Genomic Instability Induced by Mutations in *Saccharomyces cerevisiae POL1*

Pedro J. A. Gutiérrez and Teresa S.-F. Wang[1]

Department of Pathology, Stanford University School of Medicine, Stanford, California 94305-5324

Manuscript received December 5, 2002
Accepted for publication May 6, 2003

ABSTRACT

Mutations of chromosome replication genes can be one of the early events that promote genomic instability. Among genes that are involved in chromosomal replication, DNA polymerase α is essential for initiation of replication and lagging-strand synthesis. Here we examined the effect of two mutations in *S. cerevisiae POL1*, *pol1-1* and *pol1-17*, on a microsatellite $(GT)_{16}$ tract. The *pol1-17* mutation elevated the mutation rate 13-fold by altering sequences both inside and downstream of the $(GT)_{16}$ tract, whereas the *pol1-1* mutation increased the mutation rate 54-fold by predominantly altering sequences downstream of the $(GT)_{16}$ tract in a *RAD52*-dependent manner. In a *rad52* null mutant background *pol1-1* and *pol1-17* also exhibited different plasmid and chromosome loss phenotypes. Deletions of mismatch repair (MMR) genes induce a differential synergistic increase in the mutation rates of *pol1-1* and *pol1-17*. These findings suggest that perturbations of DNA replication in these two *pol1* mutants are caused by different mechanisms, resulting in various types of mutations. Thus, mutations of *POL1* can induce a variety of mutator phenotypes and can be a source of genomic instability in cells.

MUTATIONS in genes involved in DNA replication, DNA repair, or chromosome segregation can potentially induce a mutator phenotype. Among the genes involved in DNA replication, the eukaryotic replicative DNA polymerases (Polα, Polδ, and Polε) play an essential role in determining the faithful transmission of genetic information from one generation to the next. Homologs of these replicative polymerases from yeast to humans share a high degree of conservation in their protein domain organization (Delarue *et al.* 1990; Wang 1996). In addition to replication, these replicative polymerases are also involved in double-strand-break repair (Holmes and Haber 1999), nucleotide excision repair (Lindahl and Wood 1999), and telomere homeostasis maintenance (Diede and Gottschling 1999; Adams-Martin *et al.* 2000; Dahlen *et al.* 2003).

In budding yeast, a mutator phenotype has been associated with several mutant alleles of *POL2* and *POL3*, which encode the replicative polymerase ε and δ, respectively. Several of these *pol2* and *pol3* mutants exhibit an increase in frameshifts in homonucleotide runs (Tran *et al.* 1997b; Kirchner *et al.* 2000), in microsatellite instability (Strand *et al.* 1993; Kokoska *et al.* 1998, 2000), in deletions of sequence flanked by short direct repeats (Gordenin *et al.* 1992; Tran *et al.* 1995; Kokoska *et al.* 2000), or in base substitutions (Morrison *et al.* 1993; Morrison and Sugino 1994). In contrast, little is known about the effect of mutations in *POL1* (Polα), the replicative polymerase essential for both initiation at the replication origin and initiation of Okazaki fragments during the lagging-strand synthesis (Waga and Stillman 1998). Although Pol1p (Polα) does not seem to be involved in synthesizing the main bulk of cellular DNA as shown in an *in vitro* reconstituted replication assay containing an *SV40*-ori-bearing plasmid (Waga *et al.* 1994; Waga and Stillman 1994), its unique role in chromosome initiation has led us to investigate *POL1*'s contribution to mutation avoidance.

Our previous fission yeast studies identified conditional mutants in the catalytic subunit of Polα, which conferred a mutator phenotype at the *ura4*$^+$ locus characterized by an elevated rate of base substitutions and deletion of sequences flanked by short direct repeats (Liu *et al.* 1999; Kai and Wang 2003). The mutation rate was exacerbated when these replication mutators were combined with a deletion of Cds1 (Liu *et al.* 1999; Kai and Wang 2003), a checkpoint effector kinase essential in fission yeast for tolerating and recovering from replication perturbations (Walworth 2000; Boddy and Russell 2001). These studies suggest that mutations of Polα potentially affect the initiation complex at the replication origin or during Okazaki fragment synthesis, compromising the stability of replication fork and thus generating a mutator phenotype in cells.

In this study, we use the budding yeast *Saccharomyces cerevisiae* to investigate the contribution of *POL1* to mutation avoidance by analyzing two distinct conditional *pol1* mutant alleles, *pol1-1* and *pol1-17*. The *pol1-1* mutant allele contains a single missense mutation (Gly^{493} to Arg) within the N-terminal region (Pizzagalli *et al.* 1988), while *pol1-17* encodes a mutation (Thr^{1004} to Ile) buried near the most conserved domain of the B-family (α-like) polymerases (Ito and Braithwaite 1991; Heringa and

[1]*Corresponding author:* Department of Pathology, Edwards Bldg., Rm. R270, Stanford University Medical Center, 300 Pasteur Dr., Stanford, CA 94305. E-mail address: twang@pmgm2.stanford.edu

TABLE 1

Yeast strains used in this study

Strain	Relevant genotype	Source
	Haploids[a]	
AMY101	*pms1Δ::LEU2*	A. Sugino
AMY125	*MAT*α *ade5-1 his7-2 leu2-3,112 trp1-289 ura3-52*	Strand *et al.* (1993)
DSF4d/5a	*MAT***a** *ura3 trp1-1 pep4Δ::HIS3 prb1Δ::LEU2 pol1-1*	Francesconi *et al.* (1993)
EAS 18	*MAT***a** *LEU2*	Kokoska *et al.* (1998)
EAS 38	*msh6Δ::LEU2*	Sia *et al.* (1997)
GCY 140	*LEU2 msh3Δ::hisG*	Sia *et al.* (1997)
MS71-*pol3-t*	*LEU2 pol3-t*	Kokoska *et al.* (1998)
MS 72	*pol2-4*	Strand *et al.* (1993)
MS 73	*pol3-01*	Strand *et al.* (1993)
PGY 210	DSF4d/5a with *POL1*	This study
PGY 300	PGY 210 with *pol1-17*	This study
PGY 1121	DSF4d/5a with *POL1-TAP-TRP1*[b]	This study
PGY 1131	DSF4d/5a with *pol1-1-TAP-TRP1*[b]	This study
PGY 1141	DSF4d/5a with *pol1-17-TAP-TRP1*[b]	This study
PGY 2005	*MAT***a** *LEU2*	This study
PGY 2006	*LEU2*	This study
PGY 2024	*LEU2 III-*205*::URA3*[c]	This study
PGY 2026	*III-*205*::TRP1*[c]	This study
PGY 2130	*MAT***a** *LEU2 pol1-1*	This study
PGY 2131	*pol1-1*	This study
PGY 2133	*MAT***a** *pol1-1*	This study
PGY 2134	*LEU2 III-*205*::URA3 pol1-1*[c]	This study
PGY 2140	*MAT***a** *LEU2 pol1-17*	This study
PGY 2143	*MAT***a** *pol1-17*	This study
PGY 2160	*MAT***a** *LEU2 pol3-t*	This study
PGY 2170	*MAT***a** *LEU2 pol3-01*	This study
PGY 2180	*MAT***a** *LEU2 pol2-4*	This study
PGY 2221	*MAT***a** *pms1Δ::LEU2*	This study
PGY 2231	*MAT***a** *pms1Δ::LEU2 pol1-1*	This study
PGY 2241	*MAT***a** *pms1Δ::LEU2 pol1-17*	This study
PGY 2320	*LEU2 rad52Δ::hisG*	This study
PGY 2321	*MAT***a** *LEU2 rad52Δ::hisG*	This study
PGY 2330	*LEU2 rad52Δ::hisG pol1-1*	This study
PGY 2331	*MAT***a** *LEU2 rad52Δ::hisG pol1-1*	This study
PGY 2340	*LEU2 rad52Δ::hisG pol1-17*	This study
PGY 2341	*MAT***a** *LEU2 rad52Δ::hisG pol1-17*	This study
PGY 2621	*MAT***a** *LEU2 msh3Δ::hisG*	This study
PGY 2631	*MAT***a** *LEU2 msh3Δ::hisG pol1-1*	This study
PGY 2641	*MAT***a** *LEU2 msh3Δ::hisG pol1-17*	This study
PGY 2721	*MAT***a** *msh6Δ::LEU2*	This study
PGY 2731	*MAT***a** *msh6Δ::LEU2 pol1-1*	This study
PGY 2741	*MAT***a** *msh6Δ::LEU2 pol1-17*	This study
POL1-17	*MAT***a** *pol1-17 ura3-1 ura3-2 trp1-289 try1 ade2-101 gal2 can1*	Budd and Campbell (1987)
PT1	*MAT***a** *hom3 ile*$^-$ *can1*	D. Botstein
PT2	*MAT*α *hom3 ile*$^-$ *can1*	D. Botstein
RJK 396	*MAT***a** *LEU2*	Kokoska *et al.* (2000)
RJK 397	*rad52Δ::hisG-URA3-hisG*	Kokoska *et al.* (2000)

Strain	Relevant genotype	Cross
	Diploids[d]	
PGY 2002	*LEU2/LEU2*	PGY 2005 × PGY 2006
PGY 2038	*LEU2/LEU2 III-205::URA3/+*	PGY 2005 × PGY 2024
PGY 2128	*LEU2/LEU2 III-205::URA3/+ pol1-1/pol1-1*	PGY 2130 × PGY 2134
PGY 2129	*LEU2/LEU2 pol1-1/pol1-1*	PGY 2130 × PGY 2131
PGY 2139	*LEU2/LEU2 pol1-17/pol1-17*	PGY 2140 × PGY 2141
PGY 2158	*LEU2/LEU2 POL3/pol3-t*	MS71-*pol3-t* × PGY 2005
PGY 2168	*LEU2/LEU2 POL3/pol3-01*	MS73 × PGY 2005

(*continued*)

TABLE 1

(Continued)

Strain	Relevant genotype	Source
PGY 2228	*PMS1/pms1*Δ*::LEU2 POL1/pol1-1*	AMY 101 × PGY 2133
PGY 2238	*PMS1/pms1*Δ*::LEU2 POL1/pol1-17*	AMY 101 × PGY 2143
PGY 2315	*LEU2/LEU2 rad52*Δ*::hisG/rad52*Δ*::hisG*	PGY 2320 × PGY 2321
PGY 2327	*LEU2/LEU2 rad52*Δ*::hisG/rad52*Δ*::hisG pol1-1/pol1-1*	PGY 2330 × PGY 2331
PGY 2328	*LEU2/leu2-3,112 RAD52/rad52*Δ*::hisG-URA3-hisG POL1/pol1-1*	RJK 397 × PGY 2130
PGY 2337	*LEU2/LEU2 rad52*Δ*::hisG/rad52*Δ*::hisG pol1-17/pol1-17*	PGY 2340 × PGY 2341
PGY 2338	*LEU2/leu2-3,112 RAD52/rad52*Δ*::hisG-URA3-hisG POL1/pol1-17*	RJK 397 × PGY 2140
PGY 2628	*LEU2/leu2-3,112 MSH3/msh3*Δ*::hisG POL1/pol1-1*	GCY 140 × PGY 2133
PGY 2638	*LEU2/leu2-3,112 MSH3/msh3*Δ*::hisG POL1/pol1-17*	GCY 140 × PGY 2143
PGY 2728	*MSH6/msh6*Δ*::LEU2 POL1/pol1-1*	EAS 38 × PGY 2133
PGY 2738	*MSH6/msh6*Δ*::LEU2 POL1/pol1-17*	EAS 38 × PGY 2143

[a] All haploid strains (except for DSF4d/5a, PGY 1121, 1131, 1141, POL1-17, PT1, and PT2) are isogenic to AMY125 (*MAT*α *ade5-1 his7-2 leu2-3,112 trp1-289 ura3-52*) except for changes introduced by transformation. Only deviations from AMY125 genotype are shown.

