

Lesion Bypass Activities of Human DNA Polymerase μ^*

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DNA polymerase μ (Pol μ) is a newly discovered member of the polymerase X family with unknown cellular function. The understanding of Pol μ function should be facilitated by an understanding of its biochemical activities. By using purified human Pol μ for biochemical analyses, we discovered the lesion bypass activities of this polymerase in response to several types of DNA damage. When it encountered a template 8-oxoguanine, abasic site, or 1,*N*⁶-ethenoadenine, purified human Pol μ efficiently bypassed the lesion. Even bulky DNA adducts such as *N*-2-acetylaminofluorene-adducted guanine, (+)- and (–)-*trans-anti*-benzo[*a*]pyrene-*N*²-dG were unable to block the polymerase activity of human Pol μ . Bypass of these simple base damage and bulky adducts was predominantly achieved by human Pol μ through a deletion mechanism. The Pol μ specificity of nucleotide incorporation indicates that the deletion resulted from primer realignment before translesion synthesis. Purified human Pol μ also effectively bypassed a template *cis-syn* TT dimer. However, this bypass was achieved in a mainly error-free manner with AA incorporation opposite the TT dimer. These results provide new insights into the biochemistry of human Pol μ and show that efficient translesion synthesis activity is not strictly confined to the Y family polymerases.

DNA polymerase μ (Pol μ)¹ is a newly discovered member of the X family polymerases (1, 2). Additional members in this family include Pol β , Pol λ , and terminal deoxynucleotidyltransferase (1–3). During base excision repair in higher eukaryotes, Pol β is a major repair synthesis polymerase (4–6). Terminal deoxynucleotidyltransferase catalyzes nucleotide additions to DNA in a template-independent manner (7, 8). This enzyme functions during V(D)J recombination of the immunoglobulin genes and T-cell receptor genes and is restricted to lymphoid tissues (7–9). Cellular functions of Pol λ and Pol μ have not been clearly defined.

Although the biochemical activities of the X family DNA polymerases appear to be quite diverse, all of the Y family DNA polymerases share a common biochemical activity: synthesis

opposite DNA lesions (reviewed in Refs. 10–13). In eukaryotes, the Y family consists of REV1 and DNA polymerases η , ι , and κ (14). Thus, it is generally believed that a major function of the Y family DNA polymerases is to copy damaged sites of DNA during replication, a cellular process referred to as lesion bypass or translesion synthesis. Genetic studies indicate that REV1 (15–18) and Pol η (19–22) are indeed involved in lesion bypass in cells. Lesion bypass can be error-free as a result of insertion of the correct nucleotide opposite the lesion or error-prone as the result of insertion of an incorrect nucleotide opposite the lesion. Both error-free and error-prone nucleotide insertions have been observed with the Y family polymerases depending on the specific lesion and the specific polymerase (reviewed in Refs. 10–12).

Biochemical studies of purified human Pol μ have uncovered a unique property that has never been observed with any other polymerases studied so far (23). Human Pol μ is highly prone to frameshift DNA synthesis (23). At single-nucleotide repeat sequences, DNA synthesis by human Pol μ is mediated mainly by a deletion mechanism because of primer-template realignment before synthesis (23). Furthermore, when the primer 3' end contains one or a few mismatches, human Pol μ can promote primer-template realignment such that the primer 3' end can find its complementary sequences on the template several nucleotides downstream, achieving microhomology search and microhomology pairing (23). These striking biochemical properties led Zhang *et al.* (23) to propose that Pol μ may be involved in nonhomologous end joining (NHEJ) for double-strand DNA repair. The biochemical properties of human Pol μ ruled out a significant role for this polymerase in somatic hypermutation during immunoglobulin development.

One important cause of DNA double-strand breaks is DNA damage. It is conceivable that some damaged sites may contain clustered lesions or that base damage may be contained near some double-strand DNA breaks. Under those circumstances, Pol μ would encounter DNA base damage while performing microhomology search and pairing, as well as DNA synthesis, during NHEJ. Hence, we asked whether Pol μ is capable of translesion synthesis. In this report, we demonstrate that human Pol μ indeed possesses efficient lesion bypass activities in response to very different types of DNA damage, ranging from simple base modifications and baseless sites to bulky chemical DNA adducts and *cis-syn* TT dimer of UV radiation. Although *in vitro* bypass of a template TT dimer is achieved by human Pol μ in an error-free manner, bypass of the other tested lesions is mediated by a deletion mechanism that effectively avoids copying the damaged template base through primer realignment. These findings provide new insights into the biochemistry of human Pol μ and show that efficient translesion synthesis activity is not strictly confined to Y family polymerases.

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¹ The abbreviations used are: Pol, DNA polymerase; BPDE, benzo[*a*]pyrene-*trans*-7,8-dihydrodiol-9, 10-epoxide; AAF, *N*-2-acetylaminofluorene; AP, *apurinic/aprimidinic*; NHEJ, nonhomologous end joining.

