Copyright © 1999 by the Genetics Society of America

# Genetic Factors Affecting the Impact of DNA Polymerase δ Proofreading Activity on Mutation Avoidance in Yeast

**Hiep T. Tran,*[,1] Natasha P. Degtyareva,†[,2] Dmitry A. Gordenin*[,†] and Michael A. Resnick***

**Chromosome Stability Group, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 and †Department of Genetics, St. Petersburg State University, St. Petersburg, 19034, Russia*

Manuscript received October 8, 1998
Accepted for publication December 21, 1998

ABSTRACT

Base selectivity, proofreading, and postreplication mismatch repair are important for replication fidelity. Because proofreading plays an important role in error correction, we have investigated factors that influence its impact in the yeast *Saccharomyces cerevisiae*. We have utilized a sensitive mutation detection system based on homonucleotide runs of 4 to 14 bases to examine the impact of DNA polymerase δ proofreading on mutation avoidance. The contribution of DNA polymerase δ proofreading on error avoidance was found to be similar to that of DNA polymerase ε proofreading in short homonucleotide runs ($A_4$ and $A_5$) but much greater than the contribution of DNA polymerase ε proofreading in longer runs. We have identified an *intraprotein interaction* affecting mutation prevention that results from mutations in the replication and the proofreading regions, resulting in an antimutator phenotype relative to a proofreading defect. Finally, a diploid strain with a defect in DNA polymerase δ proofreading exhibits a higher mutation rate than a haploid strain. We suggest that in the diploid population of proofreading defective cells there exists a transiently hypermutable fraction that would be inviable if cells were haploids.

IN the bacterium *Escherichia coli*, the accuracy of replication is controlled by at least three steps, acting serially to ensure high fidelity: base selection, exonucleolytic proofreading, and postreplication mismatch repair (MMR). The main replicative polymerase in *E. coli* is polymerase III holoenzyme. The Pol III α subunit, encoded by the *dnaE* gene, is the catalytic subunit that is responsible for base selectivity. The proofreading exonuclease (subunit ε) is encoded by the *dnaQ* gene. The α, ε, and θ subunits (the θ subunit has an unknown function) are tightly bound together to form the polymerase III core (McHenry and Crow 1979). Replication errors are recognized and corrected by the multiprotein mutHLS mismatch repair system. The base selectivity, proofreading, and mismatch repair systems reduce the errors by $10^5$-, $10^2$-, and $10^3$-fold, respectively. The combined efficiency of these three steps reduces replication errors to $10^{-10}$ errors per replicated nucleotide (Schaaper 1988, 1993).

There are three DNA polymerases required for chromosomal replication in eukaryotes, polymerases (Pol) α, δ, and ε, which are encoded by the *POL1*, *POL3*, and *POL2* genes, respectively. Polymerase α is responsible for synthesis of primers for Okazaki fragments in the lagging strand, and the Pol δ and Pol ε have been proposed for lagging and leading DNA strand replication, although their relative roles have not been established (Sugino 1995). Unlike Pol α, which has only a polymerase catalytic function, the Pol δ and Pol ε also have a 3′ → 5′ proofreading exonuclease activity in their N-terminal region (Kesti and Syvaoja 1991; Morrison *et al.* 1991; Simon *et al.* 1991). In the yeast *S. cerevisiae*, point mutations (*pol3-01* and *pol2-4*) in the exonuclease-conserved domains eliminate proofreading activity of Pol δ and Pol ε, respectively, and result in a frameshift and a base substitution mutator phenotype (Morrison *et al.* 1991, 1993; Shcherbakova and Pavlov 1996). As in *E. coli*, replication errors are checked by an MMR system, which is composed of several proteins homologous to *E. coli* MutS and MutL (Modrich and Lahue 1996). Combined defects in proofreading and MMR can lead to mutation synergism or cell death in haploid strains. The lethal effect is possibly due to excessive mutation rates (Morrison *et al.* 1993; Morrison and Sugino 1994; Tran *et al.* 1997b, 1999).

While the interaction between proofreading and MMR in mutation prevention is well established, little is known about the potential interaction between proofreading and other DNA polymerase activities. On the basis of the model proposed by Schaaper (1993), polymerase, proofreading, and MMR exert their functions independently. Data supporting this model are provided by the *E. coli dnaE* antimutator alleles, which are antimutators either alone or in combination with defective proofreading (Fijalkowska and Schaaper 1995)

*Corresponding author:* Michael A. Resnick, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, 111 TW Alexander Dr., P.O. Box 12233, Research Triangle Park, NC 27709. E-mail: resnick@niehs.nih.gov

[1] *Present address:* Lifesensors, Inc., 271 Great Valley Pkwy., Malvern, PA 19355.

[2] *Present address:* Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599.

or defective MMR (Fijalkowska *et al.* 1993). The *dnaQ926* proofreading deficiency appears to cause lethality as a result of the loss of proofreading and subsequent saturation of DNA MMR (error catastrophe). However, *dnaQ926* strains are viable if they carry a *dnaE* antimutator allele or a multicopy plasmid carrying the *E. coli mutL* gene (Fijalkowska and Schaaper 1996). As the polymerase (α) and proofreading (ε) subunits are bound tightly together (McHenry and Crow 1979), a mutation in the α subunit could affect the editing ability of the DNA polymerase holoenzyme complex. The *dnaE173* mutation in the polymerase subunit leads to a 1,000- to 10,000-fold increase in mutation rate (Maki *et al.* 1991), and it was proposed that this allele affects the proper interaction between α and ε subunits, resulting in a defect in the proofreading capacity of the Pol III holoenzyme complex.

The interactions between the proofreading and polymerase regions and their potential role in mutation prevention are not well understood in eukaryotes. The yeast *pol2-18* mutation, located in the polymerase region of Pol ε, leads to a weak mutator phenotype as well as temperature sensitivity (Araki *et al.* 1992; Shcherbakova *et al.* 1996). In combination with the Pol ε proofreading mutation (*pol2-4*; Morrison *et al.* 1991), *pol2-18* exhibits mutation frequency synergy (Shcherbakova *et al.* 1996) consistent with the model that the polymerase and proofreading activities act in series. Moreover, *pol2-18* is an antimutator with respect to mutagenesis induced by the base analog $N^6$-hydroxylaminopurine (HAP), which is proposed to occur via a replicative misincorporation mechanism (Shcherbakova and Pavlov 1996). The authors also found *pol2-18* to be a HAP-specific antimutator in combination with the proofreading defect *pol2-4.* There have been no reports concerning interaction between polymerase and proofreading regions within DNA Pol δ.

According to the replication slippage model of Streisinger *et al.* (1966), the incidence of frameshift mutations is expected to increase with the length of a homonucleotide run. Using a sensitive system for detecting mutations in long homonucleotide runs, we previously found that both MMR and Pol ε proofreading provide efficient mutation prevention in short runs, but that only MMR is capable of preventing frameshift mutations in runs ≥8 nucleotides (Tran *et al.* 1997b). However, the impact of the Pol δ proofreading activity in correcting errors in homonucleotide runs could not be investigated, because the combination of Pol δ proofreading (*pol3-01*) and MMR (*msh2* or *pms1*) defects is lethal in a haploid strain (Morrison *et al.* 1993; Tran *et al.* 1999). Because homozygous *pol3-01 pms1* or *pol3-01 msh2* diploids are viable (Morrison *et al.* 1993; Tran *et al.* 1999) the efficiency of Pol δ proofreading and comparison with Pol ε proofreading can be addressed in diploids (see below).

In this study we examine the impact of Pol δ proofreading on mutation avoidance. Specifically, we have investigated (i) the interaction between mutations in the polymerase and the proofreading regions of Pol δ, (ii) relative contributions of Pol δ proofreading and post-replication MMR to mutation avoidance, (iii) the relative effectiveness of Pol δ and Pol ε proofreading on homonucleotide run templates, and (iv) the mutational consequences of a Pol δ proofreading defect in haploid *vs.* diploid strains. Our results show that a Pol δ proofreading defect can be influenced by a change elsewhere in the protein. Specifically, the temperature-sensitive *pol3-t* mutation, which is located in the replicative region (Tran *et al.* 1997a) and exhibits a deletion mutation phenotype, acts as an antimutator when combined with a Pol δ proofreading defect. Overall it appears that Pol δ proofreading has a much greater impact on mutation avoidance than Pol ε proofreading and has a larger contribution to error avoidance in homonucleotide runs. The mutator effect of Pol δ proofreading deficiency is further increased in diploid strains compared to the corresponding haploid strains. We suggest that a transiently hypermutable fraction exists that is revealed when diploid cells are defective in Pol δ proofreading.

## MATERIALS AND METHODS

**General genetic and molecular methods:** Yeast standard media (Sherman *et al.* 1986) and YPD containing G418 (Wach *et al.* 1994) were used. Yeast cells were grown at 30°, although strains with the temperature-sensitive polymerase mutation *pol3-t* were grown at 25° for mutation studies. Yeast transformations were performed according to Gietz and Schiestl (1991). The preparation of bacterial media and general molecular methods has been described (Sambrook *et al.* 1989).