[b] Haploid strains contain the TAP tag with the *TRP1* gene from *K. lactis* at the C terminus of *POL1* or *pol1*.

[c] *III*-205*::URA3* and *III*-205*::TRP1* signifies that *URA3* or *TRP1* were inserted at the 205-kb position (right arm) of chromosome *III*. Construction details are described in MATERIALS AND METHODS.

[d] All diploid strains are *MAT*α*/MAT***a** *ade5-1/ade5-1 his7-2/his7-2 leu2-3,112/leu2-3,112 trp1-289/trp1-289 ura3-52/ura3-52* except for noted deviations.

ARGOS 1994). By using a well-established dinucleotide repeat system (HENDERSON and PETES 1992), we investigate the effects of these *pol1* alleles on genome stability as well as their genetic interactions with double-strand-break repair and mismatch repair (MMR) genes. Our results indicate that *POL1* plays a role in ensuring genomic stability in several ways, since these mutant alleles of *POL1* can induce a range of mutator phenotypes.

MATERIALS AND METHODS

Media, growth conditions, and general methods: Standard budding yeast cultivation methods and standard media were utilized (SHERMAN 1991). Synthetic complete (SC) medium lacking tryptophan (W), leucine (L), threonine (T), and uracil (U) was used for microsatellite instability assays as described in HENDERSON and PETES (1992). For sporulation, cells were treated with 0.5% KOAc (pH 7.0) supplemented with half the normal amount of amino acids required for auxotrophic strains. Standard methods were used to switch mating type (HERSKOWITZ and JENSEN 1991), utilizing pRB1191 [*URA3 CEN4 GAL1/10-HO*] and tester strains PT1 and PT2 (plasmid and strains kindly provided by D. Botstein).

Plasmid constructions: Both pPGI11 and pPGI117 were constructed by restricting the 5.5-kb *Bam*HI-*Sph*I fragment of pPGC11 and pPGC117, which contain the entire *pol1-1* or *pol1-17* coding region in addition to upstream and downstream untranslated regions, and inserting this fragment into *Bam*HI-*Sph*I-restricted pRB1721 [pUC18-*URA3*] (kindly supplied by D. Botstein).

Yeast strain constructions: All strains used in this study are described in Table 1. Transformations were performed using the high-efficiency lithium acetate protocol described in AGATEP *et al.* (1998). PGY 2130 and PGY 2140 were generated via a two-step gene replacement, transforming EAS18 (kindly provided by T. Petes) with *Xba*I-linearized pPGI11 and pPGI117, respectively, and selecting for thermosensitive colonies after treatment with 5-fluoroorotic acid (5-FOA). The *pol1-1* and *pol1-17* mutations were confirmed by sequencing.

PGY 2170 was generated from a cross between PGY 2005 and MS 73 (kindly supplied by T. Petes). Presence of the *pol3-01* allele was determined by sequencing. PGY 2180 was constructed by transforming MS72 (kindly supplied by T. Petes) with pRB1191 and changing the mating type. PGY 2320, PGY 2321, PGY 2330, PGY 2331, PGY 2340, and PGY 2341 were all meiotic segregants from the crosses with RJK 397 (kindly provided by T. Petes), a strain that contains a disruption of the *RAD52* locus with the *hisG-URA3-hisG* cassette. Crosses are described in Table 1. After verifying that these strains were thermosensitive (as appropriate) and methyl methanesulfonate sensitive, 5-FOA was used to induce recombinogenic loss of *URA3* and one copy of *hisG*.

PGY 2221, PGY 2231, and PGY 2241 were meiotic segregants generated from crosses described in Table 1, which were verified to be Leu^+ and/or thermosensitive (the AMY101 parental strain kindly provided by A. Sugino). The presence of *pms1*Δ was confirmed by high papillation on plates containing canavanine sulfate (60 μg/ml) and by PCR with a sense primer in the upstream region and an antisense primer internal to *LEU2*. A similar strategy was used to generate PGY 2721, PGY 2731, PGY 2741, PGY 2621, and PGY 2631 (parental strains kindly provided by T. Petes). The presence of *msh6*Δ or *msh3*Δ was verified by PCR with appropriate primers as previously mentioned. Since only *MAT*α segregants were isolated for PGY 2621 and PGY 2631, the mating type was changed with pRB1191.

Diploids from a chromosome-loss assay were constructed by crossing strains specified in Table 1. Strains PGY 2024, PGY 2134, and PGY 2026, used in the *MAT* conversion assay, were constructed by transforming both PGY 2006 and PGY 2131 with a PCR-amplified fragment containing *URA3* flanked by 48 nucleotides of homologous sequence to the 205-kb region on chromosome *III* and AMY125 with a PCR fragment containing *TRP1* flanked by the same sequences.

To measure Pol1p protein levels, wild-type and *pol1* mutant strains (PGY 210, DFS4d/5a, and PGY 300) were transformed with a PCR fragment that contained the TAP tag followed by

the *TRP1* gene of *Kluyveromyces lactis* (Rigaut *et al.* 1999), which was amplified from plasmid pBS1479 using the primers 5′-ACTATATAGAATATTCATGAGATCACACAACACATAC AAAATACTTACtacgactcactataggg-3′ and 5′-GGACGTCGCT ACGTTGATATGACTAGCATATTTGATTTCATGCTAAATtc catggaaaagagaag-3′ where uppercase sequences correspond to the C terminus of *POL1*. This generated PGY 1121, PGY 1131, and PGY 1141. Correct integration was verified by PCR and Western analysis.

Microsatellite instability assays: Microsatellite assays were carried out as in Wierdl *et al.* (1997) with the following minor modifications. All assays were performed at the semipermissive temperature of 28°, the highest temperature at which viability of the thermosensitive strains was determined to be comparable to the wild type. Yeast strains were grown at the permissive temperature (25°) in liquid SC-WLTU, plated for single colonies onto solid SC-W medium, and incubated at 28°. Colonies resistant to 5-FOA (5-FOA^r) were scored after incubating 5 days at 25°.

Mutation rate analysis for microsatellite instability assays: Each experiment was calculated from 12 to 24 independent cultures and at least two independent experiments were done per strain. Mutation rate was calculated using the method of the median as described by Lea and Coulson (1949), which had been used in various other microsatellite instability studies (Henderson and Petes 1992; Strand *et al.* 1993; Johnson *et al.* 1996a; Wierdl *et al.* 1996, 1997; Kokoska *et al.* 1998). Confidence intervals were generated as described in Wierdl *et al.* (1996). Significance levels between strains were determined by using the Mann-Whitney rank test (*Analyze-it*, Microsoft Excel), a standard test of significance when populations are nonparametric and the distribution is unknown. Unless specified otherwise, all comparisons are significant with a P level of $P < 0.01$.

Analysis of poly(GT) tract lengths: Two methods were used to determine the lengths of the poly(GT) tract. Standard methods were used to directly sequence the poly(GT) tract in *Escherichia coli* colonies carrying the repeat plasmid rescued from yeast. In addition, "hot" PCR analysis of poly(GT) tracts was done directly from yeast colonies with PCR primers used in previous studies (Wierdl *et al.* 1997). Lengths were compared to standard tracts that had been previously sequenced. The 10-μl PCR reaction contained the following: 5 pmol of each primer, 200 μM of dNTP, 1 μCi of [α-^{33}P]dATP (Amersham, Buckinghamshire, UK), 1× Vent DNA polymerase buffer, and 0.2 units of *Taq* polymerase. PCR products were analyzed on a 6% denaturing polyacrylamide gel. Radioactive PCR sizing of the poly(GT) tract was done from both bacterial and yeast colonies to confirm that changes present in pSH44 did not result from mutations induced in *E. coli* during plasmid propagation. No tract length discrepancies were found throughout these studies.

Confidence intervals of 95% were derived using standard statistical methods based on the binomial distribution as described in two introductory statistics texts (Freedman *et al.* 1991, p. 348; Glantz 1992, p. 206) with the formula $2\sqrt{P(1-P)/N}$. N is the total number of tracts sequenced, and P is the percentage of tracts that are in a particular category (*i.e.*, "deletions," "insertions," and "no change"). These confidence intervals were also used in reporting rates for specific alterations [*e.g.*, rates of deletions, alterations outside of the poly(GT) tract, etc.]. Significant differences among types of alterations were determined using the chi-square test (or Fisher exact test when appropriate).

Analysis of FOA^r colonies that had no change in the poly (GT) tract: When no changes were detected in the poly(GT) tract after PCR sizing, pSH44 was isolated from the 5-FOA^r cells and digested with *Hin*dIII to detect gross sequence changes in the poly(GT)-*HIS4-URA3* substrate. These plasmids were subsequently sequenced with various sense and antisense primers running along the length of the *HIS4-URA3* reporter.

Spontaneous mutation rate and mutation spectrum in *CAN1*: Standard methods were used to determine the forward mutation rate in the *CAN1* gene, which confers resistance (Can^r) to the arginine analog canavanine sulfate (Xie *et al.* 1999). Rates were calculated from 11–15 independent cultures and two independent experiments were done per strain. Genomic DNA was isolated from Can^r colonies and the *CAN1* open reading frame was PCR amplified with primers ~100 bp upstream and downstream. These products were purified (QIAGEN, Chatsworth, CA) and restricted with *Hph*I, which produces six fragments of 480, 411, 303, 252, 249, and 207 bp. These fragments were examined using electrophoresis on 2%-TBE agarose gels.

Plasmid retention assay: Cells were inoculated into SC-WLTU and grown at room temperature to midlog phase. Approximately 200 cells were plated onto YPA medium and incubated at 28° for 2–3 days until colonies reached 2 mm in diameter. Colonies were then replica plated onto SC and SC-W plates. Sectored colonies that grew on SC-W were counted and compared to the total number of colonies. The percentage of plasmid retention was calculated using the equation $P = S/(T - T_0)$, where P is the proportion of plasmid retention, S is the number of sectored colonies, T is the total number of colonies on SC, and T_0 is the number of colonies that did not grow on SC-W (*i.e.*, did not contain the plasmid when plated). At least 12 cultures per strain were tested and the average percentage reported. Confidence intervals for percentages were generated as described above, replacing the number of tracts, N, with the average number of colonies per culture. Significance among percentages was determined using chi-square analysis.

Chromosome loss assay: The assay is based on loss of chromosome *III*. If *MAT***a**/α diploids lose chromosome *III*, which contains the mating-type locus, these cells can mate with haploid tester strains and can be scored using nutritional complementation. To qualitatively assess chromosome loss, diploids were first grown at 21° in YPA medium to exponential phase. For each diploid strain, ~1000 cells in 25 μl were dotted onto YPA medium and incubated at 28° for 2 days. Cells were then replica plated onto YPA and ~10^6 *MAT*α tester cells (PT2) were dotted on top of the replica-plated patches. These plates were incubated overnight at 25°, replica plated onto minimal media (SD), and incubated at 30° for 2 days to score for mating events.

