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Mutagenic and Recombinagenic Responses to Defective DNA Polymerase δ Are Facilitated by the Rev1 Protein in *pol3-t* Mutants of *Saccharomyces cerevisiae*

Erica Mito,*,[1] Janet V. Mokhnatkin,*,† Molly C. Steele,*,‡ Victoria L. Buettner,§ Steve S. Sommer,§ Glenn M. Manthey* and Adam M. Bailis*,[2]

*Division of Molecular Biology, Beckman Research Institute, City of Hope National Medical Center, Duarte, California 91010-3000, †Dual Admissions Program, Miami University of Ohio and University of Cincinnati College of Medicine, Oxford, Ohio 45056, ‡Program in Biological Sciences, University of Chicago, Chicago, Illinois 60637 and §Department of Molecular Genetics, City of Hope National Medical Center, Duarte, California 91010-3000

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ABSTRACT

Defective DNA replication can result in substantial increases in the level of genome instability. In the yeast *Saccharomyces cerevisiae*, the *pol3-t* allele confers a defect in the catalytic subunit of replicative DNA polymerase δ that results in increased rates of mutagenesis, recombination, and chromosome loss, perhaps by increasing the rate of replicative polymerase failure. The translesion polymerases Pol η, Pol ζ, and Rev1 are part of a suite of factors in yeast that can act at sites of replicative polymerase failure. While mutants defective in the translesion polymerases alone displayed few defects, loss of Rev1 was found to suppress the increased rates of spontaneous mutation, recombination, and chromosome loss observed in *pol3-t* mutants. These results suggest that Rev1 may be involved in facilitating mutagenic and recombinagenic responses to the failure of Pol δ. Genome stability, therefore, may reflect a dynamic relationship between primary and auxiliary DNA polymerases.

THE cell has a large arsenal of mechanisms for preventing genome instability in the form of mutations, genome rearrangements, and loss of heterozygosity (LOH). Efficient DNA replication is critical for normal cellular function, not only because of the necessity to duplicate the genetic information, but also because faulty replication influences the spontaneous frequencies of mutation, genome rearrangement, and LOH arising from nicks, gaps, and breaks in DNA (Horiuchi *et al.* 1994; Ivessa *et al.* 2000; Saleh-Gohari *et al.* 2005). While a variety of DNA repair pathways, including homologous recombination, can provide an efficient and effective means of repairing such DNA damage (Michel *et al.* 2001; Garg and Burgers 2005a), without the appropriate controls they themselves may lead to increased genome instability (Petes and Hill 1988). These controls are critically important as elevated genome instability can lead to cell death, tumorigenesis, and the development of a range of complex diseases in humans. The normal function of systems involved in DNA replication, recombination, and repair are crucial as they have interdependent responsibilities in maintaining genomic integrity.

DNA replication in yeast is catalyzed by the primary replicative polymerases α, δ, and ε. Pol α synthesizes the primers for leading and lagging strand synthesis, while Pol δ and Pol ε are responsible for the bulk of bidirectional DNA replication (Garg and Burgers 2005b; Johnson and O'Donnell 2005; Pursell *et al.* 2007; Nick McElhinny *et al.* 2008). Strains carrying mutations in the *POL1*, *POL2* (*CDC17*), and *POL3* (*CDC2*) genes, which encode the catalytic subunits of polymerases α (Budd and Campbell 1987), ε (Boulet *et al.* 1989), and δ (Morrison *et al.* 1990), respectively, display increased rates of spontaneous mutation and recombination (Aguilera and Klein 1988; Gordenin *et al.* 1992; Ruskin and Fink 1993; Zou and Rothstein 1997; Kirchner *et al.* 2000; Pavlov *et al.* 2001; Galli *et al.* 2003; Fortune *et al.* 2005), supporting the link between defective DNA replication and genome instability. In particular, mutations in the *POL3* gene that confer a temperature-sensitive growth defect, most likely by affecting the capacity of the cell to replicate its DNA, also confer elevated rates of spontaneous mutation and recombination with a variety of assays (Gordenin *et al.* 1992, 1993; Tran *et al.* 1995, 1996, 1997, 1999; Kokoska *et al.* 1998; Schweitzer and Livingston 1999; Kokoska *et al.* 2000; Jin *et al.* 2001; Galli *et al.* 2003). One of these mutations, *pol3-t*, is thought to affect the processivity of

[1]*Present address:* Anderson School at UCLA, Global Economics and Management Area, 110 Westwood Plaza; C513 Entrepreneurs Hall, Los Angeles, CA 90095-1481.

[2]*Corresponding author:* Division of Molecular Biology, Beckman Research Institute, City of Hope National Medical Center, 1500 East Duarte Rd., Duarte, CA 91010. E-mail: abailis@bricoh.edu

Pol δ (Gordenin *et al.* 1992; Tran *et al.* 1995; Kokoska *et al.* 2000), which is likely to increase the formation of daughter strand gaps that may be intermediates in the formation of spontaneous mutation and recombination events (Horiuchi *et al.* 1994; Ivessa *et al.* 2000; Michel *et al.* 2001; Minesinger and Jinks-Robertson 2005; Saleh-Gohari *et al.* 2005; Lopes *et al.* 2006).

Failure of a replicative polymerase due to an encounter with a spontaneous or induced DNA lesion that blocks its progress provokes a variety of error-free and error-prone responses mediated by a combination of Rad18- and Rad5-dependent post-replication repair and Rad51-dependent recombination repair (Liefshitz *et al.* 1998; Cejka *et al.* 2001; Minesinger and Jinks-Robertson 2005). However, in replicative polymerase-defective cells, polymerases may fail without encountering polymerase-blocking lesions, raising the possibility that the processes leading to mutation and recombination may also be different.

The translesion polymerases Pol η, Pol ζ, and Rev1 are recruited to DNA lesions that stall replication forks by blocking advancement of the replicative polymerases (Plosky and Woodgate 2004; Fischaber and Friedberg 2005). Pol η, product of the *RAD30* gene (McDonald *et al.* 1997), possesses the active site plasticity to permit accurate bypass of thymine dimers and 8-oxo guanine lesions (Johnson *et al.* 1999; Haracska *et al.* 2000; Prakash *et al.* 2005), but exhibits high rates of misinsertion at other lesions or undamaged nucleotides (Yuan *et al.* 2000). Null alleles of *RAD30* confer sensitivity to UV light, but no effect on UV-induced mutagenesis, and variable effects on spontaneous mutagenesis (McDonald *et al.* 1997; Roush *et al.* 1998), suggesting potential roles in both mutagenic and nonmutagenic lesion bypass mechanisms. Interestingly, Pol η has also been implicated in homologous recombination in chicken cells (Kawamoto *et al.* 2005), while human Pol η can catalyze DNA synthesis from strand invasion intermediates *in vitro* (McIlwraith *et al.* 2005; Rattray and Strathern 2005). Therefore, Pol η may be involved in both mutagenic and recombinagenic responses to stalled replicative polymerases.