**Strains and plasmids:** A series of isogenic strains were constructed from the original CG379, *MAT*α *ade5-1 his7-2 leu2-3,112 trp1-289 ura3-52* (Morrison *et al.* 1991) and from its *pol3-01* or *pol3-t* or *pol3-t, 01* derivatives. These strains contain modified *InsD* (Figure 1A and see below) or *InsE* inserts in the chromosomal *LYS2* gene, where the $A_4$ run was changed to $A_5$, $A_7$, $A_8$, $A_{10}$, $A_{12}$, and $A_{14}$ (Figure 1B; Tran *et al.* 1997b). The mutations *pol3-01* and *pol3-t* are point mutations in the exonuclease and the polymerase domains of the *POL3* gene, respectively (Morrison *et al.* 1993; Tran *et al.* 1997a; and see Figure 2). The mutation in *pol3-01* alters the aspartate and glutamate residues in the essential exonuclease motif, FDIEC, at amino acids 320–324; the D321A and E323A double mutation in *pol3-01* eliminates the proofreading activity of the polymerase δ (Morrison *et al.* 1993). The *pol3-t* allele is due to a single base substitution in the context GAA<u>G</u>AC to GAA<u>A</u>AC leading to loss of the *Mbo*II site. The *pol3-t* allele encodes a D641N substitution (Tran *et al.* 1997a) in the vicinity of the conserved polymerase motif VI of DNA Pol δ (Boulet *et al.* 1989). [In the revised nucleotide sequence of *POL3* (Morrison and Sugino 1992) this amino acid is at position 643 of Pol δ (Figure 2) ]. The *pol3-t* allele produces a mutant polymerase that induces replication slippage between distant short repeats as well as frameshift mutations in homopolymeric runs (Tran *et al.* 1995, 1996). The *pol3-01* mutation causes a mutator phenotype due to increased base substitution and frameshift mutation rates (Morrison *et al.* 1993). Strains with the double mutation *pol3-t, 01* were constructed from *pol3-01* strains. The *pol3-t* allele was introduced to the chromosomal

*pol3-01* allele using plasmid p171 and a gene replacement technique described previously (Kokoska *et al.* 1998). The presence of both *pol3-01* and *pol3-t* mutations was confirmed by restriction digest of appropriate PCR products (Morrison *et al.* 1993; Kokoska *et al.* 1998). These strains also contain modified *InsE* inserts in the chromosomal *LYS2* gene, where the $A_4$ run was changed to $A_5$, $A_7$, $A_8$, $A_{10}$, $A_{12}$, and $A_{14}$ homonucleotide runs (Tran *et al.* 1997b). Strain 1036 *lys2-BX*: (*MAT***a** *ade2-1 arg4-8 leu2-3,112 lys2-BX thr1-4 trp1-1 ura3-52 cup1-1*) contains a deletion of the *Bam*HI-*Xho*I region covering the *InsE* inserts in the *LYS2* gene. The *pol3-01* mutation was introduced into this strain using YIpAM26 plasmid as described previously (Morrison *et al.* 1993). The *MSH2*, *RAD27*, and *EXO1* genes were disrupted in strains using the gene disruption technique as described below.

***lys2::InsLD* construction:** The *InsD* insert in the plasmid p92 (Tran *et al.* 1995) was modified to *InsLD* (locked *InsD*) containing three additional stop codons (Figure 1A). Changing *InsD* to *InsLD* on the plasmid was done using site-directed, double-stranded mutagenesis (Chameleon mutagenesis kit; Stratagene, La Jolla, CA). Double-stranded p92 plasmid was annealed with a mix of a mutation oligonucleotide 5′-CTGAC TCTTATACACTAGTAGCTACCTGACGTGGGCAAAC-3′ (underlined nucleotides indicate differences of *InsLD* from *InsD*) and the oligonucleotide 5′-GTAATTGCAAGTGGATATCTG AACCAGTCC-3′ as a helper primer (underlined nucleotides change the unique *Eco*RI site in p92 to an *Eco*RV site). Replacement of the chromosomal wild-type *LYS2* gene with the *lys2::InsLD* allele was done by two-step gene replacement using a *Hpa*I-digested integrative version of the plasmid (without the *Cla*I-*Cla*I *ARS-CEN* cassette).

**Gene replacement and disruption:** The following genes were disrupted: *MSH2*, *RAD27*, and *EXO1*. For *MSH2* disruption we used a *Sac*I-*Pst*I *msh2::LEU2* fragment from p203 (Tran *et al.* 1997a). The *RAD27/RTH1* gene was disrupted using an *Eco*RI-*Sal*I *rth1::URA3* fragment (Johnson *et al.* 1995). The entire open reading frame of *EXO1* was deleted using PCR disruption with the kanMX module (Wach *et al.* 1994) and primers described below. The lowercase type indicates nucleotide sequences that belong to the kanMX cassette; DNA sequences belonging to the genes are written in uppercase. For the *EXO1* gene we amplified the kanMX cassette using EXO1-kanMX-3′: 5′-TTGGCTTGACTTAGTAGTTTCGATGTCCCT TTTCTTACTTatcgatgaattcgagctcg-3′ and EXO1-kanMX-5′: 5′-AGGTATGAAGGAGAAGTGTTAGCCATTGATGGCTAT GCATcgtacgctgcaggtcgac-3′ and verified by PCR using the following primers: EXO1-test-3, 5′-ATTGGGAAAGCAAGGAGA TAG-3′ and EXO1-test-5, 5′-TCTTCTTCCTCAGTTAAAGC-3′. The *RAD27/RTH1* gene disruption transformants were identified by MMS sensitivity (340 μl MMS/liter of YPD), induction of illegitimate mating, and by PCR using RTH1-1F, 5′-GGACA CCGGAAGAAAAAAT-3′ and primer RTH1-2R, 5′-AACTTCAG GGTCAAGAAACAGCA-3′.

**Construction of *msh2 pol3-01* diploid strains:** The combination of *pol3-01* mutation with null mutations in MMR genes *PMS1* or *MSH2* is lethal in haploids, but diploid strains are viable (Morrison *et al.* 1993; Tran *et al.* 1999). To study the impact of the DNA Pol δ proofreading in mutation prevention we constructed a series of isogenic diploid strains *msh2 pol3-01* containing homonucleotide runs of various sizes in the *lys2::InsE* insert. The derivative strain 1036 *lys2-BX pol3-01* was obtained from 1036 *lys2-BX* using YIpAM26 (Morrison *et al.* 1993). The 1036 *MAT***a** *lys2-BX pol3-01* strain was transformed with the replicative plasmid pBL304 (Morrison and Sugino 1994) carrying the *POL3* and the *URA3* genes. Then the *MSH2* gene in the transformant was deleted using a *Pst*I-*Sac*I *msh2::LEU2* fragment from plasmid p203 (Tran *et al.* 1997a). The resulting strain 1036 *lys2-BX pol3-01 msh2* pBL304 was mated with a series of *MAT*α *pol3-01* strains isogenic to CG379, containing homonucleotide runs ($A_4$, $A_5$, $A_7$, $A_8$, $A_{10}$, $A_{12}$, and $A_{14}$) in the *lys2::InsE* insert. After loss of plasmid pBL304 on 5-fluoroorotic acid (5-FOA) medium, the second copy of the chromosomal *MSH2* gene in the diploid was deleted using the *Spe*I-*Spe*I fragment *msh2::URA3* from plasmid pII2-Tn10-LUK7-7 (Reenan and Kolodner 1992). Deletion of *MSH2* was verified by PCR as described previously (Tran *et al.* 1996). The resulting diploid strain is *pol3-01/pol3-01 msh2::LEU2/msh2::URA3 lys2::InsE*-$A_n$/*lys2*Δ.

**Construction of isogenic homozygous diploid strains:** To investigate the impact of a ploidy on mutation rates we constructed a series of isogenic homozygous diploid strains. Haploid *leu2* strains derived from CG379 were transformed with plasmid YEpHO (gift from Dr. Chernoff) carrying the *LEU2* and the *HO* endonuclease genes. The HO endonuclease induces mating type switching in haploid strains (*MAT***a** to *MAT*α or vice versa). Haploid strains with the opposite mating types then form *MAT***a**/*MAT*α homozygous diploid strains. Transformants with YEpHO were grown on YPD media to allow loss of the plasmid. Single $Leu^-$ clones were isolated. Diploid clones were identified as being nonmaters with *MAT***a** *his3* or *MAT*α *his3* testers as well as giving a low forward mutation rate to $Can^r$ because of the presence of two *CAN1* gene copies. For strains where the *LEU2* marker could not be used, we utilized plasmid pGHO-TRP1 (Bennett *et al.* 1993) containing the *TRP1* and the *HO* gene under control of the *GAL1-10* promoter. Mating type switching was induced by incubation in galactose media for 8 hr and diploid clones were identified as described above.

**Mutation analysis:** Mutation rates were determined in at least 12 independent cultures by a fluctuation test using the median method described by Lea and Coulson (1949). The nature of the $Lys^+$ revertants was identified by sequencing the *reversion window* of the *lys2::InsE* insert, as described previously (Tran *et al.* 1995). Sequencing was performed using ABI 373 automated sequencer.

