

Lesion Bypass by Human DNA Polymerase μ Reveals a Template-dependent, Sequence-independent Nucleotidyl Transferase Activity*

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DNA polymerase μ (pol μ), which is related to terminal deoxynucleotidyl transferase and DNA polymerase β , is thought to be involved in non-homologous end joining and V(D)J recombination. Pol μ is induced by ionizing radiation and exhibits low fidelity. Analysis of translesion replication by purified human pol μ revealed that it bypasses a synthetic abasic site with high efficiency, using primarily a misalignment mechanism. It can also replicate across two tandem abasic sites, using the same mechanism. Pol μ extends primers whose 3'-terminal nucleotides are located opposite the abasic site. Most remarkably, this extension occurs via a mode of nucleotidyl transferase activity, which does not depend on the sequence of the template. This is not due to simple terminal nucleotidyl transferase activity, because pol μ is unable to add dNTPs to an oligo(dT)₂₉ primer or to a blunt end duplex oligonucleotide under standard conditions. Thus, pol μ is a dual mode DNA-synthesizing enzyme, which can act as either a classical DNA polymerase or as a non-canonical, template-dependent, but sequence-independent nucleotidyl transferase. To our knowledge, this is the first report on a DNA-synthesizing enzyme with such properties. These activities may be required for its function in non-homologous end joining in the processing of DNA ends prior to ligation.

DNA polymerase μ (pol μ)¹ is a recently identified member of the X family of DNA polymerases. Pol μ shares 41% amino acid identity with terminal deoxynucleotidyltransferase (TdT), a template-independent DNA polymerase (1, 2). In addition, both pol μ and terminal deoxynucleotidyltransferase are overexpressed in the immune system. However, unlike terminal deoxynucleotidyltransferase, which is specific to the immune system, pol μ is expressed in additional tissues (1, 2). *In vivo* analysis with pol μ -deficient mice revealed depletion of B cells in peripheral lymphoid organs in ~50% of the mice and impairment of the immunoglobulin κ light chain rearrangement (3, 4). This suggests that pol μ is essential in V(D)J recombination, at least in mice. In addition, pol μ is up-regulated in response to ionizing and UV radiation and forms complexes with DNA ligase IV and the Ku proteins (5). This indicates that pol μ partakes not only in V(D)J recombination but generally in

non-homologous end joining (NHEJ). Pol μ was shown to be an error-prone DNA polymerase (1, 6), capable of incorporating either dNTPs or rNTPs (7, 8). It has an outstanding tendency to form frameshift mutations, directed by its ability to misalign the primer terminus with short downstream homologies (microhomology search) (6). Based on this property, it was proposed that pol μ functions in NHEJ by searching homology between two broken ends, followed by filling in small gaps.

When DNA is damaged in the vicinity of a break, its processing by DNA synthesis might require lesion bypass. Here we show that, unlike most other DNA polymerases, purified pol μ bypasses a synthetic abasic site very efficiently. Surprisingly, when extending a primer terminus located opposite an abasic site, pol μ adds each of the four dNTPs, regardless of the sequence of the template. To our knowledge, this is the first report on a template-dependent but non-instructed nucleotidyl transferase activity.

MATERIALS AND METHODS

Proteins—Recombinant human pol μ was expressed in *Escherichia coli* and purified as described (9). *E. coli* Pol IV (His-tagged) was a generous gift from H. Ohmori (Kyoto University, Kyoto, Japan), and the *E. coli* pol I Klenow fragment was purchased from U. S. Biochemical Corp.

DNA Substrates—DNA oligonucleotides without a lesion were supplied by Sigma-Genosys, whereas oligonucleotides containing synthetic abasic sites (dSpacer, Glen Research, Sterlin, VA), were synthesized by the Synthesis Unit of the Biological Services Department in our institute or purchased from Metabion (Martinsried, Germany). The DNA substrates used in this study are shown in Fig. 1. They were prepared by annealing a ³²P-5'-end-labeled primer oligonucleotide to the template oligonucleotide, followed by purification on a BioSpin 30 gel-filtration column (Bio-Rad) as described previously (10–12). Analysis by electrophoresis on native gels revealed that >95% of the primers were annealed to the template oligonucleotides.

Primer Extension Reactions—A typical primer extension reaction (20 μ l) contained 20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 8 μ g/ml bovine serum albumin, 4% glycerol, 5 mM dithiothreitol, 5 mM MgCl₂, 100 μ M each of dATP, dCTP, dGTP, and dTTP, and 50 nM primed oligonucleotide substrate. Pol μ or DNA polymerase I (Klenow fragment) at 20–50 nM were added to start the reactions, which were carried out at 37 °C for 10–30 min. Reactions were stopped by adding an equal volume of a mixture of 99.5% formamide, 0.025% bromphenol blue, and 0.025% xylene cyanol. Samples were fractionated by electrophoresis on 15 or 20% polyacrylamide gels containing 8 M urea, after which they were dried, visualized, and quantified using a Fuji BAS 2500 phosphorimaging device. Primer extension was calculated by dividing the amount of all extended primers by the sum of extended and non-extended primers. The extent of bypass was calculated by dividing the amount of bypass products by the amount of all extended primers. To qualitatively examine the specificity of primer extension by pol μ , the reactions were carried out under similar conditions in the presence of a single dNTP at concentrations of 1, 10, and 100 μ M. The efficiency of extension was calculated by dividing the amount of extended primers by the total amount of primers.

Steady-state Kinetic Analyses—Analysis of kinetic parameters for primer extension from the abasic site or deoxynucleotide incorporation opposite an undamaged nucleotide template was performed as follows.

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¹ The abbreviations used are: pol μ , DNA polymerase μ ; NHEJ, non-homologous end joining.

FIG. 1. The sequence of primer-template oligonucleotide substrates used in this study. X marks the synthetic abasic site. *Cont.*, control.

