Translesion Synthesis of Abasic Sites by Yeast DNA
Polymerase ε

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Studies of replicative DNA polymerases have led to the generalization that abasic sites are strong blocks to DNA replication. Here we show that yeast replicative DNA polymerase ε bypasses a model abasic site with comparable efficiency to Pol η and Dpo4, two translesion polymerases. DNA polymerase ε also exhibited high bypass efficiency with a natural abasic site on the template. Translesion synthesis primarily resulted in deletions. In cases where only a single nucleotide was inserted, dATP was the preferred nucleotide opposite the natural abasic site. In contrast to translesion polymerases, DNA polymerase ε with 3′→5′ proofreading exonuclease activity bypasses only the model abasic site during processive synthesis and cannot reinitiate DNA synthesis. This characteristic may allow other pathways to rescue leading strand synthesis when stalled at an abasic site.

Abasic sites are considered to be the most common DNA lesions in the genome. They arise because of the chemical instability of DNA (depurination events) and during the intermediate steps of base excision repair. Estimates have suggested that 10,000–20,000 bases are lost per day in mammalian cells; in the genome of Escherichia coli, one depurination event per hour is estimated to occur (1). Abasic sites are non-instructional to DNA polymerases and highly mutagenic when encountered by the replication fork. Studies of replicative DNA polymerases from bacteriophages, bacteria, archaebacteria, and eukaryotic model systems have shown that abasic sites are strong blocks to polymerase activity (2–5). Both Family A and Family B replicative polymerases have been shown to stall prior to or opposite the abasic site, including T4 DNA polymerase, T7 DNA polymerase, and yeast DNA polymerase δ (2, 5, 6).

It has been proposed that the 3′→5′ exonuclease activity of replicative DNA polymerases suppresses the insertion of nucleotides opposite abasic sites, limiting the possibility of extending synthesis beyond the DNA lesion (7). An amino acid substitution inactivating the 3′→5′ exonuclease activity of T4 polymerase dramatically increases bypass synthesis of a tetrahydrofuran (THF)3 site when the polymerase is allowed to cycle on the primer-template (8). Later work showed that proofreading-deficient T4 polymerase cannot bypass abasic sites when restricted from cycling on and off the primer-template, perhaps because of restrictions imposed by the geometry of the active site (2). Experiments in which single-hit conditions are clearly met demonstrate that replicative DNA polymerases cannot bypass model abasic sites. Instead, models have proposed a handoff from the replicative polymerase at the abasic site to translesion polymerases. One example is yeast DNA polymerase δ (Pol δ), which inserts a dATP opposite the abasic site, followed by extension by DNA polymerase ε and a switch back to Pol δ (5, 9).

Under normal growth conditions, the yeast replication fork primarily engages Pol δ in the synthesis of the lagging strand and DNA polymerase ε (Pol ε) in the synthesis of the leading strand (10, 11). As a leading strand polymerase, Pol ε would presumably replicate a large portion of the genome and likely encounter DNA lesions if they are unrepaired when the replication fork passes the site. Although Pol δ has been extensively studied, less is known about how Pol ε functions when encountering a DNA lesion. So far, studies indicate that Pol ε stalls at cis-syn thymine dimers and a highly mutagenic benzo[a]pyrene DNA adduct (12, 13). We recently showed that Pol ε can bypass 7,8-dihydro-8-oxoguanine and O6-methylguanine (14). We have now extended our studies to abasic sites, including both a commonly used THF moiety (a model abasic site) and a natural abasic site in the template strand. We find that Pol ε has unexpectedly high bypass efficiency and that the bypass is highly mutagenic.