EXPERIMENTAL PROCEDURES

Materials—Human Pol μ , human Pol η , human Pol β , yeast Pol ζ , and the catalytic subunit of yeast Pol α were purified to near homogeneity as previously described (23–27). The Klenow fragment of *Escherichia coli* DNA polymerase I was purchased from Invitrogen. Oligonucleotides were synthesized by Operon (Alameda, CA). *N*-Acetoxy-*N*-2-acetylaminofluorene (the activated form of *N*-2-acetylaminofluorene (AAF)) was obtained from the Midwest Research Institute (Kansas City, MO).

DNA Templates Containing a Site-Specific Lesion—The 30-mer DNA template, 5'-GGATGGACTGCAGGATCCGGAGGCCGCGCG-3', contained an 8-oxoguanine at the underlined G. Four 36-mer templates, 5'-GAAGGGATCCTTAAGACYXTAACCGGTCTTCGCGCG-3', contained a tetrahydrofuran (AP site analog) at the X position and a C, T, A, or G at the Y position. The 29-mer DNA template, 5'-CCATCGCTACCTACCATCCGAATTCGCC-3', contained a 1,*N*⁶-ethenoadenine at the underlined A. These damaged DNA templates were synthesized via automated DNA phosphoramidite methods by Operon. A 33-mer DNA template containing either a (+)-*trans-anti*-benzo[*a*]pyrene (BPDE)-*N*²-dG or a (–)-*trans-anti*-BPDE-*N*²-dG was prepared as described previously (28–30). Its sequence is 5'-CTCGATCGCTAACGC-TACCATCCGAATTCGCC-3', with the modified guanine underlined. A 30-mer DNA template containing an AAF-adducted guanine was prepared as previously described (31). Its sequence is 5'-CCTTCTTA-ATAGCTTCATACCTTCTTCC-3', with the modified guanine underlined. A 49-mer DNA template containing a *cis-syn* TT dimer or a TT (6–4) photoproduct was prepared as previously described (32). Its sequence is 5'-AGTACCATGCCTGCACGAATTAAGCAATTCGTAATC-ATGGTCATAGCT-3', with the modified TT underlined.

DNA Polymerase Assays—A standard DNA polymerase reaction mixture (10 μ l) contained 25 mM KH₂PO₄, pH 7.0, 5 mM MgCl₂, 5 mM dithiothreitol, 100 μ g/ml bovine serum albumin, 10% glycerol, 50 μ M dNTPs (dATP, dCTP, dTTP, and dGTP individually or together as indicated), 50 fmol of an indicated DNA substrate containing a ³²P-labeled primer, and a purified DNA polymerase as indicated. After incubation at 30 °C for 10 min, reactions were terminated with 7 μ l of a stop solution (20 mM EDTA, 95% formamide, 0.05% bromphenol blue, and 0.05% xylene cyanol). The reaction products were resolved on a 20% polyacrylamide gel containing 8 M urea and visualized by autoradiography. DNA synthesis products were quantitated by scanning densitometry with the SigmaGel software (Sigma) for analysis.

RESULTS

Lesion Bypass of Simple Base Damage by Human Pol μ —To determine whether a strong blocking lesion such as an AP site could block the frameshift synthesis of human Pol μ , we annealed a 5'-³²P-labeled 17-mer primer that terminated just before a template AP site and performed DNA synthesis assays with purified human Pol μ . The template AP-T, in which the primer 3' A could pair with the template T 5' to the AP site by primer-template realignment, was examined first (Fig. 1). Surprisingly, DNA synthesis was observed (Fig. 1A, lane 1). To determine which nucleotide was incorporated during translesion synthesis, DNA polymerase assays were performed in the presence of only one deoxyribonucleoside triphosphate at a time. As shown in Fig. 1A, lane 5, a G was incorporated. This result is consistent with realignment of the primer 3' A with the template T two nucleotides downstream before DNA synthesis, leading to G insertion opposite the next template C. To confirm this interpretation, purified human Pol η was used for extension DNA synthesis after a 10-min reaction of Pol μ bypass of the AP-T template. Primer extension by Pol η alone from the undamaged DNA template was used as the control. The control reaction yielded the expected 36-mer DNA product (Fig. 1B, lane 3). In contrast, after DNA synthesis across from the AP site by human Pol μ , Pol η extended the synthesis to the 34-mer DNA band (Fig. 1B, lane 1), indicating that a –2 deletion had occurred during DNA synthesis by human Pol μ across from the AP site. To prove that this is a reliable method for analysis of DNA products of human Pol μ , we digested the DNA with the *Dpn*II restriction endonuclease after 10-min translesion synthesis by Pol μ and 10-min extension by Pol η . Indeed,