5' GGGTAACGCCAGGGTTTC		
3' CCCATTGCGGTCCCAAAGXGTCAGTGCTGCAACATGTCG 5'		AB1G
5' GGGTAACGCCAGGGTTTCN		AB1G-0A-T
3' CCCATTGCGGTCCCAAAGXGTCAGTGCTGCAACATGTCG 5'		
5' GGGTAACGCCAGGGTTTC		AB1G-cont
3' CCCATTGCGGTCCCAAAGTGTCAAGTGCTGCAACATGTCG 5'		
5' TGCTGCAAGGCGATTAAGT		AB2C
3' ACGACGTTCCGCTAATTCAXCCCATTGCGGTCCCAAAGG 5'		
5' TGCTGCAAGGCGATTAAGT		AB2C-cont
3' ACGACGTTCCGCTAATTCAGCCCATTGCGGTCCCAAAGG 5'		
5' GGGTAAACGCCAGGTC		AB33A
3' CCCATTTGCGGGTCCAGXACAGTGCTGCAACATG 5'		AB33C
	C	AB33G
	G	AB33T
	T	
5' GGGTAAACGCCAGGTCN		AB33A-0A-T
3' CCCATTTGCGGGTCCAGXACAGTGCTGCAACATG 5'		
5' GGGTAAACGCCAGGTC		ABXX33
3' CCCATTTGCGGGTCCAGXXCAGTGCTGCAACATG 5'		
5' GGGTAAACGCCAGGTCA		AB-(A-tail)
3' CCCATTTGCGGGTCCAGXAAAAAAAAA 5'		
5' GGGTAAACGCCAGGTC		Cont-(A-tail)
3' CCCATTTGCGGGTCCAGXAAAAAAAAA 5'		
5' GGGTAAACGCCAGGTCA		AB-(G-tail)
3' CCCATTTGCGGGTCCAGXGGGGGGGGG 5'		
5' GGGTAAACGCCAGGTC		Cont-(G-tail)
3' CCCATTTGCGGGTCCAGGGGGGGGG 5'		

Human pol μ (at 25 nM) was incubated with increasing concentrations of a single deoxynucleotide (1–2000 μ M; 6–8 concentration points) and with 50 nM DNA substrate for 20 or 30 min under standard reaction conditions. Gel band intensities of the substrates and products were quantified as described above. The percentage of primer extended was duplicated by 1000 fmol and divided by the time of the reaction to give V_{obs} values. These values were plotted using the Lineweaver-Burk double-reciprocal plot of the Michaelis-Menten equation, namely $1/v = 1/V_{\text{max}} + K_m/[dNTP] \cdot V_{\text{max}}$. Apparent K_m and V_{max} steady-state parameters were obtained from the fit and used to calculate the frequency of deoxynucleotide incorporation (f_{inc}) using the equation $f_{\text{inc}} = (V_{\text{max}}/K_m)^{\text{incorrect nucleotide}} / (V_{\text{max}}/K_m)^{\text{correct nucleotide}}$. For each substrate a set of 3–5 experiments was carried out under conditions in which primer utilization was <25%.

RESULTS

Purified Pol μ Effectively Bypasses a Single or Two Tandem Abasic Site(s)—The ability of pol μ to replicate across DNA blocking lesions was assayed using primed oligonucleotides, each carrying a site-specific synthetic abasic site. Abasic sites are common lesions in DNA, formed both spontaneously and by DNA damaging agents such as ionizing radiation (13, 14). Abasic sites were shown to severely block polymerization by purified prokaryotic (*e.g.* Refs. 10, 11, 15–18) and eukaryotic DNA polymerases (12, 17, 19–22).

Fig. 2 shows standing start primer extension assays performed with purified pol μ , using two different primer-templates. When template AB1G, containing a synthetic abasic site was used, primer extension by pol μ was similar to that obtained in the absence of the lesion (Fig. 2A, compare lanes 2 and 5). In contrast, primer extension by pol I (Klenow fragment) was strongly inhibited on this DNA substrate (Fig. 2A lane 3), consistent with previous results (11). Similar results were obtained with another DNA substrate, AB2C (Fig. 1), in

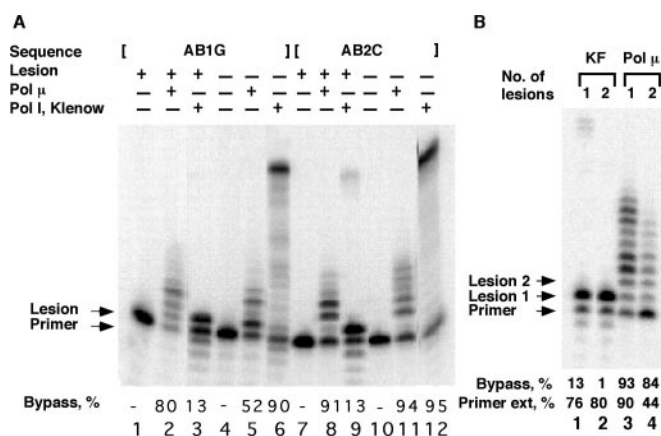


FIG. 2. Primer extension by pol μ on templates carrying one or two tandem abasic sites. A, reactions were carried out as described under "Materials and Methods" with primer-templates AB1G or AB2C and 50 nM pol μ or pol I (Klenow fragment) for 10 min at 37 °C. B, reactions were carried out with primer-template AB33A, carrying a single abasic site, or primer-template ABXX33, carrying two tandem abasic sites, and with 40 nM pol μ or pol I (KF, Klenow fragment) for 10 min at 37 °C.