EXPERIMENTAL PROCEDURES

Plasmids—The glutathione S-transferase tag (GST tag) fused with the POL2 gene was amplified from the PJL1-GST plasmid using the oligonucleotides GST forward PJL1 (5′-GTCAAG-GAGAAAAAACCGGATCCACTAGTTCTAGATGTCC-CCTATACATAGGTATA-3′) and GST reverse PJL1 (5′-GGAAGATCTCCGGTTTTTTTTCTTGCCAAACAT-CATCAGGGGCCCCTGGAACAGAACTT-3′), and purified on an agarose gel. The PJL1 exon was cleaved by NotI. The strain PY116 containing plasmid PJL6 (15) was transformed with the purified PCR product and the cleaved plasmid. The transformants were selected on SC-ura-trp. The expression of the GST-Pol ε fusion protein was confirmed by Western blot.
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Protein Purification—The purification of Pol2/Dpb2 (wild-type (WT) and exonuclease-deficient (pol2–4)) and Pol2 was carried out as described previously (16). Pol ε exo− and Pol ε exo− (pol2–4) were purified in parallel as described earlier but with the following modifications. The resuspended ammonium sulfate precipitate was dialyzed against B100, for 2 h (the suffix in all buffer names indicates the concentration of sodium acetate in the buffer; for example, buffer B300 is buffer B with 300 mM sodium acetate). The dialyzed extract was mixed with 2 ml of glutathione-Sepharose equilibrated in B300 and slowly rotated for 4 h. The glutathione-Sepharose with bound GST-Pol ε or GST-Pol ε exo− was collected on a column and washed with 15 column volumes of B300. PreScission protease was added to the column and incubated overnight at 4 °C to cleave and remove the GST tag. The following morning, the column was eluted with 10 × 0.5 column volumes. The eluted proteins were pooled and loaded onto a 1-ml Mono-Q column and separated over a linear salt gradient from B750 to B1200, followed by gel filtration on a Superose 6 gel filtration column with B400 as the running buffer (15).

Bypass Efficiency Assays—The enzyme-to-DNA ratios used in the primer-extension assays were 1:10 for Pol ε exo−; 1:7 for Pol ε exo−; 1:5 for Pol2/Dpb2 exo+ and Pol2/Dpb2 exo−; 1:1 for Pol2; and 1:50 for Klenow exo−; or a 2-fold molar excess of enzyme over DNA, unless otherwise indicated, e.g. in Fig. 4. Primer-extension assays were performed as described earlier using different primer-templates (16). 20-, 56-, and two 58-mers were annealed, respectively, to 38-, 75-, and two 76-mers that contained either a THF moiety or an undamaged nucleotide (see Table 1). Single-hit conditions were confirmed by calculating the termination probability of the undamaged templates by dividing the intensity of band N with the intensity of bands at position N and beyond. The relative bypass efficiency was calculated by dividing the bypass probability of the damaged template with the bypass probability of the undamaged template; the bypass probability for the damaged template was calculated by dividing the sum of the products after the lesion position with the sum of the products from the nucleotide before the lesion and beyond as described by Kunkel and colleagues (17). The insertion probability opposite the abasic site was divided by the insertion probability of the undamaged template to give the relative insertion efficiency (17). The extension probability of the damaged template was divided by the extension probability of the undamaged template to give the relative extension efficiency (17).

The primer-extension assay for Klenow exo− (New England Biolabs) was performed in 50 mM NaCl, 10 mM Tris-HCl, pH 7.9, 10 mM MgCl₂, 1 mM dithiothreitol, and 25, 100, or 500 μM of each dNTP (dATP, dTTP, dCTP, and dGTP) at 37 °C for 2 or 8 min. The natural abasic template was constructed by cleaving the 0.1 nmol of uracil-containing template with 1 unit of uracil-DNA glycosylase (Fermentas) at 37 °C for 70 min. To avoid breakage of the natural abasic template, the corresponding primer was heated at 55 °C for 10 min, and the natural abasic template was added to the solution and heated for an additional 1 min. The template was slowly cooled to room temperature. Primer-extension assays with Dpo4 ( Trevigen) were performed in 10 mM HEPES-NaOH, pH 7.8, 1 mM dithiothreitol, 50 mM NaCl, 100 μg/ml bovine serum albumin, 0.1% Triton X-100, 10 mM MgCl₂, 100 μM dNTP, and 2 nM primer-template (Seq1–58 nt) at 70 °C.