Pol ζ is the product of the *REV3* and *REV7* genes (Morrison *et al.* 1989; Lawrence and Hinkle 1996) and is required for most spontaneous mutagenesis (Quah *et al.* 1980; Roche *et al.* 1994; Kunz *et al.* 1998; Endo *et al.* 2007) and for all UV-induced mutagenesis (Lawrence and Christensen 1979; Lawrence and Maher 2001) in yeast. It is also important for seeing the mutations associated with double-strand break (DSB) repair events at the mating-type locus in yeast (Holbeck and Strathern 1997; Rattray *et al.* 2002) and the immunoglobulin genes in mammals (Diaz *et al.* 2001; Zan *et al.* 2001). Perhaps the most relevant biochemical property of Pol ζ is its extraordinary ability to extend from mispaired bases (Prakash *et al.* 2005; Acharya *et al.* 2006), which may make it ideal for catalyzing an extension following base insertion opposite a lesion by Pol η. The capacity to drive DNA synthesis from mismatch-containing substrates is likely to be what enables Pol ζ to function during translesion synthesis (Baynton *et al.* 1998), homologous recombination (Rattray and Strathern 2002; Rattray *et al.* 2003; Sonoda *et al.* 2003; Wu *et al.* 2003), and gross chromosomal rearrangement (Meyer and Bailis 2007). Importantly, a null allele of the *REV3* gene was also previously shown to suppress the mutagenic effect of mutations in the *POL3* gene, including *pol3-t* (Pavlov *et al.* 2001; Northam *et al.* 2006), suggesting that Pol ζ may be engaged following spontaneous replicative polymerase failure.

Rev1, product of the *REV1* gene (Larimer *et al.* 1989), is a polymerase that is required along with Pol ζ for most spontaneous and induced mutagenesis in yeast (Lawrence 2002); however, its limited deoxycytidyl transferase activity (Nelson *et al.* 1996) is not required for its function in mutagenesis (Baynton *et al.* 1999; Haracska *et al.* 2001). Instead, studies *in vitro* suggest that Rev1 enhances the capacity of Pol ζ to extend from mismatches and opposite DNA lesions, perhaps through binding to Rev3 (Acharya *et al.* 2006). Because Rev1 protein levels are 50-fold higher in the G_2/M phase of the yeast cell cycle than in the S phase (Waters and Walker 2006), Rev1/Pol ζ-mediated mutagenesis probably occurs at single-stranded regions after the bulk of replication has been completed (Lopes *et al.* 2006). Restriction of Rev1 activity to the G_2/M phase is also consistent with its potential involvement in homologous recombination as suggested by its requirement for gene conversion at immunoglobulin gene loci in chicken cells (Okada *et al.* 2005). Interestingly, the *REV3* and *REV7* genes were not required for these events, consistent with the suggestion that Rev1 can participate in Pol ζ-dependent and -independent events (Baynton *et al.* 1999; Okada *et al.* 2005).

The work presented here explores the responses by the translesion polymerases Pol η, Pol ζ, and Rev1 to defective polymerase δ in yeast strains bearing the *pol3-t* mutation. We observed that, while the *pol3-t* mutation conferred significantly elevated rates of mutation, recombination, and chromosome loss, null alleles of the *RAD30*, *REV1*, *REV3*, and *REV7* genes alone had few effects. However, combining the *pol3-t* allele with the translesion polymerase mutations revealed that loss of *REV1* consistently suppressed the elevated rates of mutation, recombination, and chromosome loss conferred by *pol3-t*. These results are consistent with Rev1 responding to replicative polymerase failure in *pol3-t* mutant cells by eliciting a broad spectrum of genome-destabilizing events, perhaps by facilitating the interaction of defective Pol δ with daughter strand nicks or gaps.

MATERIALS AND METHODS

Yeast strains, plasmids, and growth conditions: All of the yeast strains used in this study are isogenic with W303-1A (Thomas and Rothstein 1989) and derived from the strains

TABLE 1

Yeast strains used in this study

Strain	Genotype[a]
em398	*MAT***a**/α *ADE2/ade2-1 his3-11,15/his3::URA3::his3 trp1-1/TRP1 POL3/pol3-t RAD30/rad30::LEU2*
em422	*MAT***a**/α *ADE2/ade2-1 HIS3/his3-Δ200 trp1-1/trp1-1::his3-Δ3′::his3-Δ5′::URA3 REV7/rev7::hisG*
em487	*MAT***a**/α *HIS3/his3-Δ200 trp1-1/trp1-1::his3-Δ3′::his3-Δ5′::URA3 POL3/pol3-t RAD30/rad30::LEU2*
em497	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HOM3/hom3-10 pol3-t/pol3-t*
em503	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HOM3/hom3-10 rad30::LEU2/rad30::LEU2*
em508	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HOM3/hom3-10 POL3/pol3-t rad30::LEU2/rad30::LEU2*
em527	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HIS3/his3-11,15 HOM3/hom3-10 TRP1/trp1-1 URA3/ura3-1 POL3/pol3-t RAD30/rad30::LEU2*
em563	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HIS3/his3-11,15 HOM3/hom3-10 URA3/ura3-1*
em582	*MAT***a**/α *ADE2/ade2-1 HIS3/his3-Δ200 TRP1/trp1-1:: his3-Δ3′::his3-Δ5′::URA3 POL3/pol3-t RAD30/rad30::LEU2*
em622	*MAT***a**/α *CAN1/can1-100 HIS3/his3-11,15 HOM3/hom3-10 LEU2/leu2-3,112 TRP1/trp1-1 REV7/rev7::hisG*
em624	*MAT***a**/α *ADE2/ade2-1 HIS3/his3-Δ200 LEU2/leu2-3,112 TRP1/trp1-1:: his3-Δ3′::his3-Δ 5′::URA3 POL3/pol3-t REV7/rev7::hisG*
em629	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 LYS2/lys2-ΔBgl TRP1/trp1-1 POL3/pol3-t REV7/rev7::hisG*
em630	*MAT***a**/α *LEU2/leu2-3,112 TRP1/trp1-1 POL3/pol3-t REV7/rev7::hisG*
em651	*MAT***a**/α *CAN1/can1-100 HIS3/his3-11,15 HOM3/hom3-10 TRP1/trp1-1 rev7::hisG/rev7::hisG*
em658	*MAT***a**/α *ADE2/ade2-1 CAN1/can1-100 HIS3/his3-11,15 HOM3/hom3-10 LYS2/lys2-ΔBgl TRP1/trp1-1 pol3-t/pol3-t rev7::hisG/rev7::hisG*
em682	*MAT***a**/α *CAN1/can1-100 HOM3/hom3-10 LEU2/leu2-3,112 TRP1/trp1-1 URA3/ura3-1 POL3/pol3-t REV1/rev1::HIS3*
em702	*MAT***a**/α *CAN1/can1-100 HOM3/hom3-10 LEU2/leu2-3,112 TRP1/trp1-1 URA3/ura3-1 rev1::HIS3/rev1::HIS3*
em803	*MAT***a**/α *CAN1/can1-100 HOM3/hom3-10 TRP1/trp1-1 URA3/ura3-1 pol3-t/pol3-t rev1::HIS3/rev1::HIS3*
em806	*MAT***a**/α *ADE2/ade2-1 his3-11,15/his3::URA3::his3 LEU2/leu2-3,112 REV7/rev7::hisG*
em841	*MAT***a**/α *ADE2/ADE2 HIS3/his3::URA3::his3 TRP1/trp1-1 POL3/pol3-t rev7::hisG/rev7::hisG*
ABX2196	*MAT***a**/α *ADE2/ade2-1 HIS3/his3::URA3::his3 LEU2/leu2-3,112 REV1/rev1::KAN-MX*
ABX2197	*MAT***a**/α *ADE2/ade2-1 HIS3/his3::URA3::his3 LEU2/leu2-3,112 trp1-1/trp1-1::his3-Δ3′::his3-Δ5′::URA3 REV1/rev1::KAN-MX*
ABX2211	*MAT***a**/α *HIS3/his3::URA3::his3 LEU2/leu2-3,112 URA3/ura3-1 REV1/rev1::KAN-MX POL3/pol3-t*
ABX2212	*MAT***a**/α *HIS3/his3-Δ200 LEU2/leu2-3,112 112 trp1-1/trp1-1:: his3-Δ3′::his3-Δ5′::URA3 REV1/rev1::KAN-MX POL3/pol3-t*
ABX2297	*MAT***a**/α *CAN1/can1-100 HIS3/HIS3 HOM3/hom3-10 TRP1/trp1-1 REV3/rev3::hisG-URA3-hisG POL3/pol3-t*