MAT* locus conversion assay:** To determine whether colonies from the chromosome loss assay were due to *MAT* locus conversion, a genetic strategy was designed. Leu$^+$ diploids PGY 2038 and PGY 2128 were constructed to carry homozygous chromosome *III* except for the 205-kb region that was heterozygous for *URA3*. After incubation at 28°, single colonies of 2×10^6 cells were mixed with 1×10^7 cells of haploid PGY 2026 (Leu$^-$), which has *TRP1* in the 205-kb region of chromosome *III*. Cells were mated for 5 hr at 25° and plated on SC-WL plates that select for mating events. Colonies that grew on these plates were replica plated onto SC-U medium. Triploid cells that have lost chromosome *III* should be Ura$^-$ and carry the wild type and *TRP1* insertion in the 205-kb region. If recombination has resulted in a homozygous *MATa** locus, triploid cells will be Ura$^+$ and carry all three variants (wild type, *URA3*, and *TRP1* insertion) in the 205-kb region. Frequencies of Ura$^+$ cells can be used to measure the rate of *MAT* conversion using the method of the median. The 205-kb region was also amplified in these cells to verify the presence or absence of *URA3* and *TRP1*.

Analysis of Pol1p protein levels: TAP-tagged (Rigaut *et al.*

TABLE 2

Mutation rates in 33-bp poly(GT) tract

Relevant genotype/strain[a]	Rate of 5-FOA^r cells in independent experiments ($\times 10^{-6}$)[b]	Average rate of FOA^r ($\times 10^{-6}$)	Fold over wild type
Wild type/PGY 2005	0.53 (0.21–0.63) 0.28 (0.17–0.39) 0.34 (0.18–0.48)	0.39	1
pol1-1 (Polα)/PGY 2130	24 (13–34) 18 (14–21) 20 (17–22)	21	54
pol1-17 (Polα)/PGY 2140	5.5 (3.9–5.7) 5.1 (4.0–5.8) 4.9 (4.3–5.3)	5.2	13
pol3-t (Polδ)/PGY 2160	5.7 (4.6–6.3) 2.5 (1.7–3.7) 3.1 (2.0–6.4)	3.8	10
pol3-01 (Polδ)/PGY 2170	5.0 (3.8–6.1) 5.7 (3.6–6.5) 4.0 (3.3–5.2)	4.9	13
pol2-4 (Polε)/PGY 2180	0.43 (0.25–0.94) 0.42 (0.25–0.56) 0.78 (0.51–1.2)	0.54	1.4

[a] All strains have been transformed with pSH44.
[b] Numbers in parentheses denote 95% confidence limits.

1999) wild type (PGY 1121), *pol1-1* (PGY 1131), and *pol1-17* (PGY 1141) were grown at 28° to midlog phase in liquid YPA medium. Protein extracts were prepared by breaking cells using glass beads in cell lysis buffer [150 mM NaCl, 25 mM HEPES (pH 7.6), 2 mM EDTA, 2 mM dithiothreitol, 0.5% NP-40, 2× complete protease inhibitors-EDTA free (Roche Molecular Biochemicals)]. Concentrations of the soluble extract were then quantified, normalized, serially diluted twofold, fractionated on an 8% SDS-polyacrylamide gel, and transferred to a polyvinylidene difluoride membrane using standard procedures. Membranes were probed with peroxidase-anti-peroxidase (PAP) antibody (Sigma, St. Louis), which recognizes the protein A module in the TAP tag.

Viability assays: Cells were grown to exponential phase and diluted back to 6.3×10^6 cells/ml. Fivefold serial dilutions (5 μl) were spotted on YPA plates and grown at various temperatures.

RESULTS

Thermosensitive mutations in *POL1* induce a significant mutator phenotype: We employed a frequently used plasmid-based assay to measure repeat tract instability (Henderson and Petes 1992; Strand *et al.* 1993; Sia *et al.* 1997; Kokoska *et al.* 1998, 2000). In this assay, a plasmid (pSH44) containing a $(GT)_{16}$-repetitive tract inserted in frame within the *URA3* gene is transformed into polymerase mutant strains rendering these cells Ura$^+$ (Henderson and Petes 1992). Alterations occurring in the poly(GT) tract that result in out-of-frame insertions, deletions, or in the downstream *URA3* coding sequence inactivate *URA3* expression. The Ura$^-$ cells can then be selected on 5-FOA-containing medium (Boeke *et al.* 1987).

Mutation rates in two *pol1* mutants, *pol1-1* and *pol1-17*, were first compared to two *pol3* mutants (*pol3-01* and *pol3-t*) and a *pol2* mutant (*pol2-4*). The *pol3-t* allele, which is an Asp_{641}-to-Asn_{641} change (Tran *et al.* 1997a), resides between two highly conserved regions in the putative polymerase active site (Wang *et al.* 1997; Franklin *et al.* 2001). Both *pol3-01* and *pol2-4* alleles contain Ala substitutions in the FDIE exonuclease motif that eliminate exonuclease activity and are not thermosensitive (Morrison and Sugino 1994). All strains were analyzed at the semipermissive temperature of 28°. Rates of alteration in pSH44 are shown in Table 2. Mutation rates in the wild type (PGY 2005), *pol3-t* (PGY 2160), *pol3-01* (PGY 2170), and *pol2-4* (PGY 2180) mutants were ~10-fold lower compared to previously published values (Strand *et al.* 1993; Kokoska *et al.* 1998). This difference can be attributed to performing the experiments at a lower temperature of 28°. When wild-type cells were assayed at the previously reported temperature of 32°, the rate of 5-FOA^r colonies rose to levels comparable with previously published values.

As previously reported (Strand *et al.* 1993), the mutation rate in the *pol2-4* mutant is similar to wild type. The mutation rate in *pol1-17* is comparable with both *pol3* mutant alleles; interestingly, the *pol1-1* mutant displayed a four- to fivefold higher mutation rate compared to all other mutant polymerase alleles. These results indicate that *POL1* plays a role in mutation avoidance, similar to that of *POL3*.

***pol1* mutants induce two classes of mutagenic effects on the $(GT)_{16}$ tract:** We further analyzed the types of

TABLE 3

Types of alterations in 33-bp poly(GT) tract

Relevant genotype/strain	No. of tracts sequenced	No. of tracts containing indicated no. of base-pair additions (+) or deletions (−)						Total changes in poly(GT)
		−4	−2	0	+2	+4	Other[a]	
Wild type/PGY 2005	50	0	3	10	26	2	9[1(+14), 7(−14), 1(−16)]	40
pol1-1/PGY 2130	145	4	7	94	18	3	19[1(+8), 1(+10), 1(+14), 7(−10), 5(−14), 3(−16), 1(−20)]	51
pol1-17/PGY 2140	81	2	0	35	28	1	15[2(+14), 1(−8), 1(−10), 4(−14), 6(−16), 1(−20)]	46

[a] Numbers outside of brackets denote the total number of "Other" changes while numbers outside of parentheses indicate the frequency of the change enclosed in the parentheses.

mutations that accumulated in these two *pol1* mutants and compared them to the wild type (Table 3). In the wild type, 20% of the plasmids isolated from 5-FOA^r colonies (10 out of 50) did not carry any change in the poly(GT) tract, while 52% of the plasmids harbored one repeat unit additions. Neither proportion was significantly different from previously published data using the Fisher exact test (Sia *et al.* 1997).

Analysis of pSH44 isolated from 5-FOA^r colonies in *pol1-1* (PGY2130) mutant cells revealed that only 54 of 145 events occurred in the poly(GT) tract (Table 3), suggesting that microsatellite destabilization was not the principal cause for the elevated mutation rate observed in the *pol1-1* mutant (Table 2). In contrast to *pol1-1*, the *pol1-17* mutant carried similar proportions of alterations inside and outside the poly(GT) tract (35/81 outside and 46/81 inside the tract; Table 3), suggesting that the mutation in the *pol1-17* strain had a greater impact on the repeat tract than in *pol1-1*. The extent and nature of microsatellite instability was further evaluated in each strain (Figure 1). The *pol1-1* and *pol1-17* mutant exhibited a 24- and 10-fold increase in mutation rate, respectively, compared to wild type. Interestingly, the *pol1-1* mutation induced a 179-fold higher mutation rate than wild type in sequences outside of the poly(GT) tract, whereas the effect induced by the *pol1-17* mutation was 29-fold. When the rates of poly(GT) tract insertions and deletions were analyzed, the *pol1-1* mutation was found to preferentially induce deletions, while the *pol1-17* mutation induced both deletions and insertions at comparable rates (Figure 1).

These data indicate that *pol1-1* is a stronger mutator than *pol1-17*, exhibiting a twofold and sixfold higher relative mutation rate inside and outside of the repeat tract, respectively. These results also indicate that mutations in *POL1* induce two distinct types of mutations: changes in the microsatellite tract and changes outside the tract, presumably downstream in *URA3*.

***pol1-1* and *pol1-17* exhibit differential mutator activities:** To test whether changes outside of the poly(GT) tract in both *pol1-1* and *pol1-17* occurred in microsatellite sequences endogenous to the *URA3* gene, pSH44 was isolated from 5-FOA^r colonies that contain no changes in the poly(GT) tract and was digested with *Hin*dIII to produce a 3.6- and a 4.3-kb fragment (Figure 2). The majority of plasmids isolated from *pol1-17* did not exhibit any apparent size changes (right, Figure 2), indicating that alterations in the *URA3* coding region in *pol1-17* were either small changes or point mutations. In contrast, a large portion of 5-FOA^r isolates from *pol1-1* colonies had apparent size changes (left, Figure 2). These changes were grouped into two categories for both *pol1-17* and *pol1-1*: gross alterations (including deletions, insertions, and complex changes) and no detectable size change (Table 4). Thus, a greater number of plasmids in the *pol1-1* strain (38 out of 54) exhibited gross alterations compared to *pol1-17* (5 out of 24; $P <$

