Translesion Synthesis Past Estrogen-Derived DNA Adducts by Human DNA Polymerases η and κ[†]

Naomi Suzuki,[‡] Shinji Itoh,[§] Kinning Poon,[‡] Chikahide Masutani,[||] Fumio Hanaoka,[||,⊥] Haruo Ohmori,[#] Itsuo Yoshizawa,[§] and Shinya Shibutani*[,‡]

Laboratory of Chemical Biology, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794-8651, Hokkaido College of Pharmacy, Katsuraoka-cho, Otaru, Hokkaido, 047-0264, Japan, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan, Discovery Research Institute, RIKEN, and CREST, JST, Wako, Saitama 351-0198, Japan, and Department of Genetics and Molecular Biology, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan

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ABSTRACT: Newly discovered human DNA polymerase (pol) η and κ are highly expressed in the reproductive organs, such as testis, ovary, and uterus, where steroid hormones are produced. Because treatment with estrogen increases the risk of developing breast, ovary, and endometrial cancers, miscoding events occurring at model estrogen-derived DNA adducts were explored using pol η and a truncated form of human pol κ (pol $\kappa\Delta$C). These enzymes bypassed N^2-[3-methoxyestra-1,3,5(10)-trien-6-yl]-2′-deoxyguanosine (dG-N^2-3MeE) and N^6-[3-methoxyestra-1,3,5(10)-trien-6-yl]-2′-deoxyadenosine (dA-N^6-3MeE), which were embedded in site-specifically modified oligodeoxynucleotide templates. Quantitative analysis of base substitutions and deletions occurring at the lesion site showed that pol $\kappa\Delta$C was more efficient at incorporating dCMP opposite the dG-N^2-3MeE lesion than pol η. Surprisingly, the frequency of translesion synthesis beyond the dC•dG-N^2-3MeE pair was 13% of the normal dC•dG pair and was 4 and 6 orders of magnitude higher than that of dC•(+)-*trans*-dG-N^2-benzo[*a*]pyrene and dC•dG-C8-acetylaminofluorene pairs, respectively, suggesting that dG-N^2-3MeE is a natural substrate for pol κ. In contrast, the bypass frequency beyond the dT•dA-N^6-3MeE pair was 7 orders of magnitude less than that for the normal dT•dA pair. dA-N^6-3MeE is a more miscoding lesion than dG-N^2-3MeE. Pol η promoted incorporation of dAMP and dCMP at the dA-N^6-3MeE lesion, while with pol $\kappa\Delta$C, deletions were more frequently observed, along with incorporation of dAMP and dCMP opposite the lesion. These observations were also supported by steady-state kinetic studies. When taken together, the properties of pol η and κ are consistent with the mutagenic events attributed to estrogen-derived DNA adducts.

Newly discovered human DNA polymerases are associated with translesion synthesis past DNA adducts (reviewed in refs *1* and *2*). Both DNA polymerase η (pol η)[1] and κ lack the 3′−5′ exonuclease activity and, as a result, copy DNA templates with low fidelity, thereby increasing the frequency of spontaneous mutations (*3*, *4*) and catalyzing miscoding reactions at several DNA lesions. Pol η can efficiently bypass *cis-syn*-cyclobutane and thymine dimers generated by exposure to UV light (*5*, *6*), cisplatin intrastrand cross-links (*7*), 8-oxo-7,8-dihydrodeoxyguanosine (8-oxodG) (*8*), and 2-acetylaminofluorene (AAF)- (*7*) and 7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE)-derived adducts (*9*). Pol κ does not bypass thymine dimers, [6-4]photoproducts (*10*, *11*) and cisplatin intrastrand cross-links (*10*, *12*) but does conduct translesion synthesis past abasic sites (*10*), 8-oxodG (11), and 2-AAF- and BPDE-derived adducts (*10*−*14*). However, endogenous bulky DNA adducts have not been examined for translesion synthesis by these newly discovered DNA polymerases.

Interestingly, pol κ is highly expressed in human testis and ovary and mouse adrenal gland (*15*−*17*). Pol η is also expressed in mouse testis (*18*). These organs highly produce steroid hormones, including estrogen. Endogenous and synthetic estrogens have been suspected to be involved in the development of breast, ovary, and endometrial cancers (*15*, *16*). Exposure to estrogens induces several different tumors, including mammary and uterine, in laboratory animals (*19*, *20*). The mechanism of the carcinogenic effect of estrogens is unknown but may be related to the promotion and/or initiation of cancer (*21*, *22*). Treatment with estrogens forms DNA adducts in tissues of animals (*20*). The DNA damage may lead to a mutation during DNA synthesis as an

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* To whom correspondence should be addressed. Phone: 631-444-8018. Fax: 631-444-3218. E-mail: shinya@pharm.sunysb.edu.

[‡] SUNY at Stony Brook.

[§] Hokkaido College of Pharmacy.

[||] Osaka University.

[⊥] RIKEN and CREST, JST.

[#] Kyoto University.

[1] Abbreviations: dG-N^2-3MeE, N^2-[3-methoxyestra-1,3,5(10)-trien-6(α,*β*)-yl]-2′-deoxyguanosine; dA-N^6-3MeE, N^6-[3-methoxyestra-1,3,5-(10)-trien-6(α,*β*)-yl]-2′-deoxyadenosine; dNTP, 2′-deoxynucleoside triphosphate; pol η, DNA polymerase η; pol κ, DNA polymerase κ; pol $\kappa\Delta$C, a truncated form of pol κ; F_{ins}, frequency of insertion; F_{ext}, frequency of extension; PAGE, polyacrylamide gel electrophoresis.

