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

Manabu Yasui,[‡] Naomi Suzuki, Y. R. Santosh Laxmi, 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

Received April 29, 2006; Revised Manuscript Received July 28, 2006

ABSTRACT: The long-term treatment of tamoxifen (TAM), widely used for adjuvant chemotherapy and chemoprevention for breast cancer, increases a risk of developing endometrial cancer. A high frequency of K-*ras* mutations has been observed in the endometrium of women treated with TAM. Human DNA polymerase (pol) η and pol κ are highly expressed in the reproductive organs and are associated with translesion synthesis past bulky DNA adducts. To explore the miscoding properties of α-(N^2-deoxyguanosinyl)tamoxifen (dG-N^2-TAM), a major TAM−DNA adduct, site-specifically modified oligodeoxynucleotides containing a single diastereoisomer of trans or cis forms of dG-N^2-TAM were prepared by phosphoramidite chemical procedure and used as templates. The primer extension reaction catalyzed by pol $\kappa\Delta$C, a truncated form of pol κ, extended more efficiently past the adduct than that of pol η by incorporating dCMP, a correct base, opposite the adduct. With pol η, all diastereoisomers of dG-N^2-TAM promoted small amounts of direct incorporation of dAMP and deletions. With pol $\kappa\Delta$C, dG-N^2-TAM promoted small amounts of dTMP and/or dAMP incorporations and deletions. The miscoding properties varied depending on the diastereoisomer of dG-N^2-TAM adducts and the DNA pol used. Steady-state kinetic studies were also performed using either the nonspecific sequence or the K-*ras* gene sequence containing a single dG-N^2-TAM at the second base of codon 12. With pol η, the bypass frequency past the dA•dG-N^2-TAM pair positioned in the K-*ras* sequence was only 2.3 times lower than that for the dC•dG-N^2-TAM pair, indicating that dG-N^2-TAM in the K-*ras* sequence has higher miscoding potential than that in the nonspecific sequence. However, with pol $\kappa\Delta$C, the bypass frequency past the dC•dG-N^2-TAM pair was higher than that of the dT•dG-N^2-TAM pair in both sequences. The properties of pol η and pol κ are consistent with the mutagenic events attributed to TAM−DNA adducts.

Tamoxifen (TAM[1], the structure in Figure 1) is widely used as a first-line endocrine therapy for breast cancer patients who are estrogen receptor positive and is also a prophylactic agent for preventing breast cancer in women at high risk for this disease. Besides the significant benefit, long-term treatment of TAM in women increases the risk of developing endometrial cancer (*1*−*5*).

In rats, a high level of TAM−DNA adducts are produced in the liver (*6*, *7*) and initiate the development of hepatocellular carcinomas (*8*, *9*). TAM is converted to several α-hydroxylated metabolites, which are in turn O-sulfonated by hydroxysteroid sulfotransferases and react with cellular DNA, resulting in the formation of four diastereoisomers (two trans forms (fr-1 and fr-2) and two cis forms (fr-3 and fr-4)) of α-(N^2-deoxyguanosinyl)tamoxifen (dG-N^2-TAM) and/or α-(N^2-deoxyguanosinyl)-*N*-desmethyltamoxifen (dG-N^2-*N*-desTAM) adducts (Figure 1) (*10*). Among the diastereoisomers, a trans form (fr-2) of dG-N^2-TAM and dG-N^2-*N*-desTAM were detected as a major TAM−DNA adduct in rodents (*11*−*13*) and monkeys (*14*, *15*). TAM−DNA adducts were also detected in the endometrium of women treated with TAM (*16*−*18*), although there is some contradiction regarding the detection of TAM−DNA adducts in human tissues (*19*−*22*). A high frequency of K-*ras* mutations was recently observed in the endometrium of women treated with TAM (*23*); the mutational specificity was consistent with that occurring at the dG-N^2-TAM adduct (*24*). In addition to the genotoxic events, TAM has partial estrogenic activity through the estrogen receptor in endometrial cells and rat uterus (*25*). This effect may enhance the promotion of cancer. Therefore, genotoxic damage and estrogenic activity may cause TAM-induced cancers.

Several human DNA polymerases (pol) are associated with translesion synthesis past DNA adducts (*26*, *27*). Among them, pol η and pol κ lack the 3′−5′ exonuclease activity and, as a result, copy DNA templates with low fidelity, thereby increasing the frequency of spontaneous mutations (*28*, *29*) and catalyzing miscoding reactions at several DNA lesions, including UV-induced damages (*30*, *31*), 8-oxo-7,8-dihydro-deoxyguanosine (8-oxodG) (*32*), and 2-acetylami-

[†] This research was supported by Grant ES09418 from the National Institute of Environmental Health Sciences.