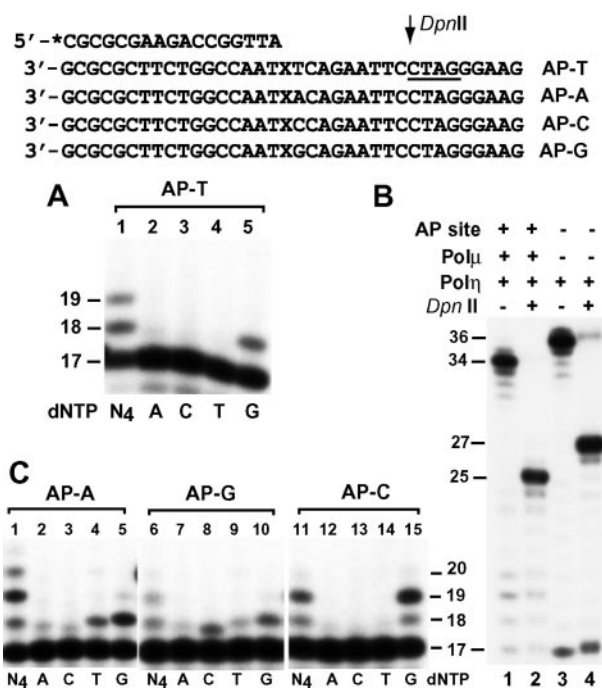


FIG. 1. DNA synthesis by human Pol μ from templates containing a site-specific AP site. A 5'-³²P-labeled (asterisk) 17-mer primer was separately annealed to four templates with the primer 3' end terminating right before the lesion as shown at the top. Each template differed by one nucleotide 5' to the AP site (X). The *Dpn*II recognition sequence is underlined, and the cleavage site on the ³²P-labeled strand is indicated. A, DNA synthesis assays were performed with 2 ng (35 fmol) of purified human Pol μ in the presence of a single dATP (A), dCTP (C), dTTP (T), or dGTP (G) or all four dNTPs (*N*₄), by using 50 fmol of the template AP-T. B, DNA synthesis was initiated with purified human Pol μ (3 ng, 53 fmol) at 30 °C for 10 min. Next purified human Pol η (2 ng, 25 fmol) was added to the reaction, and the incubation was continued for another 10 min at 30 °C (lanes 1 and 2). As the control, DNA synthesis from the undamaged template was performed with Pol η alone at 30 °C for 10 min (lanes 3 and 4). Cleavage of the DNA products by *Dpn*II is indicated (lanes 2 and 4). C, DNA synthesis assays were performed with human Pol μ (2 ng) by using templates AP-A, AP-G, and AP-C as indicated. DNA size markers in nucleotides are indicated on the sides.

the product of Pol μ contained a –2 deletion (Fig. 1B, lane 2) as compared with the normal DNA synthesis of the control (Fig. 1B, lane 4). These results show that AP site bypass by human Pol μ is mediated by a deletion mechanism as a result of primer realignment during translesion synthesis.

Lesion bypass by the deletion mechanism predicts that sequence context 5' to the lesion would significantly affect the specificity of nucleotide incorporation during translesion synthesis. When the template T 5' to the AP site was replaced by an A (AP-A template), T was also incorporated by human Pol μ in addition to G incorporation (Fig. 1C, lanes 1–5). With a template G 5' to the AP site (AP-G template), C and G were preferentially incorporated (Fig. 1C, lanes 6–10). With a template C 5' to the AP site (AP-C template), only G was incorporated (Fig. 1C, lanes 11–15). These results were precisely predicted by –1 and –2 deletions as a consequence of a shift of the primer 3' end downstream by 1 or 2 nucleotides, respectively, by human Pol μ before DNA synthesis.

To determine whether the unexpected lesion bypass activity of human Pol μ is limited to an AP site, we analyzed two more examples of simple base damage in the template: 8-oxoguanine and 1,*N*⁶-ethenoadenine. The 8-oxoguanine template contained a 5'-³²P-labeled 17-mer primer that terminated just before the lesion (Fig. 2). As shown in Fig. 2A, lane 1, human Pol μ performed translesion synthesis and predominantly incorporated

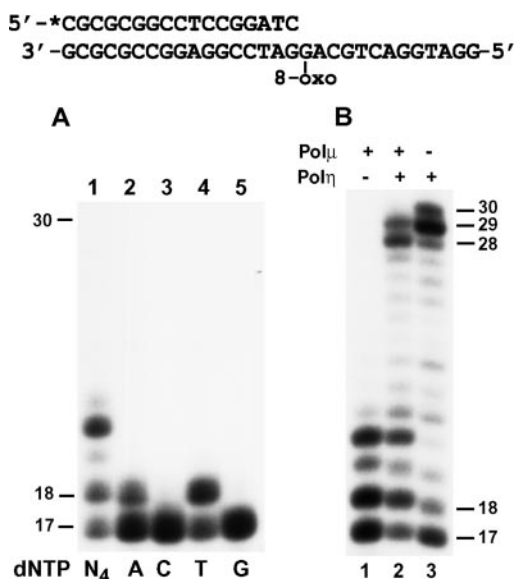


FIG. 2. DNA synthesis by human Pol μ from templates containing a site-specific 8-oxoguanine. A 17-mer primer was labeled with ^{32}P (asterisk) at its 5' end and annealed to the damaged template with the primer 3' end terminating right before the lesion as shown at the top. **A**, DNA synthesis assays were performed with 3 ng (53 fmol) of purified human Pol μ in the presence of a single dATP (**A**), dCTP (**C**), dTTP (**T**), or dGTP (**G**) or all four dNTPs (**N₄**). **B**, DNA synthesis was initiated with purified human Pol μ (3 ng) at 30 °C for 10 min. Next, purified human Pol η (2 ng, 25 fmol) was added to the reaction, and the incubation was continued for another 10 min at 30 °C (**lane 2**). **Lane 1**, DNA synthesis by human Pol μ alone; **lane 3**, DNA synthesis by human Pol η (2 ng) alone. DNA size markers in nucleotides are indicated on the sides.