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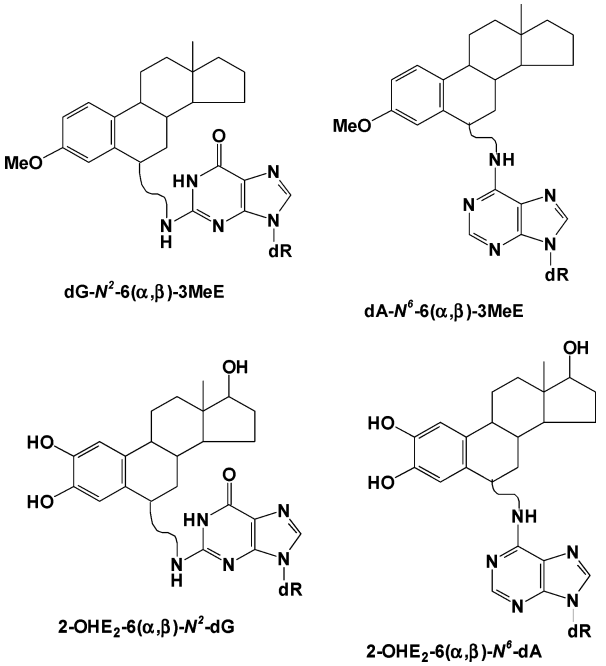

FIGURE 1: Structures of estrogen-DNA adducts derived from 3MeE-6α-sulfate and 2-OHE$_2$.

initiating event in human cancer (*23*). The newly found DNA polymerases may miscode estrogen-derived DNA adducts during DNA synthesis, leading to an increased risk of developing breast, ovary, and endometrial cancers.

2- and 4-hydroxyestradiol (2- and 4-OHE$_2$, respectively), two major estrogen metabolites, induced DNA adducts in cultured mammalian cells (*24*). These catecholestrogens are further oxidized to form corresponding semiquinones and quinones by P450, peroxidases, or autoxidization (*25*, *26*). Estrogen 2,3-quinone (E-2,3-Q) reacts with dG and dA residues in DNA, resulting in the formation of N^2-(2-hydroxyestron-6-yl)-2′-deoxyguanosine (2-OHE-N^2-dG) and N^6-(2-hydroxyestron-6-yl)-2′-deoxyadenosine (2-OHE-N^6-dA) adducts, respectively (*25*; the structures in Figure 1). Reaction of E-2,3-Q with dG and dA produces N^7-(4-hydroxyestron-1-yl)guanine and N^3-(4-hydroxyestron-1-yl)-adenine adducts, respectively, with a loss of deoxyribose (*27*).

We previously found that pyridinium 3-methoxyestra-1,3,5(10)-trien-(6α or 6*β*)-yl sulfate is highly reactive to dG and dA, forming N^2-[3-methoxyestra-1,3,5(10)-trien-6-yl]-2′-deoxyguanosine (dG-N^2-3MeE) and N^6-[3-methoxyestra-1,3,5(10)-trien-6-yl]-2′-deoxyadenosine (dA-N^6-3MeE) (Figure 1) (*28*). This reagent binds dG-N^2 and dA-N^6 in positions similar to that of E-2,3-Q. Because 2-OHE$_1$-N^2-dG and 2-OHE$_1$-N^6-dA are unstable (*29*), dG-N^2-3MeE and dA-N^6-3MeE, which are stable, are used in the present paper as model estrogen-DNA adducts for exploring miscoding specificity and frequency. Site-specific modified oligodeoxynucleotides containing a single dG-N^2-3MeE or dA-N^6-3MeE adduct were used as the DNA template for primer-extension reactions catalyzed by either pol η or a truncated form of human pol κ (pol $\kappa\Delta$C). The miscoding properties of the estrogen-DNA adducts were analyzed using an in vitro experimental system that quantifies base substitutions and deletions (*30*). Steady-state kinetic experiments were performed to determine the frequency of nucleotide insertion

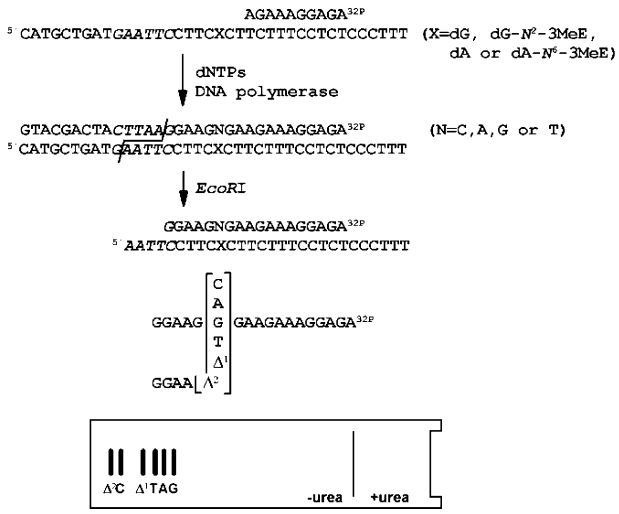

FIGURE 2: Diagram of the method used to determine the miscoding specificities. Unmodified, dG-N^2-3MeE-, or dA-N^6-3MeE-modified 38mer templates are annealed to a ^{32}P-labeled 10mer primer. Primer extension reactions catalyzed by pol η or $\kappa\Delta$C were conducted in the presence of four dNTPs. Fully extended products formed during DNA synthesis are recovered from the polyacrylamide gel (35 × 42 × 0.04 cm), annealed with a complementary 38mer, cleaved with *Eco*R I, and subjected to a two-phase PAGE (15 × 72 × 0.04 cm), as described in the Materials and Methods. To determine the miscoding specificity, mobility of the reaction products are compared with those of 18mer standards containing dC, dA, dG, or dT opposite the lesion and one (Δ^1) or two-base (Δ^2) deletions.