* Corresponding author. Phone: (631) 444-7849. Fax: (631) 444-3218. E-mail: shinya@pharm.stonybrook.edu.

[‡] Present address: Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan.

[1] Abbreviations: dNTP, 2′-deoxynucleoside triphosphate; TAM, tamoxifen; dG-N^2-TAM, α-(N^2-deoxyguanosinyl)tamoxifen; 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; HPLC, high performance liquid chromatography.

10.1021/bi0608461 CCC: $33.50
Published on Web 09/07/2006

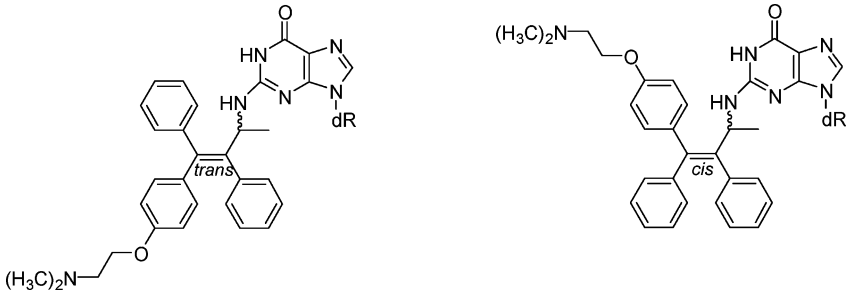

FIGURE 1: Structures of the dG-N^2-TAM adduct.

nofluorene (AAF)- (*33*) and 7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE)-derived adducts (*34*). Pol κ also conducts translesion synthesis past abasic sites (*35*), 8-oxodG (*36*) and 2-AAF- and BPDE-derived adducts (*35*−*39*). Both enzymes are highly expressed in the human ovary and uterus (*40*−*44*), which are responsible for the production of steroidal hormones. The activated metabolites of endogenous and synthetic estrogens are suspected to form DNA adducts that are miscoded by such enzymes (*40*, *41*). Due to the structural similarity, the DNA pols may also miscode TAM−DNA adducts during translesion synthesis, increasing the risk of developing cancers in reproductive organs.

In the present study, site-specifically modified oligodeoxynucleotides containing a single diastereoisomer of dG-N^2-TAM were used as the DNA template for primer extension reactions catalyzed by pol η or a truncated form of human pol κ (pol $\kappa\Delta$C). The miscoding properties of the TAM−DNA adducts were analyzed using an *in vitro* experimental system that quantifies base substitutions and deletions (*45*). A phosphoramidite chemical procedure established recently in our laboratory (*46*) makes it possible to prepare oligodeoxynucleotides containing a single diastereoisomer of the dG-N^2-TAM adduct into the second base of codon 12 in the K-*ras* gene sequence. Using the modified oligodeoxynucleotide as a template, steady-state kinetic studies were performed to determine the frequency of nucleotide insertion opposite the lesion and chain extension reactions for measuring the relative frequency of translesion synthesis past the dG-N^2-TAM adduct. Taken together, we found that dG-N^2-TAM is a miscoding adduct in reactions catalyzed by pols η and κ, suggesting the contribution of these enzymes to TAM mutagenicity.

MATERIALS AND METHODS

Synthesis of Oligodeoxynucleotides. Oligodeoxynucleotides used as the DNA template (5′CCTTCXCTTCTTTCCTCTCCCTTT and 5′CATGCTGATGAATTCCTTCXCTTCTTTCCTCTCCCTTT, where X is G), primer, and standard markers were prepared by solid-state synthesis on an automated DNA synthesizer. A 24-mer (5′CCTTCXCTTCTTTCCTCTCCCTTT, where X is dG-N^2-TAM) containing a mixture of the trans forms (fr-1 and fr-2) of dG-N^2-TAM was prepared from the *trans*-dG-N^2-TAM phosphoramidite by a chemical procedure established in our laboratory (*46*). The 24-mer oligomer containing fr-1 was isolated from that containing fr-2 by HPLC (*46*). Similarly, a 24-mer containing a mixture of the cis forms (fr-3 and fr-4) of dG-N^2-TAM was prepared from the *cis*-dG-N^2-TAM phosphoramidite, and the oligomers containing fr-3 or fr-4 were separated by HPLC. The dG-N^2-TAM-modified 24-mer containing each diastereoisomer was then ligated to a 14-mer (5′CATGCTGATGAATT) to prepare the 38-mer template (5′CATGCTGATGAATTCCTTCXCTTCTTTCCTCTCCCTTT, where X is dG-N^2-TAM) (*47*). The dG-N^2-TAM-modified and unmodified oligomers were purified on a Water's reverse-phase μBondapak C_{18} (0.39 × 30 cm), using a linear gradient of 0.05 M triethylammonium acetate at pH 7.0, containing 10−50% acetonitrile with an elution time of 60 min and a flow rate of 1.0 mL/min (*24*). The oligomers were further purified by electrophoresis on 20% polyacrylamide denaturing gel (PAGE) (35 × 42 × 0.04 cm) (*48*). Oligomers were labeled at the 5′-terminus by treating with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (*49*) and subjected to 20% PAGE containing 7 M urea (35 × 42 × 0.04 cm) to establish homogeneity. The position of the oligomers was established by β-phosphorimager analysis (Molecular Dynamics, Inc.).