[a] All strains used in this study were isogenic with W303-1A (*MAT***a**, *ade2-1 can1-100 his3-11,17 leu2-3,112 trp1-1 ura3-1 rad5-G535R*) (Thomas and Rothstein 1989) but carried the wild-type allele of the *RAD5* gene. Only deviations from this genotype are listed. All strains were constructed for this study.

listed in Table 1. All strains used in this study contain the wild-type *RAD5* allele. Standard methods were used for the construction, growth, and maintenance of yeast strains (Burke *et al.* 2000). Isolation of the *pol3-t* mutant allele has been previously described (Kokoska *et al.* 1998). The *pol3-t* allele was incorporated into the W303 strain background by pop-in–pop-out (Rothstein 1991), using the plasmid p171, the generous gift of Dmitri Gordenin, and was maintained in a heterozygous state in diploid strains. Since the *pol3-t* mutation is believed to confer rapid genome destabilization, producing secondary mutations shortly after germination, all strains containing a *pol3-t* allele used in our experiments were derived from spore colonies taken directly from dissection plates that had been maintained at 23° for no longer than 3 days. Segregants containing *pol3-t* were identified by their temperature-sensitive growth at 37°. The *rad30::HIS3*, *rev1::HIS3*, *rev3::hisG-URA3-hisG*, and *rev7::hisG-URA3-hisG* alleles were crossed into our laboratory strains using W303-derived strains that were the generous gift of John McDonald and Roger Woodgate.

The *rad30::LEU2* allele was generated by single-step gene disruption (Rothstein 1991) using a construct generated *in vitro* as described below. Primers P1 (5′-CCT TAT CGC GGC GAA AAA AGC GAC GGT CGA GGA GAA CT C-3′) and P2 (5′-GGT ACT TCG TTC TTC TTA TCG GTT CAA GAA GGT ATT GAC-3′) were used to clone the *LEU2* gene from plasmid pRS415 (Sikorski and Hieter 1989), producing fragments with ends consisting of 18 bp of homology to the genomic sequences immediately flanking the site of *HIS3* marker insertion in the *rad30::HIS3* allele. Primers P3 (5′-CCT GCC GAT CAT AGG ATA CC-3′) and P4 (5′-CTT TTT TCG CCG CGA TAA GG-3′) and primers P5 (5′-GAT AAG AAG AAC GAA GTA CC-3′) and P6 (5′-GAC TTC CAA ATC TCT ATC-3′) were used to clone 155- and 138-bp fragments homologous to sequences upstream and downstream from *rad30::HIS3*, respectively. These fragments each share homology with one end of the fragment produced from pRS415. The three PCR-generated fragments were then used as templates for primers P3 and P6 to produce a single *rad30::LEU2* fragment that was then integrated into the genome using lithium acetate transformation (Schiestl and Gietz 1989; Manthey *et al.* 2004). Segregation against the *rad30::HIS3* allele in genetic crosses and Southern blot analyses (data not shown) were carried out to verify insertion of the *rad30::LEU2* construct into the *RAD30* locus.

The *rev1::KAN-MX* allele was generated by single-step gene disruption as described below. Primers REV1-F3943 (5′-CAA TTC CCA GCT CGT CCC-3′) and REV1R-6530 (5′-GCT CAC TGT GCA ACC ATT CG-3′) were used to amplify a 2587-bp DNA fragment carrying the wild-type *REV1* sequence from genomic DNA. The ends of the fragment were made blunt

with T4 DNA polymerase and cloned into pBlueScript (Stratagene) that had been digested with *Hin*cII to create the plasmid pLAY568. pLAY568 was digested with *Hin*cII to remove 797 bp of DNA encompassing 53 bp of DNA 5′ to the initiation codon for *REV1* and 741 bp downstream. A 1483-bp DNA fragment containing the *KAN-MX* selectable marker generated by *Sma*I and *Eco*RV digestion of the plasmid pFA6-KAN-MX was inserted into *Hin*cII-digested pLAY568 to generate pLAY571. Digestion of pLAY571 with *Xba*I and *Xho*I released a 3290-bp *rev1::KAN-MX* fragment that was electroporated into yeast, followed by selection for resistance to G418. The structure of the disrupted *REV1* locus was confirmed by Southern blot analysis and segregation against the *rev1::HIS3* allele in genetic crosses (data not shown).