opposite the lesion and primer extension from the 3′ terminus with matched or mismatched dN•dG-N^2-3MeE or dN•dA-N^6-3MeE (N = C, A, G, or T) pairs. When taken together, we found that the dG-N^2-3MeE and dA-N^6-3MeE adducts promoted base substitutions and deletions during the translesion synthesis catalyzed by pol η and $\kappa\Delta$C, suggesting the contribution of these enzymes to estrogen mutagenicity.

MATERIALS AND METHODS

Synthesis of Oligodeoxynucleotides. Unmodified DNA templates (5′-CCTTCXCTTCTTTCCTCTCCCTTT and 5′-CATGCTGATGAATTCCTTCXCTTCTTTCCTCT-CCCTTT; where X is G or A), primers, and standard markers (Figure 2) were prepared by solid-state synthesis on an automated DNA synthesizer. The 24mer and 38mer oligodeoxynucleotides containing a single dG-N^2-3MeE or dA-N^6-3MeE located at the X position were prepared as described previously (*31*). The modified and unmodified oligomers were purified using HPLC and gel electrophoresis (*31*).

Primer Extension Reactions. Pol η and $\kappa\Delta$C were prepared as described previously (*5*, *10*). Pol $\kappa\Delta$C, a catalytically active fragment composed of 560 amino acids, used in this paper, lacks motifs VIIa and VIIb that denote zinc clusters from intact DINB1 protein (870 amino acids) (*10*). Although pol $\kappa\Delta$C has a lower processivity than full-length pol κ, the miscoding rates on undamaged DNA by pol $\kappa\Delta$C and κ were similar (*3*). A 10mer (5′-AGAGGAAAGA) or 12mer (5′-AGAGGAAAGAAG) was labeled at the 5′ terminus with T4 polynucleotide kinase and [γ-^{32}P]ATP (*32*). Using dG-N^2-3MeE- or dA-N^6-3MeE-modified and unmodified 38mer oligodeoxynucleotide (200 fmol) primed with a ^{32}P-labeled 10mer (5′-AGAGGAAAGA; 100 fmol) or 12mer (5′-AGAGGAAAGAAG; 100 fmol), primer extension reactions

catalyzed by pol η and $\kappa\Delta$C were conducted at 25 °C for 30 min in a buffer (10 μL) containing four dNTPs (100 μM each) (Figure 2). The reaction buffer for pol η contains 40 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 10 mM dithiothreitol, 250 μg/mL bovine serum albumin, 60 mM KCl, and 2.5% glycerol. A similar reaction buffer was used for pol $\kappa\Delta$C, using 5 mM $MgCl_2$ instead of 1 mM $MgCl_2$. Reactions were stopped with the addition of 5 μL of formamide dye. The samples were subjected to 20% polyacrylamide gel electrophoresis (PAGE) (35 × 42 × 0.04 cm). The radioactivity of extended products was measured by β phosphorimager (Molecular Dynamics).

Miscoding Analysis. Using dG-N^2-3MeE- or dA-N^6-3MeE-modified and unmodified 38mer oligodeoxynucleotide (450 fmol) primed with a ^{32}P-labeled 12mer (5′-AGAGGAAAG-TAG; 300 fmol) or a ^{32}P-labeled 13mer (5′-AGAGGAAAG-TAGN; 300 fmol; N = C, A, G, or T), primer extension reactions catalyzed by pol η or $\kappa\Delta$C (15 ng for the unmodified template and 150 ng for the modified templates) were conducted at 25 °C for 30 min in a buffer (30 μL) containing four dNTPs (100 μM each) and subjected to 20% PAGE (35 × 42 × 0.04 cm). Extended reaction products (approximately 28−32 bases long) were extracted from the gels. The recovered oligodeoxynucleotides were annealed with an unmodified 38mer, cleaved with *Eco*R I, and subjected to electrophoresis on two-phase 20% polyacrylamide gels (15 × 72 × 0.04 cm) containing 7 M urea in the upper phase and no urea in the lower phase (two-phase PAGE) (*30*) (Figure 2). To quantify base substitutions and deletions, mobility of the reaction products are compared with those of 18mer standards containing dC, dA, dG, or dT opposite the lesion and one- (Δ^1) or two-base (Δ^2) deletions.

Steady-State Kinetic Studies. Kinetic parameters associated with a nucleotide insertion opposite dG-N^2-3MeE and dA-N^6-3MeE lesion and a chain extension from the 3′ primer terminus were determined at 25 °C, using varying amounts of single dNTPs (0−500 μM). For insertion kinetics, reaction mixtures containing dNTP (0−500 μM) and either pol η (0.5−5 ng) or $\kappa\Delta$C (0.5−5 ng) were incubated at 25 °C for 2 min in 10 μL of Tris-HCl buffer (pH 8.0) using a 24mer template (150 fmol; 5′-CCTTCXCTTCTTTCCTCTCCCTTT, where X is G, A, dG-N^2-3MeE, or dA-N^6-3MeE), primed with a ^{32}P-labeled 12mer (100 fmol; 5′-AGAGGAAA-GAAG). Reaction mixtures containing a 24mer template (150 fmol) primed with a ^{32}P-labeled 13mer (100 fmol; 5′-AGAGGAAAGAAGN, where N is C, A, G, or T), with varying amounts of dGTP (0−500 μM), and either pol η (0.5−5 ng) or $\kappa\Delta$C (0.5−5 ng) were used to measure chain extension. The reaction samples were subjected to 20% denaturing PAGE (35 × 42 × 0.04 cm). The Michaelis constants (K_m) and maximum rates of reaction (V_{max}) were obtained from Hanes−Woolf plots. F_{ins} and F_{ext} were determined relative to the correct base pair (dC·dG or dT·dA) according to the equation: $F = (V_{max}/K_m)_{[wrong\ pair]}/(V_{max}/K_m)_{[correct\ pair=dC\bullet dG]}$ (*33*, *34*).