T (Fig. 2A, lane 4). This result is predicted by the mechanism of primer realignment during translesion synthesis, which would lead to -1 deletion. To confirm that -1 frameshift synthesis indeed occurred, the products were extended by purified human Pol η . As shown in Fig. 2B, lane 3, Pol η alone copied the damaged DNA template, yielding 29-mer and 30-mer DNA bands. In contrast, when the damaged DNA template was first copied by human Pol μ across from the lesion and then extended by Pol η , the products were 28-mer and 29-mer DNA bands. This result indicates that -1 deletion had occurred during synthesis by Pol μ . Less frequently, human Pol μ also incorporated A while copying the 8-oxoguanine DNA template (Fig. 2A, lane 2). The precise mechanism for this minor incorporation is unknown.

The 1,N⁶-ethenoadenine template contained a 5'- ^{32}P -labeled 20-mer primer that terminated just before the lesion (Fig. 3). Purified human Pol μ synthesized DNA from both the damaged and the undamaged templates (Fig. 3A, lanes 1 and 6). However, the specificity of nucleotide incorporation was quite different. From the undamaged template, Pol μ incorporated nucleotides in the order of T>G>C (Fig. 3A, lanes 2-5). This specificity is consistent with T incorporation opposite the template A, -2 template shift, and -3 template shift, respectively, as predicted by the unique Pol μ property of highly frequent frameshift DNA synthesis (23). From the damaged template, G was preferentially incorporated (Fig. 3, lane 10). To determine whether deletion had occurred during translesion synthesis, the products were extended by the purified catalytic subunit of yeast Pol α . Pol α was chosen for extension synthesis because of its better activity in copying the last nucleotide of the template 5' end (Fig. 3B, lane 2). Indeed, the lesion bypass products of human Pol μ contained -1 and -2 deletions, respectively (Fig. 3B, lane 1), as compared with the normal synthesis control of Pol α alone (Fig. 3B, lane 2). The -2 deletion is consistent with

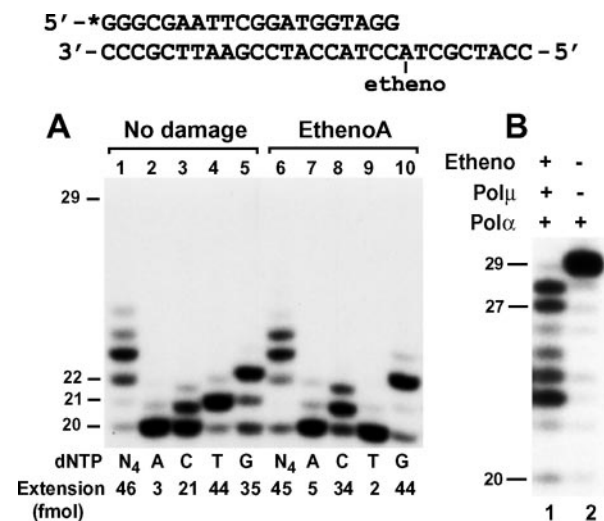


FIG. 3. DNA synthesis by human Pol μ from templates containing a site-specific 1,N⁶-ethenoadenine. A 20-mer primer was labeled with ^{32}P (asterisk) at its 5' end and annealed to the damaged template with the primer 3' end terminating right before the lesion as shown at the top. **A**, DNA synthesis assays were performed with 4 ng (70 fmol) of purified human Pol μ in the presence of a single dATP (**A**), dCTP (**C**), dTTP (**T**), or dGTP (**G**) or all four dNTPs (**N₄**), by using the undamaged or damaged templates as indicated. Quantitation of extended primers is shown below the gel. **B**, DNA synthesis was initiated with human Pol μ (3 ng) at 30 °C for 10 min. Then, the purified catalytic subunit of yeast Pol α (9 ng, 54 fmol) was added to the reaction, and the incubation was continued for another 10 min at 30 °C (**lane 1**). **Lane 2**, DNA synthesis from the undamaged template by Pol α (9 ng) alone. DNA size markers in nucleotides are indicated on the left.

a shift of the primer 3' end downstream by two nucleotides by Pol μ before DNA synthesis. The -1 deletion may have resulted from C misincorporation opposite the lesion before shift of the primer 3' end downstream by one nucleotide. Supporting this interpretation, Pol μ significantly incorporated C in response to the template 1,N⁶-ethenoadenine (Fig. 3A, lane 8). These results show that human Pol μ efficiently bypasses a template 1,N⁶-ethenoadenine by a deletion mechanism.