which the abasic site was located in a different sequence context (Fig. 2A, lanes 7–12). We have also analyzed the ability of pol μ to bypass two tandem abasic sites in a different sequence context by using template ABXX33 (Fig. 1). *E. coli* pol I (Klenow) was completely blocked by the two tandem abasic sites (Fig. 2B, lane 2). In contrast, pol μ extended the primer across the two tandem abasic sites, although at a lower efficiency than across a single abasic site (90 and 44% primer extension, re-

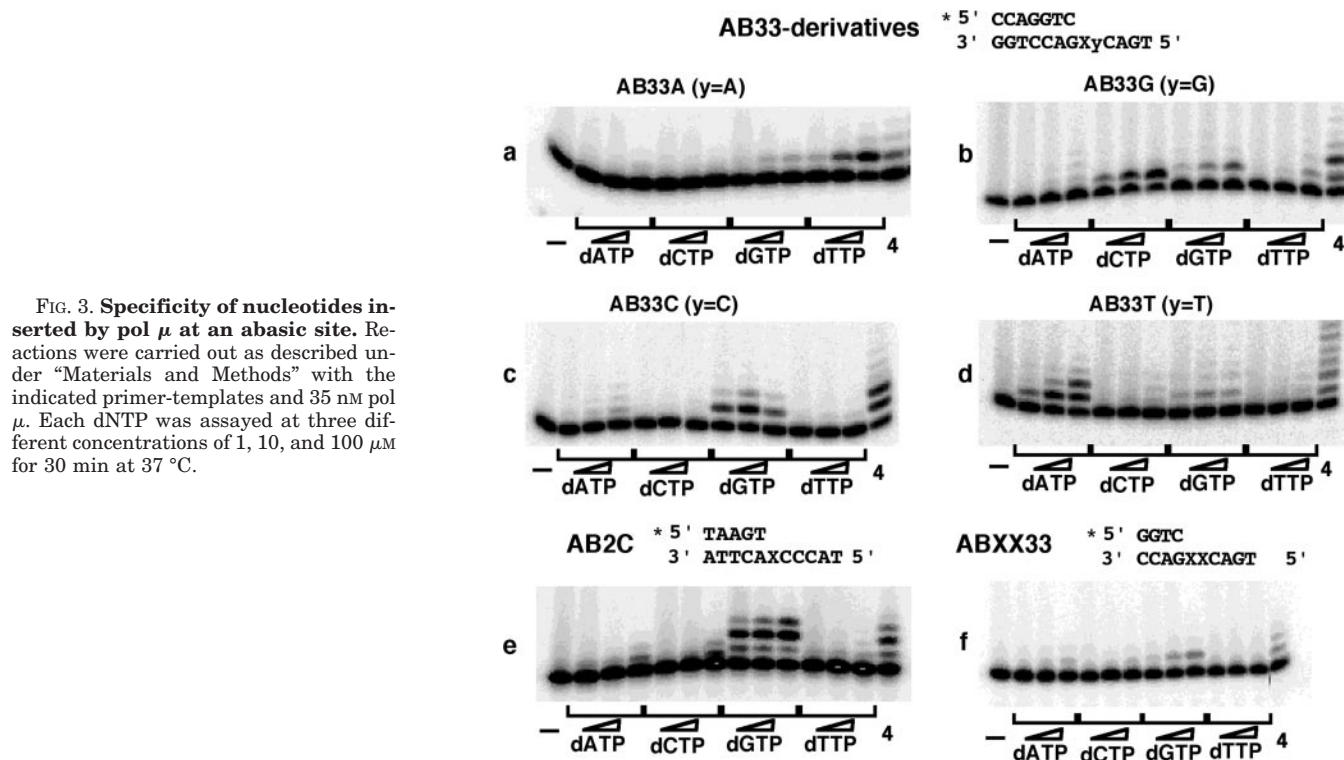


FIG. 3. Specificity of nucleotides inserted by pol μ at an abasic site. Reactions were carried out as described under "Materials and Methods" with the indicated primer-templates and 35 nM pol μ . Each dNTP was assayed at three different concentrations of 1, 10, and 100 μ M for 30 min at 37 $^{\circ}$ C.

spectively; Fig. 2B, compare lanes 3 and 4). Most remarkably, pol μ did not show any pause at the site of the lesions, and bypass *per se* was little affected (93% bypass of a single abasic site and 84% bypass of two tandem abasic sites; Fig. 2B). The decreased activity on the substrate with the two tandem abasic sites, as compared with the single lesion template, is therefore due to inhibition of synthesis initiation rather than inhibition of lesion bypass.

Pol μ Uses Misalignment to Bypass Abasic Sites—To elucidate the mechanism by which pol μ bypasses abasic sites, we examined the specificity of the nucleotide inserted opposite the abasic site. To this end, we performed standing start primer extension reactions with single dNTPs using template AB33A. As can be seen in Fig. 3a, when the template nucleotide next to the abasic site was an A, pol μ incorporated primarily a dTMP residue. We assayed three additional variants of AB33A, each containing a different template nucleotide 5' to the abasic site (Figs. 1 and 3). As can be seen in Fig. 3, a–d, the specificity of incorporation by pol μ was dependent on the template nucleotide next to the abasic site. In each case, the most frequent event was insertion of a nucleotide complementary to the template nucleotide 5' to the lesion. Pol μ also inserts dGMP on these templates, although with lower efficiency (Fig. 3, a, b, and d). This is the nucleotide complementary to the template C located two nucleotides 5' to the abasic site. Taken together, these results suggest that pol μ bypasses abasic sites by using a skipping mechanism in which the abasic site is "flipped out" and the polymerase replicates the next template nucleotide, similar to the bypass by pol β (20, 21). Consistent with this suggestion, pol μ incorporated primarily dGMP on another template (AB2C), where a template C is located 5' to the lesion (Fig. 3e).

