16

[a] Revertants were isolated in the POL$^+$ RAD$^+$ and *pol3* RAD$^+$ strains at 20, 25, and 30°C, in the *pol3 rad50* and *pol3 rad52* strains at 20 and 25°C, and in the POL$^+$ *rad52* strain at 30°C. Since there were no significant differences between distributions of revertants obtained at different temperatures, only the total numbers of revertants are shown. d1, d2, and e2 to e9 are various classes of deletions identified by sequencing (see Fig. 3). See the footnotes to Table 1 and text for a description of the terms (i.e., complete, incomplete, and NDL) corresponding to the structures identified by PCR.
[b] NA, not analyzed (appropriate PCR class did not occur or was a minor category).

TABLE 3. Revertants of InsD and InsE insertions in the *LYS2* gene in two different orientations relative to the replication origin of the CEN plasmid[a]

LYS2 orientation relative to replication origin[b]	InsD		InsE					
	−31 bp		−76 bp, BIR e1	−61 bp, BIR e2	−31 bp		NDL	
	BIR d1	BOR d2			BIR e3	BOR e4	BOR e9	−1 bp
A	10	5	0	96	5	17	0	3
B	23	0	3	75	1	0	2	4

[a] See also Fig. 2 and footnotes to Table 2.
[b] *LYS2* InsE and LYS2 InsD in the plasmids: p92 and p93, orientation A; p94 and p95, orientation B.

tion A was comparable to that obtained with the chromosomal allele (Table 2).

Nearly all the InsE revertants (Table 3) could be grouped as e2 (61-bp deletion), e3 or e4 (31-bp deletion), and NDL (1- or 7-bp deletion). (The e2 category was identified on the basis of PCR analysis and then confirmed by sequencing five isolates for each orientation.) Among 121 revertants of InsE in the A orientation, 96 were in the e2 category and 22 were in the e3 and e4 categories. In the e3 and e4 categories, 17 of 22 were e4 BOR-type deletions. The distribution of 85 revertants examined was quite different ($P < 0.001$) for the B orientation mainly because of the complete absence of e4 (BOR) deletions. As for InsD, the spectrum of revertants obtained with orientation A was most similar to that observed when InsE was present in the *LYS2* chromosomal gene.

Thus, the BOR types of InsD (d2) and InsE (e4) deletions that were frequent among chromosomal revertants were frequent only for the similar orientation A in the plasmid. Reversal of the orientation of the *LYS2* gene relative to the plasmid origin of replication resulted in loss of the BOR deletions. On the basis of these results, we suggest (i) that deletions between short direct repeats occur during semiconservative replication and (ii) that they occur preferentially in either the leading or lagging strand (see Discussion).

Excision of quasipalindrome inserts. Although deletions between small, separated direct repeats are infrequent, the frequency is greatly enhanced if the intervening region contains an IR. This was shown with the 6-kb Tn*5*, which contains long (1.5-kb) IRs (18), and with InsG and InsH quasipalindromes (i.e., 33- and 69-bp IRs separated by an 8- or 9-bp spacer): the rates were increased 10- to 100-fold in a *pol3-ts* mutant (Fig. 1, Table 4, and reference 17). Reversion of the quasipalindrome insertions occurred by deletion, as judged from on PCR analysis of 120 independent revertants (5 revertants arising at 20 or 25°C were examined for each strain in Table 4). To further establish the nature of the revertants, the region flanking the original insert was sequenced for nine InsG and nine InsH revertants isolated from among the various strains and conditions described in Table 4. Only precise deletions between the 9-bp flanking repeats were observed. These results are consistent with our previous proposal based on genetic evidence in which we suggested that most revertants of InsG and InsH arose by complete deletions and that these deletions are stimulated by the *pol3-t* mutation (17).

Role of *RAD50* and *RAD52* genes in reversion of the InsD, InsE, and quasipalindrome insertion mutations. Previously we demonstrated that deletions of long IRs (Tn*5*) involving small direct repeats in a *pol3-ts* mutant required the *RAD50* and *RAD52* genes (18). We therefore examined the possible role of these genes in reversion of InsD, InsE (Table 1), and quasipalindrome insertions (Table 4). The only effect that was observed in a POL^+ background was an increase in reversion rates for InsD and InsE due to frameshift mutator activity of *rad52*. This frameshift mutator activity of *rad52* was also observed in the *pol3-t* mutant. Previously only base substitution mutator activity had been ascribed to a *rad52* mutation (32). A *rad50* mutation had no significant effect on the overall reversion rate in a POL^+ background.