RESULTS

Miscoding Properties of Estrogen-DNA Adducts in Reactions Catalyzed by Pol η. Primer extension reactions catalyzed by pol η were conducted on unmodified, dG-N^2-3MeE- and dA-N^6-3MeE-modified 38mer templates in the presence of four dNTPs (Figure 3). Primer extensions catalyzed by pol η readily occurred on the templates containing unmodified dG or dA to form the extended products (Figure 3A). With dG-N^2-3MeE- and dA-N^6-3MeE-modified templates, primer extension was retarded slightly one-base prior and opposite the lesions. Using 100 fmol of pol η, 64 ± 5 and 35 ± 3% of the starting primer were extended past the dG-N^2-3MeE and dA-N^6-3MeE, respectively. Larger amounts of extended products were obtained by increasing the amount of this enzyme.

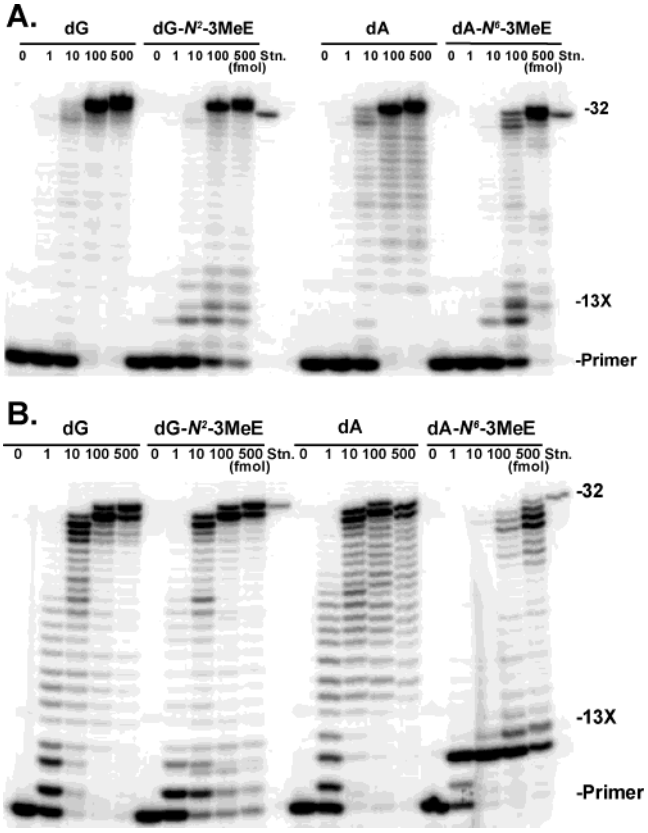

FIGURE 3: Primer extension reactions catalyzed by pol η or κ on the estrogen-derived DNA adducts. Using unmodified, dG-N^2-3MeE-, or dA-N^6-3MeE-modified 38mer templates primed with a ^{32}P-labeled 10mer, primer extension reactions were conducted at 25 °C for 30 min in a buffer containing four dNTPs (100 μM each) and variable amounts (no enzyme; 0.1 ng, 1.6 fmol; 1.0 ng, 16 fmol; 10 ng, 160 fmol; 50 ng, 800 fmol) of pol η (A) or $\kappa\Delta$C (B) as described in the Materials and Methods. One-third of the reaction mixture was subjected to PAGE (35 × 42 × 0.04 cm). The radioactivity of the extended products was measured by a β phosphorimager, and 13× shows the location opposite the dG-N^2-3MeE or dA-N^6-3MeE lesion. A 32mer (5′-AGAGGAAAGAAGC-GAAGGAATTCAACAGCATG) was used as a marker for the fully extended product [standard (Stn.)].

As shown in Figure 2, the fully extended products (approximately 28−32mers) past the unmodified or modified lesions were recovered, digested by *Eco*R I, and subjected to two-phase PAGE for quantifying base substitutions and deletions formed by the lesion. A standard mixture of six ^{32}P-labeled oligodeoxynucleotides containing dC, dA, dG, or dT opposite the lesion and containing one- and two-base deletions can be resolved by this method (Figure 4A). Incorporation of dCMP and dTMP, the correct bases, were observed opposite unmodified dG and dA, respectively; small