Analysis of Translesion Synthesis Products from Primer-extension Reactions with Pol ε—Biotinylated Acc65I overhang primer (5′-Biotin-GTACGATCTTCGAGGATACATTTACACGGGATCAGATGATGCTCTGAGACGATCTCGATCTGCTGAGAGATAC-3′) was annealed at a 1:1 molar ratio to a complementary template, EcoRI template (5′-AGATGGAATTCGTTTTACCCTGCGTAAXUTAGCACGAAGTATCTGCTCTGCTCCTAGAGCATATACTACGATCAGATGATGCTCTGAGACGATCTCGATCTGCTGAGAGATAC-3′). The X indicates A, T, C, or G, and the U indicates the position of a THF moiety or a uracil. The uracil was removed by uracil-N-glycosylase (Fermentas) to create a natural abasic site. Lower-case letters in the oligonucleotide sequence indicate positions of mismatched bases used to identify the strand after cloning into a plasmid. The primer-template (1 pmol) was incubated with WT Pol ε (0.2 μM) for 30 min at 30 °C as described for the primer-extension reactions. The product was bound to Dynabeads M-280 streptavidin (Dynal Biotech) for 3 h as recommended by the manufacturer’s protocol. Next, the product was washed five times with washing buffer (Dynal Biotech) and once with water using a Dynal Magnetic Particle Concentrator (Dynal MFC) to remove all non-biotinylated components. To remove the template containing the uracil/abasic site, the immobilized primer-template was incubated with 150 μl of 0.1 M NaOH for 5 min to denature the primer-template. The immobilized primer was collected with the Dynal MPC, and the template with the uracil/abasic site was removed in the supernatant.

The bound primer was washed once with 0.1 M NaOH and two times with TE buffer, pH 7.6, when bound to the Dynal MPC. Recombinant Pyrococcus furiosus DNA polymerase (Fermentas) was used in the PCR to amplify the purified biotinylated primer, which was previously extended by Pol ε in the primer-extension reaction. The PCR was carried out with Primer 1 (5′-CTAGAGATGATGATACGCTTCCTCAGATTCTTTACCTAGATCTGCCGTCTC-3′) and Primer 2 (5′-GATGGTTACCGATCTACGAGGAG-3′). The PCR product (129 bp) was purified and cleaved by Acc65I and EcoRI at 37 °C, followed by ligation into the Acc65I and EcoRI sites of the pBluescript II SK+ vector. The ligation mixture was transformed into chemically competent E. coli cells, colonies were amplified, and the plasmids isolated and sent to MWG Biotech (Germany) for sequencing of the insert.

RESULTS

Efficiency of Tetrahydrofuran Moiety Bypass by Pol ε—To study the ability of yeast Pol ε to bypass abasic sites, we first chose the widely used abasic site analogue, a THF moiety. This allows a comparison with reported bypass properties of other DNA polymerases. Primer-extension assays were carried out under single-hit conditions with a THF moiety in the template or an identical template with an undamaged nucleotide replacing the abasic site (Table 1). The assay conditions were empirically determined to ensure that Pol ε was not allowed to cycle to a previously extended primer (14, 16). The THF moiety or undamaged nucleotide was positioned four residues downstream of the 3′-end of the primer so that Pol ε would reach the
residue during processive synthesis. Three different templates with randomly chosen sequences: sequence 1–58 nt dsDNA (Seq1–58 nt), sequence 2–58 nt dsDNA (Seq2–58 nt), and sequence 3–57 nt dsDNA (Seq3–57 nt), were incubated with Pol\textsubscript{H9280} for 2 or 8 min (Fig. 1A and Table 1). The majority of the replication products ended one or two nucleotides before the THF moiety. Surprisingly, we found that Pol\textsubscript{H9280} also carried out translesion synthesis (Fig. 1A).

The strategy to compare two substrates that are identical with the exception of the THF moiety on the damaged template allows a relative bypass efficiency to be calculated as described by Kunkel and colleagues (17, 18): the bypass probability of the abasic site in the damaged template divided by the bypass probability of the undamaged template. As shown for other DNA polymerases, the bypass efficiency was influenced by the sequence context in which the abasic site was located (Table 2). Remarkably, the bypass efficiency of Pol\textsubscript{H9280} (6–17\%) was comparable with previously reported values for human DNA polymerase\textsubscript{H9257} (10–13\%) and Sulfolobus solfataricus Dpo4 (13–30\%), two typical Y family translesion polymerases (Table 2) (17, 19). In comparison, the replicative T7 DNA polymerase was reported to have less than 0.2\% bypass efficiency, placing it below the level of detection (Table 2) (6).