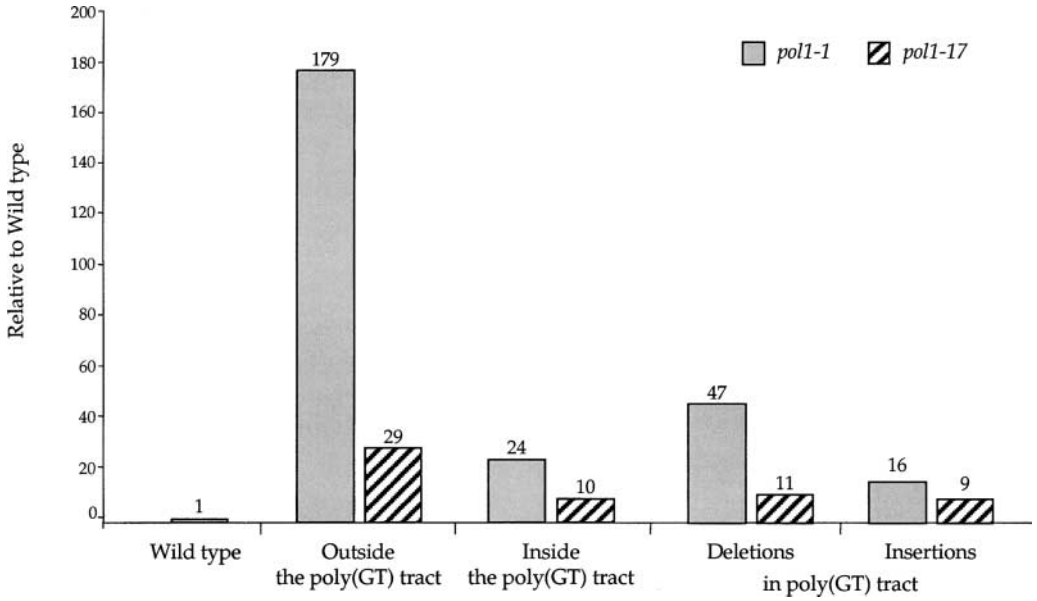


FIGURE 1.—Normalized rates of poly(GT) tract instability in *pol1* mutants. Values from Tables 2 and 3 were used to determine poly(GT) tract instability by separating the rates of alteration within the poly(GT) tract and outside it. The rate of instability outside the poly(GT) tract was calculated by multiplying the rate of 5-FOA-resistant cells in *pol1* mutants by the proportion of alterations that occurred outside the poly(GT) tract, while repeat tract instability was derived by multiplying the fraction of tracts that had alterations by the rate of 5-FOA^r cells. The analogous method was used to calculate rates of deletions and insertions in the repeat tract. Rates are calculated with 95% confidence intervals (noted in parentheses) and presented as 10^{-6}/cell division. The rate of instability outside the poly(GT) tract for wild type was 0.073 (0.032–0.11); for *pol1-1*, 13.0 (11–15); and for *pol1-17*, 2.1 (1.5–2.6). The rate of instability inside the poly(GT) tract for wild type was 0.29 (0.25–0.33); for *pol1-1*, 71.0 (55–87); and for *pol1-17*, 2.8 (2.0–3.6). The rate of deletion for the wild type was 0.081 (0.038–0.12); for *pol1-1*, 3.8 (2.5–5.1); and for *pol1-17*, 0.92 (0.54–1.4). The rate of insertion for the wild type was 0.21 (0.16–0.26); for *pol1-1*, 3.3 (2.1–4.5); and for *pol1-17*, 1.9 (1.4–2.4). The wild type, *pol1-1* mutant, and *pol1-17* mutant are denoted by the open bar, shaded bar, and striped bar, respectively. Bar graph shows rates relative to the wild type.

0.01 by the Fisher exact test). When the rates of gross alterations and "no detectable size change" alterations were calculated and compared to the wild type (Table 5), *pol1-1* and *pol1-17* displayed elevated rates of "no detectable size change" alterations that were 85- and 38-fold, respectively. The *pol1-1* mutant had an ~300-fold higher rate of gross alterations compared to the wild type (Table 5).

To further characterize the types of changes induced in *pol1-1* and *pol1-17*, we sequenced representative samples from each group. As expected, those in the "no detectable size change" category revealed base substitutions in the *URA3* gene (data not shown). Those alterations categorized as deletions or complex changes, mostly observed in *pol1-1*, were found to be either large deletions (~2–5 kb) extending into the vector or deletions within the *URA3* gene. These deletion mutations were of sequences that had been flanked by short direct repeats and were reminiscent of the changes occurring in mutants of *pol*α^+ in *Schizosaccharomyces pombe* (LIU *et al.* 1999) and *POL3* (*pol*δ) in *S. cerevisiae* (TRAN *et al.* 1995, 1996). Interestingly, all of the insertion mutations in *pol1-1* (as in lanes 7 and 10, Figure 2) were identified as Ty1 transposon sequences resulting in *URA3* gene disruptions, probably caused by a recombination event involving the *ura3-52* locus (the *ura3-52* mutation is a Ty1 transposon disruption of the *URA3* gene; ROSE and WINSTON 1984).

To determine whether the mutator phenotype induced by the *pol1-1* and *pol1-17* mutations was also observed in a genomic context, the forward mutation rate was measured at the *CAN1* locus. Mutation rates (expressed as 10^{-7}/cell division with 95% confidence intervals in parentheses) for the wild type, *pol1-1*, and *pol1-17* strains were 1.6 (1.4–1.7), 9.7 (8.5–12), and 5.0 (3.6–5.4), respectively. Although *pol1-1* and *pol1-17* exhibited only a sixfold and threefold increase over the wild type, respectively, the same trend was observed. PCR amplification of ~20 Can^r colonies from each strain followed by *Hph*I digestion (see MATERIALS AND METHODS) revealed that all Can^r isolates from the wild type and the *pol1-17* mutant showed no visible changes. In the *pol1-1* mutant, 4 of 24 isolates showed various deletions and 7 of 24 isolates did not amplify, suggesting that gross deletions occurred in the primer site required for amplification (data not shown). These studies further suggest that the *pol1-1* and *pol1-17* mutant alleles promote genomic instability through different mechanisms.

Mutations induced in *pol1-1* depend on the Rad52p: To investigate whether Rad52p activity could affect the types of mutations exhibited by *pol1-1* and *pol1-17*, double mutants with *rad52*Δ were generated. It was immediately apparent that the *pol1-1 rad52*Δ double mutant had a growth defect compared to both the *rad52*Δ and the *pol1-1* single mutant (Figure 3A). Further analysis revealed that the growth defect in the *pol1-1 rad52*Δ double mutant was due to reduced growth rate (data not shown) and decreased viability, evidenced by a reduction in plating efficiency. In contrast, the growth rates in the *pol1-17 rad52*Δ double mutant and the *pol1-*

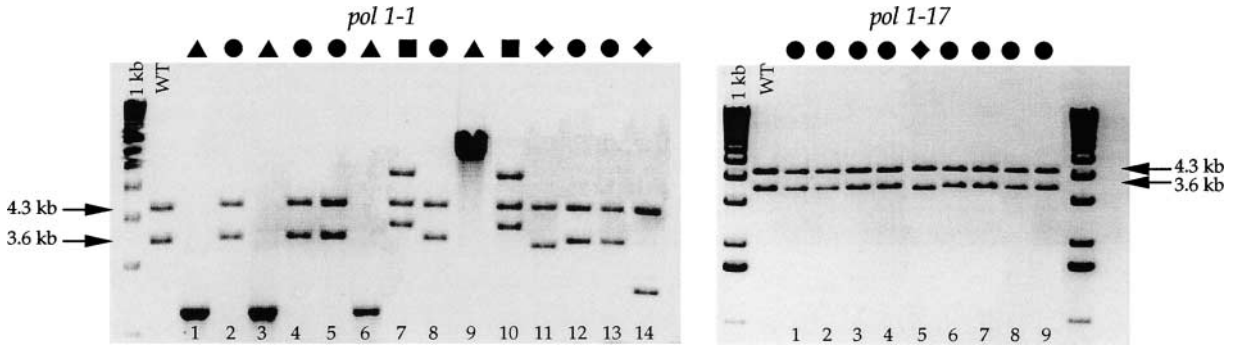


FIGURE 2.—Analysis of 5-FOA-resistant clones with no poly(GT) tract changes. For detection of alterations in 5-FOA-resistant clones that showed no frameshift in the poly(GT) tract, the pSH44 plasmid characterized in HENDERSON and PETES (1992) was digested with *Hin*dIII, producing a 4.3-kb fragment (backbone) and a 3.6-kb fragment [poly(GT)-*URA3* coding sequence]. Alterations were grouped into four classes: (●) no detectable size change (*pol1-1*: lanes 2, 4, 5, 8, 12, and 13; *pol1-17*: lanes 1–4 and 6–9); (■) insertions (*pol1-1*: lanes 7 and 10); (◆) deletions (*pol1-1*: lanes 11 and 14; *pol1-17*: lane 5); and (▲) complex changes (*pol1-1*: lanes 1, 3, 6, and 9).

17 single mutant were comparable. However, it was difficult to discern an effect at 30° since the *pol1-17* single mutant is inherently compromised at that temperature (Figure 3A).

As shown in Tables 6 and 7, the mutation rate in the *pol1-1 rad52*Δ double mutant displayed a threefold decrease when compared to the *pol1-1* single mutant (compare Table 2, 21×10^{-6} in the *pol1-1* single mutant to 6.4×10^{-6} in the *pol1-1 rad52*Δ double mutant in Table 6). This suggests that Rad52p activity affects the mutation rate in *pol1-1*. In contrast, the mutation rate in the *pol1-17 rad52*Δ double mutant did not differ from that of the *pol1-17* single mutant ($P = 0.23$) or from that of the *rad52*Δ mutant ($P = 0.16$; compare 2.7×10^{-6} in the *pol1-17 rad52*Δ double mutant in Table 6 to 5.2×10^{-6} in the *pol1-17* single mutant in Table 2 and to 1.7×10^{-6} in *rad52*Δ in Table 6), suggesting that the *pol1-17* allele was less affected by the absence of Rad52p activity.

Since the *pol1-1* mutation had a 180-fold greater effect compared to wild type on the *URA3* gene downstream of the poly(GT) tract, we analyzed the rate of outside-of-the-tract alterations in the *pol1 rad52*Δ mutants (Figure 3B). Deletion of *RAD52* resulted in a twofold decrease in mutation rate for outside-of-the-(GT)-tract alterations in the *pol1-1* strain, whereas in *pol1-17*, there was no difference (Figure 3B). These results further support the notion that loss of Rad52p activity has a greater effect on the *pol1-1* mutant than on the *pol1-17* mutant.

Restriction analysis of plasmid pSH44 carrying no changes in the poly(GT) tract from 5-FOA^r isolates of the *pol1-1 rad52*Δ double mutant showed that only 13% of plasmids had gross alterations (Table 7), in contrast to the 70% of plasmids that had this mutation type in the *pol1-1* single mutant (Table 7; $P < 0.01$ by Fisher exact test). When the rates of gross alterations and "no detectable size change" alterations were calculated and compared to the wild type, the *pol1-1 rad52*Δ double mutant had a 16-fold lower rate of gross alterations compared to the *pol1-1* single mutant (from 310-fold relative to wild type in the *pol1-1* single mutant to 19-fold in the *pol1-1 rad52*Δ double mutant), while their rates for "no detectable size change" were comparable (from 85-fold relative to wild type to 82-fold in the *pol1-1 rad52*Δ double mutant; Table 7). Taken together, these results suggest that Rad52p activity contributes to the generation of gross alterations in the *pol1-1* strain.

Mutations in *pol1* induce plasmid loss and chromosome loss: Finding that the *pol1-1 rad52*Δ double mutant had a noticeable reduction in plating efficiency when plating cells on medium for plasmid selection (data not shown) led us to investigate whether the *pol1-17* and *pol1-1* mutations could induce plasmid and chromosome loss at the semipermissive temperature (Figure 4).