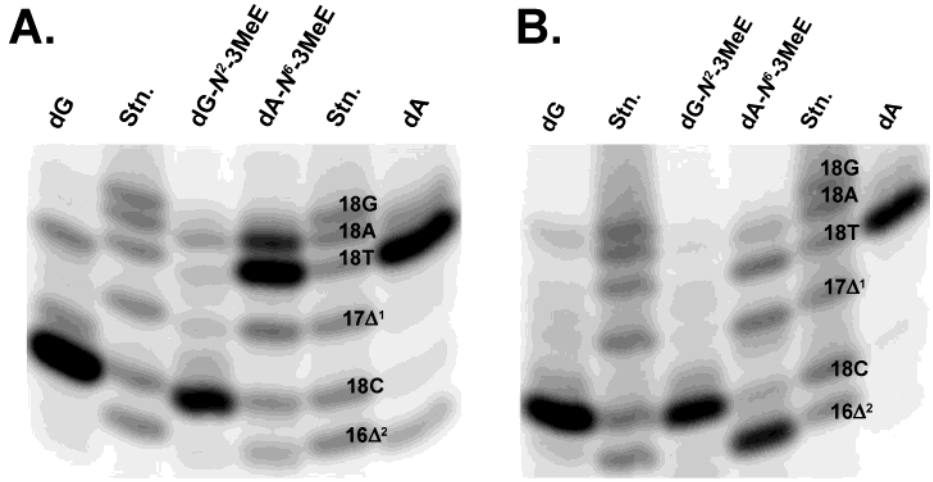

FIGURE 4: Quantitation of miscoding specificities induced by the dG-N^2-3MeE or dA-N^6-3MeE adduct. A 12mer primer (^{32}P-AGAGGAGGGTAG) was used for the primer extension reactions catalyzed by 100 fmol of pol η (A) or $\kappa\Delta$C (B), as described in Figure 3. Three of each of the independent reaction samples were combined and subjected to PAGE (35 × 42 × 0.04 cm). The fully extended products recovered from the gel were used for analysis of base substitutions and deletions, as described in the caption of Figure 2.

amounts of unknown products were observed. dG-N^2-3MeE promoted incorporation of dCMP (57 ± 5% of the starting primer) as the primary product, accompanied by small amounts of incorporations of dAMP (5.0 ± 0.4%) and dTMP (1.5 ± 0.2%) and one-base deletion (1.5 ± 0.3%). When the dA-N^6-3MeE-modified template was used, incorporation of dTMP (46 ± 4%), the correct base, was primarily observed. Significant amounts of incorporations of dAMP (17 ± 1%) and dCMP (3.7 ± 0.4%) opposite the lesion and one- (5.5 ± 0.3%) and two-base (1.6 ± 0.3%) deletions were detected.

Miscoding Properties of Estrogen-DNA Adducts in Reactions Catalyzed by Pol κ. Primer extension reactions catalyzed by pol $\kappa\Delta$C readily occurred on the templates containing unmodified dG or dA to form the extended products (Figure 3B). With dG-N^2-3MeE-modified templates, primer extension was retarded slightly one-base prior and opposite the lesion. Using 100 fmol of pol $\kappa\Delta$C, 98 ± 3% of the starting primer was extended past the dG-N^2-3MeE lesion. On the other hand, dA-N^6-3MeE was a more blocking lesion than dG-N^2-3MeE (Figure 3B); only 15 ± 2% of the starting primers were extended past the dA-N^6-3MeE lesion when 100 fmol of pol $\kappa\Delta$C was used.

As described for pol η, the extended products passed the unmodified and modified lesions were used for analysis of base substitutions and deletions using two-phase PAGE (Figure 2). When the unmodified templates were used, expected incorporations of dCMP and dTMP, the correct base, were observed opposite dG and dA, respectively (Figure 4B). dG-N^2-3MeE promoted incorporation of dCMP (67 ± 4%) as the primary product; no significant miscoding was observed at the lesion. dA-N^6-3MeE promoted incorporation of dTMP (9.0 ± 0.7%), the correct base, opposite the lesion, followed by small amounts of dAMP (3.1 ± 0.2%) and dCMP (2.7 ± 0.1%) incorporation. In addition, large amounts of two- (28 ± 2%) and one-base (8.7 ± 0.9%) deletions were detected at the lesion; however, no deletions were observed at the dG-N^2-3MeE lesion.

Formation of Deletions. The formation of deletions occurring during DNA synthesis catalyzed by pol $\kappa\Delta$C on the dA-N^6-3MeE-modified template was explored. Using dA-N^6-3MeE-modified 38mer primed with a ^{32}P-labeled 13mer containing C, A, G, or T positioned opposite the lesion [13-(N)mer], the primer extension reactions catalyzed by pol κ were conducted in the presence of four dNTPs. The fully extended products were analyzed by the two-phase PAGE assay (Figure 5). With 13(A)mer, two-base deletion was primarily formed, followed by a small amount of dAMP incorporation opposite the lesion. With 13(G)mer, one-base deletion was observed with a small amount of dGMP incorporation. No deletions were detected when the 13(C)-mer and 13(T)mer primers were used.

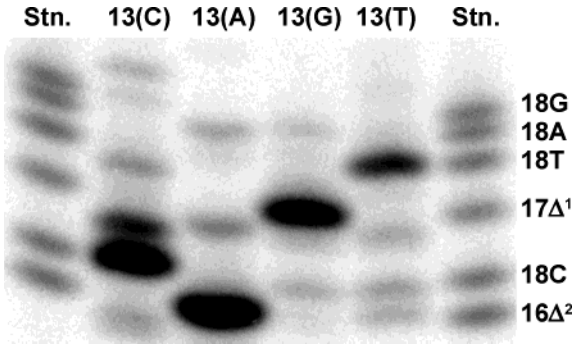

FIGURE 5: Formation of deletions during translesion synthesis catalyzed by pol κ. Using a dA-N^6-3MeE-modified 38mer template primed with a ^{32}P-labeled 13mer containing C, A, G, or T positioned opposite the lesion [13(N), ^{32}P-AGAGGAGGGTAGN; N = C, A, G, or T], primer extension reactions were conducted at 25 °C for 30 min, using pol $\kappa\Delta$C (100 fmol). Three of each of the independent reaction samples were combined and subjected to PAGE (35 × 42 × 0.04 cm). The fully extended products recovered from the gel were used for analysis of the miscoding specificity using a two-phase PAGE as described in the caption of Figure 2.