Determination of spontaneous mutation rates: Spore colonies were excised from plates containing freshly dissected tetrads incubated at 23° for 2–3 days and dispersed in dH_2O. For the *CAN1* mutation assay, aliquots of cell suspension were plated on synthetic medium lacking arginine and supplemented with 60 μg/ml canavanine and incubated for 4 days at 30°. For the *hom3-10* reversion assay, aliquots of cell suspension were plated on synthetic medium lacking threonine and incubated for 4 days at 30°. Viable counts were determined by plating appropriate dilutions of cell suspension onto synthetic complete medium and incubating for 4 days at 30°. Mutation rates were determined by the method of the median (Lea and Coulson 1949). Confidence intervals were determined as previously described (Spell and Jinks-Robertson 2004). Statistical significance was evaluated using the Mann–Whitney test.

Determination of mutation spectrum by DNA sequence analysis: Single canavanine-resistant colonies were selected from 48 independent cultures of each genotype and genomic DNA was prepared by glass bead disruption and phenol: chloroform extraction. Sequences encompassing the *CAN1* gene and its promoter were amplified from each sample by PCR using the primer pairs 298D (5′-TTT CGA GGA AGA CGA TAA GGT-3′) and 803U (5′-GCA CCT GGG TTT CTC CAA T-3′) and 679D (5′-GAG TTC TGG GTC GCT TCC ATC-3′) and 1841U (5′-GTA TGA CTT ATG AGG GTG AGA-3′). Nucleotide sequences were determined by automated fluorescence sequencing using the primers 276D (5′-TAT TGG TAT GAT TGC CCT TG-3′), 404U (5′-GAA TAT GCC AAA GAA CCC-3′), 679D (5′-GAG TTC TGG GTC GCT TCC ATC-3′), and 1150D (5′-ACA ACC ATT ATT TCT GCC GC-3′). Mutations were confirmed by reamplifying and sequencing in the opposite direction. Statistical significance of the differences in mutation spectrum was evaluated using Fisher's exact test and contingency chi-square analysis.

Determination of spontaneous unequal sister-chromatid recombination rates: Spontaneous unequal sister-chromatid recombination was assayed as previously described (Fasullo and Davis 1987). Briefly, haploid strains that carry a *trp1-1*-linked direct repeat of 5′- and 3′-deleted *his3* sequences, arranged tail-to-head around a *URA3* marker, were used to measure interchromatid recombination. Spore colonies were obtained from freshly dissected tetrads that had been incubated at 23° for 2–3 days and dispersed in dH_2O. Aliquots of suspended cells were plated on synthetic medium lacking histidine and allowed to grow at 30° for 4 days. Viable counts were determined by plating appropriate dilutions onto synthetic complete medium and incubating at 30° for 4 days. Rates of sister-chromatid recombination were determined by the method of the median (Lea and Coulson 1949). Confidence intervals were determined as previously described (Spell and Jinks-Robertson 2004). Statistical significance was evaluated using the Mann–Whitney test.

Determination of intrachromosomal recombination rates: Spontaneous intrachromosomal recombination was assayed using a construction that was previously described (Maines *et al.* 1998). Spore colonies carrying 3′- and 5′-deleted *his3* segments that share 415 bp of *HIS3* coding sequence flanking a *URA3* marker at the *HIS3* locus were dispersed in dH_2O. Aliquots of suspended cells were plated on synthetic medium lacking histidine and incubated at 30° for 4 days to select for recombinants with a complete *HIS3* allele. Viable counts were determined by plating appropriate dilutions on synthetic complete medium and incubating at 30° for 4 days. Recombination rates, confidence intervals, and statistical significance were determined as described above.

Determination of spontaneous chromosome loss and interhomolog recombination rates: Spontaneous loss of chromosome V and interhomolog recombination were assayed as previously described (Klein 2001). Briefly, individual zygotes were micromanipulated onto selective medium to ensure diploidy and allowed to grow at 23° for 3–4 days. Colonies were excised from the plates and dispersed in dH_2O. Aliquots of suspended cells were plated on synthetic medium lacking arginine and supplemented with 60 μg/ml canavanine and incubated at 30° for 3–4 days to determine the number of canavanine-resistant cells. Canavanine-resistant colonies were replica plated to synthetic medium lacking threonine, and the replicas were incubated at 30° for 2 days to determine the fractions of colonies that had acquired their canavanine resistance through interhomolog recombination ($Can^R\ Thr^+$) or loss of chromosome V ($Can^R\ Thr^-$). Viable counts were determined by survival on synthetic complete medium after incubation at 30° for 3–4 days. Recombination and chromosome loss rates, confidence intervals, and statistical significance were determined as described above.

RESULTS

Elevated mutation rates in the *pol3-t* mutant are suppressed by *rev1*Δ: Several studies have documented significantly increased rates of mutation in *pol3-t* mutant strains with a variety of assays (Gordenin *et al.* 1992; Tran *et al.* 1995, 1996; Gordenin and Resnick 1998; Kokoska *et al.* 1998; Galli *et al.* 2003). We observed an ~10-fold increase in the rate of mutation of the *CAN1* gene (Table 2), indicative of a general mutator effect (Whelan *et al.* 1979). The *pol3-t* allele had only a 2.5-fold effect on the rate of reversion of the *hom3-10* allele ($P = 0.001$), a measure of the propensity toward frameshift mutation (Flury *et al.* 1976; Marsischky *et al.* 1996). The modest effect of the *pol3-t* allele on frameshifting in our assays suggests that its effect on the general mutation rate may not be primarily due to slippage of Pol δ during DNA synthesis (Tran *et al.* 1996).

Loss of the translesion polymerases Rev1 and ζ themselves had no significant effect on mutation of *CAN1* or *hom3-10*, as the mutation rates in the *rev1*Δ ($P = 0.8$ or 0.2), *rev3*Δ ($P = 0.6$ or 0.2), and *rev7*Δ ($P = 0.4$ or 0.3) single mutants were not significantly different from wild type (Table 2). Loss of Pol η had a slight but significant effect on mutation of *CAN1* ($P = 0.009$) but no significant effect on the reversion of *hom3-10* ($P = 0.5$). These results suggest that these polymerases have minimal individual impact on spontaneous mutagenesis in our strains. When combined with *pol3-t*, however, loss of Rev1 completely suppressed the elevated rate of