Plasmid retention in the *pol1-1* and *pol1-17* single mutants was ~90% and comparable to the wild type. The reduced level (69%) of plasmid retention in the *rad52*Δ mutant was epistatic to the *pol1-17 rad52*Δ double mutant (75%), suggesting that lack of Rad52p activity did not promote additional plasmid loss in the *pol1-17* mutant. In contrast, the *pol1-1 rad52*Δ double mutant (46%) displayed a decrease in plasmid retention compared to the *pol1-1* (92%) and *rad52*Δ (69%) single mutants. This suggests that Rad52p activity helps to maintain plasmid stability in the *pol1-1* mutant. Thus, results from the plasmid retention assay revealed further differences between the *pol1-1* and *pol1-17* mutant alleles.

Chromosome *III* loss was then analyzed in these mutants as described in MATERIALS AND METHODS. In the single mutants, the level of chromosome stability seems to correlate with their respective mutation rates (Tables 2 and 3). The weaker mutator (*pol1-17*) shows a lower level of chromosome loss, whereas the stronger mutator (*pol1-1*) shows a higher degree of chromosome instability (top row, middle and right, Figure 4B). Interestingly,

TABLE 4

Types of alterations in *pol1* mutants that have no change in the poly(GT) tract

Relevant genotype/strain	Total no. analyzed	No. of plasmids carrying size changes: gross alterations[a]				No detectable size change
		Deletions	Insertions	Complex	Total	
Wild type/PGY 2005	10	1	3	0	4	6
pol1-1/PGY 2130	54	11	10	17	38	16
pol1-17/PGY 2140	24	2	3	0	5	19

[a] The number in the "Total" column is the sum of alterations in the "Deletions, Insertions, and Complex" columns.

chromosome loss in *pol1-1* did not seem to depend on *RAD52* function (right in Figure 4B; compare top and bottom rows), even though *RAD52* was required for plasmid stability (Figure 4A). Furthermore, finding that the *pol1-17 rad52*Δ double mutant displayed an elevated level of chromosome loss suggests that Rad52p activity is required for preventing chromosome loss, despite the fact that its activity is not required for maintaining plasmid stability (Figure 4A). These data again suggest the intrinsic mechanistic differences between the *pol1-1* and *pol1-17* alleles.

Mutation at the same amino acid in *pol1-1* (Gly^{493}) to Glu has been shown to associate with a hyper-recombination phenotype (Aguilera and Klein 1988). Hence, higher papillation observed for the *pol1-1* mutant in the chromosome loss assay could possibly result from an increase in recombination activity that generates homozygous diploids for the *MAT* locus. To test this possibility, a genetic strategy was designed to measure the rate of *MAT* conversion in the *pol1-1* mutant. Wild type (PGY 2038) and *pol1-1* (PGY 2128) diploids heterozygous for a *URA3* insertion at the 205-kb region of chromosome *III* were generated. Mating these diploids with PGY 2026, which contains a *TRP1* insertion in the 205-kb region of chromosome *III*, can differentiate chromosome loss and *MAT* locus conversion. Cells that have lost chromosome *III* will be Ura^-, while cells that have undergone *MAT* conversion will be Ura^+. We found that the rate of *MAT* conversion in the *pol1-1* mutant was 7.6×10^{-7} while the rate of chromosome loss was 4.5×10^{-5} (Figure 4C). These results support the notion that the high papillation is due primarily to chromosome loss.

Levels of Pol1p in *pol1-1* and *pol1-17*: It has previously been reported that reduced expression of DNA polymerase δ in a cell can lead to a mutator phenotype that is manifested by an increase in deletion mutations (Kokoska *et al.* 2000). To test whether the deletion mutations observed in *pol1-1* and *pol1-17* are caused by a decrease of the mutant Pol1p level, the levels of TAP-tagged Pol1p were measured in these mutants and compared to the wild-type TAP-tagged Pol1p (Figure 5). A slight decrease of Pol1p was noted in the *pol1-17* mutant. However, there was no apparent difference in Pol1p levels between the *pol1-1* mutant and the wild type. These data suggest that the mutator effects exhibited in the *pol1-1* mutant are not due to a reduction in Pol1p expression.

Genetic interactions between *pol1* mutants and MMR genes: In budding yeast, there are two heterotetrameric complexes of mismatch repair proteins. One is composed of the *MSH2*, *MSH6*, *PMS1*, and *MLH1* gene products and primarily repairs single-base mismatches but also recognizes small insertion/deletion loops (Johnson *et al.* 1996b; Marsischky *et al.* 1996). The other complex, in which *MSH6* is replaced with *MSH3*, repairs

TABLE 5

Rates of alterations in *pol1* mutants that have no change in the poly(GT) tract

Relevant genotype/strain	No detectable size change		Gross alterations	
	Rate/division[a]	Relative to WT	Rate/division[a]	Relative to WT
Wild type/PGY 2005	4.7×10^{-8}	1	3.1×10^{-8}	1
pol1-1/PGY 2130	4.0×10^{-6}	85	9.6×10^{-6}	310
pol1-17/PGY 2140	1.8×10^{-6}	38	4.7×10^{-7}	15

[a] Rates of "no detectable size change" and "gross alterations" were calculated by first multiplying the rate of 5-FOA^r colonies by the fraction of changes outside of the poly(GT) tract (data from Tables 2 and 3) and then multiplying this value by the fraction of changes observed as "no detectable size change" or "gross alterations" (data from Table 4).

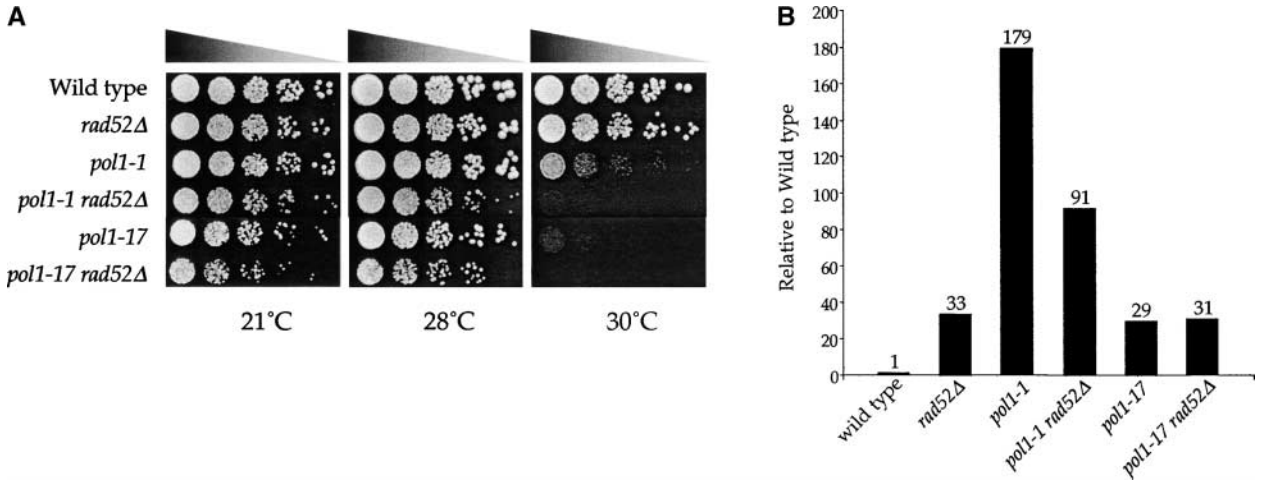


FIGURE 3.—Viability and mutation rates in *pol1 rad52*Δ double mutants. (A) Fivefold serial dilutions of each strain, starting from a cell density of 6.3×10^6 cells/ml, were spotted on YPA plates and incubated as described in MATERIALS AND METHODS. (B) Genomic instability outside of the poly(GT) tract. Mutation rates for alterations outside of the poly(GT) tract were derived as described above in Figure 1 using frequency and rate data from Tables 6 and 7 with 95% confidence intervals noted in parentheses and presented as 10^{-6}/cell division. The rate for *rad52*Δ was 2.4 (2.1–2.7); for *pol1-1 rad52*Δ 6.7 (5.9–7.5); and for *pol1-17 rad52*Δ 2.2 (1.8–2.6). These rates were divided by the wild-type rate and presented in the bar graph.

small loops up to eight bases, but is unable to correct loops that are ≥20 bp (SIA *et al.* 1997). Finding that the majority of the changes in *pol1-1* and in *pol1-17* were either frameshift or base substitution mutations (as shown by summing together the fraction of frameshifts from Table 3 with the fraction of "no detectable size change" from Table 4) and that a putative active site *pol1* mutant (*pol1-Y869A*) had a strong mutator effect when combined with *pms1* (PAVLOV *et al.* 2001) led us to investigate whether various defects in DNA mismatch repair could alter the mutation rate and spectra of these *pol1* mutants.

Both *pol1-17* and *pol1-1* alleles were independently combined with deletions in *PMS1, MSH6*, or *MSH3* to generate double mutants. There was no difference in the growth rate or viability between any of these double mutants and the wild-type or the single-mutant strains, except for a slight but reproducible decrease in viability in the *pol1-1 pms1*Δ mutant at 32° (data not shown). The mutation rate (Table 8) and mutation spectra (Table 9) in these mutants were then analyzed.

The mutation rate in the *pol1-17 pms1*Δ double mutant was similar to that of the *pms1*Δ single mutant, both exhibiting a 620-fold increase over the wild type. In contrast, the 2700-fold mutation rate increase in the *pol1-1 pms1*Δ double mutant over the wild type was synergistic with respect to either of the single mutants (Table 8). When the mutation spectrum was determined in *pms1*Δ and *pol1-1 pms1*Δ (Table 9), almost all changes occurred in the poly(GT) tract (for changes outside of the GT tract, 0/71 in *pol1-1 pms1*Δ *vs.* 5/71 in *pms1*Δ is not significant). Moreover, both *pms1*Δ and *pol1-1 pms1*Δ displayed similar proportions of insertions (33/71 and 32/71) and deletions (33/71 and 39/71), respectively

TABLE 6

Rates and types of alterations in *pol1 rad52*Δ double mutants within poly(GT) tract

Relevant genotype/strain[a]	Rate of 5-FOA^r cells in independent expts ($\times 10^{-6}$)[b]	Average rate of FOA^r ($\times 10^{-6}$)[c]	No. of tracts containing indicated no. of base-pair additions (+) or deletions (−)						Total tracts analyzed
			−4	−2	0	+2	+4	Other	
*rad52*Δ/PGY 2321	1.3 (0.90–2.5) 2.1 (1.7–3.4)	1.7 (4)	0	0	72	20	2	3 [1(−8), 2(−10)]	97
*pol1-1 rad52*Δ/PGY 2331	7.8 (5.9–13) 5.0 (3.0–7.4)	6.4 (17)	0	3	87	10	1	24 [2(−8), 1(−10), 8(−14), 5(−16), 5(−20), 3(−22)]	125
*pol1-17 rad52*Δ/PGY 2341	1.8 (0.68–2.0) 3.6 (2.6–4.8)	2.7 (7)	2	4	47	22	2	9[1(+8), 2(+14), 3(−10), 1(−14), 1(−16), 1(−22)]	86

[a] All strains were transformed with pSH44.
[b] Numbers in parentheses denote 95% confidence limits.
[c] Number in parentheses denote rates of 5-FOA resistance relative (fold) to the wild-type strain.

(Table 9). These data suggest that Pol1p carrying the *pol1-1* mutation induces alterations in the poly(GT) tract that can ordinarily be corrected by postreplication MMR.