The increased frequency of deletions observed in the *pol3-t* mutant was generally reduced in the *rad52* or *rad50* mutants although not to the same extent as previously reported for deletions involving Tn*5* (18). For InsD, the complete deletions were decreased approximately two- to threefold in the *rad50* and *rad52* mutants. While similar reductions were found for the shorter, incomplete deletions of InsE, there was as much as a 10-fold reduction for the larger InsE complete deletions (61 bp) (Table 1). For the short quasipalindrome InsG, there was no significant effect of the *rad50* and *rad52* mutations (Table 4); however, there was as much as a 10-fold reduction of the InsH quasipalindrome deletions (156 bp) in the *rad50* or *rad52* strains. These results may indicate that the effect of *rad50* and *rad52* is greater as the distance between repeats is increased. While the *rad50* and *rad52* mutations were antimutators for

TABLE 4. Reversion rates of quasipalindrome insert mutations

Genotype	Reversion rate (10^{-6})			
	InsG		InsH	
	20°C	25°C	20°C	25°C
POL^+ RAD^+	0.5 (0.3–0.6[a])	0.4 (0.2–0.8)	0.2 (0.1–0.4)	0.3 (0.1–0.6)
POL^+ *rad50*	0.5 (0.2–0.9)	1.0 (0.2–1.5)	0.3 (0.02–0.5)	0.6 (0.2–0.8)
POL^+ *rad52*	0.3 (0.1–0.4)	0.9 (0.5–1.6)	0.6 (0.5–0.8)	0.2 (0.16–0.34)
pol3-t RAD^+	1.6 (1.2–2.4)	3.7 (1.6–5.1)	3.5 (2.1–9.4)	11 (9.4–14)
pol3-t rad50	0.4 (0.3–1.7)	2.5 (1.8–5.8)	0.8 (0.5–2.3)	2.6 (0.8–6.0)
pol3-t rad52	1.1 (0.3–3.0)	1.5 (0.8–5.4)	1.6 (1.3–3.5)	1.6 (1.3–3.5)

[a] 95% confidence interval.

extended deletions in the *pol3-t* background, the *rad52* mutator phenotype for 1-bp deletions was still retained.

DISCUSSION

Replication slippage as a mechanism for deletion between direct repeats. We have demonstrated with our model system that deletions involving many bases occur exclusively between small direct repeats. Two mechanisms are usually invoked to explain deletions in apparently random DNA sequences: breakage, followed by possible degradation and joining of nonhomologous double-stranded ends or replication slippage between short direct repeats that are common in all genomes (see reviews in references 13 and 36). End joining of DSBs, as an alternative to recombinational repair, has been demonstrated in various systems, including *S. cerevisiae* (see reference 28 and references therein). The replication slippage hypothesis (50) was developed to explain frameshift mutations clustering in homonucleotide runs. For both prokaryotes and eukaryotes, this hypothesis has been expanded to include slippage between separated small direct repeats stimulated by intervening long or short IRs (8, 9, 12, 16, 18) as well as deletions in apparently random DNA sequences (11, 14). On the basis of the results presented above, we suggest that replication slippage is the primary source of spontaneous extended deletions in *S. cerevisiae* that lead to loss of many bases (7 bp or more in these experiments).

Evidence that deletions arise by replication slippage includes short direct repeats at the deletion breakpoints and orientation dependence (or polarity) of deletions. All precise and imprecise deletions of the InsD and InsE inserts identified in this study involved 3- to 9-bp repeats at the breakpoints. This was not due to functional constraints on the mutations, since −1-bp and +2-bp frameshift mutations that restore reading frame were found at several sites in these inserts (Fig. 3). The stimulation of deletions in the polymerase δ mutant (discussed below) further implicates replication in the deletion process.

The incidence of various types of deletions depended on orientation relative to replication direction, suggesting not only that there is a role for replication in deletion formation but that there might be strand preference. Similar observations led to the proposal that point mutations induced by 2-acetylaminofluorene (55) and deletions of short palindromic sequences (53) arose during replication of the lagging strand in *E. coli*. Previously, we demonstrated that imprecise excision of the Tn*5* IR from the chromosomal *LYS2* gene in the *pol3-t* mutant involved small direct repeats and that there was polarity to the breakpoints (17).

In this study, we found orientation dependence for deletions in *pol3* strains, even in the absence of IRs. While all deletions had breakpoints in short direct repeats, the repeats need not be identical, as found for many of the revertants in the *pol3* mutant. Thus, it was possible to identify sets of deletions (d1-d2, e3-e4, e5-e6, and e7-e8-e9) in which repeat sequence was retained and the breakpoint was either within (BIR) or external to (BOR) the run of exactly matched bases (Fig. 3). The frequencies of the different BOR and BIR deletions depended on the orientation of the gene relative to the replication origin (Tables 2 and 3 and Results). The BOR types d2 (for InsD) and e4 (for InsE) were observed only for orientation A. The distribution of BIR- and BOR-type deletions among InsD and InsE revertants in the plasmid *LYS2* orientation A was similar to that for the chromosomal *LYS2* gene (the direction of replication through the *LYS2* gene is the same as for orientation A). The distribution of deletions was significantly different when the gene on the plasmid was in the opposite orientation relative to the origin of replication.