Lesion Bypass of Bulky DNA Adducts by Human Pol μ —To determine whether human Pol μ can respond to bulky adducts in DNA, we examined synthesis from a template containing an AAF-adducted guanine and a (+)- or (-)-*trans-anti*-BPDE-N²-dG adduct. The AAF-damaged template contained a 5'- ^{32}P -labeled 17-mer primer that terminated just before the lesion (Fig. 4). As shown in Fig. 4A, DNA synthesis from the damaged template was observed (Fig. 4A, lane 1), and G was most frequently incorporated by human Pol μ (Fig. 4A, lane 5). Less frequently, A was also incorporated, and C was rarely incorporated (Fig. 4A, lanes 2 and 3). G incorporation is consistent with a -1 deletion mechanism resulting from a shift of the primer 3' end downstream by one nucleotide and copying of the undamaged template C 5' to the lesion. An incorporation is consistent with a -2 template shift synthesis and copying of the undamaged template T two nucleotides downstream. Supporting this conclusion, Pol μ bypass followed by Pol η extension resulted in bypass products of 28-mer and 29-mer DNA bands, as compared with the 29-mer and 30-mer bypassed DNA bands by Pol η alone (Fig. 4B, compare lanes 2 and 3).

For DNA synthesis from templates containing the (+)- and (-)-*trans-anti*-BPDE-N²-dG adducts, a ^{32}P -labeled 19-mer primer that terminated right before the lesion was annealed. As shown in Fig. 5, lanes 1-5, human Pol μ predominantly incorporated a C opposite the undamaged template G. Minor T incorporation was also observed, probably as a result of realignment of the primer 3' G to pair with the template C two nucleotides downstream (-2 template shift). In the presence of

the BPDE adducts, DNA synthesis was observed, although Pol μ was more active on the template containing the (+)-*trans-anti*-BPDE- N^2 -dG adduct (Fig. 5A, lanes 6 and 11). In contrast to the undamaged template, C incorporation was barely detectable, whereas T incorporation became predominant during bypass of the BPDE adducts (Fig. 5A, lanes 6–15). These results are precisely predicted by the -2 deletion mechanism that resulted from a shift of the primer 3' end downstream by two nucleotides before DNA synthesis. The bypass products of hu-

man Pol μ indeed contained -2 deletion as analyzed by the Klenow extension after Pol μ translesion synthesis (Fig. 5B, compare lanes 1 and 3 with lanes 2 and 4). The Klenow DNA polymerase was chosen for extension synthesis because of its better activity in copying the last nucleotide of the template 5' end (Fig. 5B, lanes 2 and 4).

Accurate Translesion Synthesis by Human Pol μ Opposite a Template *cis-syn* TT Dimer—Cyclobutane pyrimidine dimers and (6–4) photoproducts are the major DNA lesions of UV radiation. The *cis-syn* TT dimer is a widely studied cyclobutane pyrimidine dimer. To determine whether human Pol μ is able to bypass a *cis-syn* TT dimer or a TT (6–4) photoproduct, we annealed a 5'- 32 P-labeled 15-mer primer to the template; the primer 3' end terminated just before the lesion (Fig. 6). Next, DNA synthesis assays were performed. A higher Pol μ concentration (15 ng, 263 fmol) was needed to achieve DNA synthesis of four nucleotides or longer from the undamaged template at this sequence context (Fig. 6, A and B, lane 1). Purified human Pol μ was unable to bypass the template TT (6–4) photoproduct (Fig. 6A, lane 3). In contrast, Pol μ catalyzed DNA synthesis to a similar extent in the absence or presence of the template TT dimer (Fig. 6, A, lanes 1 and 2, and B, lanes 1 and 6). With the undamaged template, AA was incorporated by Pol μ opposite the template TT sequence (Fig. 6B, lane 2). T was also significantly incorporated, probably as a result of -2 frameshift synthesis (Fig. 6B, lane 4). With the damaged template, surprisingly, T incorporation was barely detectable (Fig. 6B, lane 9). Instead, AA was predominantly incorporated during translesion synthesis across from the TT dimer (Fig. 6B, lane 7). Less frequently, C was also incorporated (Fig. 6B, lane 8), which could be explained by a realignment of the primer 3' TT to pair with the template AA four nucleotides downstream to copy the next template G (-4 deletion).

To confirm that AA was indeed predominantly incorporated by human Pol μ opposite the TT dimer, we extended the bypassed products by purified yeast Pol ζ . As the control, primer extension by Pol ζ was performed by using the undamaged DNA template. As shown in Fig. 6C, lane 1, Pol ζ alone extended the primer near the end of the undamaged template, forming a 35-mer DNA band. Extension of the Pol μ -bypassed products also yielded the 35-mer DNA band (Fig. 6C, lane 2). Furthermore, the mobility of the various DNA bands between the