## RESULTS

**Experimental systems:** To investigate genetic controls of extended deletions and small (−1 nt, +1 nt) frameshift mutations, we used the previously described *InsD* (31-bp) and *InsE* (61-bp) insertion mutations in the chromosomal *LYS2* gene (Tran *et al.* 1995). These insertions at a common position in the *LYS2* gene are flanked by 7- and 6-bp direct repeats, respectively. (One direct repeat belongs to the *LYS2* gene, and another belongs to the insert.) Both inserts shift the reading frame of the *LYS2* gene and generate a TGA stop codon (Figure 1). Reversions can arise by either extended or small (−1- or +2-bp) deletions/insertions that restore the original *LYS2* gene reading frame (Tran *et al.* 1995, 1996). Extended deletions appear to occur via replication slippage (Tran *et al.* 1995). We generated another sensitive mutation detection insert, *InsLD*, which is derived from *InsD* by site-directed mutagenesis by adding three stop codons in all three possible reading frames (Figure 1A). This *InsLD* mutation detection system allows the identification of only extended deletions, because reversion is observed only if all three stop codons are removed.

To investigate the mutational consequences of a DNA

**A**

```
____________LYS2_________ _________ InsLD ________________ ________LYS2________________
                                   †††        †††       †††                         †††
TACAGTTTCAAAGTGTTTGCCCACGTCAGGTAGCTACTAGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAGCTGCATTTG
```

**B**

```
_________LYS2______ _____________________________InsE________________________________ _________LYS2________________
                          ****                                                                      †††
TTTCAAAGTGTTTGCCCACGTCAGATCCTGGAAAACGGGAAAGGTTCCGTTCAGGACGCTACTTGTGTATAAGAGTCAGCGTCAGGGCCAAGGATGAAGCTGCATTTG
                                                                                                    **

      original insE: A4   ctggAAAAcggg               Lys- (+1 run)

   modified A  runs: A5   ctggAAAAAcggg              Lys- (-1 run)
                     A7   ctggAAAAAAAcggg            Lys- (+1 run)
                     A8   ctggAAAAAAAAcggg           Lys- (-1 run)
                     A10  ctggAAAAAAAAAAcggg         Lys- (+1 run)
                     A12  ctTAAAAAAAAAAAAcggg        Lys- (-1 run)
                     A14  ctTAAAAAAAAAAAAAAcggg      Lys- (+1 run)
```

Figure 1.—(A) The *InsD* insert described previously (Tran *et al.* 1995) was modified to *InsLD* by site-directed mutagenesis to generate three stop codons (†††). The insert is flanked by two direct repeats (underlined); one belongs to the insert and the other belongs to the *LYS2* gene. The insert causes a +1-nt shift of the *LYS2* reading frame, generating a TGA stop codon (†††). Three additional stop codons in *insLD* block all three possible open reading frames so reversion can occur only via extended deletions that restore the original *LYS2* reading frame. (B) Series of homonucleotide runs in the *lys2::InsE* insert in the *LYS2* gene described previously (Tran *et al.* 1997b). The *InsE* insert shifts the *LYS2* reading frame, generating a TGA stop codon (†††). The $A_4$ homonucleotide run hot spot for −1 deletions (****) was increased up to $A_{14}$. The hot spot for −1-nt deletions in the *pol3-01* haploids and diploids is denoted (**).

Pol δ proofreading defect (*pol3-01*), a DNA Pol δ polymerase mutation (*pol3-t*), and a defect in MMR, we used a series of homonucleotide runs in the *InsE* insert described previously (Tran *et al.* 1997b). The longest homonucleotide run, $A_4$ in *InsE*, was changed to $A_7$, $A_{10}$, and $A_{14}$ resulting in +1-bp frameshifts and to $A_5$, $A_8$, and $A_{12}$ leading to −1-bp frameshifts (Figure 1B). To evaluate the nature of the mutations in the homonucleotide runs, a sample of $Lys^+$ revertants from the +1 and −1 frameshift allele detection systems was sequenced in the reversion window region. The mutator phenotype was also examined using a *his7-2* reversion assay. While the nature of the *his7-2* mutation is not known, it has been used in several studies investigating proofreading and MMR defects. It exhibits high reversion rates with either MMR or proofreading mutators (Morrison *et al.* 1991, 1993; Morrison and Sugino 1994).

The instability of the homonucleotide runs and the deletion of the insertion *InsLD* were examined in strains with the proofreading (*pol3-01*) defect or with the putative polymerase defect (*pol3-t*) or with defects in both functions *pol3-t, 01.* (Construction of the double mutation *pol3-t, 01* is described in materials and methods.) The locations of the *pol3-01* and *pol3-t* mutations in DNA Pol δ are shown in Figure 2. Since only in the absence of MMR can errors generated during replication be accurately measured, the role of DNA Pol δ proofreading in mutation prevention in homonucleotide runs was examined in $MMR^-$ (*msh2*) strains. Because the double mutation *pol3-01 msh2* is lethal for haploid strains (Tran *et al.* 1999), the impact of the proofreading defect *pol3-01* on mutation rates was investigated in diploid strains lacking postreplication MMR.

**Effect of DNA Pol δ proofreading and polymerase mutations on extended deletions:** Previously, we described the replication defective mutant of DNA Pol δ, *pol3-t*, whose defect is due to a mutation in the vicinity of the conserved polymerase VI motif (Figure 2). At the permissive temperature, this mutant polymerase exhibits increased replication slippage, resulting in both extended deletions and −1- and +2-bp frameshifts (Tran *et al.* 1995, 1996). As shown in Table 1, the *pol3-t* strain has a >50-fold higher rate of reversion of the *InsLD* mutation than the isogenic wild type and has only a modest effect (up to 11-fold increase) on frameshift mutations in homonucleotide runs (Tables 1 and 2).

Figure 2.—Location of the proofreading (*pol3-01*) and polymerase (*pol3-t*) mutations in polymerase δ of *S. cerevisiae.* The EXO, POL, and ZnF boxes identify exonuclease, polymerase, and Zn-finger conserved motifs, respectively, in the polymerase δ alignment (Hindges and Hubscher 1997). The proofreading mutation *pol3-01* is due to a change from FDIEC to FAIAC in the EXO1 domain at positions 320–324 (Morrison *et al.* 1993). The *pol3-t* mutation is due to a nucleotide change G to A that eliminates the *Mbo*II recognition site, producing amino acid substitution D643N in the vicinity of the polymerase motif VI of *S. cerevisiae* polymerase δ. [Note that previously the *pol3-t* mutation was reported as a D641N substitution (Tran *et al.* 1997a), where the amino acid position was determined on the basis of the initially published sequence (Boulet *et al.* 1989)]. The new D643N amino acid substitution position is based on a revised *POL3* gene sequence [EMBL accession no. X61920 (Morrison and Sugino 1992) as well as the Stanford University *S. cerevisiae* database at http://genome-www2.stanford.edu/cgi-bin/SGD/getSeq?map=pmap&seq=YDL102W&flankl=0&flankr=0&rev=]. S.c., *Saccharomyces cerevisiae*; S.p., *Schizosaccharomyces pombe*; C.a., *Candida albicans.*

The *InsLD* revertants were due entirely to extended deletions. In contrast, there was no increase in extended deletions in the *pol3-01* proofreading mutant, although it is a strong mutator for *CAN1* forward mutations (Table 1). All but three reversions of the *lys2::InsLD* allele obtained in the proofreading mutant *pol3-01* were due to precise deletions between the distant short repeats identified in Figure 1A. (Those three reversions were due to point mutations that eliminated only one stop codon. Because the original reading frame of the *LYS2* gene was not restored in these revertants, the revertants might be due to secondary suppressor mutations that suppress a second stop codon in *InsLD.*)

The double mutant *pol3-t, 01* provided the opportunity to investigate possible interactions between the replication and proofreading defects of Pol δ in the same polypeptide. The double mutant *pol3-t, 01* demonstrates growth properties similar to the *pol3-t* mutant and it has the same deletion rate as a *pol3-t* mutant (Table 1). However, the high canavanine resistance mutation rate of the *pol3-01* mutant is reduced (Table 1). On the basis of this and results described below, we suggest that *pol3-t* is an antimutator when combined with the *pol3-01* mutator allele.

**Mutator and antimutator phenotype of the polymerase mutation *pol3-t*:** In the *E. coli* Pol III holoenzyme, proofreading and polymerase activities are encoded by the *dnaQ* and *dnaE* genes, respectively. These two activities presumably act in series (Schaaper 1993); therefore, defects in both activities may result in mutator synergism relative to the single mutator phenotypes. We investigated the possible interaction between the proofreading and replication activities in yeast using the single and double mutants described in the previous section.

In an MMR$^+$ background *pol3-t* has only a modest

**TABLE 1**

**Effect of the DNA Pol δ proofreading *pol3-01* and the polymerase *pol3-t* mutations on reversion of the *lys2::InsLD* mutation by replication slippage between distant short repeats**

| Strain | Lys$^+$ rate ($\times 10^9$) | Total Lys$^+$ sequenced | Number of Lys$^+$ due to deletions | Can$^r$ rate ($\times 10^7$) |
|---|---|---|---|---|
| POL$^+$ | 5.1 (3.1–7.0)[a] | 10 | 10 | 2.5 (1.7–3.0)[a] |
| *pol3-01* | 6.1 (3.4–7.2) | 14 | 11 | 106 (51–131) |
| *pol3-t* | 275 (151–399) | 19 | 19 | 18.8 (14.8–28.6) |
| *pol3-t, 01* | 267 (189–304) | 12 | 12 | 33.6 (23–74) |

[a] 95% confidence interval.