This ability of Pol\textsubscript{H9280} to bypass a model abasic site under single-hit conditions is unprecedented among Family B polymerases. To investigate the influence of the 3′–5′ exonuclease activity of Pol\textsubscript{ε} on bypass efficiency for a THF moiety, we overexpressed and purified an exonuclease-deficient form of Pol\textsubscript{ε} (the pol2–4 allele). The exonuclease-deficient Pol\textsubscript{ε} (Pol\textsubscript{ε} exο) was used in primer-extension assays with the undamaged and damaged templates as described for WT Pol\textsubscript{ε}. The Pol\textsubscript{ε} exο could bypass the lesion (Fig. 1B) with only a slightly increased bypass efficiency compared with the WT Pol\textsubscript{ε} (Table 2). In all cases, the insertion efficiency was greater for Pol\textsubscript{ε} exο compared with WT Pol\textsubscript{ε}. In contrast, the extension efficiency was lower for Pol\textsubscript{ε} exο compared with WT Pol\textsubscript{ε}. Again, sequence context influenced the bypass efficiency, and in this case, a more inhibitory sequence context benefited more from the inactivation of the proofreading activity (Table 2).

As a control, we added an exonuclease-deficient Klenow fragment (Kf exο) in primer-extension assays with the Seq1–58 nt, Seq2–58 nt, and Seq3–57 nt templates. The synthesis on the undamaged template was distributive when compared with Pol\textsubscript{ε} (supplemental Fig. S1). No translesion synthesis was found with the 2-min incubation time, but a nucleotide was efficiently incorporated opposite the abasic site (supplemental Fig. S1 and Table 1) (17). The inability to bypass the THF moiety was unaffected by dNTP concentrations, and extended reaction times did not yield measurable bypass products (supplemental Fig. S1). These findings verified that the translesion synthesis by Pol\textsubscript{ε} was carried out on the model abasic site and demonstrated that Pol\textsubscript{ε} has a unique property that allows translesion synthesis in the absence of a processivity clamp.

**Influence of Processivity on Bypass Efficiency**—Previous work showed that the intrinsically high processivity of Pol\textsubscript{ε} depends, in part, on the interaction between an extended tail domain and the primer-template (16). These results suggested that the tail domain consists of three subunits (Dpb2, Dpb3, and Dpb4). To examine if the extended tail domain of Pol\textsubscript{ε} influences translesion synthesis capacity, we purified three variants of Pol\textsubscript{ε} that lacked subunits from the tail domain: an exonuclease-proficient Pol2/Dpb2 complex (Pol2/Dpb2), an exonuclease-defi-
ciant Pol2/Dpb2 complex (Pol2/Dpb2 exo\textsuperscript{−}), and the exonuclease-proficient Pol2 subunit (Pol2). These complexes lacked two or three non-catalytic subunits, and we expected that a fully functional interaction between the enzyme and the primer-template duplex would not be established (16). Again, primer-extension assays were carried out under single-hit conditions with the three primer-template duplexes. However, the empirically determined ratio of polymerase to DNA was changed because of inefficient primer utilization. The bypass efficiency of the Pol2/Dpb2 complex and Pol2 was slightly reduced compared with Pol ε on templates Seq1–58 nt and Seq2–58 nt (see Fig. 1, C and E, and Table 2). However, no bypass was detected when Seq3–57 nt was used as the template (Table 2). The bypass efficiency of Pol2/Dpb2 exo\textsuperscript{−} was clearly reduced when

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**FIGURE 1.** Primer-extension assays with a tetrahydrofuran moiety in the template strand. The primer-extension reactions were carried out on an undamaged template and a template with a tetrahydrofuran moiety positioned as indicated in the figure to calculate bypass efficiency. Three separate templates (2 nM) were used together with (A) 0.2 nM Pol ε, (B) 0.29 nM Pol ε exo\textsuperscript{−}, (C) 0.4 nM Pol2/Dpb2, (D) 0.4 nM Pol2/Dpb2 exo\textsuperscript{−}, and (E) 2 nM Pol2. In F, Pol ε or Pol ε exo\textsuperscript{−} were added, as indicated in the figure, to a shorter primer-template that does not support fully processive synthesis.
TABLE 2
Properties of Pol ε and its derivatives when encountering a model abasic site