Kinetic Studies on dG-N^2-3MeE- and dA-N^6-3MeE-Modified DNA Templates. Using steady-state kinetic methods, F_{ins} by pol η was measured opposite dG-N^2-3MeE and dA-N^6-3MeE within the linear range of the reaction. The F_{ins} value for dCTP (3.97×10^{-2}), the correct base, opposite the dG-N^2-3MeE lesion was 24, 28, and 60 times higher than that of dATP, dGTP, and dTTP, respectively (Table 1). Because F_{ext} for the dC•dG-N^2-3MeE pair was also higher than that for any of other nucleotides paired with dG-N^2-3MeE, the

Table 1: Kinetic Parameters for Nucleotide-Insertion and Chain-Extension Reactions Catalyzed by Pol η and κ[a]

	dNTP ↓GAAGAAAGGAGA^{32P} 5'CCTTCXCTTCTTTCCTCTCCCTTT			dGTP ↓NGAAGAAAGGAGA^{32P} 5'CCTTCXCTTCTTTCCTCTCCCTTT			
N/X	K_m (M)	V_{max} (% min^{-1})	F_{ins}	K_m (M)	V_{max} (% min^{-1})	F_{ext}	$F_{ins} \times F_{ext}$
			Pol η				
			X = dG				
C/G	1.18 ± 012[b]	44.2 ± 0.19	1.0	0.36 ± 0.12	41.2 ± 2.3	1.0	1.0
			X = dG-N^2-3MeE				
C/X	12.4 ± 0.7	18.6 ± 1.1	3.97×10^{-2}	4.88 ± 0.95	15.7 ± 0.7	2.40×10^{-2}	9.50×10^{-4}
A/X	39.9 ± 5.3	2.49 ± 0.10	1.67×10^{-3}	12.3 ± 0.2	3.08 ± 0.18	1.83×10^{-3}	3.06×10^{-6}
G/X	22.4 ± 6.4	1.14 ± 0.07	1.41×10^{-3}	19.7 ± 5.3	4.62 ± 0.11	1.77×10^{-3}	2.50×10^{-6}
T/X	78.1 ± 5.0	1.94 ± 0.02	0.66×10^{-3}	5.92 ± 0.91	4.87 ± 0.45	6.05×10^{-3}	3.99×10^{-6}
			X = dA				
T/A	0.66 ± 0.16[b]	62.1 ± 0.78	1.0	1.34 ± 0.07	34.4 ± 3.1	1.0	1.0
			X = dA-N^6-3MeE				
C/X	37.5 ± 1.4	4.38 ± 0.02	1.21×10^{-3}	21.8 ± 0.4	6.16 ± 0.05	1.10×10^{-2}	1.33×10^{-5}
A/X	31.4 ± 1.5	6.33 ± 0.03	2.09×10^{-3}	13.8 ± 2.4	13.1 ± 0.12	3.77×10^{-2}	7.88×10^{-5}
G/X	32.0 ± 1.4	3.19 ± 0.12	1.03×10^{-3}	50.2 ± 8.2	8.03 ± 0.16	6.30×10^{-3}	6.49×10^{-6}
T/X	34.3 ± 0.1	28.9 ± 2.7	8.73×10^{-3}	5.38 ± 0.8	25.0 ± 1.0	1.80×10^{-1}	1.57×10^{-3}
			Pol κ				
			X = dG				
C/G	3.12 ± 0.64[b]	11.2 ± 0.36	1.0	3.13 ± 0.05	105.4 ± 5.6	1.0	1.0
			X = dG-N^2-3MeE				
C/X	25.7 ± 1.1	30.9 ± 1.1	0.33	6.78 ± 0.42	90.9 ± 0.2	0.40	0.133
A/X	17.3 ± 2.3	0.04 ± 0.01	6.48×10^{-4}	54.1 ± 8.2	3.13 ± 0.10	1.73×10^{-3}	1.12×10^{-6}
G/X	80.2 ± 15.3	0.16 ± 0.01	5.63×10^{-4}	92.9 ± 22.2	2.34 ± 0.11	0.77×10^{-3}	0.43×10^{-6}
T/X	235 ± 31	0.69 ± 0.07	8.38×10^{-4}	3.19 ± 0.74	3.11 ± 0.04	2.96×10^{-2}	2.48×10^{-5}
			X = dA				
T/A	4.45 ± 0.44[b]	74.7 ± 0.01	1.0	2.60 ± 0.71	177.6 ± 1.5	1.0	1.0
			X = dA-N^6-3MeE				
C/X	159 ± 4.6	0.13 ± 0.01	0.49×10^{-4}	21.0 ± 4.2	1.00 ± 0.07	0.69×10^{-3}	3.38×10^{-8}
A/X	140 ± 25	0.26 ± 0.02	1.11×10^{-4}	25.9 ± 0.9	0.48 ± 0.05	0.26×10^{-3}	2.89×10^{-8}
G/X	88.8 ± 6.8	0.047 ± 0.01	0.32×10^{-4}	64.4 ± 3.1	0.48 ± 0.10	0.11×10^{-3}	0.35×10^{-8}
T/X	227 ± 5.0	0.063 ± 0.04	0.17×10^{-4}	12.3 ± 1.1	4.53 ± 0.40	5.39×10^{-3}	9.16×10^{-8}