**TABLE 2**

**Mutator and antimutator effects of the *pol3-t* mutation on the frameshift mutation rate in homonucleotide runs**

| | Wild type[a] | *pol3-t* | | *msh2*[a] | *pol3-t msh2* | | | *pol3-01*[a] | *pol3-t, 01* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Length of run | Rate in run | Rate in run (× $10^9$) | Fold increase over wt | Rate in run (× $10^9$) | Rate in run (× $10^9$) | Fold increase over: *pol3-t* | Fold increase over: *msh2* | Rate in run (× $10^9$) | Rate in run (× $10^9$) | Fold decrease over *pol3-01* |
| $A_4(-1)$ | 0.4 (4/55)[b] | ≤2.5 (0/38)[b] | ≤6.0 | 31 (20/30)[b] | 221 (22/32)[b] | ≥88 | 7.1 | 15 (4/37)[b] | 4.7 (1/36)[b] | 3.2 |
| $A_7(-7)$ | 3.8 (9/24) | 41 (5/17) | 11 | 1,550 (31/31) | 8,830 (11/11) | 215 | 5.7 | 170 (9/26) | 77.3 (10/15) | 2.2 |
| $A_{10}(-1)$ | 47 (9/9) | 161 (17/24) | 3.4 | 314,000 (10/10) | 253,000 (10/10) | 1,571 | 0.81 | 3,520 (20/20) | 203 (19/25) | 17.3 |
| $A_{14}(-1)$ | 186 (10/10) | 614 (12/12) | 3.3 | 1,760,000 (8/8) | 5,100,000 (9/9) | 8,306 | 2.89 | 10,000 (10/10) | 1,240 (15/15) | 8.1 |
| $A_5(+1)$ | 1.1 (7/21) | ≤4.6 (0/21) | ≤4.0 | 37 (16/30) | 320 (42/48) | ≥70 | 8.6 | 93 (19/22) | 8.3 (3/36) | 11.2 |
| $A_8(+)$ | 10 (17/27) | 24 (14/37) | 2.4 | 3,440 (34/34) | 10,600 (14/14) | 442 | 3.1 | 2840 (17/18) | 85.4 (27/31) | 33 |
| $A_{12}(+1)$ | 140 (8/8) | 164 (7/11) | 1.2 | 173,000 (8/8) | 389,000 (9/9) | 2,372 | 2.2 | 2,780 (10/10) | 1,092 (13/15) | 2.5 |

The mutation rate in the run ($R_r$) was calculated as follows: $R_r = R_t\ (N_r/N_t)$, where $R_t$ is the total reversion rate determined by fluctuation test. The $N_r/N_t$ ratio is described in footnote *b*. wt, wild type.

[a] Data from Tran *et al.* (1997b, 1999) are given for comparison.

[b] The ratio of the number of revertants with a frameshift mutation in the run ($N_r$) to the total number of revertants analyzed by sequencing ($N_t$).

influence on the frameshift mutation rate in runs of various lengths (Table 2 and Tran *et al.* 1995, 1996). However, on the basis of previous results (Tran *et al.* 1996) and the data in Table 2, the *pol3-t* mutant generates a large number of premutational changes that are efficiently corrected by MMR. Similar to the observation with a Pol ε proofreading mutant (Tran *et al.* 1997b), the impact of the *pol3-t* mutator decreases with increased length of the homonucleotide run. For −1 frameshifts, the mutation rate increases of *pol3-t msh2* mutants in the $A_4$ and $A_{14}$ runs are, respectively, 7- and 3-fold those of *msh2* mutants alone; similarly, for +1-bp frameshifts, the mutation rate increases of *pd3-t msh2* mutants in the $A_5$ and $A_{12}$ runs are, respectively, 8.6- and 2.2-fold those of *msh2* mutants alone (Table 2).

As described previously (Tran *et al.* 1997b) and in Tables 1 and 2, the proofreading (*pol3-01*) and the polymerase (*pol3-t*) mutations increase the Can[r] forward mutation rate and the mutation rate in homonucleotide runs. Surprisingly, the *pol3-t, 01* double mutant does not exhibit a synergistic mutator phenotype. Instead of the expected mutation enhancement, there is a 2- to 33-fold decrease in the mutation rate for various homonucleotide runs in the double mutant, relative to strains defective only in proofreading (Table 2). There also appears to be an antimutator effect on the Can[r] forward mutation rate of *pol3-t* in the *pol3-01* background (Table 1).

**Impact of DNA Pol δ proofreading defect on mutation in homonucleotide runs:** Previously we examined the interaction between mutations in the Pol ε proofreading function and MMR on instability of long homonucleotide runs. Both Pol ε proofreading and MMR are efficient in preventing errors in short runs ($A_4$ and $A_5$), while only MMR prevents frameshift mutations in runs of ≥8 nucleotides (Tran *et al.* 1997b). It was not possible to investigate the interaction between Pol δ proofreading defect (*pol3-01*) and MMR in the haploid double mutant *pol3-01 msh2*, because it is inviable (Tran *et al.* 1999). Here we analyze the interaction between Pol δ proofreading and MMR on homonucleotide runs in diploid isogenic strains. The diploid strains have one copy of the chromosomal *LYS2* gene deleted in the *Bam*HI-*Xho*I region covering the *InsE* insert and the other copy of the *LYS2* gene contains homonucleotide runs of various lengths in the *InsE* (see materials and methods).

Similar to observations in haploid strains, the *msh2* and *pol3-01* diploid strains also generally exhibit an exponential increase in the mutation rate with increased length of homonucleotide run (Tables 2 and 3). Increasing the homonucleotide run length greatly increased the incidence of −1-nt and +1-nt mutations for all diploid strains tested (Tables 3 and 4). For wild-type strains, the proportion of reversions that were specifically due to deletions or additions in the homonucleotide runs increased from 18% (6/32) for the $A_4$ and 20% (9/44) for the $A_5$ to almost 100% for the $A_{10}$, $A_{12}$, and $A_{14}$ homonucleotide runs (Table 3). The wild-type and *msh2* strains exhibited comparable homonucleotide run mu-

**TABLE 3**

**Overall Lys$^+$ reversion rates in diploid MMR and/or proofreading defective strains containing *lys2* alleles with various lengths of homonucleotide runs**

| Length of run[a] | Wild type | | | msh2/msh2 | | | pol3-01/pol3-01 | | | msh2/msh2 pol3-01/pol3-01 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total rate ($\times 10^9$) | Ratio[b] | CI[c] | Total rate ($\times 10^9$) | Ratio | CI | Total rate ($\times 10^9$) | Ratio | CI | Total rate ($\times 10^9$) | Ratio | CI |
| $A_4$ (−1) | 3.9 | 6/32 | 3.3–8.5 | 28.6 | 12/18 | 23.5–44.8 | 816 | 0/42 | 725–2,910 | 7,860 | 6/39 | 3,980–12,220 |
| $A_7$ (−1) | 9.6 | 20/21 | 6.7–18.4 | 1,610 | 16/16 | 906–2,450 | 5,410 | 6/23 | 2,650–8,220 | 105,000 | 15/15 | 62,600–143,000 |
| $A_{10}$ (−1) | 41.6 | 13/14 | 27.5–117 | 152,000 | 10/10 | 93,000–203,000 | 9,950 | 12/12 | 6,980–22,200 | 471,000 | 8/8 | 238,000–1,040,000 |
| $A_{14}$ (−1) | 126 | 10/10 | 95.2–165 | 1,770,000 | 10/10 | 1,340,000–1,870,000 | 180,000 | 9/9 | 61,100–266,000 | 3,720,000 | 8/8 | 2,450,000–7,740,000 |
| $A_5$ (+1) | 3.7 | 9/44 | 3.5–6.7 | 64.9 | 11/20 | 55.6–150 | 588 | 15/15 | 425–1,340 | 2,500 | 12/12 | 1,510–3,820 |
| $A_8$ (+1) | 12.4 | 12/14 | 6.9–25 | 4,490 | 16/16 | 2,310–6,570 | 29,200 | 9/9 | 18,800–41,400 | 189,000 | 8/8 | 164,000–233,000 |
| $A_{12}$ (+1) | 57.5 | 8/9 | 27–92 | 145,000 | 10/10 | 112,000–215,000 | 52,800 | 7/7 | 36,700–72,700 | 1,310,000 | 8/8 | 945,000–1,640,000 |

All diploid strains are isogenic and derived from mating the 1036 lys-ΔBX-derivative strains with the CG379-derivative strains that contain homonucleotide runs of various lengths. These diploid strains are either wild type, or homozygous mutant for msh2 (msh2::LEU2/msh2::URA3), homozygous mutant for pol3-01, or combined double mutations msh2/msh2 pol3-01/pol3-01. For details of strain construction see materials and methods.

[a] Reversion rate was determined for −1 runs ($A_5$, $A_8$, and $A_{12}$) and for +1 runs ($A_4$, $A_7$, $A_{10}$, and $A_{14}$) strains. The predominant type of frameshift mutation in the run is given in parentheses (in nucleotides), determined as described in materials and methods.

[b] The ratio of the number of revertants with the frameshift mutation in the run ($N_r$) to the total number of revertants analyzed by sequencing ($N_t$).

[c] CI, 95% confidence interval for mutation rate ($\times 10^9$).

tation rates in both haploid (Tran *et al.* 1997b) and diploid strains.