TABLE 2

Mutation rate analysis in wild-type and polymerase mutant strains

Genotype	Mutation rate[a]			
	Can^r ($\times 10^{-7}$)	Fold wild type	Hom$^+$ ($\times 10^{-9}$)	Fold wild type
Wild type	2.5 (1.7–3.1)	1.0	6.4 (4.0–9.0)	1.0
pol3-t	25.9 (17.0–53.7)	10.4	15.8 (11.6–32.7)	2.5
*rad30*Δ	4.1 (2.6–5.1)	1.6	7.0 (5.9–10.1)	1.1
*rev1*Δ	1.9 (1.5–6.0)	0.8	8.5 (7.3–10.9)	1.3
*rev3*Δ	2.0 (1.2–2.7)	0.8	9.0 (5.0–16.0)	1.4
*rev7*Δ	2.2 (1.3–3.4)	0.9	7.6 (6.1–9.7)	1.2
*pol3-t rad30*Δ	31.0 (22.4–39.8)	12.4	60.0 (53.8–76.3)	9.4
*pol3-t rev1*Δ	3.4 (1.9–4.6)	1.4	13.4 (9.3–17.5)	2.1
*pol3-t rev3*Δ	25.2 (17.3–28.0)	10.1	68.0 (46.1–130.0)	10.6
*pol3-t rev7*Δ	25.7 (23.3–38.0)	10.3	73.1 (47.3–125.0)	11.4

[a] Median mutation rates were determined from a minimum of 10 independent cultures of each genotype using the method of the median (Lea and Coulson 1949). Each culture was derived from an independent spore colony obtained by sporulating and dissecting the diploid strains em527, em622, em629, em630, em682, and ABX2297. Confidence intervals (95%) are indicated in parentheses.

CAN1 mutation conferred by *pol3-t*, as the mutation rate in the *pol3-t rev1*Δ double mutant was not significantly different ($P = 0.17$) from that in wild type, indicating that Rev1 facilitates *CAN1* mutagenesis in the presence of a defective Pol δ. In contrast, no significant effect of Rev1 was observed on *hom3-10* reversion in *pol3-t* mutants, as the rate in the *pol3-t rev1*Δ double mutant was not significantly different ($P = 0.84$) from that in the *pol3-t* single mutant, suggesting that Rev1 does not facilitate replicative polymerase slippage. Interestingly, the potent suppressive effect of the *rev1*Δ allele on stimulation of *CAN1* mutation by *pol3-t* was not observed for the *rad30*Δ, *rev3*Δ, or *rev7*Δ alleles, as the rates in the *pol3-t rad30*Δ ($P = 0.98$), *pol3-t rev3*Δ ($P = 0.72$), and *pol3-t rev7*Δ ($P = 0.79$) double mutants were not significantly different from those in *pol3-t* single-mutant cells. This runs counter to the results of previous studies that indicated that *rev3*Δ can suppress the effects of *pol3* alleles, including *pol3-t* (Pavlov *et al.* 2001; Northam *et al.* 2006), on mutagenesis of *CAN1*, suggesting that Pol ζ may exert different effects on mutagenesis in different yeast strains. The *rad30*Δ, *rev3*Δ, and *rev7*Δ alleles, however, do exert an effect on reversion of *hom3-10* in *pol3-t* mutant cells, as the rates are four- to fivefold higher in the *pol3-t rad30*Δ, *pol3-t rev3*Δ, and *pol3-t rev7*Δ double mutants than in the *pol3-t* single mutants. This suggests that Pol η and Pol ζ may suppress slippage of Pol δ in *pol3-t* mutant cells or may promote repair responses that oppose other mechanisms of frameshift formation (Tran *et al.* 1996).

The *pol3-t* mutation confers a distinct mutation spectrum that is not suppressed by *rev1*Δ: The nucleotide sequences of 48 independent *can1* mutations obtained from wild-type and *pol3-t* mutant cells revealed distinct mutation spectra (Table 3, supplemental Table 1). While the distributions of mutations among transitions, transversions, and deletions/insertions were not significantly different ($P = 0.16$), the fraction of deletions >3 bp in length was much greater in the *pol3-t* mutants (29/32) than in the wild type (2/17; $P < 0.0001$). Further, 25 of the 29 deletions in the *pol3-t* mutants were flanked by three to eight nucleotide repeats, whereas only one of the wild-type deletions shared this feature. These results are consistent with previous results demonstrating that *pol3-t* stimulates deletions between repetitive sequences (Gordenin *et al.* 1992; Tran *et al.* 1995, 1996; Kokoska *et al.* 1998, 2000; Galli *et al.* 2003).

While the *rev1*Δ allele had no significant effect on the *CAN1* mutation rate (Table 2), it had a significant effect on the distribution of mutations among transitions, transversions, and deletions/insertions (Table 3; $P = 0.03$). However, *rev1*Δ did not significantly affect the fraction of deletions that were >3 bp in length (1/20; $P = 0.20$). Interestingly, while *rev1*Δ nearly completely suppressed the elevated *CAN1* mutation rate conferred by *pol3-t* (Table 2), it restored neither the distribution of mutations among transitions, transversions, and deletions/insertions ($P = 0.008$) nor the elevated fraction of deletions >3 bp (30/37; $P < 0.0001$) to wild type. Additionally, the ratio of long deletions from the *pol3-t rev1*Δ double mutants that were bounded by 3- to 8-bp repeats (29/30) was not significantly different from that observed for the *pol3-t* single mutant ($P = 0.92$). These results suggest that *rev1*Δ may suppress the incidence of mutation in *pol3-t* mutant cells, but has little effect on the mechanism.

Increased rates of direct repeat recombination in *pol3-t* mutant strains are suppressed by *rev1*Δ: Frequent replicative polymerase failure brought about by decreased processivity might be expected to increase mitotic recombination by promoting the strand inva-

TABLE 3

Characterization of *can1* mutations from wild-type and polymerase mutant strains

	Transitions			Transversions			Deletion[a]		
Genotype	GC > AT	AT > GC	GC > CG	GC > TA	TA > GC	TA > AT	(1–3)	(4–227)	Insertion
Wild type	10 (20)	0 (0)	7 (14)	8 (16)	2 (4)	1 (2)	15 (31)	2 (4)	4 (8)
pol3-t	3 (6)	3 (6)	4 (7)	6 (11)	1 (2)	5 (9)	3 (5)	29 (52)	1 (2)
*rev1*Δ	13 (27)	2 (4)	0 (0)	8 (17)	0 (0)	0 (0)	19 (40)	1 (2)	3 (6)
*pol3-t rev1*Δ	5 (11)	1 (2)	0 (0)	1 (2)	1 (2)	1 (2)	7 (15)	30 (67)	0 (0)

The nucleotide sequences of the *CAN1* gene from 48 independent canavanine-resistant mutants of each gentoype were determined. The numbers of transition, transversion, deletion, and insertion mutations are listed. Percentages of the total are in parentheses.

[a] Deletion mutations were segregated into classes on the basis of the length of sequence deleted. Those in which 1–3 nucleotides were deleted are in the group marked "(1–3)" and those in which 4–227 nucleotides were deleted are in the group marked "(4–227)."

sion of the sister chromatid and repair synthesis (Navarro *et al.* 2007). Alternatively, polymerase failure may increase recombination by leading to the generation of DSBs through endonuclease processing at daughter strand nicks or gaps (Tishkoff *et al.* 1997) or upon collision between a daughter strand nick or gap and a replication fork in the next round of DNA synthesis (Navarro *et al.* 2007). Processes dependent on the presence of homologous sequences on the sister chromatid must occur subsequent to their generation in the S phase of the cell cycle, while other processes may also occur in G_1, utilizing homologous sequences on the same chromatid.