The same mechanism was probably used when pol μ bypassed two tandem abasic sites, because mostly dGMP was incorporated by pol μ on primed template ABXX33, where the first template nucleotide 5' to the two abasic sites is a C (Fig. 3f). In light of these results, the inhibition of synthesis initiation but not elongation by the two tandem abasic sites may be

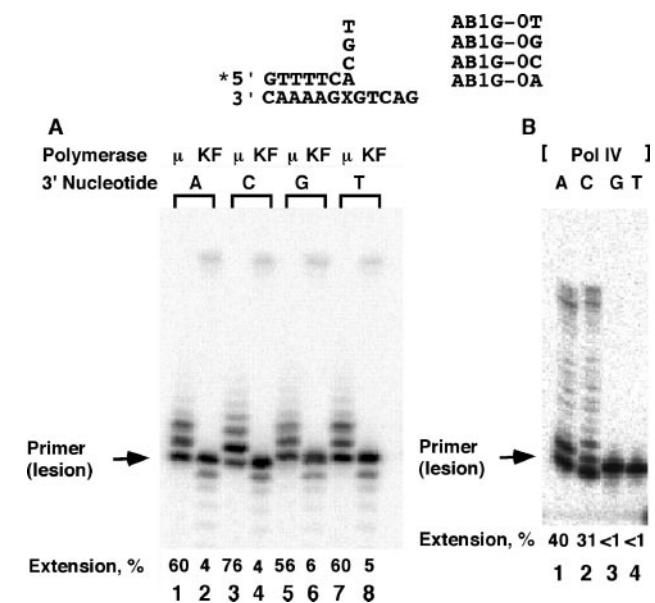
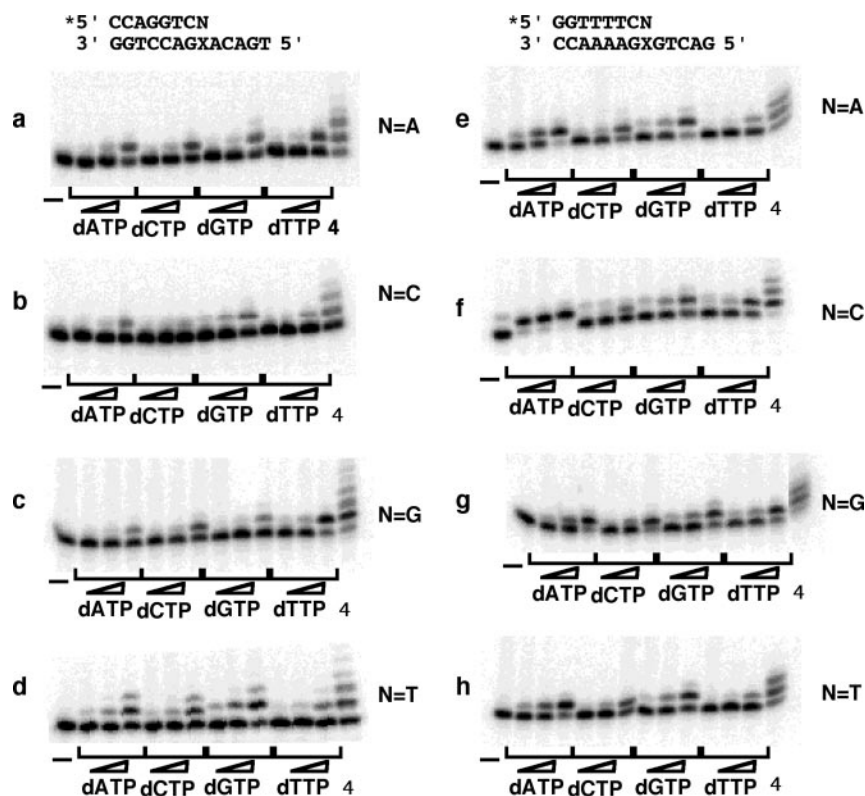


FIG. 4. Extension by pol μ of a primer terminus located opposite an abasic site. Reactions were carried out as described under "Materials and Methods" with the indicated primer-templates and 50 nM pol μ , *E. coli* pol IV, or pol I (KF, Klenow fragment) for 10 min at 37 $^{\circ}$ C.

explained by the difficulty in skipping two abasic sites rather than only one.

Pol μ Extends Primers Located Opposite an Abasic Site in a Sequence-independent Manner—The high bypass efficiency of pol μ prompted us to analyze its ability to elongate primers located opposite the lesion. This was done using template AB1G, with a primer terminated opposite the abasic site. As expected, Pol I (Klenow fragment) was ineffective in extending such primers (Fig. 4A, lanes 2, 4, 6, and 8). In contrast, pol μ extended all (abasic site:base) mismatches quite effectively, regardless of the identity of the 3'-terminal base located oppo-

FIG. 5. Specificity of extension by pol μ of a primer terminus located opposite an abasic site. Reactions were carried out as described under "Materials and Methods" with the indicated primer-templates and 25 nM pol μ . Each dNTP was assayed at three different concentrations of 0.2, 1, and 10 μ M for 10 min at 37 $^{\circ}$ C.



site the lesion (Fig. 4A, lanes 1, 3, 5, 7). This was somewhat surprising, because only the extension of C was expected to be effective if the polymerase uses a misalignment mechanism, as shown above. In contrast to pol μ , the *E. coli* DNA polymerase IV, which was shown to use template misalignment frequently (23, 24), could elongate only the primers terminated with C or A (Fig. 4B). These bases are complementary to the first or second template bases, respectively, 5' to the abasic site, consistent with a mechanism of primer misalignment. Therefore, the ability of pol μ to extend effectively all four bases suggests that pol μ uses a mechanism other than primer misalignment when extending a nucleotide present opposite an abasic site.