When analyzing the two *pol1* mutants in the *MSH6* deletion background, both *pol1-1 msh6*Δ and *pol1-17 msh6*Δ double mutants showed an ~300-fold increase in mutation rate compared to wild type (Table 8). When comparing rates to their corresponding single mutants, the *pol1-17 msh6*Δ double mutant displays a greater than multiplicative effect, implying that deletion of *MSH6* has a greater effect on the *pol1-17* allele (Table 9). This is consistent with the *pol1-17* mutation's preference for inducing base substitutions (Tables 4 and 5). The mutation spectra in *msh6*Δ, *pol1-1 msh6*Δ, or *pol1-17 msh6*Δ did not differ from each other in the fraction of alterations occurring within or outside the poly(GT) tract (see Table 9). More than 60% of the alterations in these double mutants occurred outside of the poly(GT) tract (compare number in "0" column with number of tracts sequenced in Table 9). Since this was the signature spectrum for the *pol1-1* mutant as well, ~30 pSH44 isolates from FOA^r *msh6*Δ, *pol1-1 msh6*Δ, and *pol1-17 msh6*Δ colonies were digested with *Hin*dIII to detect large changes in the poly(GT)-*URA3* sequence. None of the isolates showed any detectable size changes. Hence, these results strongly suggest that *MSH6* deletion induces an increase in point mutations in both *pol1-1* and *pol1-17*, with *pol1-17* exhibiting a greater effect.

In the *MSH3* deletion background, both *pol1-1 msh3*Δ and *pol1-17 msh3*Δ exhibited an increase in mutation rate compared to their respective single mutants ($P < 0.01$; Table 8). Combining the *pol1-17* and *msh3*Δ mutations had an additive effect on the mutation rate, whereas in the *pol1-1 msh3*Δ double mutant the increase in mutation rate was four times that of an additive effect. This suggests that deletion of *MSH3* has a greater impact on the mutator phenotype of *pol1-1* than on the *pol1-17* mutant. The mutation spectra in the *pol1-1 msh3*Δ and *pol1-17 msh3*Δ double mutants were very similar to *msh3*Δ alone, carrying no alterations outside of the poly(GT) tract (Table 9).

Taken together, these results indicate that deletion of mismatch repair genes produces a synergistic effect on the mutation rates in *pol1* mutants, thus suggesting an interplay between Pol1p and the MMR system.

DISCUSSION

Analyses of the mutator phenotypes induced in two distinct *pol1* conditional mutants have shown that (i) compromising *POL1* function can induce genome instability by displaying elevated mutation rates and by promoting plasmid and chromosome loss (Tables 2 and 3; Figure 4); (ii) specific mutations of *POL1* can generate distinct types of mutations resulting in gross alterations, frameshifts, and base substitutions (Tables 3, 4, and 5; Figures 2 and 3); and (iii) the distinct types of genomic

TABLE 7

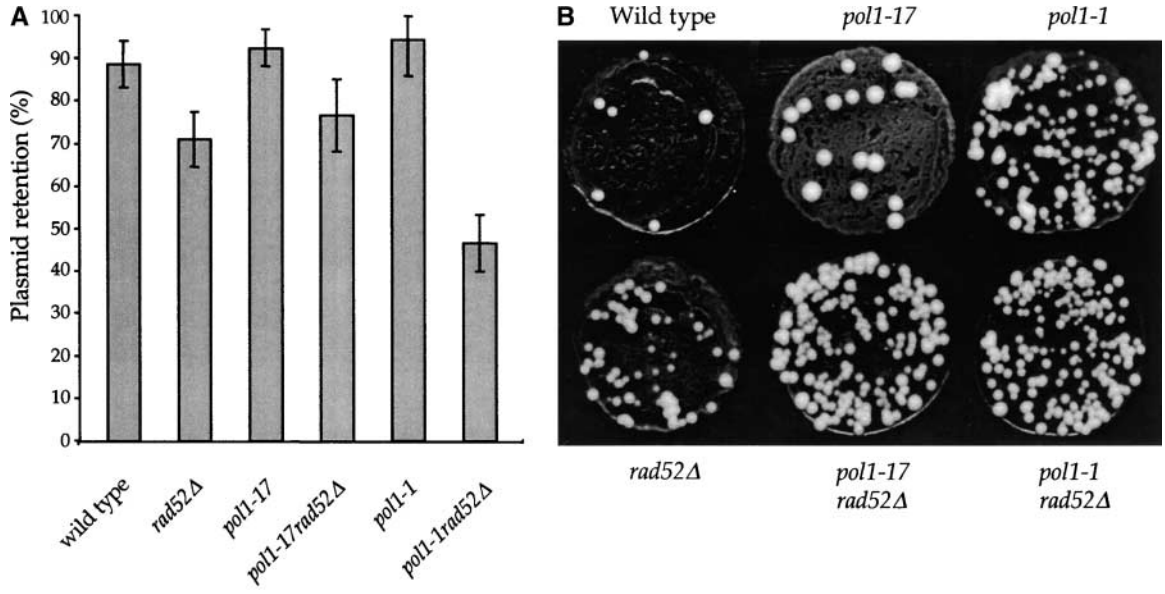
Rates and types of alterations in *pol1 rad52*Δ double mutants outside of poly(GT) tract

Relevant genotype/strain	No. of plasmids analyzed	Gross alterations								
		Plasmids with specific changes						No detectable size change		
		Deletions	Insertions	Complex	Total[a]	Rate/division[c]	Relative to WT	Plasmids with no changes	Rate/division[c]	Relative to WT
*rad52*Δ/PGY 2321	7	0	0	0	0	$<1.8 \times 10^{-7}$	<5.8	7	1.3×10^{-6}	27
pol1-1/PGY 2130	54	11	10	17	38	9.6×10^{-6}	310	16	4.0×10^{-6}	85
*pol1-1 rad52*Δ/PGY 2331	23	1	0	2	3	5.8×10^{-7}	19 [116][b]	20	3.9×10^{-6}	82

[a] The number in the "Total" column is the sum of alterations in the "Deletions, Insertions, and Complex" columns.

[b] Numbers in brackets indicate fold decrease *vs. pol1-1*.

[c] Rates of "no detectable size change" and "gross alterations" were calculated as in Table 5 using data from Tables 2, 3, and 6. The minimal value was calculated by multiplying the rate of 5-FOA^r colonies by the fraction of changes outside of the poly(GT) tract (data from Tables 2 and 3) and then multiplying by the fraction obtained by dividing 1 by the total of number of tracts analyzed (1/7).



C

Relevant genotype/ Strain	Rate of *MAT* conversion ($\times 10^{-6}$)	Fold over wild type	Rate of chromosome loss ($\times 10^{-6}$)	Fold over wild type
POL1/POL1 URA3/- PGY 2038	N.D.	N.D.	<0.45	1
pol1-1/pol1-1 URA3/- PGY 2128	0.76 (0.45-0.85)	N.D.	45 (40-48)	100

FIGURE 4.—Plasmid and chromosome instability in *pol1 rad52*Δ mutants. (A) Each strain was transformed with the pSH44 plasmid to test for plasmid retention as described in MATERIALS AND METHODS. Error bars represent 95% confidence intervals (see MATERIALS AND METHODS). Average percentages of plasmid retention for the wild type, *rad52*Δ, *pol1-1*, *pol1-17*, *pol1-1 rad52*Δ, and *pol1-17 rad52*Δ were 87, 69, 92, 90, 46, and 75%, respectively. (B) Chromosome *III* loss in *pol1 rad52*Δ mutants was analyzed in exponentially growing homozygous diploid strains (genotype shown in Table 1) as described in MATERIALS AND METHODS. Mating events appeared as papillae after 2 days of incubation at 30°. (C) Rate of *MAT* locus conversion and chromosome *III* loss. The rate of chromosome *III* loss was overestimated in the wild type by taking one colony as the median value. Numbers in parentheses denote 95% confidence intervals. No colonies in the wild type exhibited a conversion event; thus the rate of *MAT* conversion was not determined (ND). *URA3/−* indicates that one chromosome carries the *URA3* insertion while the other contains the intact locus.

instability induced by mutations in *POL1* differentially require *MSH3* or *MSH6* in postreplicative MMR (Tables 8 and 9).

Induction of repeat tract alterations in *pol1* mutants by polymerase slippage: Studies analyzing several mutator alleles of *POL3* have reported dinucleotide repeat instability using the assay system employed in this study (STRAND *et al.* 1993; KOKOSKA *et al.* 1998, 2000). The poly(GT) tract instability in *pol3* mutants is thought to reflect catalytic defects that induce polymerase slippage, since these *pol3* mutant alleles mapped to regions in either the polymerase active site or the exonuclease domain. In contrast to *pol3* mutations, a mutation in *POL2* has shown nominal effects on dinucleotide frameshift (STRAND *et al.* 1993). Both *pol1* mutations described in this study destabilize the microsatellite tract (Figure 1), suggesting that these mutations in *POL1* induce an increased polymerase slippage in the repeat tract similar to that proposed in the *pol3* studies (KOKOSKA *et al.* 1998, 2000).

In vitro reconstituted *SV40* replication experiments have indicated that Pol1p's synthetic contribution is limited to the initiator DNA (iDNA; ~25 nucleotides) synthesis. Moreover, these biochemical studies have suggested that during lagging-strand synthesis, the RNA-iDNA is completely removed by RNase H and Fen-1. These *in vitro* results suggest that Pol1p contibutes little to the main bulk of genomic synthesis (WAGA and STILLMAN 1998). Thus, errors caused by the catalytic function of Pol1p are thought to have a negligible contribution to maintaining the integrity of the genome. Here, we showed that mutations in *POL1* could have an *in vivo* effect comparable to *POL3* (see Tables 2 and 3; Figure 1).