| DNA polymerase | Seq1–58 nt 3’-TXC-5’ | Seq2–58 nt 3’-CXA-5’ | Seq3–57 nt 3’-AXT-5’ | Seq1–20 nt 3’-TXC-5’ |
|----------------|-----------------------|----------------------|---------------------|---------------------|
| Pol ε relative | Insertion efficiency (%) | 22                    | 16                  | 15                  | 3                   |
|                | Extension efficiency (%) | 79                    | 40                  | 38                  | <0.2                |
|                | Bypass efficiency (%)   | 17                    | 6                   | 6                   | <0.2                |
| Pol ε exo⁻ relative | Insertion efficiency (%) | 67                    | 66                  | 94                  | 22                  |
|                | Extension efficiency (%) | 31                    | 15                  | 20                  | 2                   |
|                | Bypass efficiency (%)   | 21                    | 10                  | 18                  | 0.5                 |
| Pol2/Dpb2 relative | Insertion efficiency (%) | 17                    | 13                  | 10                  | ND                  |
|                | Extension efficiency (%) | 72                    | 48                  | <0.2                | ND                  |
|                | Bypass efficiency (%)   | 12                    | 6                   | <0.2                | ND                  |
| Pol2/Dpb2 exo⁻ relative | Insertion efficiency (%) | 38                    | 51                  | 76                  | ND                  |
|                | Extension efficiency (%) | 27                    | 13                  | 7                   | ND                  |
|                | Bypass efficiency (%)   | 11                    | 7                   | 6                   | ND                  |
| Pol2 relative | Insertion efficiency (%) | 18                    | 12                  | 9                   | ND                  |
|                | Extension efficiency (%) | 78                    | 44                  | <0.2                | ND                  |
|                | Bypass efficiency (%)   | 14                    | 5                   | <0.2                | ND                  |
| Klenow exo⁻ relative | Insertion efficiency (%) | 69                    | 54                  | 82                  | ND                  |
|                | Extension efficiency (%) | <0.2                  | <0.2                | <0.2                | ND                  |
|                | Bypass efficiency (%)   | <0.2                  | <0.2                | <0.2                | ND                  |
| XATT           |                       |                       |                     |                     |
| GXTT           |                       |                       |                     |                     |
| GAXT           |                       |                       |                     |                     |
| Dpo4 relative | Insertion efficiency (%) | 38                    | 55                  | ND                  |
|                | Extension efficiency (%) | 31                    | 60                  | ND                  |
|                | Bypass efficiency (%)   | 13                    | 30                  | ND                  |
| T7 exo⁻ relative | Insertion efficiency (%) | <0.2                  | 24.7                | <0.2                |
|                | Extension efficiency (%) | <0.2                  | <0.2                | <0.2                |
|                | Bypass efficiency (%)   | <0.2                  | <0.2                | <0.2                |

a X indicates the position of the tetrahydrofuran moiety.

b Insertion, extension, and bypass efficiencies were calculated from single-hit reactions as previously described (17). The values are those of lesion-containing templates compared with undamaged DNA (i.e., relative insertion, relative extension, and relative bypass). Values <0.2 represent the limits of detection on phosphorimaging screens.

c ND, no data.

d Data from S. solfataricus Dpo4 (17) are shown for comparison.

e Data from T7 DNA polymerase (6) are shown for comparison.

compared with Pol ε exo⁻ on all three templates (see Fig. 1, B and D, and Table 2).

Earlier results showed that Pol ε requires a minimal primer length of 40 base pairs of dsDNA for processive DNA synthesis. A 20-nt primer-template duplex did not support fully processive synthesis by Pol ε (16). For this reason, we chose a shorter version of Seq1, which had the highest bypass efficiency of all templates used, with only a 20-nt dsDNA (Seq1–20 nt) instead of 58 nt. We added Pol ε and Pol ε exo⁻ to Seq1–20 nt and empirically determined the ratio of polymerase to DNA, which allowed us to perform the experiments under single-hit conditions. Again, because of inefficient primer utilization, we used a higher ratio of polymerase to DNA compared with the longer primer-template duplexes (16). We found that Pol ε mainly stalled before the abasic site and that only a minor fraction of the products had a nucleotide inserted opposite the abasic site (Fig. 1F, lanes 4 and 5). Pol ε exo⁻ frequently added nucleotides opposite the abasic site but extended only a minor fraction of the insertions before dissociating (Fig. 1F, lanes 8 and 9). Pol ε exo⁻ could not extend synthesis beyond one nucleotide after the abasic site, even when equimolar amounts of enzyme and DNA were used (Fig. 1F, lanes 10 and 11).