Next we performed a qualitative analysis to determine the identity of the nucleotide extended by pol μ from specific abasic site:base mismatches. This was done using template AB33A primed with an oligonucleotide whose 3'-terminal nucleotide was located opposite the abasic site. Surprisingly, pol μ incorporated each one of the four dNMPs with apparently similar efficiencies (Fig. 5a). Similar results were obtained for substrates with a primer 3'-terminal A, C, G, and T (Fig. 5, a-d). This suggested a mode of DNA synthesis that is independent of the sequence of the template.

To quantify these results, we performed quantitative kinetic experiments in which we determined the K_m and V_{max} values for extension from abasic site:base mismatches in three different substrates, AB33A-0A, AB33G-0A, and AB33T-0C (Table I). As a control, we used AB33A with no lesion. As can be seen in Table I, when extending an abasic site:A mismatch on substrate AB33A0-A, pol μ essentially lost its ability to discriminate against insertion of the incorrect nucleotide. All four dNTPs were inserted with comparable efficiencies (within 2-fold from the correct nucleotide). A similar decrease in the ability to discriminate against insertion of the incorrect nucleotide was also observed with the other lesion-containing substrates that were used (Table I). In contrast, pol μ did show clear discrimination against insertion of the incorrect nucleotides when the control substrate without the lesion was used

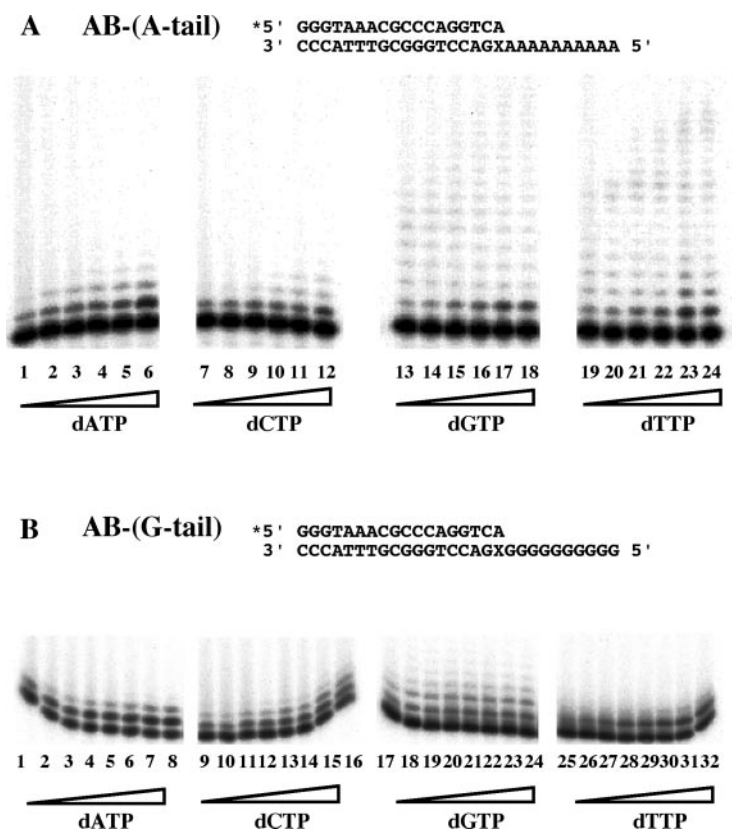
TABLE I
Kinetic measurement of nucleotide incorporation by pol μ when elongating from an abasic site:nucleotide mismatch

Reactions were carried out as described under "Materials and Methods" with 25 nM primer-templates, 20 nM pol μ , for 3–30 min at 37 $^{\circ}$ C. The fidelity of nucleotide incorporation is indicated by the f_{inc} values, calculated as $(V_{max}/K_m)_{incorrect}/(V_{max}/K_m)_{correct}$.

dNTP	V_{max} fmol/min	K_m μ M	V_{max}/K_m	f_{inc}
AB33A0-A dNTP 5'-GGTCA ↓ 3'-CCAGXACAG				
dATP	4 ± 1.2	52 ± 16	0.077	0.48
dCTP	6 ± 1.2	42 ± 6	0.143	0.9
dGTP	2.4 ± 0.6	8 ± 0.9	0.3	1.8
dTTP	5.5 ± 1	34 ± 11.0	0.160	1
AB33G0-A dNTP 5'-GGTCA ↓ 3'-CCAGXGCAG				
dATP	7.5 ± 0.8	42 ± 4	0.178	0.7
dCTP	2.5 ± 0.3	10 ± 1.6	0.250	1
dGTP	2 ± 0.2	5.5 ± 0.1	0.363	1.4
dTTP	3 ± 0.4	41 ± 1.7	0.073	0.3
AB33T0-C dNTP 5'-GGTCC ↓ 3'-CCAGXTCAG				
dATP	6 ± 3.5	202 ± 92	0.030	1
dCTP	3 ± 1.2	475 ± 195	0.006	0.2
dGTP	2.3 ± 0.6	15.6 ± 4.2	0.147	5
dTTP	2.5 ± 1.7	338 ± 36	0.007	0.25
AB33A control dNTP 5'-GGTCC ↓ 3'-CCAGGACAG				
dATP	0.9 ± 0.3	564 ± 22	1.6 × 10 ⁻³	3.5 × 10 ⁻⁴
dCTP	1 ± 0.5	218 ± 37	4.6 × 10 ⁻³	1 × 10 ⁻³
dGTP	10 ± 1.6	114 ± 32	0.09	0.02
dTTP	18 ± 4	4 ± 1.7	4.5	1

(Table I, AB33A control). To examine the specificity of extension in a different sequence context, a qualitative primer extension analysis was performed with primed template AB1G. As can be seen in Fig. 5, e-h, a similar picture was obtained;