We examined the rates of unequal sister chromatid recombination (USCR) (Fasullo and Davis 1987) in wild-type and polymerase mutant strains to determine the impact of altered polymerase activity on mitotic recombination events that are restricted to the S and G_2 phases in haploid strains. Both the *rev1*Δ ($P = 0.017$) and *rev7*Δ ($P = 0.005$) alleles had significant effects on the rate of USCR, reducing it by four- and sevenfold, respectively (Table 4). This suggests that Rev1 and Pol ζ are required to propagate normal levels of USCR. Interestingly, despite having no significant effect ($P = 0.065$) on its own, the *pol3-t* allele increased the rate of USCR to wild-type levels when combined with *rev1*Δ ($P = 0.084$) and *rev7*Δ ($P = 0.69$). This suggests that the reduced levels of USCR observed in the absence of Rev1 or Pol ζ are observed only when normal Pol δ is present. The *rad30*Δ allele had no significant effect on USCR, either alone ($P = 0.25$) or in combination with *pol3-t* ($P = 0.42$), suggesting that, unlike Rev1 and Pol ζ, Pol η does not play a role in spontaneous USCR.

Deletions by recombination between nontandem direct repeats are thought to occur by a variety of mechanisms, including USCR, intrachromatid crossing over, single-ended invasion, and single-strand annealing (Schiestl and Prakash 1988; Lin *et al.* 1990; Belmaaza and Chartrand 1994). Unlike USCR, the other mechanisms do not require that DNA replication has proceeded through the recombination substrate, suggesting that direct repeat recombination (DRR) may not be restricted to S and G_2 phases. The results of our experiments were similar to those from a number of studies that have demonstrated that the *pol3-t* mutation can increase the rate of DRR (Tran *et al.* 1997; Lobachev *et al.* 1998,

TABLE 4

Recombination rates in wild-type and polymerase mutant haploids

Genotype	USCR[a] ($\times 10^{-5}$)	Fold wild type	DRR[a] ($\times 10^{-4}$)	Fold wild type
Wild type	3.0 (1.9–3.5)	1.0	1.1 (0.9–1.4)	1.0
pol3-t	4.6 (1.9–10.8)	1.5	9.4 (6.9–10.5)	9.4
*rad30*Δ	1.1 (0.3–2.5)	0.4	3.8 (2.1–4.8)	3.5
*rev1*Δ	0.7 (0.5–0.9)	0.2	1.1 (0.9–1.7)	1.0
*rev7*Δ	0.4 (0.1–1.1)	0.1	2.2 (1.3–3.4)	2.0
*pol3-t rad30*Δ	2.4 (0.6–7.0)	0.8	8.2 (4.9–10.9)	7.5
*pol3-t rev1*Δ	4.3 (2.9–5.2)	1.4	6.0 (5.0–6.5)	5.5
*pol3-t rev7*Δ	2.0 (1.2–2.8)	0.7	17.1 (13.0–26.0)	15.6

[a] Median rates of USCR and DRR were determined from a minimum of 10 independent cultures of each genotype using the method of the median (Lea and Coulson 1949). Each culture was derived from an independent spore colony obtained by sporulating and dissecting the diploid strains em398, em422, em487, em582, em624, em806, em841, ABX2211, and ABX2212. Confidence intervals (95%) are indicated in parentheses.

TABLE 5

Chromosome loss and interhomolog recombination rates in wild-type and polymerase mutant diploids

Genotype	CL[a] ($\times 10^{-6}$)	Fold wild type	IHR[a] ($\times 10^{-5}$)	Fold wild type
Wild type	4.5 (3.9–8.9)	1.0	1.7 (1.2–2.2)	1.0
pol3-t	57.0 (26.8–76.4)	12.7	34.6 (23.5–45.5)	20.4
*rad30*Δ	6.8 (4.3–8.7)	1.5	2.4 (1.9–4.7)	1.4
*rev1*Δ	12.5 (10.7–16.2)	2.8	1.0 (0.8–1.7)	0.6
*rev7*Δ	14.7 (11.6–16.0)	3.3	6.2 (4.9–7.2)	3.7
*pol3-t rad30*Δ	49.4 (41.3–61.8)	11.0	20.8 (18.7–28.3)	12.2
*pol3-t rev1*Δ	18.8 (9.3–24.1)	4.2	9.6 (6.7–11.4)	5.7
*pol3-t rev7*Δ	18.2 (13.5–21.9)	4.0	17.1 (14.7–25.0)	10.1

[a] Median rates of CL and IHR were determined from a minimum of 10 independent cultures of each genotype using the method of the median (Lea and Coulson 1949). Each culture was derived from a freshly isolated diploid having the same genotype as those listed for em563, em497, em503, em508, em651, em658, em702, or em803. Confidence intervals (95%) are indicated in parentheses.

2000; Kokoska *et al.* 2000; Galli *et al.* 2003) as the rate was increased approximately ninefold (Table 4). The *rad30*Δ mutation also stimulated DRR by about fourfold, suggesting that Pol η may suppress DRR. While the *rev1*Δ ($P = 0.28$) and *rev7*Δ ($P = 0.07$) mutations alone had no significant effect on the rate of DRR, the *rev1*Δ mutation suppressed the hyperrecombinagenic effect of *pol3-t* nearly twofold ($P = 0.032$), while the *rev7*Δ mutation stimulated it nearly twofold ($P = 0.001$). These results suggest that Rev1 is required to observe the full stimulatory effect of *pol3-t* on DRR, while the presence of Pol ζ inhibits it.

The *rev1*Δ mutation suppresses the stimulatory effects of the *pol3-t* mutation on chromosome loss and interhomolog recombination: Defects in the DNA replication apparatus have been shown previously to increase both chromosome loss (CL) and interhomolog recombination (IHR), presumably in response to an accumulation of lesions such as daughter strand nicks and gaps and DSBs (Haber 1999; Daigaku *et al.* 2006; Navarro *et al.* 2007). Increases in spontaneous IHR have previously been observed in *pol3-t* mutant diploids, consistent with an increase in recombinational responses to replicative polymerase failure (Galli *et al.* 2003). Similarly, we observed an ~20-fold increase in the rate of IHR in *pol3-t*/*pol3-t* homozygous diploid cells (Table 5). This correlated closely with a nearly 13-fold increase in CL in the same strains, consistent with the *pol3-t* mutation causing an increase in recombinagenic lesions that can also disrupt chromosomal transmission, such as DSBs.