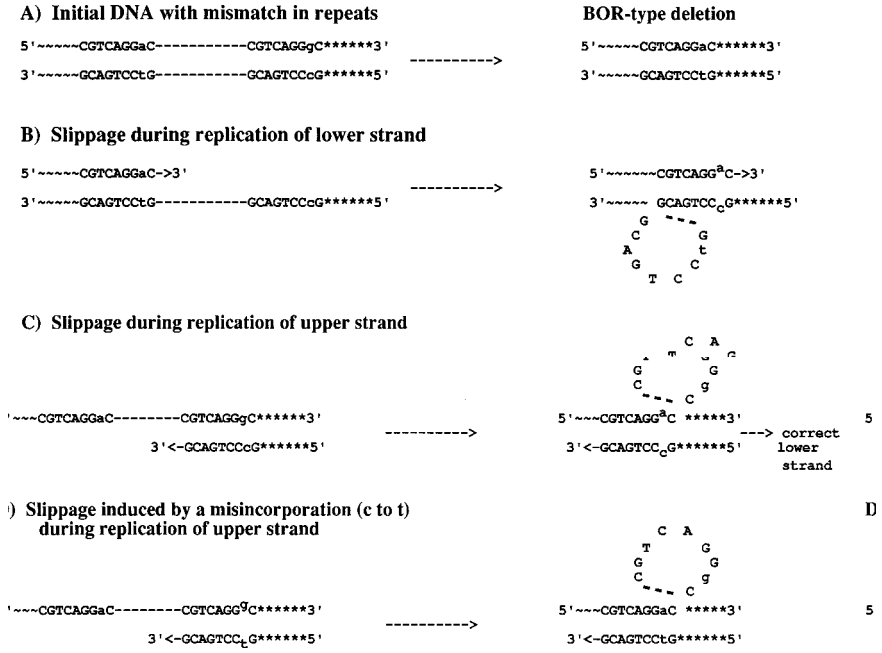
We propose that not only are the deletions due to replication slippage but that the orientation dependence for the spectrum of deletions is due to strand specificity (i.e., lagging or leading strand). Three mechanisms of replication slippage between repeats that can account for the change in BOR-type deletions when orientation relative to the origin of replication is changed are presented in Fig. 4. Figures 4B and C describe the consequences of slippage during the replication of either strand, with no assumptions about initiating events. A third mechanism (Fig. 4D) assumes that an initiating event—incorporation of an incorrect base—stimulates the slippage. (Presented in Fig. 4 are d2 deletions; the models also apply to the e4 deletions.) Figure 4C differs from Fig. 4B in that a mismatch correction is additionally required for the generation of a BOR-type deletion. (However, we note that the correction might destabilize the slippage intermediate, thereby preventing formation of a deletion.) In Fig. 4D, deletions are proposed to be initiated by misincorporation during replication, resulting in a mismatch in the nascent strand. After slippage, the repeats would be precisely matched, resulting in an increased length of paired region (i.e., there are no mismatches). In support of this latter mechanism, a mismatch at or near a 3′ end of a primer has been shown to block or slow nascent-strand extension in vitro (57; K. A. Johnson, unpublished observation cited in reference 26). This inhibition of strand extension might lead to subsequent slippage in a manner similar to that proposed for 1-bp deletions arising by misalignment following misincorporation (31).

In the present experiments, the d2 and e4 BOR categories of deletions were orientation dependent. They were observed only when the origin of replication was to the left of the repeats described in Fig. 4, corresponding to plasmid orientation A and to the chromosomal orientation of the *LYS2* gene. Thus, slippage during replication of the lower strand, as described in Fig. 4B, corresponds to slippage in the leading strand, while slippage during replication of the upper strand, as described in Fig. 4C and D, corresponds to events in the lagging strand.

While the mechanisms described in Fig. 4C and D require at least one more step in addition to replication slippage, there is insufficient information to conclude which of them is more likely. Assuming that replication slippage is responsible for the deletions, the orientation dependence leads us to conclude that deletions occur during semiconservative replication from an origin in a strand-specific manner.

The consequences of a defect in DNA polymerase δ on deletions. In agreement with our previous results for deletions of the Tn*5* long IR (17, 18) and with results of vonBorstel et al. for spontaneous *ura3* mutations (56), a temperature-sensitive mutation in the *POL3* gene increased the frequency and changed the spectrum of extended deletions. The effects of the *pol3* mutation on the rates of appearance of the various kinds of mutations are summarized in Table 5, and these rates are compared with rates in the POL^+ background. To simplify the table, only results obtained at 25°C were included. (As shown in Tables 1 and 4, observed effects are comparable to effects at the other two temperatures examined.)

The effect of the *pol3-t* mutation on deletions was smallest for those stimulated by the InsG and InsH quasipalindromes. The small increase over the wild-type level may be due to the already high deletion rates in the POL^+ strain. The high rates in the POL^+ strain, which were 100-fold higher than for deletions involving Tn*5* or the nonpalindromic InsE and InsD inserts, may be due to a greater likelihood for secondary structure in the short quasipalindrome IRs during replication. For



example, if Okazaki-size single-stranded DNA regions comparable in size to the short quasipalindromes arise during replication, stem-like structures could form even if replication is normal. While this would also explain why *pol3-t* and *pol1* (*cdc17-1*) had no effect on rates of deletion of a 160-bp perfect palindrome, two other *pol1* mutants exhibited 3- to 14-fold increases in deletion rates of this perfect palindrome (45).