Primer Extension Reactions. Pol η and a truncated form of pol κ (pol $\kappa\Delta$C) were prepared as described previously (*30*, *35*). Although pol $\kappa\Delta$C has lower processivity than that of full-length pol κ, the miscoding rate on the undamaged DNA by pol $\kappa\Delta$C was similar to that of pol κ (*28*). A 10-mer (5′AGAGGAAAGA) or 12-mer (5′AGAGGAAAGAAG) was labeled at the 5′-terminus with T4 polynucleotide kinase and [γ-^{32}P]-ATP (*49*). Using dG-N^2-TAM-modified or unmodified 38-mer oligodeoxynucleotide (200 fmol) primed with a ^{32}P-labeled 10-mer (5′AGAGGAAAGA; 100 fmol) or 12-mer (5′AGAGGAAAGAAG; 100 fmol), primer extension reactions catalyzed by pol η or pol $\kappa\Delta$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 DTT, 250 μg/mL BSA, 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% PAGE (35 × 42 × 0.04 cm). The radioactivity of extended products was measured by β-phosphorimager (Molecular Dynamics).

Miscoding Analysis. Using dG-N^2-TAM-modified or unmodified 38-mer oligodeoxynucleotide (450 fmol) primed with a ^{32}P-labeled 12-mer (5′AGAGGAAAGTAG; 300 fmol), primer extension reactions catalyzed by pol η or pol $\kappa\Delta$C (15 ng for the unmodified template; 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 38-mer, cleaved with *Eco* RI,

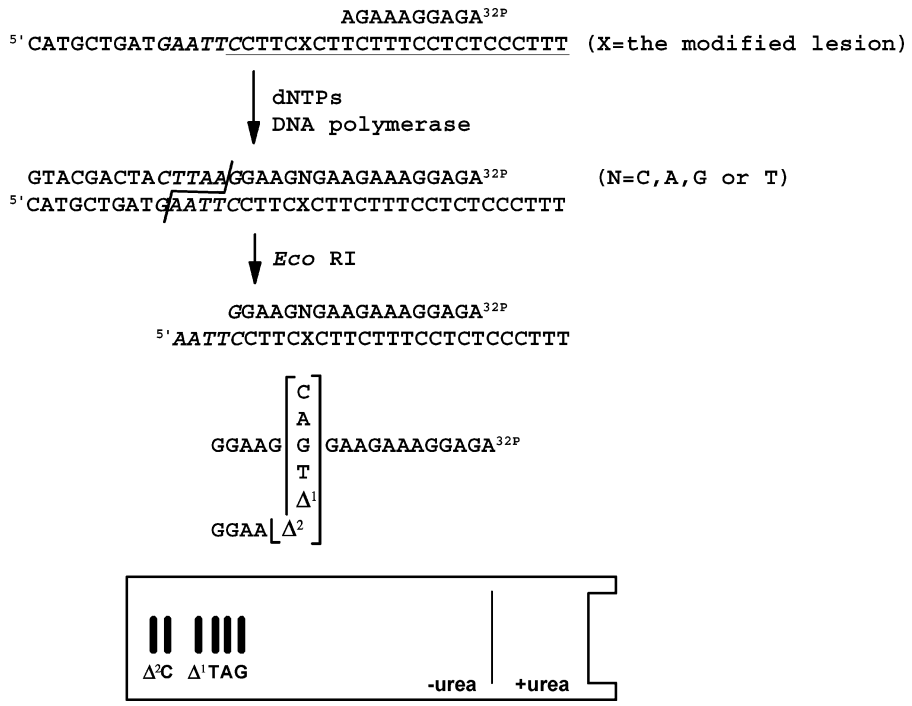

FIGURE 2: Diagram of the method used to determine miscoding specificities. Unmodified or dG-N^2-TAM-modified 38-mer templates are annealed to a ^{32}P-labeled 10-mer primer. Primer extension reactions catalyzed by pol η or pol $\kappa\Delta$C were conducted in the presence of four dNTPs. Fully extended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