FIG. 6. Specificity of extension by pol μ of a primer terminus situated opposite an abasic site, which is located upstream to (A)₁₀ or (G)₁₀ runs. Reactions were carried out as described under "Materials and Methods" with the indicated primer-templates and 25 nM pol μ for 30 min at 37 °C. **A**, reactions with the DNA substrate AB-(A-tail). dATP concentrations were 10, 20, 50, 100, 250, and 500 μ M in lanes 1–6, respectively. The same concentrations were used for dCTP (lanes 7–12), dGTP (lanes 13–18), and dTTP (lanes 19–24). **B**, reactions with the DNA substrate AB-(G-tail). dATP concentrations were 20, 40, 50, 70, 100, 150, 250, and 350 μ M in lanes 1–8, respectively. dCTP concentrations were 20, 40, 50, 60, 70, 80, 100, and 150 μ M in lanes 9–16, respectively. dGTP concentrations were 10, 20, 50, 70, 100, 115, 130, and 150 μ M in lanes 17–24, respectively. dTTP concentrations were 2, 4, 10, 15, 20, 30, 40, and 50 μ M in lanes 25–32, respectively.



Pol μ inserted all four dNMPs on each primer-template configuration. Only in the case of the 3'-terminal C in the primer strand was there preferential incorporation of dAMP, most likely due to misalignment, and template-directed incorporation opposite the next base (T). Still, even in this case, all other three dNTPs were incorporated by pol μ (Fig. 5, e–h).

The ability of pol μ to incorporate all four dNTPs when extending a primer terminus located opposite an abasic site may stem from local loss of template instruction, or from misalignment and instruction by template nucleotides at various positions 5' to the lesion. To distinguish between these two possibilities, we performed a quantitative kinetic analysis of the specificity of extension in a substrate in which all 10 template nucleotides 5' to the lesion were A (AB-(A-tail); Figs. 1 and 6A). This strongly biased one-nucleotide extensions to the insertion of dTMP, because any template-directed nucleotide incorporation, be it a direct extension or any misalignment event, is expected to yield dTMP incorporation on substrate AB-(A-tail). As can be seen in Fig. 6A, each of the four dNTPs was incorporated on this template. Moreover, multiple insertions were observed. Thus, with dATP as the only dNTP, extension by four nucleotides was observed, and a similar result was observed with dCTP as the only dNTP. Quite remarkably, when either dTTP or dGTP were used as the only dNTPs, there were multiple extension steps leading to a nascent DNA strand whose length exceeded the length of the template (Fig. 6). In the case of dTTP, this can be explained by template-instructed incorporation accompanied by primer slippage. However, in the case of dGTP, this indicates that long-range sequence-independent polymerization has occurred, as is discussed below. Primer extension analysis was performed also with another substrate, AB-(G-tail), which contains 10 G nucleotides 5' to the abasic site (Figs. 1 and 6B). Results obtained with AB-(G-tail) were generally similar to those obtained with AB-(A-tail), except that the numbers of dNTP additions did not exceed the size of the G-tail (Fig. 6B).

We determined the kinetic parameters for the insertion of each of the four dNTPs on substrates AB-(A-tail) and AB-(G-tail) and on the control substrates without a lesion (Fig. 1 and Table II). As can be seen in Table II, all four nucleotides were inserted with similar efficiencies on the AB-(A-tail) substrate. In the case of the AB-(G-tail) substrate, dTMP and dGMP were incorporated best, and, most remarkably, the complementary dCMP was inserted with the lowest efficiency, primarily due to a high K_m value (Table II). Pol μ did discriminate against insertion of the wrong nucleotides in the control substrates without the lesion, although its fidelity was low, especially in the G-tailed substrate (Table II). These results indicate that pol μ can act as a template-dependent, sequence-independent DNA polymerase.

DISCUSSION

All known DNA polymerases, with the exception of one, function mainly as template-dependent DNA polymerases. The exception is terminal deoxynucleotidyl transferase, which adds nucleotides to a 3'-primer terminus in the absence of a template (25). The REV1 protein, which is a member of the Y family of DNA polymerases, was initially thought to be a dCMP transferase (26, 27). However, subsequent studies showed that it is a G template-specific DNA polymerase (28–30). The results presented above show that, given a primer terminus opposite an abasic site, pol μ adds each of the four dNTPs with no obvious preference, regardless of the sequence of the DNA template. This is, to the best of our knowledge, the first example of a DNA polymerase that is basically template-dependent but can act in a mode that is non-instructed by the sequence of the template. It is noteworthy that this nucleotidyl transferase activity occurs in the context of a primer-template, suggesting that it is a template-dependent dNMP transferase activity, unlike terminal deoxynucleotidyltransferase, which has a template-independent dNMP transferase activity. Interestingly, the transferase activity of pol μ seems to be activated only at

TABLE II
Kinetic measurement of nucleotide incorporation by pol μ when elongating from an abasic site: nucleotide mismatch in homo-nucleotide-tailed substrates

Reactions were carried out as described under "Material and Methods" with 50 nM primer-templates, 25 nM pol μ , for 20–30 min at 37 °C. The fidelity of nucleotide incorporation is indicated by the f_{inc} values, calculated as $(V_{max}/K_m)_{incorrect}/(V_{max}/K_m)_{correct}$.