Unlike the wild-type and *msh2* strains, the *pol3-01* diploid exhibits an important difference from the haploid. As shown in Table 5, the diploid has a much higher mutation rate (3- to 19-fold) as compared with the haploid strain. There are also differences in the mutation spectra between *pol3-01* haploid and diploid strains. Among 37 Lys$^+$ revertants of the *lys2::InsE*-$A_4$ allele in the haploid *pol3-01* strain, only 7 were at the GG hotspot position (indicated in Figure 1B) and 4 were in the $A_4$ run. However, in the *pol3-01/pol3-01* diploid strain 37 of 42 Lys$^+$ revertants were at the GG hot spot and none were in the $A_4$ run.

As MMR effectively corrects errors generated during replication, replication infidelity can be measured adequately only in the absence of the postreplication MMR. Similar to previous reports for DNA Pol ε proofreading (Tran *et al.* 1997b), there is a synergistic interaction between the *msh2* and DNA Pol δ proofreading deficiencies (Tables 3 and 4). However, there are differences in the mutation spectra between *pol2-4 msh2* and *pol3-01 msh2* strains. Among 39 revertants examined from the *pol3-01 msh2 lys2::InsE*-$A_4$/*pol3-01 msh2 lys2*Δ diploid strain, 14 were at the GG hot spot and 6 revertants occurred in the $A_4$ run. This differs from the spectrum of the haploid *pol2-4 msh2 lys2::InsE*-$A_4$ strain where all but 3 among 29 revertants were in the $A_4$ run (Tran *et al.* 1997b). [We did not investigate the *pol2-4 msh2/pol2-4 msh2* mutation spectrum because the *pol2-4/pol2-4* and *msh2/msh2* diploids did not exhibit any differences in mutation rates in comparison with *pol2-4* and *msh2* haploids, respectively (Table 6).] It is interesting that for the *msh2/msh2* diploid strain, there were no revertants because of changes at the GG hot spot and 12 among 18 Lys$^+$ revertants occurred in the $A_4$ run. These results are similar to those found for the *msh2* haploid, in which 20 of the 30 Lys$^+$ revertants arose in the $A_4$ run (Tran *et al.* 1997b). The high mutation frequency at the GG hot spot in the *pol3-01/pol3-01* diploid strain is likely dependent on the DNA sequence context because mutation hot spots are not observed at two other GGG sites within the reversion window.

In the MMR$^-$ background, the efficiency of DNA Pol δ proofreading for −1-nucleotide (nt) frameshift mutations is sharply reduced as the homonucleotide run length is increased from $A_7$ to $A_{10}$. The $A_4$ and $A_7$ mutation rates in the double mutant *pol3-01 msh2/pol3-01 msh2* are more than 63-fold higher than those in the *msh2/msh2* strain, but less than 3-fold higher for the $A_{10}$ and $A_{14}$ runs (Table 4; last column). For +1-nt insertions the efficiency of Pol δ proofreading is also decreased with increasing length of the homonucleotide run. The mutation rate in the $A_5$ run in *msh2 pol3-01/msh2 pol3-01* is 70-fold higher than in the single *msh2/msh2* mutant; in contrast, when the mutation rate in the $A_{12}$ run in the same two strains is compared, the difference is 9-fold

**TABLE 4**

**Lys$^+$ reversion rates attributable to frameshift mutations in homonucleotide runs in diploid MMR and/or proofreading defective strains**

| Length of run[a] | Wild type | *msh2/msh2* | | *pol3-01/pol3-01* | | *msh2/msh2 pol3-01/pol3-01* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fold increase over | | |
| | Rate in run ($\times 10^9$)[b] | Rate in run ($\times 10^9$) | Fold increase over wt[c] | Rate in run ($\times 10^9$) | Fold increase over wt | Rate in run ($\times 10^9$) | wt | *pol3-01* | *msh2* |
| $A_4$ (−1) | 0.7 | 19.1 | 27.3 | ≤19.4 | ≤28 | 1,209 | 1,727 | ≥62.3 | 63.3 |
| $A_7$ (−1) | 9.1 | 1,610 | 177 | 1,411 | 155 | 105,000 | 11,538 | 74.4 | 65.2 |
| $A_{10}$ (−1) | 38.6 | 152,000 | 3,938 | 9,950 | 258 | 471,000 | 12,200 | 47.3 | 3.1 |
| $A_{14}$ (−1) | 126 | 1,770,000 | 14,048 | 180,000 | 1,429 | 3,720,000 | 29,524 | 20.7 | 2.1 |
| $A_5$ (+1) | 0.8 | 35.7 | 44.6 | 588 | 735 | 2,500 | 3,125 | 4.3 | 70 |
| $A_8$ (+1) | 10.6 | 4,490 | 424 | 29,200 | 2,755 | 189,000 | 17,830 | 6.5 | 42.1 |
| $A_{12}$ (+1) | 51.1 | 145,000 | 2,838 | 52,800 | 1,033 | 1,310,000 | 25,636 | 24.8 | 9.0 |

All diploid strains are isogenic and derived as described in the footnote of Table 3. wt, wild type.

[a] The predominant type of frameshift mutation in the run is given in parentheses (in nucleotides).

[b] The mutation rate in the run ($R_r$) was calculated as follows: $R_r = R_t (N_r/N_t)$, where $R_t$ is the total rate of reversions. Values of $R_t$ and $N_r/N_t$ are taken from Table 3.

[c] Fold increase corresponds to the ratio of the rate for a given genotype to the rate of the wild-type or mutant strain with poly(dA) run of the same sizes.

(Table 4; last column). Thus, despite the reduced proofreading efficiency of Pol δ within longer homonucleotide runs, DNA Pol δ proofreading is still active during replication of the $A_{12}$ run, strongly affecting the mutation rate for +1-nt insertions. This is different from the influence of Pol ε proofreading, which is eliminated as the run length is extended from $A_5$ to $A_8$. The mutation rate in *msh2 pol2-4* is 321-fold higher than in *msh2* for the $A_5$ run, but there are only 2.3- and 1.6-fold increases for the $A_8$ and $A_{12}$ runs, respectively (see Table 2 in Tran *et al.* 1997b, 1999).

**Differences in hypermutability of the Pol δ proofreading mutant between diploid and haploid strains:** We found that the diploid *pol3-01* mutant exhibits a higher mutation rate than the haploid *pol3-01* strain. This ploidy effect appeared specific to *pol3-01* and was not observed in wild-type, *msh2*, or *exo1* mutants. As shown in Table 5, the differences in rate were observed for homonucleotide runs of different lengths. No differences were found between haploid and diploid wild-type, *msh2*, or *exo1* mutants.

**TABLE 5**

**Relative Lys$^+$ reversion rates of the *lys2*-homonucleotide-run mutations in diploid *vs.* haploid mutants**

| Length of runs | Relative rates of Lys$^+$ reversion in diploid[a] *vs.* haploid strains | | | |
|---|---|---|---|---|
| | Wild type | *msh2* | *exo1* | *pol3-01* |
| $A_4$ (−1) | 0.7 | 0.62 | 1.67 | 5.8 |
| $A_7$ (−1) | 0.96 | 1.04 | ND | 10.7 |
| $A_{10}$ (−1) | 0.89 | 0.48 | ND | 2.8 |
| $A_{14}$ (−1) | 0.68 | 1.1 | 1.1 | 17.9 |
| $A_5$ (+1) | 1.1 | 0.88 | 1.7 | 5.0 |
| $A_8$ (+1) | 0.78 | 1.3 | ND | 9.7 |
| $A_{12}$ (+1) | 0.41 | 1.7 | 0.83 | 18.9 |

ND, not determined.

[a] Diploid strains are described in the legend to Table 3. The *MAT*α haploid strains containing homonucleotide runs of various lengths are isogenic to strain CG 379. These haploid strains were mated to haploid strain derivatives of 1036 *lys2-ΔBX* that were not isogenic with the *MAT*α strains.

Because the diploid strains were constructed by mating strains from different backgrounds (Table 5), some of the differences between haploid and diploid strains could have arisen from strain background variation. Therefore, we constructed a series of homozygous diploid strains by HO endonuclease-induced autodiploidization of haploid strains (see materials and methods). Spontaneous mutation reversion rates were measured for the *his7-2*, *lys2::InsE*-$A_{12}$, and *lys2::InsE*-$A_{14}$ mutants. (Note that because the diploid strains have two alleles, the mutation rate in the diploid strains might be expected to be twofold higher than in haploids.) As shown in Table 6, there is no significant difference in the haploid and diploid mutation rates for the three loci (*his7-2*, *lys2::InsE*-$A_{12}$, or *lys2::InsE*-$A_{14}$) in wild-type strains and in mutator strains *pol2-4*, *msh2*, *exo1*, and *rad27*. However, the *pol3-01* diploid strain exhibits an 8.5- to 48-fold higher mutation rate than the haploid strain. The *EXO1* and *RAD27* genes code for 5′ to 3′ exonucleases. The former is implicated in the excision of mismatches (Tran *et al.* 1999) and the latter is required for the removal of flaps during lagging strand replication.