Sensing the Model Abasic Site—Earlier studies found that polymerases are more successful at bypassing an abasic site if allowed repeated attempts to extend a primer (6, 8). To allow Pol ε to repeatedly cycle onto the primer-template, we carried out reactions with a 2-fold molar excess of enzyme over DNA for 10 min. To quantify the capacity of Pol ε to bypass the abasic site when given multiple opportunities, we first measured the sum of products after the lesion (nt +1 and longer; Fig. 2) divided by the sum of products starting from the nucleotide upstream of the abasic site (nt −1 and longer; Fig. 2). The obtained value for the damaged template was divided by the corresponding value for the undamaged template. The resulting number allowed us to compare the capacity of Pol ε, Pol ε exo⁻, Pol2/Dpb2, Pol2/Dpb2 exo⁻, and Pol2 to perform translesion synthesis under the given conditions.

We found that exonuclease-deficient Pol ε and Pol2/Dpb2 exo⁻ had greater capacities to extend the synthesis past the abasic site (Fig. 2). In contrast, the exonuclease-proficient enzymes did not improve their bypass capacity compared with the bypass efficiency at single-hit conditions (see Fig. 2 and Table 1). This observation suggests that the WT Pol ε has only one opportunity to bypass the abasic site: when an abasic site enters the polymerase site during the processive synthesis of new DNA.

The primer-extension assays with excess enzyme revealed that proofreading-deficient Pol ε had strong pause sites at the
first two nucleotides downstream of the abasic site (nt + 1 and +2; Fig. 2). In contrast, no such pause sites were found when proofreading-proficient Pol ε was used. This difference led us to investigate whether this also was true for Pol δ and Pol ε exo under single-hit conditions (Fig. 1, A and B). We plotted the termination probability for Pol ε and Pol ε exo on both undamaged and damaged templates across the area of interest in the experiments from Fig. 1, A and B (Fig. 3). The results show that Pol ε has the highest termination probability at the nucleotide preceding the abasic site, followed by a slightly decreased termination probability at the abasic site. The termination probability reached a steady state at the first nucleotide positioned downstream of the abasic site. In contrast, we found that Pol ε exo has the highest termination probability at the abasic site and maintained a high termination probability for three to four nucleotides downstream of the abasic site on the Seq2–58 nt and Seq3–57 nt templates (Fig. 3). This difference suggests that Pol ε can identify the abasic site for the same distance as single-base deletions in homonucleotide runs during normal DNA replication (20).

**Bypass of a Natural Abasic Site**—The structures of natural abasic sites and THF moieties differ slightly, and their geometry could influence the translesion synthesis properties of Pol ε when they are located in the active site. We constructed natural abasic sites by replacing the THF moiety in Seq1–58 nt with deoxyuracil, followed by treatment with uracil-N-glycosylase to remove the uracil base. The natural abasic site-containing template was incubated with WT Pol ε under single-hit conditions. The bypass efficiency was even higher than with the model abasic site (Fig. 4). A side-by-side comparison with Dpo4 revealed that the relative bypass efficiency was similar between Pol ε and Dpo4 (68 and 69%, respectively). The relative bypass efficiency was 24% for Dpo4 with a THF moiety in the same experiment, comparable with previously reported values (see Table 1). The uracil was efficiently removed from the template, as shown by the shorter replication products (Fig. 4 A).

**Fidelity of Natural Abasic Site Bypass by Pol ε**—To examine the fidelity of the bypass we performed primer-extension assays on a larger scale. After that the extension products were purified on magnetic beads, PCR amplified with a limited number of cycles, subcloned into a plasmid, and sequenced (supplemental Fig. S2). The untreated uracil-containing template resulted in 100% dATP incorporation opposite the uracil. The uracil-N-glycosylase-treated template generated a variety of mutations that could be divided into groups, either the insertion of a nucleotide opposite the abasic site or generation of a deletion (Table 3). The nucleotide context of Seq1–58 nt, with the highest bypass efficiency, resulted in mostly insertions, where dATP was the most common insertion. We exchanged the nucleotides flanking the natural abasic site and found that the vast majority of the bypass products resulted in deletions (Table 3). Still, dATP was the most frequently inserted nucleotide when the bypass resulted in the insertion of a nucleotide. In contrast, dGTP was the most frequently inserted nucleotide when a THF moiety was positioned on Seq1–58 nt (see Table 3). This suggested that Pol ε may use the same bypass mechanism as Dpo4, where the nucleotide located 5′ of the THF moiety determines what will be inserted across the abasic site (21). Exchanging the 5′-nucleotide resulted in bypass products that predominantly carried a 1 nt deletion (see Table 3). We conclude that the bypass of natural abasic sites by Pol ε is highly mutagenic and the large number of frameshifts is a serious threat to the integrity of the genome unless repaired.