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) (*45*) (Figure 2). To quantify base substitutions and deletions, the mobility of the reaction products were compared with those of 18-mer standards containing dC, dA, dG, or dT opposite the lesion and one (Δ^1) or two-base (Δ^2) deletions. The detection limit on the gel was 0.03% of the labeled oligomers.

Steady-State Kinetic Studies. Kinetic parameters associated with nucleotide insertion opposite the dG-N^2-TAM lesion and 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 pol $\kappa\Delta$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 24-mer template (150 fmol; 5′CCTTCXCTTCTTTCCTCTCCCTTT, where X is G or dG-N^2-TAM) primed with a ^{32}P-labeled 12-mer primer (100 fmol; 5′AGAGGAAAGAAG). Reaction mixtures containing a template (150 fmol) primed with a ^{32}P-labeled 13-mer primer (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 pol $\kappa\Delta$C (0.5−5 ng) were used to measure chain extension. Another 21-mer template containing a codon 12 of the K-*ras* sequence (150 fmol; 5′GGAGCTGXTGGCGTAGGCTGT (codons 10−16), where X is G or dG-N^2-TAM) was also used for determining the frequency of dNTP insertion opposite the lesion using a ^{32}P-labeled 13-mer primer (100 fmol; 5′ACAGCCTACGCCA). Reaction mixtures containing a template (150 fmol) primed with a ^{32}P-labeled 14-mer primer (100 fmol; 5′ACAGCCTACGCCAN, where N is C, A, G, or T), with varying amounts of dCTP (0−500 μM), and either pol η (0.5−5 ng) or pol $\kappa\Delta$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. Frequencies of dNTP insertion (F_{ins}) and chain extension (F_{ext}) were determined relative to the dC•dG base pair according to the equation: $F = (V_{max}/K_m)_{[wrong\ pair]}/(V_{max}/K_m)_{[correct\ pair=dC•dG]}$ (*50*, *51*).

RESULTS

Miscoding Properties of dG-N^2-TAM DNA Adducts in Reactions Catalyzed by pol η. Primer extension reactions catalyzed by pol η were conducted on unmodified or dG-N^2-TAM-modified 38-mer template in the presence of four dNTPs (Figure 3). On the templates containing a single unmodified dG, primer extensions catalyzed by this enzyme readily occurred to form the extended products. With the modified templates containing a single diastereoisomer (fr-1, fr-2, fr-3, and fr-4) of the dG-N^2-TAM adduct, primer extensions were retarded one-base prior to the adduct, opposite the adduct, and one-base past the adduct. The extended products past the dG-N^2-TAM lesions were obtained by increasing the amount of the enzyme. When 500 fmol of pol η was used, approximately 4.5 ± 1.0% for fr-1, 47 ± 3.4% for fr-2, 33 ± 2.6% for fr-3, and 17 ± 1.9% for fr-4 of the starting primer were extended past the adducts to form the fully extended products (28−34 mers). Blunt-end addition to the fully extended product (33−34 mers) was observed (*52*, *53*). The primer extension past the fr-2 or fr-3 was more efficient than that past fr-1 and fr-4, indicating that the frequency past the adducts varied depending on the diastereoisomers.