Moreover, the synergistic increase in microsatellite destabilization in the absence of MMR (Table 8 and 9), particularly in *pol1-1*, suggests that a great number of the frameshift alterations induced in these two *pol1* mutants are corrected by postreplication MMR. This suggests that Pol1p has a synthetic contribution in genomic

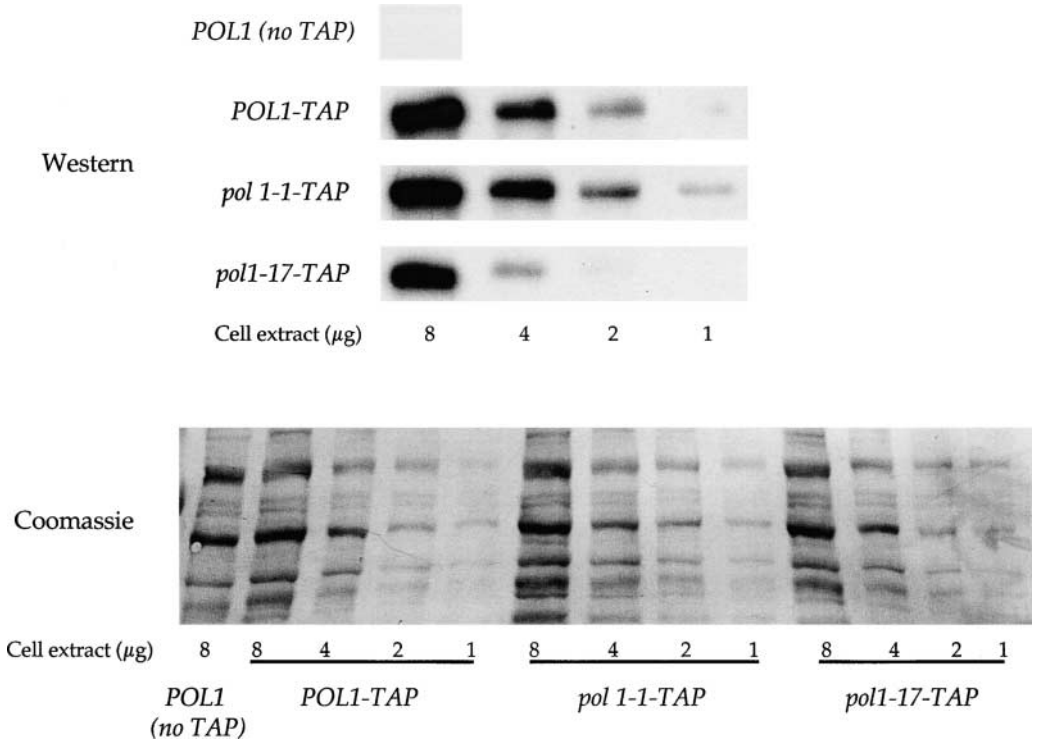


FIGURE 5.—Levels of Pol1p in wild-type, *pol1-1*, and *pol1-17* strains. Cell extracts were prepared from cultures that had been grown at 28°, the temperature used in all genetics assays. Total protein concentration in lysates was normalized and twofold serial dilutions were performed. TAP-tagged Pol1p was detected with the PAP antibody as described in MATERIALS AND METHODS. Coomassie staining of the total protein on the membrane is shown as a loading control.

replication. Alternatively, mutations in *pol1* may lead to lower processivity of Pol1p, hence facilitating primer-template misalignments, which could have an indirect effect on the synthetic activity of Pol3p and/or Pol2p and result in alterations in the poly(GT) tract.

Possible mechanisms that induce the *pol1-1* mutator phenotype: The mutator phenotype exhibited in *pol1-1* suggests that the *pol1-1* mutation causes both polymerase slippage and double-strand breaks (DSBs). Previous studies have also suggested that the *pol1-1* mutant is prone to polymerase slippage since it displays an elevated level of CAG tract instability and an increase in excision of 80-bp hairpins (RUSKIN and FINK 1993; SCHWEITZER and LIVINGSTON 1998). A large fraction of alterations in the pSH44 plasmids with no (GT)-tract changes isolated from *pol1-1* exhibited gross alterations (Table 4). Of these, many were deletions of sequence flanked by short direct repeats that were dependent on Rad52p for their generation (Table 7). As previously proposed in studies with *pol3*, these deletions are likely the result of Rad52p-dependent polymerase slippage and/or the decrease in cellular Pol3p levels (TRAN *et al.* 1995; KOKOSKA *et al.* 2000). As shown in this study (Figure 5), the mutation in the *pol1-1* strain does not affect the cellular Pol1p level. Hence, the deletion mutator phenotype observed in the *pol1-1* mutant is a result of an intrinsic defect and not due to a decrease in Pol1p levels as seen in Pol3p (KOKOSKA *et al.* 2000). It is possible that the mutation in *pol1-1* causes a conformational change in Pol1p that compromises protein-protein interactions, thus resulting in a mutator phenotype.

Another portion of the mutations seen in *pol1-1* were insertions resulting from recombination with the *ura3-52* locus, which normally is the consequence of DSBs. Since induced DSBs are not repaired in a *rad52*Δ background, they can be indirectly reflected in plasmid retention and chromosome loss assays. Consistent with the notion that DSBs are occurring in *pol1-1*, the *pol1-1 rad52*Δ double mutant shows a decreased rate of plasmid retention compared to the *pol1-1* single mutant.

pol1-1, however, displays a comparable extent of chromosome loss independent of *RAD52* (compare *pol1-1* and *pol1-1 rad52*Δ in Figure 4B). It is unlikely this is due to the hyper-recombination properties of *pol1-1* (AGUILERA and KLEIN 1988; LUCCHINI *et al.* 1990) since the rate of *MAT* locus conversion was 100-fold lower than the rate of chromosome loss (Table 8). The similar extent of chromosome loss observed in *pol1-1* and *pol1-1 rad52*Δ mutants may in turn reflect a saturation of chromosome loss events that are unable to be differentiated in the assay.

Although the *pol1-1* mutation is remotely located from the polymerase active site, there is an 85-fold induction of base substitutions in *pol1-1* (Table 5). Several scenarios could explain this result. It is possible that the Gly-to-Arg substitution in *pol1-1* may induce a conformational change that affects its catalytic function, thus resulting in base substitutions. Alternatively, the strong positive charge of this Arg residue could alter the accessibility or affinity of polymerase to the DNA backbone, thus indirectly affecting the polymerase DNA synthetic function.

These base substitutions could also be generated by activation of translesion synthesis. A recent fission yeast study has shown that mutagenic synthesis by DinB, a

TABLE 8

Rate of FOA-resistant colonies in *pol1* and mismatch repair mutants

Relevant genotype/strain[a]	Rate of 5-FOA^r cells in independent experiments ($\times 10^{-6}$)[b]	Average rate of FOA^r ($\times 10^{-6}$)	Fold increase over wild type
*pms1*Δ/PGY 2221	230 (220–270) 320 (260–380) 170 (130–220)	240	620
*pol1-1 pms1*Δ/PGY 2231	1200 (1000–1480) 1400 (980–1990) 530 (430–640)	1040	2700
*pol1-17 pms1*Δ/PGY 2241	100 (100–210) 240 (220–280) 380 (320–420)	240	620
*msh6*Δ/PGY 2721	6.9 (4.5–7.6) 6.0 (4.5–7.8)	6.5	17
*pol1-1 msh6*Δ/PGY 2731	63 (36–79) 150 (88–170) 120 (88–170)	110	290
*pol1-17 msh6*Δ/PGY 2741	130 (87–160) 57 (26–68) 180 (150–200)	120	320
*msh3*Δ/PGY 2621	41 (31–44) 28 (22–32) 34 (31–42)	33	87
*pol1-1 msh3*Δ/PGY 2631	220 (170–310) 250 (230–280) 270 (220–300)	240	630
*pol1-17 msh3*Δ/PGY 2641	57 (48–74) 70 (31–93) 30 (28–45)	52	140

[a] All strains were transformed with pSH44.
[b] Numbers in parentheses denote 95% confidence limits.

translesion polymerase, accounts for the elevated mutation rate and accumulation of point mutations in a *pol*α mutant when the cell activates checkpoint function in response to replication stress (Kai and Wang 2003). Thus, mutation in *pol1-1* could cause a replication perturbation, thereby inducing the checkpoint response that activates mutagenic translesion synthesis, resulting in base substitution mutations.

The possible mechanisms that induce the *pol1-17* mutator phenotype: The *pol1-17* mutant exhibits a higher base substitution mutator phenotype than the *pol1-1* mutant does. Consistent with this mutator phenotype in *pol1-17*, the *pol1-17 msh6*Δ double mutant exhibited a synergistic increase in mutation rate and the expected mutation spectrum (Tables 8 and 9). This suggests that the *pol1-17* mutation induces base substitutions that are often corrected by *MSH6*. This effect may reflect the *pol1-17* mutation's location in the polymerase structure, since the *pol1-17* mutation maps to the active site of Polα between the metal-activator-binding region I and nucleotide-binding region III (Copeland *et al.* 1993; Copeland and Wang 1993; Dong and Wang 1995). A mutation in the Pol1p catalytic domain may compromise the primer-template interaction, resulting in a decrease of fidelity and thus generating base substitutions. The ability of the *pol1-17* mutation to generate base substitutions supports the notion that Pol1p contributes to the synthesis of genomic DNA sequence. As discussed above for the mechanisms that induce *pol1-1* mutator phenotype, the *pol1-17* mutation could also cause replication stress and induce mutagenic synthesis via the checkpoint response, generating base substitutions (Kai and Wang 2003).

The *pol1-17* mutant also displays a small fraction of deletion mutations (Table 4) that suggest a polymerase slippage mechanism. The slightly reduced level of Pol1p in the *pol1-17* strain (Figure 5) may promote these deletion mutations as seen in Pol3p (Kokoska *et al.* 2000). The plasmid retention assay supports the notion that the *pol1-17* mutation does not seem to induce a large level of DSBs (compare *rad52*Δ, *pol1-17*, and *pol1-17 rad52*Δ in Figure 4A), which correlates with the reduction of gross alterations in this mutant. However, the incidence of DSBs reflected in the chromosome loss assay is surprisingly high (*pol1-17 rad52*Δ, Figure 4B). This difference may reflect that DSBs occur more fre-

TABLE 9

Types of poly(GT) tract alterations in mismatch repair *pol1* mutants

Relevant genotype/strain	No. of tracts sequenced	No. of tracts containing indicated no. of base-pair additions (+) or deletions (−)					
		−4	−2	0	+2	+4	Other[a]
*pms1*Δ PGY 2221	71	3	30	5	33	0	0
*pol1-1 pms1*Δ PGY 2231	71	2	37	0	32	0	0
*msh6*Δ PGY 2721	26	0	1	17	7	0	1(−8)
*pol1-1 msh6*Δ PGY 2731	26	0	2	22	2	0	
*pol1-17 msh6*Δ PGY 2741	25	0	0	15	7	0	3(−14)
*msh3*Δ PGY 2621	42	7	24	0	11	0	0
*pol1-1 msh3*Δ PGY 2631	43	6	20	0	11	5	1(−10)
*pol1-17 msh3*Δ PGY 2641	42	3	20	1	16	0	1(−8), 1(−16)

[a] Numbers outside of parentheses indicate the frequency of the change enclosed in the parentheses.

quently in a genomic context than in a plasmid, since there are many more origin and Okazaki fragment initiations in chromosome *III* than in the pSH44 plasmid, which contains only one replication origin. Hence, a subtle initiation defect in *pol1-17* may be amplified as the chance for generating DSB increases in the context of the chromosome.

***POL1*'s role in mutation avoidance:** Results of this study suggest that *POL1* participates in mutation avoidance in several ways: by suppressing gross alterations, frameshift mutations, and base substitutions. Because Pol1p has an essential role in initiation and lagging-strand synthesis (Waga and Stillman 1998; Bell and Dutta 2002), it is required to interact with a wide variety of proteins involved in these processes. Dysfunction of the replication complex induced by a mutation in *POL1* can stall the replication fork, resulting in mutagenic single-strand DNA. Thus the induction of DSBs or polymerase slippage in the *pol1-1* mutant may reflect perturbations in the replication complex.

Studies in fission yeast have shown that a mutation in *pol*α results in upregulation of DinB for mutagenic synthesis (Kai and Wang 2003). Furthermore, a mutant allele of Polα, *pol*α*ts13*, is able to activate checkpoint effector Cds1 kinase activity and displays synthetic lethality in a *cds1* deletion background (Bhaumik and Wang 1998). These fission yeast studies suggest that mutations in Polα perturb the integrity of either the replication complex or the replication fork movement (Bhaumik and Wang 1998; Liu *et al.* 1999; Kai and Wang 2003). Interestingly, fission yeast *pol*α*ts13* carries a three-amino-acid deletion that is localized three residues upstream of the *pol1-1* allele in a highly conserved N-terminal region of Polα.