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**FIGURE 2.** Exonuclease-proficient Pol ε was unable to reinitiate synthesis if dissociated from the template at the abasic site. In primer-extension assays, 4 nM of each polymerase was added as indicated with 2 nM of either undamaged or damaged Seq1–58 nt. The primer-extension assays were incubated at 30 °C for 10 min to allow recycling at the primer ends if the polymerase dissociated at some position. The products were separated on an 8% denaturing polyacrylamide gel and analyzed by phosphorimaging.
DISCUSSION

We present evidence that the Family B DNA polymerase /H9280 has, for replicative DNA polymerases, unprecedented translesion capacity across abasic sites and that the bypass primarily results in deletions. When inserting a nucleotide opposite the natural abasic site, Pol /H9280 does follow the “A-rule.” In either case, the bypass of abasic sites by Pol /H9280 is highly mutagenic, but genetic experiments in yeast have shown that damage-induced mutagenesis depends on the Pol32 subunit of Pol /H9254, Pol /H9256, and Rev1 (5, 22–24).

The ability of Pol /H9280 to carry out error-prone translesion synthesis has several implications. One possibility could be that abasic sites are efficiently repaired so that the replication fork and Pol /H9280 rarely encounter them. This hypothesis is supported by the fact that in vivo studies of translesion synthesis across abasic sites on plasmids or in chromatin were carried out in strains with deleted apurinic/apyrimidinic endonucleases (APN1 and APN2), which are important for base excision repair (5, 23, 25).

Another, perhaps more likely, possibility is the existence of leading strand-specific, error-free repair pathways that protect the genome from error-prone translesion synthesis by Pol /H9280 or other DNA polymerases acting on the leading strand. Several pathways have been suggested that involve homologous recombination with the sister chromatid and are virtually error-free (26).

The mechanisms by which the leading and lagging strands synthesize new DNA differ and may also influence how DNA lesions are bypassed. Studies on the effect of UV lesions have...
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TABLE 3
Fidelity of translesion synthesis by Pol ε across a natural abasic site or a THF moiety
The products from Pol ε translesion synthesis were analyzed as described in supplemental Fig. S2 and under “Experimental Procedures.” The sequence of the template used in this analysis was identical to Seq1–58 nt, except for a variation in the nucleotide 5’ or 3’ to the abasic site, as indicated.

| Template 3’–5 | No. of sequence colonies | Number of nucleotides inserted during TLS (%) | Number of deletions during TLS (%) | Other mutations |
|--------------|--------------------------|---------------------------------------------|----------------------------------|----------------|
|               |                          | A   | G   | C   | T   | Single | Double | Triple | Larger |                |
| Natural abasic site |                         |     |     |     |     |        |        |        |         |                |
| TXC          | 79                       | 37 (47) | 9 (11) | 5 (6) | 7 (9) | 14 (18) | 2 (2) | 0      | 2 (2)   | 3 (4)       |
| TXT          | 70                       | 13 (19) | 0 | 0 | 0 | 10 (14) | 9 (13) | 5 (7) | 28 (40) | 5 (7)       |
| TXA          | 64                       | 5 (8) | 0 | 0 | 0 | 15 (23) | 9 (11) | 4 (5) | 23 (28) | 5 (6)       |
| TXG          | 81                       | 18 (22) | 2 (2) | 1 (1) | 0 | 19 (23) | 9 (11) | 8 (7) | 28 (40) | 5 (7)       |
| AXG          | 69                       | 8 (12) | 0 | 0 | 0 | 20 (29) | 6 (9) | 7 (10) | 25 (36) | 3 (4)       |
| Uracil       |                          |     |     |     |     |        |        |        |         |                |
| TUC          | 27                       | 27 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TUT          | 30                       | 30 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TUA          | 30                       | 30 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TUG          | 30                       | 30 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AUG          | 30                       | 30 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| THF moiety   |                          |     |     |     |     |        |        |        |         |                |
| TXC          | 64                       | 1 (2) | 41 (64) | 0 | 1 (2) | 13 (20) | 3 (5) | 1 (2) | 0 | 4 (6) |
| TXT          | 43                       | 0 | 0 | 0 | 0 | 26 (60) | 3 (7) | 1 (2) | 9 (21) | 3 (7) |
| TXA          | 47                       | 0 | 0 | 0 | 0 | 33 (70) | 7 (15) | 1 (2) | 0 | 6 (13) |
| TXG          | 52                       | 0 | 0 | 0 | 0 | 41 (79) | 3 (6) | 1 (2) | 2 (4) | 5 (10) |

a TLS, translesion synthesis.