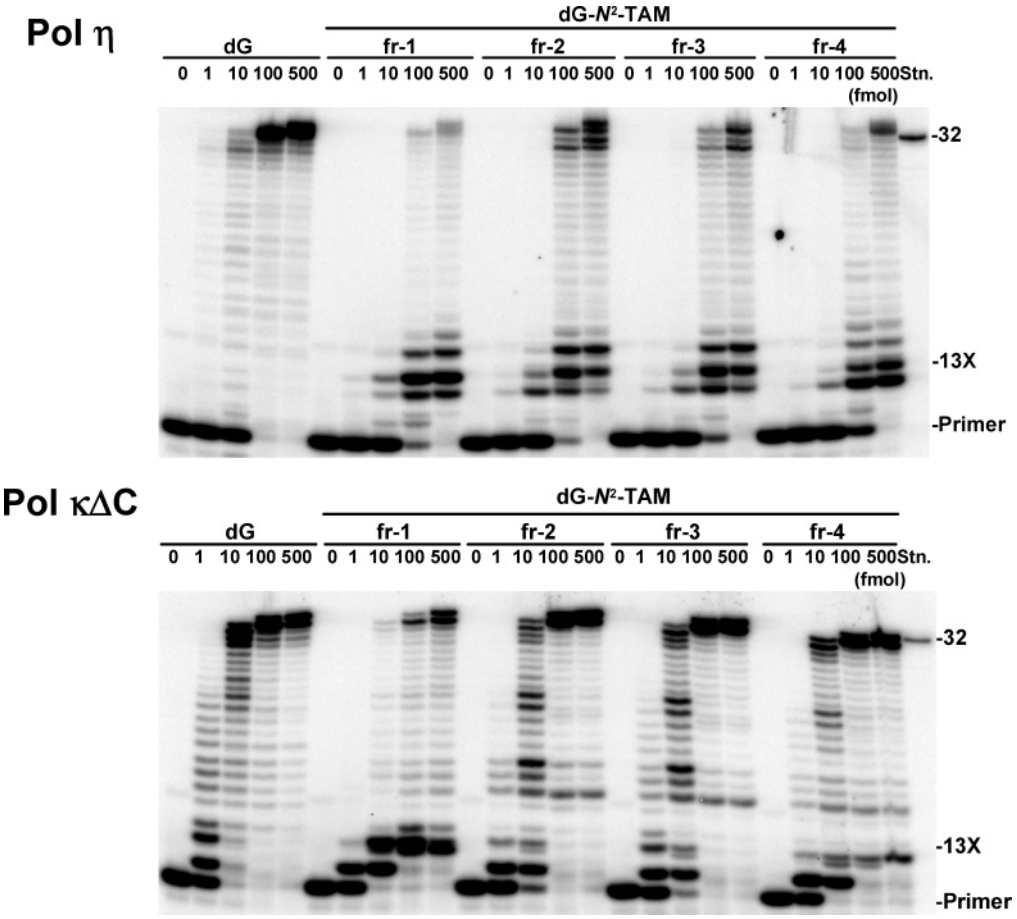

FIGURE 3: Primer extension reactions catalyzed by pol η or pol $\kappa\Delta$C on the dG-N^2-TAM-modified templates. Using unmodified or dG-N^2-TAM-modified 38-mer templates primed with a ^{32}P-labeled 10-mer (^{32p}-AGAGGAAAGA), primer extension reactions were conducted at 25 °C for 30 min in a buffer containing four dNTPs (100 μM each) and variable amounts (0, 1.0, 10, 100, and 500 fmol) of pol η or pol $\kappa\Delta$C as described in Materials and Methods. One-third of the reaction mixture was subjected to PAGE (35 × 42 × 0.04 cm). The radioactivity of extended products was measured by a β-phosphorimager. 13X shows the location opposite the dG-N^2-TAM lesion. A 32-mer (5′AGAGGAAAGAAGCGAAGGAATTCATCAGCATG) was used as a marker of the fully extended product (Stn.).

The fully extended products (approximately 28−34-mers) past the unmodified or modified adducts were recovered, digested by *Eco* RI, and subjected to two-phase PAGE for quantitative analysis of base substitutions and deletions (Figure 2). A standard mixture of six ^{32}P-labeled oligodeoxynucleotides containing dC, dA, dG, or dT opposite the adduct and those containing one- and two-base deletions can be resolved by this method (Figure 4). Preferential incorporation of dCMP, the correct bases, opposite the unmodified dG was observed. As indicated by the arrowheads in Figure 4, small amounts of unknown products were detected; the migration of these products differed from those of standard markers. Because the amount of the fully extended products past dG-N^2-TAM were less than that past the unmodified dG, the fully extended products past each diastereoisomer of dG-N^2-TAM were recovered from several reactions and used for the two-phase PAGE analysis (Figure 4). The amounts of dNMP incorporation opposite each diastereoisomer were normalized for the amounts of their starting primer. dG-N^2-TAM promoted the preferential incorporation of dCMP (4.3% for fr-1; 32.0% for fr-2; 22.5% for fr-3; and 13.8% for fr-4) opposite the adducts. A small amount of dAMP incorporation (0.04% for fr-1; 1.2% for fr-2; 2.3% for fr-3; and 1.1% for fr-4) opposite the adduct was observed in addition to significant amounts of one-base (0.08−2.4%) and two-base (0.06−9.9%) deletions. As observed for the unmodified dG, small amounts of unknown products were observed.