In a haploid strain, a *pol3-01* mutation combined with the Pol ε proofreading defect *pol2-4* (or with either mutation *exo1*, *msh2*, or *pms1*) is lethal, while the equivalent diploid strain is viable (Morrison *et al.* 1993; Morrison and Sugino 1994; Tran *et al.* 1999). The inviabil-

**TABLE 6**

**Spontaneous reversion rate for *his7-2*, *lys2::InsE-A*$_{12}$, and *lys2::InsE-A*$_{14}$ in isogenic haploid and homozygous diploid strains**

| | Relative reversion rate[a] | | |
|---|---|---|---|
| Strain | *his7-2* | *lys2::InsE-A*$_{12}$ | *lys2::InsE-A*$_{14}$ |
| *POL* absolute rate ($\times 10^8$) | 2.6 | 14 | 19 |
| *POL* relative rate | 1 | 1 | 1 |
| *POL/POL* | 0.5 | 1.1 | 3 |
| *pol2-4* | 5 | 1.1 | 2.1 |
| *pol2-4/pol2-4* | 2.6 | 3.6 | 7 |
| *pol3-01* | 27 | 24 | 53 |
| *pol3-01/pol3-01* | 227 | 1,142 | 632 |
| *exo1* | 2.4 | 2.2 | 97 |
| *exo1/exo1* | 5 | 6 | 121 |
| *msh2* | 24 | 1,214 | 9,500 |
| *msh2/msh2* | 42 | 2,500 | 18,947 |
| *rad27* | 8 | 14 | 74 |
| *rad27/rad27* | 17 | 47 | 105 |

Isogenic homozygous diploids were derived from haploids in which the mating types were switched (see materials and methods).

[a] Fold increase relative to haploid wild type.

ity of these mutant combinations in the haploid is considered to be due to the accumulation of excessive mutation. It is possible that the increased mutation rate in diploid *pol3-01* mutants is due to the existence of a *highly mutable* fraction of cells that would be inviable if the cells were haploid (see discussion). If this is true, then the coincidence of reversion of two independent mutations should be higher than expected on the basis of the single reversion rates. In the *pol3-01* diploid, the reversion rate for *lys2::InsE*-A$_{12}$ to Lys$^+$ is $1.58 \times 10^{-4}$ [95% confidence interval (CI) of the mutation rate: $0.55 \times 10^{-4}$–$2.31 \times 10^{-4}$] and for *his7-2*, it is $5.9 \times 10^{-6}$ (CI: $2.03 \times 10^{-6}$–$17.7 \times 10^{-6}$) to His$^+$. If reversion to His$^+$ and to Lys$^+$ were independent events, then the expected rate of simultaneous reversion to Lys$^+$ His$^+$ would be the product of the two reversion rates or $9.3 \times 10^{-10}$ (CI: $1.1 \times 10^{-10}$–$4.1 \times 10^{-9}$). Instead, we observed that the rate of appearance of double mutants was $3.39 \times 10^{-7}$ (CI: $1.14 \times 10^{-7}$–$4.37 \times 10^{-7}$), or 365-fold higher than the expected reversion rate.

If variation of MMR protein expression or activity is the source of a hypermutable cell fraction in the population, then loss of MMR should result in a homogeneously mutable cell population. We therefore measured the reversion rate to Lys$^+$, to His$^+$, and to His$^+$ Lys$^+$ in the homozygous strain *pol3-01 msh2/pol3-01 msh2.* The Lys$^+$ and His$^+$ reversion rates are $1.25 \times 10^{-3}$ (CI: $0.7 \times 10^{-3}$–$2.0 \times 10^{-3}$) and $2.7 \times 10^{-4}$ (CI: $1.8 \times 10^{-4}$–$5.9 \times 10^{-4}$), respectively. The double His$^+$ Lys$^+$ reversion rate is $9.7 \times 10^{-6}$ ($5.0 \times 10^{-6}$–$16.4 \times 10^{-6}$), which is only 29-fold higher than the expected reversion rate of $3.4 \times 10^{-7}$ (CI: $1.3 \times 10^{-7}$–$1.2 \times 10^{-6}$) as compared with a 365-fold increase in the *MSH2* strain. Thus, while elimination of MMR reduced much of the proposed mutational heterogeneity, these results suggest that MMR is not the sole source of heterogeneity in the *pol3-01* cell population.

If there is a hypermutable cell fraction in the population, the effect may be only transient. We therefore examined 48 independent Lys$^+$ revertants from the diploid strain *pol3-01 lys2::InsE*-A$_{12}$ *his7-2* for increased mutability. On the basis of a replica-plating assay for His$^+$ reversion, these isolates and the original strain had comparable His$^+$ reversion rates indicating that the proposed hypermutability is transient.

## DISCUSSION

Replication fidelity is dependent on many factors that include base selectivity, proofreading, and postreplication MMR as well as the DNA sequence being replicated. We have developed systems to address the impact of MMR and DNA Pol δ proofreading and polymerase defects during the replication of a variety of DNA templates that include at-risk motifs (*i.e.*, ARMs; Gordenin and Resnick 1998), which are prone to the generation of errors. Because replication and postreplication MMR complexes are highly conserved from yeast to human, and ARMs such as homonucleotide runs are found in the genomes of all organisms, the present data provide insight into mutation avoidance mechanisms during replication in higher eukaryotes. Because of its importance to replication fidelity and its possible role in MMR (Longley *et al.* 1997; Tran *et al.* 1999), we have analyzed the DNA Pol δ proofreading function in relation to several factors that influence replication accuracy.

(While the pol3-01 mutation results in loss of proofreading, it is conceivable that some aspect of the results are due to this mutation disturbing an as-yet-undefined function of this domain.) Because a Pol δ proofreading defect (*pol3-01*) is haploid lethal in combination with an *msh2* mutation while an *msh2 pol2-4* double mutant is viable, it appears that Pol δ plays a greater role in mutation avoidance than DNA Pol ε.

**Interaction between Pol δ proofreading and polymerase domains:** Using the construct *lys2::InsLD*, which allows for specific detection of large deletion mutations, we show here that the polymerase δ proofreading defect *pol3-01* did not increase replication slippage over long distances (Table 1). Moreover, this mutation did not alter replication slippage induced by the polymerase mutation *pol3-t* when these two mutations were combined in the same gene. Because *pol3-01* is a mutator for both frameshifts and base substitutions (Morrison *et al.* 1993) we suggest that the proposed misalignment induced by nucleotide misincorporation (Kunkel and Soni 1988) does not play a large role in replication slippage between distant repeats. Also, the observation that DNA polymerase proofreading does not repair loops formed between 7-nt repeats separated by 24 bp indicates that proofreading does not act on a loop that is located 7 nt from the 3′ end of a replication fork.

While *pol3-01* does not affect *pol3-t*-associated replication slippage, *pol3-t* acts as an antimutator with respect to *pol3-01* (Table 2), indicating an *interaction* between the corresponding regions. This was surprising, because synergy might be expected from the combination of these two mutators, both of which affect frameshift mutations in homonucleotide runs. The reason for the antimutator effect of the *pol3-t* mutation is not clear, but could relate to replication processivity. As suggested in our previous studies, which demonstrate that the *pol3-t* mutation induces deletions in inverted repeats (Gordenin *et al.* 1993), this mutant polymerase may replicate DNA more slowly or with decreased processivity, resulting in more single-stranded DNA regions in the lagging strand. Earlier studies demonstrate that decreased polymerase processivity can influence the fidelity of replication. For example, in the absence of its processivity cofactor, thioredoxin, T7 DNA polymerase creates more insertions (mutator) and fewer deletions (antimutator) in homopolymeric runs *in vitro* (Kunkel *et al.* 1994). The phenotype of the *pol3-t* mutant, induction of replication slippage over long distances, is consistent with the idea that this mutant polymerase dissociates from the DNA template often. It is possible that dissociation at a site of misalignment in a homonucleotide run results in replication arrest and loss of the premutational event. Previously, it was shown that the Pol δ can participate in MMR, and we proposed that its exonuclease is directly involved in the mismatch excision step (Longley *et al.* 1997; Tran *et al.* 1999). One possible explanation is that in the *pol3-t, 01* mutant MMR can be more efficient than in the *pol3-01* strain. Another possibility is that the *pol3-t* mutation partially restores the proofreading defect. For example, several mutations have been identified (Y. Pavlov and A. Sugino, personal communication) in the Pol δ polymerase region that act as antimutators in the *pol3-01* background and restore viability to *pol3-01 msh2* and *pol3-01 pms1* double mutant haploid strains.

In summary, our analysis and comparison of *pol3-t* and *pol3-01* mutants demonstrates that they have strikingly different mutator effects. The *pol3-t* mutation has a large impact on replication slippage between separated small repeats, but a relatively small effect on frameshift mutations (Tables 1 and 2). In contrast, the proofreading exonuclease-deficient *pol3-01* polymerase does not induce replication slippage between distant repeats, but it greatly increases the frameshift mutation rate in both short and long homonucleotide runs (Tables 3 and 4).

**Interaction between MMR and Pol δ proofreading and polymerase activities in mutation avoidance:** MMR has an important role in preventing mutations in homonucleotide runs and particularly in long runs, because the longer the homonucleotide run, the greater the role it plays. The *pol3-t* mutation results in mostly large deletions and a small number of frameshift mutations in a MMR$^+$ background (Tran *et al.* 1996). As the postreplication MMR effectively corrects errors generated during replication, replication infidelity can be measured adequately only in a MMR$^-$ background. The fraction of frameshift mutations increases dramatically in a MMR$^-$ background, which indicates a synergistic interaction between *pol3-t* and *msh2* with respect to frameshifts. However, this applies primarily to shorter homonucleotide runs, because with increased length of the run the mutator impact of *pol3-t* decreases (Table 2). A similar pattern is observed for the *pol3-01* proofreading mutant. Thus, for both the polymerase (*pol3-t*) and the proofreading (*pol3-01*) mutations in an MMR$^-$ background, the impact on mutation rate is greatest in shorter homonucleotide runs. Synergy between polymerase ε defects and postreplication MMR was also demonstrated previously in studies of haploid *pol2-4* mutants (Morrison and Sugino 1994; Tran *et al.* 1997b) and in diploid double mutants *pol3-01 pms1* and *pol3-01 msh2* (Morrison *et al.* 1993; Tran *et al.* 1999).