Substrate	V_{max}	K_m	V_{max}/K_m	f_{inc}
	fmol/min	μM		
AB-(A-tail) dNTP				
5'GGTCA ↓				
3'CCAGX(A) ₁₀				
dATP	4 ± 0.3	40 ± 4	0.10	1
dCTP	11 ± 3	117 ± 27	0.09	0.8
dGTP	2 ± 0.1	30 ± 9	0.07	0.6
dTTP	8 ± 0.8	76 ± 7	0.11	1
Cont-(A-tail) dNTP				
5'GGTC ↓				
3'CCAGA(A) ₁₀				
dATP	0.3 ± 0.07	150 ± 113	2 × 10 ⁻³	4.6 × 10 ⁻³
dCTP	0.6 ± 0.2	215 ± 49	2.8 × 10 ⁻³	5.8 × 10 ⁻³
dGTP	ND ^a	ND	ND	ND
dTTP	12 ± 1	25 ± 6	0.48	1
AB-(G-tail) dNTP				
5'GGTCA ↓				
3'CCAGX(G) ₁₀				
dATP	15 ± 4	78 ± 13	0.19	1.6
dCTP	25 ± 4	202 ± 49	0.12	1
dGTP	11 ± 1	15 ± 1	0.73	6
dTTP	16 ± 2	10 ± 1.5	1.6	13.3
Cont-(G-tail) dNTP				
5'GGTC ↓				
3'CCAGG(G) ₁₀				
dATP	0.5 ± 0.2	500 ± 132	1 × 10 ⁻³	2.3 × 10 ⁻³
dCTP	20 ± 4	46 ± 11	0.43	1
dGTP	1.6 ± 0.4	46 ± 3	0.03	0.07
dTTP	1 ± 0.8	83 ± 8	0.01	0.02

^a ND, not determined.

special DNA structures, because it was not observed when some "regular" base-base mismatches were studied by us (data not shown) and others (6). However, it could be that this nucleotidyl transferase activity is provoked by other mismatches in a sequence context-dependent manner.

It was reported previously that pol μ possesses a very weak terminal deoxynucleotidyltransferase activity that, under its optimal conditions (in the presence of Mn²⁺), is 370-fold lower than its template-dependent polymerase activity (1). Under our reaction conditions, using as substrates oligo(dT)₂₉ or a blunt-end, 32-base pair duplex oligonucleotide, we were unable to see terminal deoxynucleotidyltransferase activity of pol μ (data not shown). Thus, the nucleotidyl transferase activity requires a DNA template, although the polymerase performs the addition of the dNTP without reading it. This extends the activity repertoire of the polymerase to enable tasks that might be specific to lesions located at DNA ends, where pol μ is thought to operate (see below).

To date, the only DNA structures known to promote this activity of pol μ are primer terminus nucleotides located opposite an abasic site. There is a DNA sequence context effect, which influences the transferase activity. Thus, on template series AB33A, all four dNMP residues were inserted at comparable frequencies, and the same was observed for template AB1G, with A, G, and T located opposite the abasic site. However, when a C was located opposite the abasic site there was a clear preference for inserting dAMP (although the other three dNMPs were inserted as well). In this sequence context, there might be a stabilization of a misalignment intermediate in which the 3'-terminal C pairs with the template G present next to the lesion. In such a situation the first template base is a T, which might explain the preference for insertion of dAMP. This

is in agreement of the described ability of pol μ to perform homology search (6, 31). A similar but much less pronounced effect is seen with template AB33A. Thus, both nucleotidyl transferase and DNA polymerase reactions can occur in the same template sequence context. In addition, whereas in the AB33A and AB33G substrates (see Table I) there was no nucleotide discrimination, in AB33T the polymerase modestly discriminates for dGTP, which is in agreement with skipping the adjacent nucleotide and copying the next one (Table I). Even so, the ability of pol μ to insert all four nucleotides on templates containing a tail of either 10 A or 10 G residues 5' to the lesion, with no preference for the complementary nucleotide (Table II), strongly supports a mode of action that is template-dependent but sequence independent. In some cases we have observed extension of the nascent strand beyond the length of the template. Such a product can be formed by the template-dependent, sequence-independent activity of pol μ , as described above, if the newly synthesized strand can loop-out during polymerization. Alternatively, a traditional mode of terminal nucleotidyl transferase activity of pol μ might have been activated under these conditions. These possibilities are under investigation.

Although the DNA polymerases of the Y family are specialized for lesion bypass, they are not the only ones capable of replicating across DNA lesions. For example, even high fidelity DNA polymerases can bypass lesions aided by their processivity clamp (19, 21, 32, 33), and this may also occur *in vivo* (34, 35). Our results clearly show that pol μ bypasses abasic sites by using primarily a misalignment mechanism in which the abasic site is skipped-over, leading to a minus one deletion at the site of the lesion (data not shown). This is consistent with the tendency of pol μ to form frameshifts on undamaged DNA (6). The same mechanism is used by pol β (20), another member of the X family of DNA polymerases, for bypassing the same lesion. What is remarkable, though, is the very high efficiency of lesion bypass across the abasic site by pol μ . There is little pausing at the abasic site or even at the two tandem abasic sites, indicating bypass at a catalytic efficiency comparable with that at undamaged DNA. Nonetheless, bypass by pol μ creates a -1 deletion (31), which is different from the insertion specificity opposite the abasic site *in vivo* (35). Our results are in agreement with recent reports on the ability of pol μ to bypass a variety of DNA lesions (31, 36).

There is accumulating evidence that pol μ functions in NHEJ. The enzyme is induced by ionizing radiation, and it appears to accumulate in nuclear foci, which are sites of double strand breaks. Moreover, it requires the end joining repair protein Ku and the XRCC4-ligase IV complex to form a stable complex on DNA *in vitro* (5). The DNA termini of double strand breaks caused by ionizing radiation are often chemically modified and, therefore, non-ligatable. One way to process these termini prior to ligation is by the action of exonucleases. However, processing broken DNA ends by DNA synthesis, when possible, is advantageous over exonucleolytic processing, because it avoids loss of genetic material. Recently, it was suggested that pol μ functions in NHEJ through microhomology search (6), a mechanism that might result in formation of deleterious deletions. The *in vitro* results indicate that pol μ uses a skipping mechanism to bypass damaged DNA. Such a mechanism may be inhibited in the vicinity of a double strand DNA because of end effects. Therefore, the ability of pol μ to perform a nucleotidyl transferase activity from mismatched nucleotides located opposite a lesion might function in a pre-ligation step in NHEJ, where damaged DNA ends are converted to ligatable ends without loss of genetic material. This possibility is currently under study.