The *pol3-t* mutation increased considerably the deletion rates of all types of extended deletions: 61, 31, 16, and 7 bp (observed only in the NDL class for the *pol3-t* strains). The rates of the 61-bp (InsE) and 31-bp (InsD) deletions were elevated more than 100-fold in *pol3* strains.

The increase in extended deletions contrasts with the lack of a statistically significant effect of *pol3-t* on the incidence of −1-bp frameshift mutations. The most likely mechanism for the 1-bp deletions is simple replication slippage between adjacent identical bases (50) or replication slippage combined with misincorporation (31). We found that most deletions occurred in or adjacent to 2- to 4-bp homopolymeric runs, with the strongest hot spot residing in a run of four adenines (Fig. 3). If both long deletions and 1-bp deletions occur via replication slippage, the *pol3-t* mutator effect observed only for extended deletions could reflect different capabilities of the mutant DNA polymerase δ for slippage over long and short distances. For example, T7 DNA polymerase in the absence of the processivity cofactor thioredoxin exhibits in vitro mutator activity for addition mutations in homopolimeric runs and antimutator activity for deletion mutations (30). Another possibility is that the 1-bp loops that lead to single-base deletions are recognized much more efficiently by the exonuclease proofreading activity of the yeast DNA polymerase δ and/or by the mismatch repair system than bigger loops formed as a result of slippage between more distant repeats. In support of this view, a *pol3-exo* mutation is reported to increase frameshifts along with base substitutions in the yeast *URA3* gene (39), and *pms1* is a mutator increasing reversions of a single-base *hom3* frameshift mutation (28). The smaller effect of *PMS1* on deletions of the unstable 94-bp palindrome in *S. cerevisiae* (23) compared with the dramatic increase of 2-bp additions and deletions in poly(GT) runs caused by a mismatch correction defect (49) supports the idea of efficient removal of only small loops by mismatch correction in *S. cerevisiae*.

Role of the *RAD50* and *RAD52* genes in the deletion process. Genes involved in recombination and repair have been shown to play a role in the generation of large deletions in bacteria and *S. cerevisiae*. For *E. coli*, various mutations increase deletion rates, and these rates may be reduced by a RecA mutation in the case of Tn*10* and Tn*5* excision (34, 35) and for some deletions not associated with IRs (58).

In *S. cerevisiae*, the genes *RAD50* and *RAD52*, which are involved in DSB repair and in many other aspects of chromosome metabolism, including recombination, were shown to be required for the high level of Tn*5* excision in a *pol3* mutant (18). The possibility that *rad50* and *rad52* mutations decrease Tn*5* excision in a POL^+ strain could not be examined because of the low level of excision in POL^+ cells. However, the effects of *rad52* and *rad50* mutations in a POL^+ strain could be examined in the present experiments because of the higher deletion rates of quasipalindromes and InsD. As summarized in Table 5, these repair genes appeared to play no role in quasi-

TABLE 5. Summary of the relative effects of the *pol3 rad50* and *rad52* mutations on the incidence of various types of deletion revertants

Genotype	Relative rate of mutation[a]							
	Simple inserts					IR inserts		
	InsD		InsE					
	Complete, −31 bp	NDL, −1 bp[b]	Complete, −61 bp	Incomplete, −31 and −16 bp	NDL, −1 bp (*POL*$^+$), −1 and −7 bp (*pol3*)	InsG complete, 83 bp	InsH, complete, 156 bp	Tn*5* complete, −5827 bp
POL$^+$ RAD$^+$	1.0	1.0	1.0	ND[c]	1.0	1.0	1.0	1.0
POL$^+$ *rad50*	0.14 <POL$^+$ RAD$^+$	1.2 NC[d]	ND NC	NC	1.9 NSD	2.5 NSD	2.0 NSD	ND NC
POL$^+$ *rad52*	0 NC	8.5 >POL$^+$ RAD$^+$	0 NC	NC	5.3 >POL$^+$ RAD$^+$	2.3 NSD	0.7 NSD	ND NC
pol3 RAD$^+$	155 >POL$^+$ RAD$^+$	ND NC	460 >POL$^+$ RAD$^+$	>POL$^+$ RAD$^+$	1.6 NC	9.3 >POL$^+$ RAD$^+$	37 >POL$^+$ RAD$^+$	800 >POL$^+$ RAD$^+$
pol3 rad50	47 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	0.8 NC NC	70 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	>POL$^+$ RAD$^+$ NC	5.0 NC NC	6.3 >POL$^+$ RAD$^+$ ≃*pol3* RAD$^+$	8.7 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	155 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$
pol3 rad52	50 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	8.3 >POL$^+$ RAD$^+$ NC	55 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	>POL$^+$ RAD$^+$ NC	28 >POL$^+$ RAD$^+$ >*pol3* RAD$^+$	3.8 >POL$^+$ RAD$^+$ ≃*pol3* RAD$^+$	5.3 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$	50 >POL$^+$ RAD$^+$ <*pol3* RAD$^+$