[a] Kinetics of nucleotide-insertion and chain-extension reactions were determined as described in the Materials and Methods. F_{ins} and F_{ext} were estimated by the equation: $F = (V_{max}/K_m)_{[wrong\ pair]}/(V_{max}/K_m)_{[correct\ pair]}$. X = dG, dG-$N^2$-3MeE, dA, or dA-$N^6$-3MeE lesion. [b] Data are expressed as the mean ± standard deviation obtained from the three independent experiments.

relative frequency of translesion synthesis ($F_{ins} \times F_{ext}$) past the dC•dG-N^2-3MeE pair was at least 2 orders of magnitudes higher than that of other dN•dG-N^2-3MeE pairs. Unlike the dG-N^2-3MeE lesion, the F_{ins} for dTTP, the correct base, opposite the dA-N^6-3MeE lesion was only 4.2, 7.2, and 8.5 times higher than that of dATP, dCTP, and dGTP, respectively (Table 1). The $F_{ins} \times F_{ext}$ for the dT•dA-N^6-3MeE pair was only 20 times higher than that for the dA•dA-N^2-3MeE pair and was 2 orders of magnitude higher than that of the dC•dA-N^2-3MeE and dG•dA-N^2-3MeE pairs.

When pol $\kappa\Delta$C was used, the F_{ins} value for dCTP opposite the dG-N^2-3MeE lesion was 33% of that for dCTP opposite the unmodified dG, and the F_{ext} value for the dC•dG-N^2-3MeE pair was 40% of the dC•dG pair (Table 1). As a result, the $F_{ins} \times F_{ext}$ for the dC•dG-N^2-3MeE pair was 13% of the dC•dG pair. However, the F_{ins} value for dCTP opposite the dG-N^2-3MeE lesion was at least 400 times higher than that for any of the other dNTP. Therefore, the $F_{ins} \times F_{ext}$ for the dC•dG-N^2-3MeE pair was 3 to 5 orders of magnitude higher than that of the other dN•dG-N^2-3MeE pairs. In contrast, the F_{ins} value for dATP (1.11×10^{-4}) opposite the dA-N^6-3MeE lesion was 6.5 times higher than that of dTTP, the correct base, although the dT•dA-N^6-3MeE pair was extended more efficiently than any of the other nucleotides paired with the dA-N^6-3MeE. Therefore, the $F_{ins} \times F_{ext}$ for the dT•dA-N^6-3MeE pair was only 3 times higher than that of the dA•dA-N^2-3MeE and dC•dA-N^2-3MeE pairs.

DISCUSSION

When monomeric 2-OHE-N^2-dG and 2-OHE-N^6-dA produced by E-2,3-Q were incubated in buffer, these adducts were degraded rapidly, resulting in the formation of dG and dA, respectively[2]. Although the rate of degradation for 2-OHE-N^2-dG- and 2-OHE-N^6-dA-modified oligomers was slower than that for the monomeric adducts, the conversion of the adducts to dG and dA in the template oligomers may affect the miscoding frequency and specificity. Therefore, the stable model estrogen-DNA adducts, dG-N^2-3MeE and dA-N^6-3MeE, were used in this paper.

Pol η and κ are highly expressed in the reproductive organs, such as testis and ovaries (*15*−*18*). Kawamura et al. has recently observed the high expression of both enzymes in the human uterus (*35*). Because these organs produce a high level of steroid hormones, pol κ and η may contribute to translesion synthesis past DNA adducts derived from

[2] I. Terashima, N. Suzuki, and S. Shibutani, unpublished data.

Table 2: $F_{ins} \times F_{ext}$ Past DNA Adducts by Pol κ

	X = dG-N^2-3MeE	(+)*trans*-dG-N^2-BPDE[a]	dG-C8-AAF[b]
C/X	0.133	1.09×10^{-5}	8.69×10^{-8}
A/X	1.12×10^{-6}	3.37×10^{-10}	6.84×10^{-9}
G/X	0.43×10^{-6}	8.24×10^{-12}	4.10×10^{-10}
T/X	2.48×10^{-5}	5.20×10^{-10}	8.41×10^{-7}

[a] Data are taken from ref *14*. [b] Data are taken from ref *13*.

steroids. In fact, pol η and κ bypassed estrogen-derived DNA adducts, as demonstrated in the present paper, while the primer extension reactions catalyzed by pol α and δ were strongly blocked at the lesion sites (*31*, *36*). Pol η and $\kappa\Delta$C bypassed the dG-N^2-3MeE lesion by efficiently incorporating dCMP, the correct base, opposite the lesion. With pol η, small amounts of dAMP incorporation, along with dTMP incorporation, were observed opposite the lesion, indicating that G → T and G → A mutations are produced. This observation was supported by steady-state kinetic studies. Like 2-OHE-N^2-dG adducts (*29*), dG-N^2-3MeE promoted primarily G → T transversions in simian kidney (COS-7) cells (37). The mutagenic specificity was consistent with that observed with pol η. Pol η may contribute the miscoding events induced by estrogen-derived dG adducts although this enzyme is known to bypass *cis-syn*-cyclobutane thymine dimers very efficiently and accurately by incorporating two dAMPs opposite the lesion (*5*, *6*). On the other hand, with pol $\kappa\Delta$C, the F_{ins} for dCTP was 510 times higher than that for dATP; while with pol η, the F_{ins} for the ratio of dCTP•dATP was reduced to 24. The $F_{ins} \times F_{ext}$ past the dC•dG-N^2-3MeE pair using pol $\kappa\Delta$C was 13% of the dC•dG pair and was approximately 140 times higher than that for pol η (9.50×10^{-4}). Consistent with miscoding analysis using two-phase PAGE, pol $\kappa\Delta$C showed higher fidelity for translesion synthesis past estrogen-derived dG adducts, as compared with pol η.