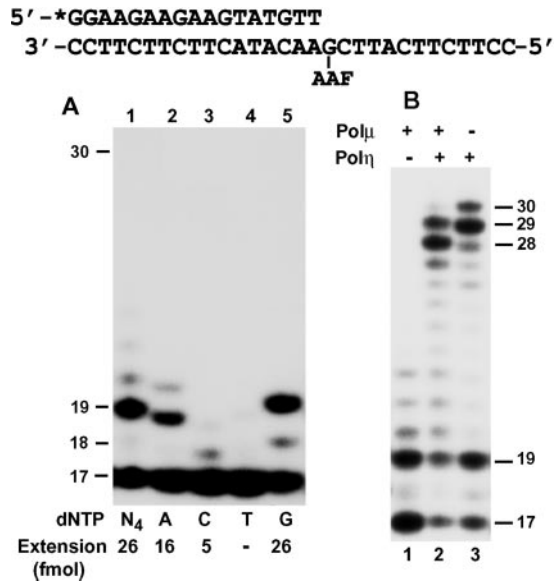


FIG. 4. DNA synthesis by human Pol μ from templates containing a site-specific AAF-guanine. A 32 P-labeled 17-mer primer was annealed to the damaged template with the primer 3' end terminating right before the lesion as shown at the top. A, DNA synthesis assays were performed with purified human Pol μ (3 ng, 53 fmol) in the presence of a single dATP (A), dCTP (C), dTTP (T), or dGTP (G) or all four dNTPs (N_4), by using 50 fmol of the damaged template. Quantitation of extended primers is shown below the gel. B, after initial DNA synthesis by human Pol μ (3 ng) at 30 °C for 10 min, purified human Pol η (2 ng, 25 fmol) was added to the reaction, and the incubation was continued for another 10 min (lane 2). Lane 1, DNA synthesis by human Pol μ alone; lane 3, DNA synthesis by human Pol η alone. DNA size markers in nucleotides are indicated on the sides.

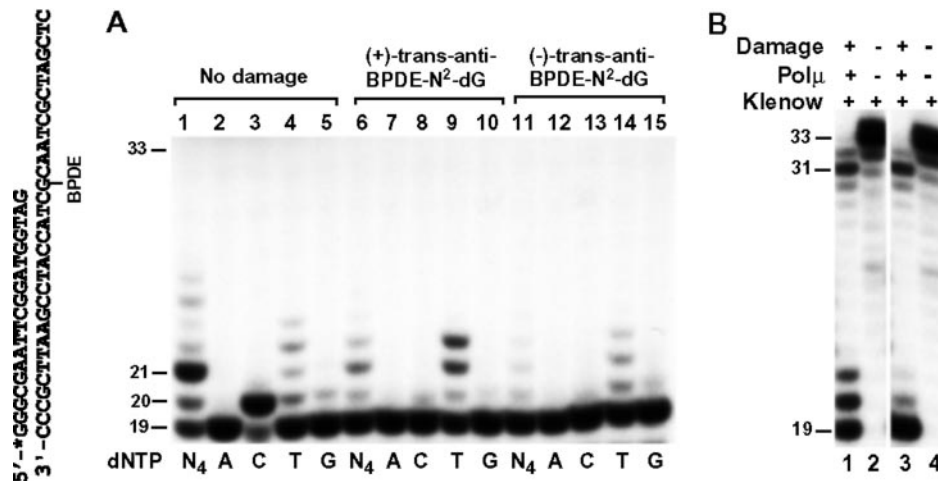


FIG. 5. DNA synthesis by human Pol μ from templates containing a site-specific (+)- or (-)-*trans-anti*-BPDE- N^2 -dG adduct. A 32 P-labeled 19-mer primer was annealed to the damaged template with the primer 3' end terminating right before the lesion as shown on the left. A, DNA synthesis assays were then performed with 4 ng (70 fmol) of purified human Pol μ in the presence of a single dATP (A), dCTP (C), dTTP (T), or dGTP (G) or all four dNTPs (N_4), by using the undamaged or damaged templates as indicated. B, DNA synthesis was initiated with human Pol μ (10 ng) at 30 °C for 10 min. Next purified Klenow DNA polymerase (1 unit) was added to the reaction, and the incubation was continued for another 10 min at 30 °C (lanes 2 and 4). Lanes 2 and 4, DNA synthesis from the undamaged template by the Klenow polymerase (1 unit) alone. Lane 1, the (+)-*trans-anti*-BPDE- N^2 -dG adduct; lane 3, the (-)-*trans-anti*-BPDE- N^2 -dG adduct. DNA size markers in nucleotides are indicated on the left.

primer and the 35-mer DNA was identical between DNA synthesis from the undamaged template by Pol ζ alone and the Pol ζ -extended Pol μ products (Fig. 6C). Because the 3' T of the TT dimer completely blocks yeast Pol ζ (25), the 35-mer DNA band could only result from extension of the Pol μ -synthesized bypass products. These results show that human Pol μ possesses error-free lesion bypass activity in response to a tem-

plate *cis-syn* TT dimer.