[a] Rates for reversion events of different insertion mutations (Fig. 1 and 3) relative to the corresponding rate in the POL$^+$ RAD$^+$ strain. Except for the rates of Tn*5* excision, values given are based on the rates presented in Table 1 and 4. The rates of Tn*5* excision were as follows: POL$^+$ RAD$^+$, 0.02×10^{-8}; *pol3* RAD$^+$, 16×10^{-8}; *pol3 rad50*, 3.1×10^{-8}; and *pol3 rad52*, 1.0×10^{-8}. These rates were determined by fluctuation tests and are comparable to those previously reported (18). All rates were determined at 25°C. The 1-bp deletions and extended deletions were classified by using PCR analyses and sequencing (see Tables 2 and 4, Fig. 3, and text). The InsE NDL revertants obtained in *pol3* background were a mix of 1- and 7-bp deletions. Too few NDL revertants were sequenced to allow us to draw conclusions about relative rates of 1- and 7-bp deletions. Therefore, all conclusions about −1-bp frameshift mutations in *pol3* background are based primarily on results obtained for InsD reversions. This calculation of a relative mutation rate was not possible for the InsE incomplete deletions, as no revertants of this category were found in the POL$^+$ RAD$^+$ strain. Statistical comparisons were made between pairs (A and B) of rates for various genotypes. Nearly all of the InsG, InsH, and Tn*5* Lys$^+$ reversions were due to precise excision (reference 17 and this paper), and all InsE reversions in a POL$^+$ background were due to NDL events (Tables 1 and 4). Therefore, the conclusion that A > B could be made if the 95% confidence intervals of the corresponding total rates did not overlap. The conclusion that A did not differ from B (i.e., no statistically significant differences [NSD or ≃]) was made if the 95% confidence limits were overlapping. For the InsD and InsE reversions (except for InsE in the POL$^+$ background), revertants were generated by more than one category of event. Therefore, comparisons could not be based on total rates (TR). To compare rates for a given category, the fraction F of the given category among the total revertants was determined. The conclusion that A > B was made if $TR_A \geq TR_B$ and $F_A > F_B$ or $TR_A > TR_B$ and $F_A \simeq F_B$. The TR values were compared on the basis of the confidence limits given in Table 1. Comparisons of F values were based on either χ^2 for contingency tables or Fisher's exact test.

[b] There was also a small fraction of +2-bp revertants (3 of 18) in the POL$^+$ background.

[c] ND, not determined, because no revertants were detected.

[d] NC, no conclusion could be made when the rate and/or F was too low to allow us to draw a statistically meaningful conclusion.

palindrome excision. The rate of InsD deletions was reduced in the *rad50* mutant. However, no conclusion could be drawn about *RAD52* involvement in InsD deletion because the high rate of reversion in the *rad52* strain, due predominantly to frameshift mutations, precluded identification of deletions.

The *rad50* and *rad52* mutations reduced the high levels of all deletions between separated repeats observed in *pol3* mutants except the InsG category (Table 5). For both the *rad50* and *rad52* mutations, the decrease generally varied 3- to 8-fold, although there was a 15-fold decrease in Tn*5* excision in the *rad52* mutant (Table 5 and reference 18). The suppressing effect of the *rad50* and *rad52* mutations appeared greater with increase in deletion length between small repeats for both IRs and non-IR deletions.

While loss of either *RAD50* or *RAD52* function results in antimutator effects on deletions between separated repeats, there are quite different effects on base pair substitutions and on −1-bp frameshift mutations. We have demonstrated that a *rad52* mutant, but not a *rad50* mutant, is a frameshift (−1-bp) mutator in both *pol3-t* and POL$^+$ backgrounds; previously *rad52* was shown to be a mutator for base substitutions (21, 22, 32). Although the −1-bp frameshifts occurred predominantly in runs of two to four identical bases (Fig. 3), the observation that there is a *rad52* mutator effect on both frameshifts and base substitutions suggests that the underlying mechanism differs from that responsible for the *rad52* and *rad50* antimutator effects on extended deletions that presumably arise by slippage between short repeats. Channeling of premutational lesions has been invoked to explain the mutator activity of *rad52* mutants (21); however, this hypothesis does not appear to apply directly to the deletion mutations described in this study.