The *rad30*Δ allele had no significant effect on the rates of CL or IHR, either alone ($P = 0.66$ or 0.07) or in combination with *pol3-t* ($P = 0.52$ or 0.92), suggesting that Pol η may play no significant role in such events in diploid cells (Table 5). Interestingly, the *rev7*Δ mutation alone led to nearly equivalent, three- to fourfold increases in CL and IHR, suggesting that the absence of Pol ζ may increase the level of recombinagenic lesions in diploid cells. However, combining the *rev7*Δ mutation with the *pol3-t* allele did not have equivalent effects on CL and IHR. The rate of CL in the *pol3-t rev7*Δ double mutant was not significantly different from those in the *rev7*Δ single mutants ($P = 0.08$), indicating that *rev7*Δ was epistatic to *pol3-t*, while the rates of IHR in the *pol3-t rev7*Δ double mutants were not significantly different from those in the *pol3-t* single mutants ($P = 0.58$), indicating that *pol3-t* was epistatic to *rev7*Δ. This suggests that Pol ζ is required to fully stimulate CL in *pol3-t* mutant cells, but does not significantly affect the impact of *pol3-t* on IHR.

In contrast to *rev7*Δ, the *rev1*Δ allele itself did not have equivalent effects on CL and IHR (Table 5), increasing CL by nearly threefold, but having no significant effect on IHR ($P = 0.07$). However, *rev1*Δ did have nearly equivalent, suppressive effects on the stimulation of CL and IHR by *pol3-t*, as the rates of both events were reduced between three- and fourfold in *pol3-t rev1*Δ double-mutant diploids relative to those in *pol3-t* single-mutant diploids. Therefore, unlike Pol ζ, Rev1 is required to fully stimulate both CL and IHR in response to the defective Pol δ encoded by *pol3-t*.

DISCUSSION

The connection between dysfunctional DNA replication and genome instability has been firmly established in yeast by the elevated rates of many types of mutagenic and clastogenic events observed in a variety of DNA replication mutants (Aguilera and Klein 1988; Gordenin *et al.* 1992; Ruskin and Fink 1993; Reagan *et al.* 1995; Ohya *et al.* 2002; Meyer and Bailis 2007). In particular, mutations that disrupt the polymerase function of Pol δ confer increased rates of mutation and recombination (Gordenin *et al.* 1992; Tran *et al.* 1995, 1996, 1997; Kokoska *et al.* 1998, 2000; Lobachev *et al.* 1998, 2000; Galli *et al.* 2003), consistent with incom-

pletely or improperly replicated DNA serving as a source for spontaneous mutation and genome rearrangement. Importantly, Pol δ has also been implicated in post-replication repair (Giot *et al.* 1997; Torres-Ramos *et al.* 1997; Galli *et al.* 2003), suggesting that it could be involved in facilitating mutagenic and recombinagenic responses to lesions created by replicative polymerase failure. The dual role of the DNA replication apparatus in replication and repair suggests that altered levels of mutation and recombination observed in DNA replication mutants may reflect defects in DNA replication, DNA repair, or both.

The *pol3-t* mutation confers a temperature-sensitive growth defect and increased rates of intrachromosomal deletion consistent with a decrease in the processivity of Pol δ (Tran *et al.* 1995; Lobachev *et al.* 1998, 2000; Kokoska *et al.* 2000). Decreased processivity might be expected to lead to an increase in polymerase pausing during replication, which could yield an increase in daughter strand nicks and gaps that have been associated with increased genome instability (Cox 1999; Lehmann and Fuchs 2006; Nagaraju and Scully 2007). This is consistent with the increased rates of mutation, recombination, and chromosome loss observed in this (Tables 2, 4, and 5) and previous studies (Gordenin *et al.* 1992; Tran *et al.* 1995, 1996, 1997; Kokoska *et al.* 1998, 2000; Lobachev *et al.* 1998, 2000; Galli *et al.* 2003). However, Pol δ has also recently been implicated in the repair of DSBs by homologous recombination (Lydeard *et al.* 2007; Maloisel *et al.* 2008), and certain homologous recombination mutants display increased rates of spontaneous mutation, recombination, and chromosome loss (Mortimer *et al.* 1981; Klein 2001; Yoshida *et al.* 2003; Navarro *et al.* 2007). Therefore, the increased genome instability observed in *pol3-t* mutant strains instead may result from increased steady-state levels of DSBs and other lesions that accumulate due to a defect in their repair.

Assaying mutation at the *CAN1* locus is advantageous because it permits the quantitation and characterization of a variety of mutation types (Whelan *et al.* 1979; Tishkoff *et al.* 1997). We observed that the mutation frequency at *CAN1* in the *pol3-t* mutant was elevated ~10-fold (Table 2), consistent with previously published results (Galli *et al.* 2003; Northam *et al.* 2006). The increase in mutation rate was accompanied by a striking increase in the frequency of deletions >3 bp that were bordered by repetitive sequences of 3–8 bp. This is highly reminiscent of the results of previous experiments documenting increases in reversion events involving deletions between short repeats (Tran *et al.* 1995, 1996; Kokoska *et al.* 1998) and is consistent with the view that *pol3-t* promotes increased Pol δ failure and promiscuous reassociation with the template during replication or repair synthesis. Further, it establishes that, like mutants defective for the gene encoding the lagging strand maturation and DNA repair factor Rad27 (Tishkoff *et al.* 1997), *pol3-t* mutants display a signature mutation.

The results of our recombination assays (Tables 4 and 5) generally reflect those of previous experiments that documented substantial increases in DRR and IHR in the *pol3-t* mutant (Gordenin *et al.* 1992; Kokoska *et al.* 2000; Galli *et al.* 2003). This is consistent with *pol3-t* conferring an increase in daughter strand nicks and gaps and other recombinagenic lesions. Interestingly, we observed no significant change in the rate of USCR in the *pol3-t* mutant (Table 4). While nuclease-catalyzed DSBs can stimulate USCR (Fasullo *et al.* 1998), spontaneous USCR has been proposed to occur by strand invasion and repair synthesis from the sister chromatid subsequent to replicative polymerase pausing (Navarro *et al.* 2007). Gangavarapu *et al.* (2007) have suggested that *RAD52*-dependent post-replication repair following disruption of lagging strand synthesis may occur by a similar mechanism. Although Pol δ has been implicated in lagging strand synthesis (Garg and Burgers 2005b; Nick McElhinny *et al.* 2008), the *pol3-t* mutant is unlike *rad27*Δ mutants that display a 46-fold increase in the rate of USCR (Navarro *et al.* 2007) along with increased levels of daughter strand nicks and gaps (Vallen and Cross 1995; Parenteau and Wellinger 1999). This suggests that the *pol3-t* mutation may result in the accumulation of fewer daughter strand nicks and gaps than *rad27*Δ or that it inhibits the utilization of these lesions for USCR. The wild-type rates of USCR observed when *pol3-t* was combined with the *rev1*Δ and *rev7*Δ alleles (Table 4) suggests that *pol3-t* may channel these lesions away from Rev1- and Pol ζ-dependent USCR into other repair pathways.