The fission yeast *pol*α^+ mutational studies, together with the results from this study, suggest that mutations in this highly conserved N-terminal region of Pol1p, thought to map to the surface of the polymerase, will compromise interactions with other cellular factors during initiation and lagging-strand synthesis, thus affecting genome stability. Thus mutations in the protein-protein interaction domains of replication proteins not only may provide a larger mutational target for mutator phenotype induction, but also may affect a variety of cellular processes essential for mutation avoidance.

We thank Tom Petes and David Botstein for providing us various plasmids and yeast strains for our studies. We particularly thank members of our lab for helpful discussions, Carlos Perez and Rose Borbely for their excellent technical help, and Ekaterina Schwartz for helpful advice in budding yeast work. This work is supported by a grant (no. CA14835) from the National Cancer Institute of the National Institutes of Health. P.J.A.G. is a recipient of a Predoctoral Fellowship from the Howard Hughes Medical Institute and Cancer Biology Predoctoral Training Program (grant no. CA09302) from Stanford University.

LITERATURE CITED

Adams-Martin, A., I. Dionne, R. J. Wellinger and C. Holm, 2000 The function of DNA polymerase α at telomeric G tails is important for telomere homeostasis. Mol. Cell. Biol. **20:** 786–796.

Agatep, R., R. D. Kirkpatrick, D. L. Parchaliuk, R. A. Woods and R. D. Gietz, 1998 Transformation of *Saccharomyces cerevisiae* by the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAC/ss-DNA/PEG) protocol. Technical Tips Online (www.umanitoba.ca/faculties/medicine/units/gietz/Trafo.html).

Aguilera, A., and H. L. Klein, 1988 Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae.* I. Isolation

and genetic characterization of hyper-recombination mutations. Genetics **119:** 779–790.

Bell, S. P., and A. Dutta, 2002 DNA replication in eukaryotic cells. Annu. Rev. Biochem. **71:** 333–374.

Bhaumik, D., and T. S.-F. Wang, 1998 Mutational effect of fission yeast Polα on cell cycle events. Mol. Biol. Cell **9:** 2107–2123.

Boddy, M. N., and P. Russell, 2001 DNA replication checkpoint. Curr. Biol. **11:** R953–R956.

Boeke, J. D., J. Trueheart, G. Natsoulis and G. R. Fink, 1987 5-Fluoroorotic acid a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175.

Budd, M., and J. L. Campbell, 1987 Temperature-sensitive mutation in the yeast DNA polymerase 1 gene. Proc. Natl. Acad. Sci. USA **84:** 2838–2842.

Copeland, W. C., and T. S.-F. Wang, 1993 Mutational analysis of the human DNA polymerase α. The most conserved region in α-like DNA polymerases is involved in metal-specific catalysis. J. Biol. Chem. **268:** 11028–11040.

Copeland, W. C., N. K. Lam and T. S.-F. Wang, 1993 Fidelity studies of the human DNA polymerase α. The most conserved region among α-like DNA polymerases is responsible for metal-induced infidelity in DNA synthesis. J. Biol. Chem. **268:** 11041–11049.

Dahlen, M., P. Sunnerhagen and T. S. Wang, 2003 Replication proteins influence the maintenance of telomere length and telomerase protein stability. Mol. Cell. Biol. **23:** 3031–3042.

Delarue, M., O. Poch, N. Tordo, D. Morase and P. Argos, 1990 An attempt to unify the structure of polymerases. Protein Eng. **3:** 461–467.

Diede, S. J., and D. E. Gottschling, 1999 Telomerase-mediated telomere addition *in vivo* requires DNA primase and DNA polymerase α and δ. Cell **99:** 723–733.

Dong, Q., and T. S.-F. Wang, 1995 Mutational studies of human DNA polymerase α: Lysine^{950} in the third most conserved region of α-like DNA polymerase is involved in binding deoxynucleoside triphosphate. J. Biol. Chem. **270:** 21563–21570.

Francesconi, S., W. C. Copeland and T. S.-F. Wang, 1993 In vivo species specificity of DNA polymerase α. Mol. Gen. Genet **241:** 457–466.

Franklin, M. C., J. Wang and T. A. Steitz, 2001 Structure of the replicating complex of a Pol α family DNA polymerase. Cell **105:** 657–667.

Freedman, F., R. Pisani, R. Purves and A. Adhikari, 1991 *Statistics*, pp. 343–360. W. W. Norton, New York.

Glantz, S. A., 1992 *Primer of Biostatistics*. McGraw-Hill, New York.

Gordenin, D. A., A. L. Malkova, A. Peterzen, V. N. Kulikov, Y. I. Pavlov *et al.*, 1992 Transposon Tn5 excision in yeast: influence of DNA polymerases α, δ, and ε and repair genes. Proc. Natl. Acad. Sci. USA **89:** 3785–3789.

Henderson, S. T., and T. D. Petes, 1992 Instability of simple sequence DNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 2749–2757.

Heringa, J., and P. Argos, 1994 Evolution of viruses as recorded by their polymerase sequences, pp. 87–103 in *The Evolutionary Biology of Viruses*, edited by S. S. Morse. Raven Press, New York.

Herskowitz, I., and R. E. Jensen, 1991 Putting the *HO* gene to work: practical uses for mating-type switching. Methods Enzymol. **194:** 132–146.

Holmes, A. M., and J. E. Haber, 1999 Double-strand break repair in yeast requires both leading and lagging strand DNA polymerases. Cell **96:** 415–424.

Ito, J., and D. K. Braithwaite, 1991 Compilation and alignment of DNA polymerase sequences. Nucleic Acids Res. **19:** 4045–4057.

Johnson, R. E., G. K. Kovvali, S. N. Guzder, N. S. Amin, C. Holm *et al.*, 1996a Evidence for involvment of yeast proliferating cell nuclear antigen in DNA mismatch repair. J. Biol. Chem. **271:** 27987–27990.

Johnson, R. E., G. K. Kovvali, L. Prakash and S. Prakash, 1996b Requirement of the yeast *MSH3* and *MSH6* genes for *MSH2*-dependent genomic stability. J. Biol. Chem. **271:** 7285–7288.

Kai, M., and T. S.-F. Wang, 2003 Checkpoint activation regulates mutagenic translesion synthesis. Genes Dev. **1:** 64–76.

Kirchner, J. M., H. Tran and M. A. Resnick, 2000 A DNA polymerase ε mutant that specifically causes +1 frameshift mutations within homonucleotide runs in yeast. Genetics **155:** 1623–1632.

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 δ (*pol3-t*). Mol. Cell. Biol. **18:** 2779–2788.

Kokoska, R. J., L. Stefanovic, J. DeMal and T. D. Petes, 2000 Increased rates of genomic deletions generated by mutations in the yeast gene encoding DNA polymerase δ or by decreases in the cellular levels of DNA polymerase δ. Mol. Cell. Biol. **20:** 7490–7504.

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

Lindahl, T., and R. D. Wood, 1999 Quality control by DNA repair. Science **286:** 1897–1904.

Liu, V. F., D. Bhaumik and T. S.-F. Wang, 1999 Mutator phenotype induced by aberrant replication. Mol. Cell. Biol. **19:** 1126–1135.

Lucchini, G., M. M. Falconi, A. Pizzagalli, A. Aguilera, H. L. Klein *et al.*, 1990 Nucleotide sequence and characterization of temperature-sensitive *pol1* mutants of *Saccharomyces cerevisiae*. Gene **90:** 99–104.

Marsischky, G. T., N. Filosi, M. F. Kane and R. D. Kolodner, 1996 Redundancy of *Saccharomyyces cerevisiae MSH3* and *MSH6* in *MSH2*-dependent mismatch repair. Genes Dev. **10:** 407–420.

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 *Saccharomyces cerevisiae*. Mol. Gen. Genet. **242:** 289–296.

Morrison, A., A. L. Johnson, L. H. Johnston and A. Sugino, 1993 Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. EMBO J. **12:** 1467–1473.

Pavlov, Y. I., P. V. Shcherbakova and T. A. Kunkel, 2001 *In vivo* consequences of putative active site mutations in yeast DNA polymerases α, ε, δ and ζ. Genetics **159:** 47–64.

Pizzagalli, A., P. Valsasnini, P. Plevani and G. Luccini, 1988 DNA polymerase I gene of *Saccharomyces cerevisiae*: nucleotide sequence, mapping of a temperature-sensitive mutation, and protein homology with other DNA polymerases. Proc. Natl. Acad. Sci. USA **85:** 3772–3776.

Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann *et al.*, 1999 A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. **17:** 1030–1032.

Rose, M., and F. Winston, 1984 Identification of a Ty insertion within the coding sequence of the *S. cerevisiae URA3* gene. Mol. Gen. Genet. **193:** 557–560.

Ruskin, B., and G. R. Fink, 1993 Mutations in *POL1* increase the mitotic instability of tandem inverted repeats in *Saccharomyces cerevisiae*. Genetics **134:** 43–56.

Schweitzer, J. K., and D. M. Livingston, 1998 Expansions of CAG repeat tracts are frequent in a yeast mutant defective in Okazaki fragment maturation. Hum. Mol. Genet. **7:** 69–74.

Sherman, F., 1991 Getting started with yeast, pp. 3–21 in *Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego.

Sia, E. A., R. I. 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.

Strand, M., T. A. Prolla, R. M. Liskay and T. D. Petes, 1993 Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature **365:** 274–276.

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

Tran, H., 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.

Waga, S., and B. Stillman, 1994 Anatomy of a DNA replication

fork revealed by reconstitution of SV40 DNA replication in vitro. Nature **369:** 207–212.

Waga, S., and B. Stillman, 1998 The DNA replication fork in eukaryotic cells. Annu. Rev. Biochem. **67:** 721–751.

Waga, S., G. Bauer and B. Stillman, 1994 Reconstitution of complete SV40 DNA replication with purified replication factors. J. Biol. Chem. **269:** 10923–10934.

Walworth, N. C., 2000 Cell-cycle checkpoint kinases: checking in on the cell cycle. Curr. Opin. Cell Biol. **12:** 697–704.

Wang, J., A. K. M. A. Sattar, C. C. Wang, J. D. Karam, W. H. Konigsberg *et al.*, 1997 Crystal structure of a pol α family replication DNA polymerase from bacteriophage RB69. Cell **89:** 1087–1099.

Wang, T. S.-F., 1996 Cellular DNA polymerases, pp. 461–493 in *DNA Replication in Eukaryotic Cells*, edited by M. L. DePamphilis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Wierdl, M., C. N. Greene, A. Datta, S. Jinks-Robertson and T. D. Petes, 1996 Destabilization of simple repetitive DNA sequences by transcription in yeast. Genetics **143:** 713–721.

Wierdl, M., M. Dominska and T. D. Petes, 1997 Microstatellite instability in yeast: dependence on the length of the microsatellite. Genetics **146:** 769–779.

Xie, Y., C. Counter and E. Alani, 1999 Characterization of the repeat-tract instability and mutator phenotype conferred by a Tn3 insertion in *RFC1*, the large subunit of the yeast clamp loader. Genetics **151:** 499–509.

Communicating editor: F. Winston