**The impact of homonucleotide run length on mutation rates in proofreading and polymerase mutants:** The accuracy of DNA replication is dependent not only on MMR, proofreading, and base selectivity, but also on the sequence of the DNA template. Using an *in vitro* system, Kroutil *et al.* (1996) showed that proofreading prevents many frameshifts in short homonucleotide runs, but the proofreading effect decreases with length of the homonucleotide run. This same effect was confirmed for Pol ε proofreading *in vivo* (Tran *et al.* 1997b). In the present work, we examined the impact of Pol δ proofreading on the mutation rate in homonucleotide

runs in diploid strains. We observed that in *pol3-01 msh2* double mutant strains, the frameshift mutation rate (−1 and +1 frameshifts) in shorter runs (4–8 nucleotides) was 40- to 70-fold higher than in the *msh2* single mutant. This difference is reduced to 2- to 9-fold in runs 10, 12, or 14 nucleotides in length. It is possible that frameshift intermediates in longer runs have a greater chance to escape Pol δ proofreading during replication. This result is consistent with earlier studies carried out both *in vitro* (Kroutil *et al.* 1996) and *in vivo* (Tran *et al.* 1997b) for other polymerases.

In general, it appears that Pol ε proofreading has less of an impact on the mutation rate than Pol δ proofreading. This conclusion is based on (i) *pol2-4*, but not *pol3-01*, being haploid viable in combination with an MMR defect; (ii) differences in rates for various DNA templates in the presence of either Pol δ or ε proofreading mutations; and (iii) Pol ε proofreading having effects over a shorter distance than Pol δ (Table 4 and Table 2 in Tran *et al.* 1997b). For short runs, $A_4$ and $A_5$, *pol2-4 msh2* (Tran *et al.* 1997b) and *pol3-01 msh2* mutation rates are comparable. However, unlike *pol3-01*, the *pol2-4* has little effect as the run increases to $A_7$ or $A_8$. Furthermore, Pol δ, but not Pol ε, proofreading can act on +1-nt frameshift intermediates in the $A_{12}$ run (Table 4, last column).

The difference between the mutation rates for −1 and +1 frameshifts in *pol3-01* mutants is interesting and could reflect differences in the interaction of the −1 or +1 frameshift intermediate with the mutant polymerase during replication of long homonucleotide runs. Previous results showed that defective MMR increases both −1-nt and +1-nt frameshifts (Sia *et al.* 1997; Tran *et al.* 1997b); however, the relative increase of −1-nt frameshifts is much greater than that of +1-nt frameshifts. It is possible that the efficiency of MMR is greater for repair of −1-nt frameshift intermediates. Alternatively, −1-nt frameshifts may be generated more often than +1 frameshifts during replication. Our results favor the second possibility. It is possible that wild-type DNA Pol δ may correct +1 frameshift intermediates with higher efficiency than it corrects −1 frameshift intermediates, because −1 frameshift errors appear to be insensitive to *pol3-01* in long runs (Table 4). In the double mutant *pol3-01 msh2* (Table 4) we have observed comparable mutation rates for both −1-nt and +1-nt.

The *pol3-t* mutation also increases the frameshift mutation rate nearly 8-fold in $A_4$ and $A_5$ homonucleotide runs, when strains are *msh2* defective. With longer runs, the effect is reduced; the frameshift mutation rate increases only 2.2- and 2.9-fold for the $A_{12}$ to $A_{14}$ runs, respectively (Table 2). Possibly the *pol3-t* polymerase mutation increases DNA misalignment during replication. Its overall impact may become less for long homonucleotide runs, where misalignment events would greatly increase [as suggested by the model of Streisinger *et al.* (1966)]. Another possibility is that the *pol3-t* mutation in the polymerase region partially impairs the proofreading activity of Pol δ. As described above for the Pol δ proofreading defect *pol3-01*, there is a reduced impact on mutation with increasing homonucleotide run length.

**Hypermutability of diploid *pol3-01* strains:** For several strains examined, the mutation rate is similar for both haploid and diploid cells (there is a generally small increase in diploids as expected for the additional second allele copy; Table 6). While no ploidy dependence was observed for wild-type, *msh2*, *exo1*, *rad27*, or *pol2-4* strains, the *pol3-01* strain was an exception to this pattern. The diploid *pol3-01* mutant has a much higher frameshift mutation rate than a haploid (Tables 5 and 6) for homonucleotide runs of various lengths as well as for the *his7-2* allele. Similar observations were also made for the base substitution mutation rate (P. Shcherbakova and Y. Pavlov, personal communication).

Hypermutability in diploids as compared to haploids was also found for mutagenesis by the base analog $N^6$-hydroxylaminopurine (HAP; Pavlov *et al.* 1988, 1991). The HAP-induced forward mutation rate at the *LYS2* gene in a diploid strain was nearly 100-fold higher than expected on the basis of the mutation rate in a haploid strain. As the *lys2* mutations induced by HAP were in most cases different for each of the two *lys2* alleles in the diploid, the two mutations must be due to independent mutational events. It is possible that many cells treated with HAP as haploids die because of multiple mutations that inactivate essential genes. Because most mutations in yeast are recessive, multiple mutants would be viable in diploid cells, resulting in more *lys2* mutants being recovered. Thus, the full impact of HAP mutagenesis can only be fully revealed in diploid cells (Pavlov *et al.* 1988, 1991).

To explain the differences in mutability between haploid and diploid *pol3-01* strains, we have proposed that the *pol3-01* diploid population includes a fraction of hypermutable cells that is absent in the haploid *pol3-01* population. This could occur if the cell population includes cells with transiently or permanently reduced expression of MMR genes or any other gene that would create hypermutability in combination with *pol3-01.* Several observations provide support for this idea. The *pol3-01* mutation when combined with either a MMR defect (*pms1* or *msh2*), an *exo1* mutation, or a DNA Pol ε proofreading defect is inviable in a haploid because of excessive mutation errors, whereas the diploid double mutants are viable and exhibit hypermutability (Morrison *et al.* 1993; Morrison and Sugino 1994; Tran *et al.* 1999). Thus, a hypermutable fraction of *pol3-01* cells with transient inactivation of one of these functions would be eliminated in a haploid, but would survive if the cells are diploid. By analogy with proofreading-defective *E. coli* strains, which display variable amounts of MMR deficiencies because of saturation (Fijalkow-

ska and Schaaper 1995, 1996; Schaaper 1988), the yeast *pol3-01* mutants may have reduced MMR capacity. This is supported by the significantly lower mutator effect of *msh2* in *pol3-01* strains than in POL$^{+}$ strains (*e.g.*, 20-fold *vs.* 14,000-fold for the $A_{14}$ run; Table 4). Complete loss of MMR in *E. coli dnaQ926* is associated with loss of viability (Fijalkowska and Schaaper 1996) as in the yeast *pol3-01 msh2* and *pol3-01 pms1* double mutants (Morrison *et al.* 1993; Tran *et al.* 1999).

The concept of a hypermutable cell fraction in *pol3-01* mutants is also supported by the high frequency of coincident mutations in separate loci. The rate of simultaneous reversion for the two alleles *his7-2* and *lys2::InsE*-$A_{12}$ in a homozygous *pol3-01* diploid is 365 times higher than expected if the two events occur independently. A hypermutable state might also be revealed as a fraction of cells that exhibit higher frequencies of recessive lethals when diploids undergo meiosis. This could be examined in the diploid cells that exhibited multiple mutations. If a reduced level of MMR activity is responsible for the hypermutable cell fraction, then loss of MMR should render the population homogenous with regard to mutation. In the diploid *pol3-01 msh2* strain the rate of simultaneous reversion for the two loci was much closer to the expected rate for two independent events. The lack of complete independence in coincident mutations in a MMR$^{-}$ background may indicate that factors other than MMR could also play a role in the formation of the hypermutable cell population. Alternatively, the *pol3-01 msh2* diploid strain, like the *pol3-01* diploid, may also demonstrate the ability, although less severe, to accumulate a subfraction of hypermutable cells that increase the occurrence of coincident double reversion events in the population.

Because the Lys$^{+}$ revertants from a *pol3-01* diploid were no more mutable than the original strain, the proposed hypermutability is likely to be a transient phenomenon, and could be due to epigenetic changes in a portion of the population. The possibility that epigenetic change may cause hypermutability, on either a transient or permanent basis, is relevant to understanding the etiology of cancer in mammalian cells. For example, analysis of the *lacI* mutation spectrum from thymic tumor DNA of mouse *Msh2*−/− revealed a fraction of *lacI* genes that had multiple mutations (Baross *et al.* 1998). It was suggested that an additional mutator activity, such as an error-prone DNA polymerase, leads to increased genomic instability in these MMR-deficient tumors. Epigenetic changes were observed in several tumor suppressor genes and *hMLH1.* For these genes, an epigenetic process involving promoter hypermethylation-induced repression of transcription has been demonstrated in association with cancer development (Herman *et al.* 1994, 1995, 1998; Merlo *et al.* 1995).