To explain the possible functions of *RAD50* and *RAD52* in deletions of the long (1.5-kb) IRs of the Tn*5*, we had proposed (18) that *RAD50* and *RAD52* either directly affect slippage or stabilize interaction of single-stranded IRs, bringing small repeats close together. Mutants with defects in these genes were originally isolated on the basis of sensitivity to ionizing radiation, and it was proposed that sensitivity was due to an inability to repair DSBs via recombination (42). Since the reduction of deletion rates in *rad50* and *rad52* mutants does not relate directly to the effects that these mutations have on spontaneous recombination, which is strongly reduced in a *rad52* mutant but is only slightly reduced or even increased in a *rad50* mutant (22), it is unlikely that their effect on deletion is through re-

combination. Nonhomologous recombinational DSB repair observed in the *rad52* mutants seems unlikely, since it often involves two or fewer base pairs of matching sequences at the breakpoints (28), which is smaller than the shortest direct repeats associated with the presently described deletions.

While the *RAD52* and *RAD50* genes have not been shown to be involved directly in replication, this possibility could also explain their role in the production of extended deletions. Both *rad50* and *rad52* reduce the permissible growth temperature of a *pol3-t* mutant (18), and *rad52* has been shown genetically to interact with a gene involved in DNA replication, *CHL15* (27). Possibly the stimulated slippage in the *pol3-t* mutant is enhanced by the *RAD50* and *RAD52* gene products. They could also facilitate the subsequent DNA synthesis which would stabilize the slippage event. For example, in the case of recombinational repair between diverged DNAs, synthesis following strand invasion of multiply mismatched DNA was proposed to stabilize the recombinants (41). The novel feature of these explanations is that they propose interactions between *RAD50* or *RAD52* and the DNA replication machinery without involvement of DSBs.

ACKNOWLEDGMENTS

We are grateful to L. Golovanova for assistance in experiments and to J. Kirchner, T. Kunkel, T. Petes, and R. Schaaper for discussion and critical comments on the manuscript.

Support was provided for different parts of this work by the Russian national program Frontiers in Genetics, by the Russian Foundation for Basic Research (grant 93-04-6032), and by the International Science Foundation (grant R2X000) (to D.A.G.). Partial support was provided by a grant from the Department of Energy (Interagency Agreement DE-A105-94ER61940).