Human Pol β Is Unable to Bypass a Variety of DNA Lesions—To evaluate whether the lesion bypass activity of human Pol μ is unique among X family DNA polymerases, we performed translesion synthesis assays with purified human Pol β using the same DNA templates under identical reaction conditions as in the Pol μ experiments. Purified human Pol β was active in copying the undamaged template (Fig. 7, lane 2). In contrast, human Pol β was unable to perform translesion synthesis opposite a template AP site regardless of the sequence context 5' to the lesion, even when a large excess of the polymerase (520 fmol) was used (Fig. 7, lanes 3–6). Similarly, human Pol β was completely unresponsive to a template 1, N^6 -ethenoadenine, an AAF-adducted guanine, a TT dimer, or a TT (6–4) photoproduct (Fig. 7, lanes 7–13). Purified human Pol β was also unable to perform translesion synthesis opposite a template (+)- or (-)-*trans-anti*-BPDE- N^2 -dG adduct, as we demonstrated recently (26). Thus, lesion bypass activity is not a common feature among the X family DNA polymerases.

DISCUSSION

Previously, we proposed that Pol μ may be involved in NHEJ of double-strand DNA breaks through its microhomology searching and pairing activities (23). Most recently, Mahajan *et al.* (33) reported that cellular levels of human Pol μ protein are increased by ionizing radiation, and that Pol μ is associated with the NHEJ proteins Ku and XRCC4-ligase IV, further supporting a role of this polymerase in NHEJ. In this study, we found that human Pol μ possesses DNA lesion bypass activities in response to various types of DNA damage. Thus, efficient translesion synthesis activity is not strictly limited to the Y family of DNA polymerases. Under similar experimental conditions, we did not detect any lesion bypass activities of purified human Pol β (Fig. 7), except for 8-oxoguanine (34), which is a miscoding rather than a strong blocking lesion (35). Because Pol μ and Pol β share sequence homologies and they both belong to the X family of DNA polymerases (1, 2), the lesion bypass activity of human Pol μ appears to be unique among X family members.

In response to the template AP site 8-oxoguanine, 1, N^6 -ethenoadenine, AAF-adducted guanine, and (+)- and (-)-*trans-anti*-benzo[*a*]pyrene- N^2 -dG, human Pol μ bypasses the lesion predominantly by a deletion mechanism. The specificity of nucleotide incorporation during translesion synthesis indicates that deletion is a result of primer realignment. Because these

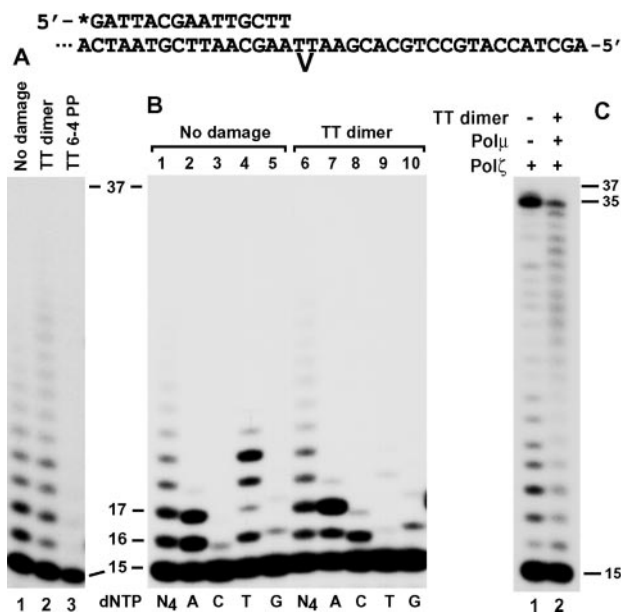
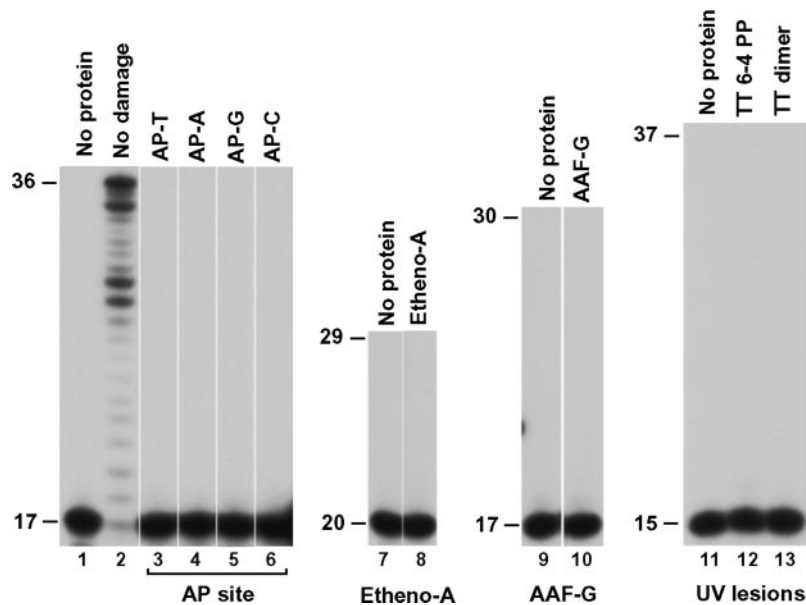