Miscoding Properties of dG-N^2-TAM Adducts in Reactions Catalyzed by pol $\kappa\Delta$C. Primer extension reactions catalyzed by pol $\kappa\Delta$C readily occurred on the templates containing unmodified dG to form the extended products (Figure 3). When 10 and 100 fmol of this enzyme were used, 68% and 92% of the primer, respectively, were extended past dG to form the fully extended products. Unlike pol η, the primer extension reactions were retarded one-base prior to the fr-1 of dG-N^2-TAM and opposite the adduct; with 100 fmol of pol $\kappa\Delta$C, only 9.0% of primers were extended past this adduct to form the fully extended products. However, using the same amount of enzyme, a majority of the primers (81−90%) were readily extended past the fr-2, fr-3, or fr-4 of dG-N^2-TAM to form the fully extended products.

The extended products past the unmodified and modified adducts were used for miscoding analysis using two-phase PAGE. When the unmodified template was used, an expected incorporation of dCMP was observed opposite the dG (Figure 4). All diastereoisomers of dG-N^2-TAM promoted the incorporation of dCMP (36.7% for fr-1; 83.3% for fr-2; 82.3% for fr-3; and 72.7% for fr-4) as a primary product. When fr-1 was used, small amounts of dAMP (0.7%) and dTMP (0.55%) incorporation and one-base deletion (1.0%) were observed at the adduct. With other diastereoisomers

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

Insertion dNTP **Extension dGTP**

↓GAAGAAAGGAGA^{32P} **↓NGAAGAAAGGAGA^{32P}**

5'CCTTCXCTTCTTTCCTCTCCCTTT **5'CCTTCXCTTCTTTCCTCTCCCTTT**

N:X	K_m (μM)	V_{max}(%min^{-1})	F_{ins}	K_m (μM)	V_{max}(%min^{-1})	F_{ext}	F_{ins}xF_{ext}
Pol η							
X = dG							
C:G	0.87± 0.08[b]	47.8 ± 0.19[b]	**1.0**	0.47± 0.12[b]	54.8 ± 4.1[b]	**1.0**	**1.0**
X = dG-N^2-TAM							
C:X	2.44 ± 0.04	3.28 ±0.39	**2.45 x 10^{-2}**	7.61 ± 0.53	5.05 ± 0.45	**5.69 x 10^{-3}**	**1.39 x 10^{-4}**
A:X	22.5 ± 1.0	17.8 ± 0.09	**1.44 x 10^{-2}**	13.1 ± 2.4	0.24 ± 0.03	**1.57 x 10^{-4}**	**2.26 x 10^{-6}**
G:X	23.2 ± 1.4	2.37 ± 0.01	**1.86 x 10^{-3}**	14.3 ± 1.4	0.23 ± 0.01	**1.38 x 10^{-4}**	**2.57 x 10^{-7}**
T:X	69.5 ± 6.2	2.31 ±0.07	**6.05 x 10^{-4}**	16.7 ± 1.9	0.08 ± 0.01	**4.10 x 10^{-5}**	**2.48 x 10^{-8}**
Pol κΔC							
X = dG							
C:G	1.68 ± 0.23[b]	39.1 ± 3.46[b]	**1.0**	1.61 ± 0.08[b]	111.0 ± 7.7[b]	**1.0**	**1.0**
X = dG-N^2-TAM							
C:X	12.5 ± 0.1	5.47 ± 0.01	**1.88 x 10^{-2}**	5.06 ± 1.17	2.42 ± 0.01	**6.94 x 10^{-3}**	**1.30 x 10^{-4}**
A:X	32.3 ± 6.4	0.41 ± 0.02	**5.45 x 10^{-4}**	33.9 ± 0.7	0.59 ± 0.01	**2.53 x 10^{-4}**	**1.38 x 10^{-7}**
G:X	N.D.	N.D.	**N.D.**	115 ± 26	1.21 ± 0.10	**1.53 x 10^{-4}**	**N.D.**
T:X	19.3 ± 2.2	0.80 ± 0.11	**1.78 x 10^{-3}**	6.65 ± 1.06	0.67 ± 0.01	**1.46 x 10^{-3}**	**2.60 x 10^{-6}**