REFERENCES

1. **Aaltonen, L. A., P. Peltomaki, F. S. Leach, P. Sistonen, L. Pykkanen, J.-P. Mecklin, H. Jarvinen, S. M. Powell, J. Jen, S. R. Hamilton, G. M. Petersen, K. W. Kinzler, B. Vogelstein, and A. de la Chapelle.** 1993. Clues to the pathogenesis of familial colorectal cancer. Science **260:**812–816.
2. **Albertini, A. M., M. Hofer, M. P. Calos, and J. H. Miller.** 1982. On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. Cell **29:**319–328.
3. **Auerswald, E.-A., G. Ludwig, and H. Schaller.** 1981. Structural analysis of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45:**107–113.
4. **Bonneaud, N., O. Ozier-Kalogeropulos, G. Li, M. Labouesse, L. Minvielle-Sebastia, and F. Lacroute.** 1991. A family of low and high copy replicative, integrative and single stranded *S. cerevisiae*/*E. coli* shuttle vectors. Yeast **7:**609–615.
5. **Brewer, B. J., and W. L. Fangman.** 1987. The localization of replication origins on ARS plasmids in S. cerevisiae. Cell **51:**463–471.
6. **Campbell, J. L., and C. S. Newlon.** 1991. Chromosomal DNA replication, p. 41–146. *In* J. R. Broach et al. (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. **Caskey, T. C., A. Pizzuti, Y. H. Fu, R. G. Fenwick, Jr., and D. L. Nelson.** 1992. Triplet repeat mutations in human desease. Science **256:**784–789.
8. **Collins, J.** 1980. The instability of palindromic DNA. Cold Spring Harbor Symp. Quant. Biol. **45:**409–416.
9. **Collins, J., G. Volckaert, and P. Nevers.** 1982. Precise and nearly precise excision of the symmetrical inverted repeats of Tn*5*; common features of *rec*A-independent deletion events in *E. coli*. Gene **19:**139–146.
10. **Dixon, W. J., and F. J. Massey, Jr.** 1969. Introduction to statistical analysis, p. 349. McGraw-Hill, Inc., New York.
11. **Efstratiadis, A., J. W. Posakony, T. Maniatis, R. M. Lawn, C. O'Connel, R. A. Spritz, J. K. DeReil, B. G. Forget, S. M. Weissman, J. L. Slightom, A. E. Blechl, O. Smithies, F. E. Baralle, C. C. Shoulders, and N. J. Proudfoot.** 1980. The structure and evolution of the human β-globin gene family. Cell **21:**653–668.
12. **Egner, C., and D. E. Berg.** 1981. Excision of transposon Tn*5* is dependent on the inverted repeats but not the transposase function of Tn*5*. Proc. Natl. Acad. Sci. USA **78:**459–463.
13. **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.
14. **Farabaugh, P. J., and J. H. Miller.** 1978. Genetic studies of the lac repressor. VII. On the molecular nature of spontaneous hotspots in the lacI gene of *E. coli*. J. Mol. Biol. **126:**847–863.
15. **Fischel, R., M. K. Lescoe, M. R. S. Rao, N. G. Copeland, N. A. Jenkins, J. Garber, and R. Kolodner.** 1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis cancer. Cell **75:**1027–1038.
16. **Foster, T. J., V. Lundblad, S. Hanley-Way, S. M. Halling, and N. Kleckner.** 1981. Three Tn*10* associated excision events: relationship to transposition and role of direct and inverted repeats. Cell **23:**215–227.
17. **Gordenin, D. A., K. S. Lobachev, 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.
18. **Gordenin, D. A., A. L. Malkova, A. Peterzen, V. N. Kulikov, Y. I. Pavlov, E. Perkins, and M. A. Resnick.** 1992. Transposon Tn*5* excision in yeast: the influence of DNA polymerases alpha, delta, epsilon and repair genes. Proc. Natl. Acad. Sci. USA **89:**3785–3789.
19. **Gordenin, D. A., Y. Y. Proscyavichus, A. L. Malkova, M. V. Trofimova, and A. Peterzen.** 1991. Yeast mutants with increased transposon Tn*5* excision. Yeast **7:**37–50.
20. **Gordenin, D. A., M. V. Trofimova, O. N. Shaburova, Y. I. Pavlov, Y. O. Chernoff, Y. V. Chekuolene, Y. Y. Proscyavichus, K. V. Sasnauskas, and A. A. Janulaitis.** 1988. Precise excision of bacterial transposon Tn*5* in yeast. Mol. Gen. Genet. **213:**388–393.
21. **Hastings, P. J., S.-K. Quah, and R. C. von Borstel.** 1976. Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA. Nature (London) **264:**719–722.
22. **Haynes, R. H., and B. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), a Molecular biology of the Yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Henderson, S. T., and T. D. Petes.** 1993. Instability of a plasmid borne inverted repeats in *S. cerevisiae*. Genetics **133:**57–62.
24. **Huberman, J. A., L. D. Spotila, K. A. Nawotka, S.-M. El-Assouli, and L. R. Davis.** 1987. The *in vivo* replication origin of the yeast 2μm plasmid. Cell **51:**473–481.
25. **Ionov, Y. M., A. Peinado, S. Malhosyan, D. Shibata, and M. Perucho.** 1993. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (London) **363:**558–561.
26. **Johnson, K. A.** 1993. Conformational coupling in DNA polymerase fidelity. Annu. Rev. Biochem. **62:**685–713.
27. **Kouprina, N., E. Kroll, V. Bannikov, V. Bliskovsky, R. Gizatulin, A. Kirillov, V. Zakharyev, P. Hieter, F. Spencer, and V. Larionov.** 1992. *CTF4* (*CHL15*) mutants exhibit defective DNA metabolism in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**5736–5747.
28. **Kramer, K. M., J. A. Brock, K. Bloom, J. K. Moor, and J. Haber.** 1994. Two different types of double-strand breaks in *S. cerevisiae* are repaired by similar *RAD52*-independent, nonhomologous recombination events. Mol. Cell. Biol. **14:**1293–1301.
29. **Krawczak, M., and D. N. Cooper.** 1991. Gene deletions causing human genetic disease: mechanism of mutagenesis and the role of the local DNA sequence environment. Hum. Genet. **86:**425–441.
30. **Kunkel, T. A., S. S. Patel, and K. A. Johnson.** 1994. Error prone replication of repeated DNA sequences by T7 DNA polymerase in the absence of its processivity subunit. Proc. Natl. Acad. Sci. USA **91:**6830–6834.
31. **Kunkel, T. A., and A. Soni.** 1988. Mutagenesis by transient misalignment. J. Biol. Chem. **263:**14784–14789.
32. **Kunz, B. A., M. G. Peters, S. E. Kohalmi, J. D. Armstrong, M. Glattke, and K. Badiani.** 1989. Disruption of the *RAD52* gene alters the spectrum of spontaneous *SUP4-o* mutations in *Saccharomyces cerevisiae*. Genetics **122:**535–542.
33. **Leach, F. S., N. N. Nickolaiades, N. Papadopulos, B. Liu, J. Jen, R. Parsons, P. Peltomaki, P. Sistonen, L. A. Aaltonen, M. Nistrom-Lahti, X.-Y. Guan, J. Zhang, P. S. Meltzer, J.-W. Yu, F.-T. Kao, D. J. Chen, K. M. Cerosaletti, R. E. K. Fournier, S. Todd, T. Lewis, R. J. Leach, S. L. Naylor, J. Weissenbach, J.-P. Mecklin, H. Jarvinen, G. M. Petersen, S. P. Hamilton, J. Green, J. Jass, P. Watson, H. T. Lynch, J. M. Trent, A. de la Chapelle, K. W. Kinzler, and B. Vogelstein.** 1993. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. Cell **75:**1215–1225.
34. **Lundblad, V., and N. Kleckner.** 1985. Mismatch repair mutations in *E. coli* K12 enhance transposon excision. Genetics **109:**3–19.
35. **Lundblad, V., A. F. Taylor, G. R. Smith, and N. Kleckner.** 1984. Unusual alleles of *recB* and *recC* stimulate excision of inverted repeat transposons Tn*10* and Tn*5*. Proc. Natl. Acad. Sci. USA **81:**824–828.
36. **Meuth, M.** 1989. Illegitimate recombination in mammalian cells, p. 833–860. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
37. **Moore, G. P., A. R. Moore, and L. I. Grossman.** 1984. The frequency of matching sequences in DNA. J. Theor. Biol. **108:**111–122.
38. **Morris, M. E., and S. Jinks-Robertson.** 1991. Nucleotide sequence of the *LYS2* gene of *Saccharomyces cerevisiae*: homology to *Bacillus brevis* tyrocidine synthetase 1. Gene **98:**141–145.
39. **Morrison, A., and A. Sugino.** 1994. The 3′-5′ exonucleases of both DNA