To compare the behavior of pol κ on dG-N^2-3MeE adduct with that on BPDE- or AAF-derived DNA adducts embedded, a similar sequence context was used (*13*, *14*) (Table 2). Studies using pol κ-deficient mouse embryonic stem cells indicated that pol κ acts to suppress mutations at DNA lesions generated by B[*a*]P (38). Like the dC•(+)-*trans*-dG-N^2-BPDE pair, $F_{ins} \times F_{ext}$ for the dC•dG-N^2-3MeE pair was 3 to 5 orders of magnitude higher than that of any of the nucleotides paired with dG-N^2-3MeE. Suprisingly, $F_{ins} \times F_{ext}$ for the dC•dG-N^2-3MeE pair was 12 000-fold higher than that for the dC•(+)-*trans*-dG-N^2-BPDE pair. These indicate that pol κ bypasses the dG-N^2-3MeE lesion more efficiently and accurately than the (+)-*trans*-dG-N^2-BPDE lesion by incorporating dCMP opposite the lesion. Pol κ may have evolved to bypass dG-N^2-3MeE and similar adducts in the reproductive organs, suggesting that dG-N^2-estrogen is a natural lesion for pol κ.

The behavior of pol κ during the bypass of dG-N^2-3MeE and (+)-*trans*-dG-N^2-BPDE differs from that of the AAF-derived DNA adduct in which a high level of miscoding occurs during DNA synthesis (*13*) (Table 2). Thus, the miscoding property varies depending on the nature of adduct. The differences may be particularly due to the adducts that are positioned at the N^2 and C8 position of the respective base, which may influence the miscoding frequency and specificity.

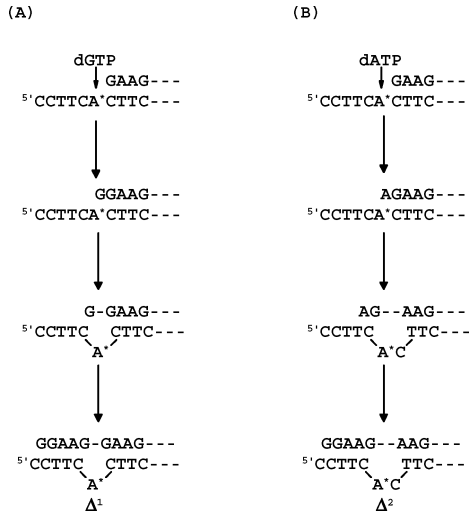

FIGURE 6: Proposed mechanism of one- and two-base deletions formed at the dA-N^6-3MeE adduct. The A with an asterisk represents the dA-N^6-3MeE adduct.

For the dA-N^6-3MeE adduct, both pol κ and η miscoded by incorporating dAMP, and less frequency of dCMP, opposite the lesion, resulted in A → T transversions and A → G transitions. The miscoding specificities detected with these enzymes were consistent with mutagenic events induced by dA-N^6-3MeE in a single-strand vector propagated in COS-7 cells (*37*). Similarly observed in this paper, the mutagenic frequency for dA-N^6-3MeE lesion was much higher than that of dG-N^2-3MeE. The estrogen-dA adduct may be more mutagenic than the estrogen-dG adduct.

Frameshift deletions were formed by the dA-N^6-3MeE adduct during translesion synthesis catalyzed by pol $\kappa\Delta$C. When 13mer primers containing C, A, G, or T positioned opposite the lesion were used for the primer extension reactions, one- and two-base deletions were formed only when 13(G)mer and 13(A)mer primers were used, respectively. Because the F_{ins} for dATP was 6.5 times higher than that of dTTP, the correct base, the majority of dAMP inserted opposite the dA-N^6-3MeE lesion may result in two-base deletions; the remaining small amount of dAMP inserted opposite the lesion may yield G → T transversion. Similary, because the F_{ins} for dGTP was 2.0 times higher than that of dTTP, the dGMP inserted opposite the lesion may result in one-base deletions. This indicates that the newly inserted dGMP opposite the lesion pairs with C 5′ to the lesion promoted one-base deletions and that the newly inserted dAMP (Figure 6A) and G 5′-flanking base could be paired with TC 5′ to the lesion, promoting two-base deletions (Figure 6B). The mechanism described previously (*39*, *40*) may account for the one- and two-base shortened reaction products observed in studies of pol κ with dA-N^6-3MeE.

Human breast, ovary, and uterine cancers display a high frequency of G → T, G → A, and A → G mutations in the *p53* tumor suppressor gene (*41*, *42*). These mutagenic spectra are consistent with that observed for the dG-N^2-3MeE and dA-N^6-3MeE adducts in the present paper. Because 8-oxodG lesions are produced by estrogen metabolites (*43*) and generate G → T transversions by several DNA polymerases including pol η and κ (*8*, *44*, *45*), oxidative lesions may also be involved in the mutagenic events.

In conclusion, pol κ and η can efficiently bypass estrogen-derived DNA adducts; estrogen-derived dA is a much more miscoding lesion than estrogen-derived dG. These newly discovered DNA polymerases are more likely to be associated with mutagenic events generated by estrogen.

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