FIG. 6. DNA synthesis by human Pol μ from templates containing a site-specific *cis-syn* TT dimer or TT (6–4) photoproduct. A 15-mer primer was labeled with 32 P (asterisk) at its 5' end and annealed to the template with the primer 3' end terminating right before a TT dimer or a TT (6–4) photoproduct (TT 6–4 PP) as shown at the top. A, DNA synthesis assays were performed with 15 ng (263 fmol) of purified human Pol μ by using 50 fmol of undamaged and damaged templates as indicated. B, DNA synthesis assays were performed in the presence of a single dATP (A), dCTP (C), dTTP (T), or dGTP (G) or all four dNTPs (N_4) by using 15 ng of purified human Pol μ and 50 fmol of undamaged and damaged templates as indicated. C, DNA synthesis was initiated with purified human Pol μ (15 ng) at 30 °C for 10 min using the damaged template. Then, purified yeast Pol ζ (26 ng, 149 fmol) was added to the reaction, and the incubation was continued for another 10 min at 30 °C (lane 2). Lane 1, DNA synthesis by human Pol ζ alone with an undamaged template of the same sequence. DNA size markers in nucleotides are indicated on the sides.

FIG. 7. Effect of various DNA lesions on polymerase activity of human Pol β . The 32 P-labeled 15-, 17-, 19-, and 20-mer primers were annealed to the damaged templates with the primer 3' ends terminating right before the lesion as shown in Figs. 1–6. DNA synthesis assays were then performed with purified human Pol β (20 ng, 520 fmol) at 30 °C for 10 min in the presence of all four dNTPs. Site-specific DNA lesions in the templates are AP site (lanes 3–6), 1, N^6 -ethenoadenine (lanes 7 and 8), AAF-guanine (lanes 9 and 10), TT (6–4) photoproduct (lane 12), and TT dimer (lane 13). Control reactions without DNA damage (lane 2) or without Pol β (lanes 1, 7, 9, and 11) are also shown. Lane 2, DNA synthesis by human Pol β (4 ng, 104 fmol) from a 36-mer undamaged template. DNA size markers in nucleotides are indicated on the left.



DNA lesions differ dramatically in structure, we propose that bypass of these lesions by human Pol μ may be achieved by looping out the template lesion, thus avoiding a direct copying of the damaged template base. The exact deletion size appears to depend on the sequence context of the lesion. For example, if the primer 3' end can pair with a template base 5' to the lesion, such realignment would be preferred by human Pol μ . Thus, when human Pol μ encounters a lesion, if the coding capacity of the modified base is lost or significantly altered, Pol μ simply realigns the primer-template strands to continue DNA synthesis by skipping the lesion. Most recently, it was reported that human cell extracts supplemented with purified human Pol μ are able to extend a primer from opposite an AAF-adducted guanine by adding a ladder of as many as 15 guanines in an apparently nontemplated reaction (36). This activity is different from the lesion bypass activity of human Pol μ reported here, and its functional significance remains unknown.

On the basis of mutation spectra of several DNA lesions, base substitutions rather than deletions appear to be the major mutational events in mammalian cells (37–40). Therefore, the prevailing deletion mechanism of lesion bypass by human Pol μ suggests that this polymerase is unlikely to play a major role in translesion synthesis during replication in normal cells and under normal growth conditions. It is now clear that single nucleotide repeats, mismatched primer 3' ends, and many DNA lesions greatly promote the primer-template realignment by human Pol μ . These biochemical properties support the role of Pol μ in NHEJ. Furthermore, the lesion bypass activities of Pol μ would make it possible for this polymerase to perform microhomology search, microhomology pairing, and DNA synthesis during NHEJ even in the presence of base lesions. After NHEJ is complete, the base lesion can then be removed by an excision repair mechanism.

Remarkably, the effective bypass of a *cis-syn* TT dimer by human Pol μ is error-free. Because no template TT sequence is present anywhere 5' to the lesion or near the lesion on the 3' side (Fig. 6), AA insertion during the bypass must result from the direct copying of the TT dimer by human Pol μ . A template TT (6–4) photoproduct, however, completely blocks human Pol μ . It is possible that because of covalent linkage between the two thymine bases, the TT dimer and the TT (6–4) photoproduct may not be flexible enough to allow loop-out by human Pol μ . Unlike the TT dimer, the TT (6–4) photoproduct may be too distorting to DNA structure to allow Pol μ nucleotide insertion opposite the lesion. The only other eukaryotic DNA polymerase known to perform error-free bypass of a TT dimer is Pol η (22, 41). Human xeroderma pigmentosum variant cells that lack Pol η activity are sensitive to and hypermutable by UV radiation (19–21,42); thus they establish an important *in vivo* role for this polymerase in error-free bypass of UV lesions. However, it is not known how Pol η would respond to other cyclobutane pyrimidine dimers such as the C-containing dimers. Therefore, it is unknown to what extent loss of the TT dimer bypass by Pol η contributes to UV-induced sensitivity and mutagenesis in xeroderma pigmentosum variant cells. With this uncertainty, we are unable to assess at the present time the *in vivo* importance of the error-free TT dimer bypass by Pol μ . Nevertheless, our results raised the possibility that Pol μ may participate in the error-free bypass of TT dimers in

cells, especially when the Pol η function is compromised, as in the case of the xeroderma pigmentosum variant cells.

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