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REFERENCES

- Dominguez, O., Ruiz, J. F., de Lera, T. L., Garcia-Diaz, M., Gonzalez, M. A., Bernad, A., and Blanco, L. (2000) *EMBO J.* **19**, 1731–1742
- Aoufouchi, S., Flatter, E., Dahan, A., Faili, A., Bertocci, B., Storck, S., Delbos, F., Cocea, L., Gupta, N., Weill, J. C., and Reynaud, C. A. (2000) *Nucleic Acids Res.* **28**, 3684–3693
- Bertocci, B., De Smet, A., Flatter, E., Dahan, A., Bories, J. C., Landreau, C., Weill, J. C., and Reynaud, C. A. (2002) *J. Immunol.* **168**, 3702–3706
- Bertocci, B., De Smet, A., Berek, C., Weill, J. C., and Reynaud, C. A. (2003) *Immunity* **19**, 203–211
- Mahajan, K. N., Nick McElhinny, S. A., Mitchell, B. S., and Ramsden, D. A. (2002) *Mol. Cell. Biol.* **22**, 5194–5202
- Zhang, Y., Wu, X., Yuan, F., Xie, Z., and Wang, Z. (2001) *Mol. Cell. Biol.* **21**, 7995–8006
- Nick McElhinny, S. A., and Ramsden, D. A. (2003) *Mol. Cell. Biol.* **23**, 2309–2315
- Ruiz, J. F., Juarez, R., Garcia-Diaz, M., Terrados, G., Picher, A. J., Gonzalez-Barrera, S., Fernandez de Henestrosa, A. R., and Blanco, L. (2003) *Nucleic Acids Res.* **31**, 4441–4449
- Duvauchelle, J. B., Blanco, L., Fuchs, R. P., and Cordonnie, A. M. (2002) *Nucleic Acids Res.* **30**, 2061–2067
- Paz-Elizur, T., Takeshita, M., Goodman, M., O'Donnell, M., and Livneh, Z. (1996) *J. Biol. Chem.* **271**, 24662–24669
- Paz-Elizur, T., Takeshita, M., and Livneh, Z. (1997) *Biochemistry* **36**, 1766–1773
- Daube, S. S., Arad, G., and Livneh, Z. (2000) *Biochemistry* **39**, 397–405
- Lindahl, T. (1993) *Nature* **362**, 709–715
- Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, pp. 19–24, ASM Press, Washington, D. C.
- Schaaper, R. M., Kunkel, T. A., and Loeb, L. A. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 487–491
- Sagher, D., and Strauss, B. (1983) *Biochemistry* **22**, 4518–4526
- Takeshita, M., Chang, C.-N., Johnson, F., Will, S., and Grollman, A. P. (1987) *J. Biol. Chem.* **262**, 10171–10179
- Hevroni, D., and Livneh, Z. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 5046–5050
- Mozzherin, D. J., Shibutani, S., Tan, C. K., Downey, K. M., and Fisher, P. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 6126–6131
- Efrati, E., Tocco, G., Eritja, R., Wilson, S. H., and Goodman, M. F. (1997) *J. Biol. Chem.* **272**, 2559–2569
- Daube, S. S., Tomer, G., and Livneh, Z. (2000) *Biochemistry* **39**, 348–355
- Haracska, L., Washington, M. T., Prakash, S., and Prakash, L. (2001) *J. Biol. Chem.* **276**, 6861–6866
- Kobayashi, S., Valentine, M. R., Pham, P., O'Donnell, M., and Goodman, M. F. (2002) *J. Biol. Chem.* **277**, 34198–34207
- Maor-Shoshani, A., Hayashi, K., Ohmori, H., and Livneh, Z. (2003) *DNA Repair* **2**, 1227–1238
- Kornberg, A., and Baker, T. (1991) *DNA Replication*, 2nd Ed., W. H. Freeman and Company, New York
- Nelson, J. R., Lawrence, C. W., and Hinkle, D. C. (1996) *Nature* **382**, 729–731
- Lin, W., Xin, H., Zhang, Y., Wu, X., Yuan, F., and Wang, Z. (1999) *Nucleic Acids Res.* **27**, 4468–4475
- Masuda, Y., Takahashi, M., Fukuda, S., Sumii, M., and Kamiya, K. (2002) *J. Biol. Chem.* **277**, 3040–3046
- Haracska, L., Prakash, S., and Prakash, L. (2002) *J. Biol. Chem.* **277**, 15546–15551
- Zhang, Y., Wu, X., Rechkoblit, O., Geacintov, N. E., Taylor, J. S., and Wang, Z. (2002) *Nucleic Acids Res.* **30**, 1630–1638
- Zhang, Y., Wu, X., Guo, D., Rechkoblit, O., Taylor, J. S., Geacintov, N. E., and Wang, Z. (2002) *J. Biol. Chem.* **277**, 44582–44587
- Tomer, G., Reuven, N. B., and Livneh, Z. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14106–14111
- Maga, G., Villani, G., Ramadan, K., Shevelev, I., Le Gac, N. T., Blanco, L., Blanca, G., Spadari, S., and Hubscher, U. (2002) *J. Biol. Chem.* **277**, 48434–48440
- Haracska, L., Unk, I., Johnson, R. E., Johansson, E., Burgers, P. M., Prakash, S., and Prakash, L. (2001) *Genes Dev.* **15**, 945–954
- Avkin, S., Adar, S., Blander, G., and Livneh, Z. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 3764–3769
- Havener, J. M., McElhinny, S. A., Bassett, E., Gauger, M., Ramsden, D. A., and Chaney, S. G. (2003) *Biochemistry* **42**, 1777–1788