Insertion dNTP **Extension dCTP**

↓ACCGCATCCGACA^{32P} **↓NACCGCATCCGACA^{32P}**

5'GGAGCTGXTGGCGTAGGCTGT **5'GGAGCTGXTGGCGTAGGCTGT**

N:X	K_m (μM)	V_{max}(%min^{-1})	F_{ins}	K_m (μM)	V_{max}(%min^{-1})	F_{ext}	F_{ins}xF_{ext}
Pol η							
X = dG							
C:G	0.82 ± 0.13[b]	436 ± 27[b]	**1.0**	0.33 ± 0.10[b]	260 ± 4.8[b]	**1.0**	**1.0**
X = dG-N^2-TAM							
C:X	3.99 ± 0.69	2.93 ± 0.02	**1.50 x 10^{-3}**	0.85 ± 0.17	1.97 ± 0.01	**2.85 x 10^{-3}**	**4.26 x 10^{-6}**
A:X	8.18 ± 0.1	2.47 ± 0.04	**5.59 x 10^{-4}**	0.88 ± 0.19	2.42 ± 0.10	**3.37 x 10^{-3}**	**1.89 x 10^{-6}**
G:X	17.9 ± 0.8	2.57 ± 0.09	**2.66 x 10^{-4}**	3.94 ± 0.22	1.17 ± 0.01	**3.58 x 10^{-4}**	**9.52 x 10^{-8}**
T:X	29.1 ± 5.5	1.88 ± 0.16	**1.20 x 10^{-4}**	6.26 ± 0.86	0.06 ± 0.01	**1.15 x 10^{-5}**	**1.38 x 10^{-9}**
Pol κΔC							
X = dG							
C:G	2.31 ± 0.22[b]	136 ± 18[b]	**1.0**	1.02 ± 0.03[b]	127 ± 3[b]	**1.0**	**1.0**
X = dG-N^2-TAM							
C:X	11.6 ± 2.0	3.24 ± 0.03	**4.80 x 10^{-3}**	3.41 ± 0.83	1.11 ± 0.01	**2.69 x 10^{-3}**	**1.29 x 10^{-5}**
A:X	N.D.	N.D.	**N.D.**	5.62 ± 0.21	0.94 ± 0.01	**1.34 x 10^{-3}**	**N.D.**
G:X	N.D.	N.D.	**N.D.**	31.8 ± 9.4	2.84 ± 0.16	**7.42 x 10^{-4}**	**N.D.**
T:X	34.6 ± 3.4	0.21 ± 0.01	**1.01 x 10^{-4}**	32.2 ± 2.01	1.19 ± 0.06	**2.95 x 10^{-4}**	**2.99 x 10^{-8}**

[a] The kinetics of nucleotide insertion and chain extension reactions were determined as described in Materials and Methods. Frequencies of nucleotide insertion (F_{ins}) and chain extension (F_{ext}) were estimated by the equation $F = (V_{max}/K_m)_{[wrong\ pair]}/(V_{max}/K_m)_{[correct\ pair\ =dC:dG]}$. X = dG or dG-N^2-TAM lesion. [b] Data expressed as mean ± S.D. obtained from three independent experiments.

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

		dG-N^2-TAM	dG-N^2-3MeE[a]	dG-N^2-AAF[b]	(+)*trans*-dG-N^2-BPDE[c]
pol η	C:X	1.39×10^{-4}	9.50×10^{-4}	1.53×10^{-3}	
	A:X	2.26×10^{-6}	3.06×10^{-6}	9.34×10^{-7}	
	G:X	2.57×10^{-7}	2.50×10^{-6}	3.24×10^{-10}	
	T:X	2.48×10^{-8}	3.99×10^{-6}	1.70×10^{-7}	
pol κ	C:X	1.30×10^{-4}	1.33×10^{-1}	1.05×10^{-3}	1.09×10^{-5}
	A:X	1.38×10^{-7}	1.12×10^{-6}	7.35×10^{-9}	3.37×10^{-10}
	G:X	N.D.	4.30×10^{-7}	1.07×10^{-8}	8.24×10^{-12}
	T:X	2.60×10^{-6}	2.48×10^{-5}	4.90×10^{-6}	5.20×10^{-10}

[a] Data are taken from ref 54. [b] Data are taken from ref 57. [c] Data are taken from ref 39; N.D., not detectable.

(fr-2, fr-3, and fr-4), only a small amount of dTMP incorporation (0.6% for fr-2; 1.8% for fr-3; and 1.4% for fr-4) was observed.