suggested that leading strand synthesis is blocked, whereas the lagging strand synthesis continues (27, 28). This difference would be natural because the lagging strand polymerase always has a new primer available further upstream from the DNA lesion. A gap remains behind the replication fork that can later be filled in by, for example, Pol δ and Pol ζ in a collaborative effort. A DNA lesion on the leading strand may have a dual effect, however. It is likely that a DNA-damage checkpoint is activated as the helicase is uncoupled from the replication fork and long stretches of single-stranded DNA are generated (29). Another consequence is that a replication restart is required to complete leading strand synthesis. The mechanism of achieving the restart is not clear in eukaryotes but could involve fork reversal, sister-chromatid exchange, or perhaps a new primer synthesized downstream of the DNA lesion, as shown for the E. coli replisome (26, 28, 30–32). Repriming would leave a gap with single-stranded DNA that other DNA polymerases could later fill in, presumably Pol δ, Pol ζ, and Rev1.

We have shown that Pol ε bypasses abasic sites with comparable efficiency to Dpo4 when first encountering a natural abasic site. In contrast to Dpo4, Pol ε seems unable to reinitiate synthesis at the abasic site when dissociated from the template. The proofreading activity of Pol ε limits the possibility for Pol ε to bypass the abasic site, whereas Dpo4 can bypass most abasic sites given multiple opportunities at the lesion. In most cases, Pol ε will dissociate, most likely affecting replication fork progression. The stalled replication fork will activate a DNA-damage checkpoint, and the free 3’-termini of the nascent strand may be accessible to translesion synthesis by other DNA polymerases.

We have earlier shown that Pol ε and Pol δ are loaded onto the proliferating cell nuclear antigen-primer ternary complex by different mechanisms, which might in part explain the division of labor at the replication fork under normal DNA replication (33). The work also demonstrated that Pol δ depends on a functional proliferating cell nuclear antigen-interaction motif, which also can be found in translesion polymerases, for the loading onto the proliferating cell nuclear antigen primer-ternary complex. Genetic experiments in which the proliferating cell nuclear antigen-interaction motif located in the Pol δ subunit Pol32 was deleted yielded decreased DNA damage-induced mutagenesis (24). A plasmid-based assay recently showed that Pol32 was required for mutagenic translesion synthesis on both the leading and lagging strands (23). In addition, translesion synthesis opposite the abasic site occurred with a frequency of only about 6%. This finding suggested that the majority of bypass events were processed via other error-free mechanisms. Damage-induced mutagenesis would then appear when the error-free systems are saturated, allowing Pol δ, Pol ζ, and Rev1 to carry out error-prone gap-filling synthesis on both the leading and lagging strands. Elegant experiments with inducible endogenous AP sites in apn1Δ strains support this model as the bypass depended on REV7 and REV1, with essentially no difference between leading and lagging strands (25). Furthermore, Pol ε often generates frameshifts when bypassing the natural abasic site. This would most likely be recognized by the mismatch repair system and the strand that was synthesized by Pol ε would be replaced by new DNA. Presumably Pol δ, Pol ζ and Rev1 would fill in the gap, leaving their typical error signature and mask the error-prone bypass synthesis by Pol ε. Again, high levels of AP sites would potentially saturate the systems, Pol δ, Pol ζ, and Rev1 would participate on both leading and lagging strands and there would not be a significant difference between leading and lagging strands.

These models explain why damage-induced mutagenesis depends on Pol32, even if Pol32 acts on the lagging strand during normal DNA replication. The capacity of Pol ε to bypass abasic sites and the dependence of Pol32, Pol ζ, and Rev1 in damage-induced mutagenesis suggests that DNA lesions on the leading strand are processed by error-free repair pathways. The error-prone bypass by Pol δ, Pol ζ, and Rev1 would then rescue the replication fork when the error-free pathways are saturated under massive DNA damage.
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