Translesion polymerases have been proposed to function in circumstances where the replicative polymerases cannot function because of rigid constraints on their ability to use altered or damaged DNA templates (Prakash *et al.* 2005). The translesion polymerases have been proposed to replace the replicative polymerase at the stalled replication fork in a carefully orchestrated process (Friedberg *et al.* 2005), allowing the bypass of lesions in a manner that frequently introduces mutations (Kunkel 2003). However, it is unclear what role these auxiliary polymerases may play in *pol3-t* mutants, whose defective Pol δ may affect primarily lagging strand synthesis (Garg and Burgers 2005b; Nick McElhinny *et al.* 2008), the interruption of which could generate daughter strand nicks or gaps, but may not result directly in replication fork stalling.

Pol η was been tentatively implicated in spontaneous mutagenesis in yeast (McDonald *et al.* 1997; Roush *et al.* 1998), as has Pol ζ (Quah *et al.* 1980; Roche *et al.* 1994; Kunz *et al.* 1998; Endo *et al.* 2007). However, the extent to which this is a response to wild-type levels of spontaneous DNA lesions and/or spontaneous polymerase failure is unclear. Pol η, Pol ζ, and Rev1 have not been reported to be required for spontaneous recom-

bination in yeast, which is in contrast to studies with higher eukaryotic systems (Gan *et al.* 2008). Our data are consistent with Pol η, Pol ζ, and Rev1 playing minor roles in spontaneous mutation, recombination, and chromosome loss (Tables 2, 4, and 5), as rates of these events were, for the most part, similar to wild type in the *rev1*Δ, *rev3*Δ, *rev7*Δ, and *rad30*Δ single-mutant strains. The most interesting observation was the four- and sevenfold decreased levels of USCR displayed by the *rev1*Δ and *rev7*Δ mutants, consistent with Rev1 and Pol ζ facilitating spontaneous recombination events between sisters. This may be the first evidence of a requirement for translesion polymerases in the propagation of spontaneous recombination in yeast. Given that USCR can occur only subsequent to completion of replication through the USCR substrate, this result strongly suggests that Rev1 and Pol ζ participate in spontaneous recombination in the S or G_2 phases of the cell cycle, consistent with data concerning the abundance and/or likely function of these proteins in the G_2 phase (Waters and Walker 2006). These results are also consistent with data supporting the function of Rev1 and Pol ζ during sister-chromatid exchange in vertebrate cells (Okada *et al.* 2005).

The mutation and recombination data reported here indicate that null mutations in the translesion polymerase genes *RAD30*, *REV3*, and *REV7* do not suppress the genome-destabilizing effects of *pol3-t* (Tables 2, 4, and 5). This contradicts the results of previous studies that demonstrated that a *rev3*Δ mutation is able to suppress the elevated mutation rates conferred by certain *pol3* alleles, including *pol3-t* (Pavlov *et al.* 2001; Northam *et al.* 2006). These contradictory results are likely to reflect differences in the genetic backgrounds of the strains used in the different studies. However, the current results suggest that *pol3-t* is, at least in certain contexts, capable of exerting its mutagenic and recombinagenic effects independently of the action of Pol η and Pol ζ.

In contrast to Pol η and Pol ζ, the epistasis interactions between *rev1*Δ and *pol3-t* indicate that Rev1 is required to fully observe the genome-destabilizing effects of the *pol3-t* allele, as the increased rates of mutation, recombination, and chromosome loss observed in the *pol3-t* single mutants are all significantly suppressed in the *pol3-t rev1*Δ double mutants (Tables 2, 4, and 5). This requirement is most clearly demonstrated by the *CAN1* mutation rate, where the substantial stimulatory effect of *pol3-t* is nearly completely suppressed by *rev1*Δ in the *pol3-t rev1*Δ double mutant. Significantly, Rev1 is seen acting independently of Pol ζ in promoting spontaneous mutagenesis, where previously they had been known to function together (Lawrence and Maher 2001; Lawrence 2002) and with Rev1 playing a structural role (Acharya *et al.* 2005; Prakash *et al.* 2005). Interestingly, although translesion replication is believed to be native to all eukaryotes, *Caenorhabditis elegans* lacks a *REV3* gene but possesses a *REV1* gene, suggesting that Rev1 may collaborate with another polymerase, such as Pol δ, to propagate translesion synthesis (Lawrence and Maher 2001). It is clear from studies of the mouse that Rev1 has the capacity to interact with multiple polymerases (Guo *et al.* 2003). In keeping with this scenario, it is tempting to speculate that Rev1 may facilitate mutagenesis in *pol3-t* mutant yeast strains by aiding the association of the defective Pol δ with daughter strand nicks and gaps during repair synthesis. However, the very similar *can1* mutation spectra displayed by the *pol3-t* single and *pol3-t rev1*Δ double mutants (Table 3, supplemental Table 1) suggest that the absence of Rev1 may reduce but not eliminate the association of Pol δ with these lesions.

We suggest that increased mutation, recombination, and chromosome loss may have a common mechanistic origin in *pol3-t* mutant cells, where each is the result of Rev1 helping to engage a dysfunctional Pol δ for the repair of daughter strand nicks or gaps during G_2. Mutations may result when Pol δ that is prone to promiscuous reassociation with the template is engaged to repair the lesions. Recombination and chromosome loss may result when Pol δ that is prone to premature failure is engaged and falls off before completing repair, leading to the persistence of nicks and gaps that are transformed into recombinagenic DSBs during the subsequent S phase (Navarro *et al.* 2007).

This study suggests that the genome instability that results from elevated levels of spontaneous replicative DNA polymerase failure is the result of the tandem action of primary replicative and auxiliary translesion polymerases. These observations may have clinical significance as high levels of spontaneous replicative polymerase failure may also occur as the result of the administration of chemotherapeutic drugs such as hydroxyurea, which drive down levels of dNTPs by inhibiting ribonucleotide reductase activity (Elford 1968; Cory *et al.* 1980). Perhaps the elevated levels of secondary cancers observed in patients that have received such drugs are due to an increase in genome instability from Rev1-mediated responses to replicative polymerase failure. In such a scenario, pharmacological disruption of Rev1 activity during the administration of chemotherapeutic drugs may reduce genome instability and the incidence of secondary cancers.

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