We are grateful to Drs. P. Shcherbakova, Y. Pavlov, and A. Sugino for providing unpublished data, and to Drs. Y. Pavlov, Yong Hwan Jin, M. Longley, R. Schaaper, and M. Sander for comments on the manuscript.

*Note added in proof*: The sequence of the his7-2 allele used in this study was recently described (P. V. Shcherbakova and T. A. Kunkel 1999, Mutator phenotypes conferred by MLH1 overexpression and by heterozygosity for mlh1 mutations. Mol. Cell Biol. **19:** 3177–3183). This mutation is due to a deletion of one A nucleotide in a run of eight adenines, so that reversions of the his7-2 allele can arise by −1 or +2 frameshifts.

## LITERATURE CITED

Araki, H., P. A. Ropp, A. L. Johnson, L. H. Johnston, A. Morrison *et al.*, 1992 DNA polymerase II, the probable homolog of mammalian DNA polymerase epsilon, replicates chromosomal DNA in the yeast *Saccharomyces cerevisiae.* EMBO J. **11:** 733–740.

Baross, F. A., S. E. Andrew, J. E. Penney and F. R. Jirik, 1998 Tumors of DNA mismatch repair-deficient hosts exhibit dramatic increases in genomic instability. Proc. Natl. Acad. Sci. USA **95:** 8739–8743.

Bennett, C., A. Lewis, K. Baldwin and M. Resnick, 1993 Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc. Natl. Acad. Sci. USA **90:** 5613–5617.

Boulet, A., M. Simon, G. Faye, G. Bauer and P. Burgers, 1989 Structure and function of the *Saccharomyces cerevisiae CDC2* gene encoding the large subunit of DNA polymerase III. EMBO J. **8:** 1849–1854.

Fijalkowska, I. J., and R. M. Schaaper, 1995 Effects of *Escherichia coli dnaE* antimutator alleles in a proofreading-deficient *mutD5* strain. J. Bacteriol. **177:** 5979–5986.

Fijalkowska, I. J., and R. M. Schaaper, 1996 Mutants in the Exo I motif of *Escherichia coli dnaQ*: defective proofreading and inviability due to error catastrophe. Proc. Natl. Acad. Sci. USA **93:** 2856–2861.

Fijalkowska, I. J., R. L. Dunn and R. M. Schaaper, 1993 Mutants of *Escherichia coli* with increased fidelity of DNA replication. Genetics **134:** 1023–1030.

Gietz, R. D., and R. H. Schiestl, 1991 Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. Yeast **7:** 253–263.

Gordenin, D. A., and M. A. Resnick, 1998 Yeast ARMs (DNA at-risk motifs) can reveal sources of genome instability. Mutat. Res. **400:** 45–58.

Gordenin, D. A., K. S. Lobachev, N. P. Degtyareva, A. L. Malkova, E. Perkins *et al.*, 1993 Inverted DNA repeats: a source of eukaryotic genomic instability. Mol. Cell. Biol. **13:** 5315–5322.

Herman, J. G., F. Latif, Y. Weng, M. I. Lerman, B. Zbar *et al.*, 1994 Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc. Natl. Acad. Sci. USA **91:** 9700–9704.

Herman, J. G., A. Merlo, L. Mao, R. G. Lapidus, J. P. Issa *et al.*, 1995 Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res. **55:** 4525–4530.

Herman, J. G., A. Umar, K. Polyak, J. R. Graff, N. Ahuja *et al.*, 1998 Incidence and functional consequences of *hMLH1* promoter hypermethylation in colorectal carcinoma. Proc. Natl. Acad. Sci. USA **95:** 6870–6875.

Hindges, R., and U. Hubscher, 1997 DNA polymerase δ, an essential enzyme for DNA transactions. Biol. Chem. **378:** 345–362.

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

Kesti, T., and J. E. Syvaoja, 1991 Identification and tryptic cleavage of the catalytic core of HeLa and calf thymus DNA polymerase epsilon. J. Biol. Chem. **266:** 6336–6341.

Kokoska, R. J., L. Stefanovic, H. T. Tran, M. A. Resnick, D. A. Gordenin *et al.*, 1998 Destabilization of yeast micro- and minisatellite DNA sequences by mutations affecting a nuclease involved in Okazaki fragment processing (*rad27*) and DNA polymerase delta (*pol3-t*). Mol. Cell. Biol. **18:** 2779–2788.

Kroutil, L. C., K. Register, K. Bebenek and T. A. Kunkel, 1996

Exonucleolytic proofreading during replication of repetitive DNA. Biochemistry **35:** 1046–1053.

Kunkel, T. A., and A. Soni, 1988 Mutagenesis by transient misalignment. J. Biol. Chem. **263:** 14784–14789.

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.

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

Longley, M. J., A. J. Pierce and P. Modrich, 1997 DNA polymerase delta is required for human mismatch repair in vitro. J. Biol. Chem. **272:** 10917–10921.

Maki, H., J. Y. Mo and M. Sekiguchi, 1991 A strong mutator effect caused by an amino acid change in the alpha subunit of DNA polymerase III of *Escherichia coli.* J. Biol. Chem. **266:** 5055–5061.

McHenry, C. S., and W. Crow, 1979 DNA polymerase III of *Escherichia coli.* Purification and identification of subunits. J. Biol. Chem. **254:** 1748–1753.

Merlo, A., J. G. Herman, L. Mao, D. J. Lee, E. Gabrielson *et al.*, 1995 5′ CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1 in human cancers. Nature Med. **1:** 686–692.

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

Morrison, A., and A. Sugino, 1992 Nucleotide sequence of the *POL3* gene encoding DNA polymerase III (delta) of *Saccharomyces cerevisiae.* Nucleic Acids Res. **20:** 375.

Morrison, A., and A. Sugino, 1994 The 3′→5′ exonucleases of both DNA polymerase δ and ε participate in correcting errors of DNA replication in *S. cerevisiae.* Mol. Gen. Genet. **242:** 289–296.

Morrison, A., J. B. Bell, T. A. Kunkel and A. Sugino, 1991 Eukaryotic DNA polymerase amino acid sequence required for 3′→5′ exonuclease activity. Proc. Natl. Acad. Sci. USA **88:** 9473–9477.

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.

Pavlov, Y. I., V. N. Noskov, Y. Chernoff and D. A. Gordenin, 1988 Mutability of *LYS2* gene in diploid Saccharomyces yeasts. II. Frequency of mutants induced by N6-hydroxylaminopurine and propiolactone. Genetika **24:** 1752–1760.

Pavlov, Y. I., V. N. Noskov, E. K. Lange, E. V. Moiseeva, M. R. Pshenichnov *et al.*, 1991 The genetic activity of N6-hydroxyadenine and 2-amino-N6-hydroxyadenine in *Escherichia coli*, *Salmonella typhimurium* and *Saccharomyces cerevisiae.* Mutat. Res. **253:** 33–46.

Reenan, R. A., and R. D. Kolodner, 1992 Characterization of insertion mutations in the *Saccharomyces cerevisiae MSH1* and *MSH2* genes: evidence for separate mitochondrial and nuclear functions. Genetics **132:** 975–985.

Sambrook, J., E. F. Fritsch and J. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schaaper, R. M., 1988 Mechanisms of mutagenesis in the *Escherichia coli* mutator *mutD5*: role of DNA mismatch repair. Proc. Natl. Acad. Sci. USA **85:** 8126–8130.

Schaaper, R. M., 1993 Base selection, proofreading, and mismatch repair during DNA replication in *Escherichia coli.* J. Biol. Chem. **268:** 23762–23765.

Shcherbakova, P. V., and Y. I. Pavlov, 1996 3′→5′ exonucleases of DNA polymerases ε and δ correct base analog induced DNA replication errors on opposite DNA strands in *Saccharomyces cerevisiae.* Genetics **142:** 717–726.

Shcherbakova, P. V., V. N. Noskov, M. R. Pshenichnov and Y. I. Pavlov, 1996 Base analog N6-hydroxylaminopurine mutagenesis in the yeast *Saccharomyces cerevisiae* is controlled by replicative DNA polymerases. Mutat. Res. **369:** 33–44.

Sherman, F., G. F. Fink and J. B. Hicks, 1986 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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

Simon, M., L. Giot and G. Faye, 1991 The 3′→5′ exonuclease activity located in the DNA polymerase delta subunit of *Saccharomyces cerevisiae* is required for accurate replication. EMBO J. **10:** 2165–2170.

Streisinger, G., Y. Okada, J. Emrich, J. Newton, A. Tsugita *et al.*, 1966 Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31:** 77–84.

Sugino, A., 1995 Yeast DNA polymerases and their role at the replication fork. Trends Biochem. Sci. **20:** 319–323.

Tran, H. T., N. P. Degtyareva, N. N. Koloteva, A. Sugino, H. Masumoto *et al.*, 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.

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

Tran, H. T., N. Degtyareva, D. Gordenin and M. A. Resnick, 1997a Altered replication and inverted repeats induce mismatch repair-independent recombination between highly diverged DNAs in yeast. Mol. Cell. Biol. **17:** 1027–1036.

Tran, H. T., J. D. Keen, M. Kricker, M. A. Resnick and D. A. Gordenin, 1997b Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. Mol. Cell. Biol. **17:** 2859–2865.

Tran, H. T., D. A. Gordenin and M. A. Resnick, 1999 The 3′→5′ exonucleases of DNA polymerases δ and ε and the 5′→3′ exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **19:** 2000–2007.

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

Communicating editor: L. S. Symington