polymerases δ and ε participate in correcting errors of DNA replication in *S. cerevisiae*. Mol. Gen. Genet. **242:**289–296.
40. **Parsons, R., G.-M. Li, M. J. Longley, W. Fang, N. Papadopulos, J. Jen, A. de la Chapelle, K. W. Kinzler, B. Vogelstein, and P. Modrich.** 1993. Hypermutability and mismatch repair deficiency in RER$^+$ tumor cells. Cell **75:**1227–1236.
41. **Priebe, S. C., 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.
42. **Resnick, M. A.** 1976. The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59:**97–106.
43. **Richards, R. I., and G. R. Sutherland.** 1994. Simple repeat is not replicated simply. Nat. Genet. **6:**114–116.
44. **Ripley, L. S.** 1990. Frameshift mutation: determinants of specificity. Annu. Rev. Genet. **24:**189–213.
45. **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.
46. **Shoffner, J. M., M. T. Lott, A. S. Voljavec, S. A. Soueidan, D. A. Costigan, and D. C. Wallace.** 1989. Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc. Natl. Acad. Sci. USA **86:**7592–7956.
47. **Sinden, R. R., and R. D. Wells.** 1992. DNA structure, mutations, and human genetic disease. Curr. Opin. Biotechnol. **3:**612–622.
48. **Stotz, A., and P. Linder.** 1990. The ADE2 gene from *Saccharomyces cerevisiae* sequence and new vectors. Gene **95:**91–98.
49. **Strand, M., T. A. Prolla, R. M. Liskay, and T. D. Petes.** 1993. Destabilisation of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature (London) **365:**274–276.
50. **Streisinger, G., Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzagi, and M. Inouye.** 1966. Frameshift mutations and genetic code. Cold Spring Harbor Symp. Quant. Biol. **31:**77–84.
51. **Thibodeau, S. N., G. Bren, and D. Schaid.** 1993. Microsatellite instability in cancer of the proximal colon. Science **260:**816–819.
52. **Tran, T. H., V. N. Kulikov, V. N. Noskov, G. I. Sizonenko, Y. O. Chernoff, and Y. I. Pavlov.** 1993. The 5′-aminoimidasole ribonucleotide-carboxylase structural gene of methylotrophic yeast *Pichia methanolica*: cloning, sequencing and homology analysis. Yeast **9:**1251–1258.
53. **Trinh, T. Q., and R. R. Sinden.** 1991. Preferential DNA secondary structure mutagenesis in the lagging strand of replication in *E. coli*. Nature (London) **352:**544–547.
54. **Umar, A., J. C. Boyer, D. C. Thomas, D. C. Nguyen, J. I. Risinger, J. Boyd, Y. Ionov, M. Perucho, and T. A. Kunkel.** 1994. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J. Biol. Chem. **269:**14367–14370.
55. **Veaute, X., and R. P. P. Fuchs.** 1993. Greater susceptibility to mutations in lagging strand of DNA replication in *E. coli* than in leading strand. Science **261:**598–600.
56. **von Borstel, R. C., R. W. Ord, S. P. Steward, R. G. Ritzel, G. S.-F. Lee, U. G. G. Hennig, and E. A. Savage.** 1993. The mutator *mut7-1* of *Saccharomyces cerevisiae*. Mutat. Res. **289:**97–106.
57. **Weiss, S. J., and P. A. Fisher.** Interaction of *Drosophila* DNA polymerase α holoenzyme with synthetic template-primers containing mismatched primer bases or propanodeoxyguanosine adducts at various positions in template and primer regions. J. Biol. Chem. **267:**18520–18526.
58. **Whoriskey, S. K., M. A. Schofield, and J. H. Miller.** 1991. Isolation and characterization of *E. coli* mutants with altered rates of deletion formation. Genetics **127:**21–30.
59. **Zhu, J., C. S. Newlon, and J. A. Huberman.** 1992. Localization of a DNA replication origin and termination zone on chromosome III of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**4733–4741.