Kinetic Studies on dG-N^2-TAM-Modified DNA Templates. The frequency of dNTP incorporation (F_{ins}) opposite a trans form (fr-2) of dG-N^2-TAM and the frequency of chain extension (F_{ext}) from dN•dG-N^2-TAM pairs were measured within the linear range of the reaction, using the same sequence context that was used for the two-phase PAGE assay (Table 1). With pol η, the F_{ins} value for dCTP (2.45×10^{-2}), the correct base, opposite the dG-N^2-TAM was only 1.7 times higher than that of dATP (1.44×10^{-2}) and was 13 and 41 times higher than that of dGTP and dTTP, respectively. Because the F_{ext} for the dC•dG-N^2-TAM pair was 36 times higher than that for the dA•dG-N^2-TAM pair, the relative bypass frequency ($F_{ins} \times F_{ext}$) past the dC•dG-N^2-TAM pair was approximately 62 times higher than that for the dA•dG-N^2-TAM pair. The $F_{ins} \times F_{ext}$ past the dC•dG-N^2-TAM pair was 2 to 3 orders of magnitude higher than those of the other dN•dG-N^2-TAM pairs. When pol $\kappa\Delta$C was used, the F_{ins} for dCTP opposite the dG-N^2-TAM was 11 and 35 times higher than that of dTTP and dATP, respec-

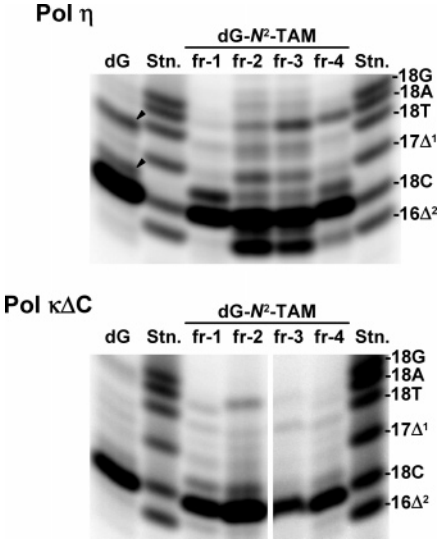

FIGURE 4: Quantitation of miscoding specificities induced by the dG-N^2-TAM adduct. A 12-mer primer (^{32P}-AGAGGAAAGAAG) was used for the primer extension reactions catalyzed by 100 fmol of pol η or pol $\kappa\Delta$C, as described in Figure 3. Three independent reaction samples were combined and subjected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

tively (Table 1). The F_{ins} for dGTP was not detected. The $F_{ins} \times F_{ext}$ for the dC•dG-N^2-TAM pair was 50 and 940 times higher than that for the dT•dG-N^2-TAM and dA•dG-N^2-TAM pairs, respectively.

Steady-state kinetic studies were also performed using the K-*ras* sequence containing a single dG-N^2-TAM (fr-2) positioned at the second base of codon 12 (Table 1). With pol η, the F_{ins} value for dCTP opposite the dG-N^2-TAM was 2.7, 5.6, and 13 times higher than that of dATP, dGTP, and dTTP, respectively. Interestingly, the F_{ext} for the dA•dG-N^2-TAM pair (3.37×10^{-3}) was slightly higher than that of the dC•dG-N^2-TAM pair (2.85×10^{-3}), resulting in the $F_{ins} \times F_{ext}$ for the dA•dG-N^2-TAM pair being only 2.3 times lower than that of the dC•dG-N^2-TAM pair. The $F_{ins} \times F_{ext}$ for the dC•dG-N^2-TAM pair was 45 and 3100 times higher than that of the dG•dG-N^2-TAM and dT•dG-N^2-TAM pairs, respectively. When pol $\kappa\Delta$C was used, only the F_{ins} for dCTP and dTTP was detected; the F_{ins} for dTTP was 48 times lower than that of dCTP. The $F_{ins} \times F_{ext}$ for dT•dG-N^2-TAM was 430 times lower than that of the dC•dG-N^2-TAM pair.

DISCUSSION

Pol η and pol κ are highly expressed in the reproductive organs, including the ovary and uterus (*40*−*44*), where steroidal hormones are produced. Because TAM has a partial structure of estrogen, these DNA pols may contribute to translesion synthesis past DNA adducts derived from TAM. In fact, the primer extension reactions catalyzed by pol $\kappa\Delta$C occurred more rapidly past the dG-N^2-TAM adduct than those by pol η by preferentially incorporating dCMP, the correct base, opposite the adduct. With pol η, a small amount of direct dAMP incorporation was observed with deletions, indicating that G → T mutations and deletions were produced. With pol $\kappa\Delta$C, dG-N^2-TAM promoted small amounts of dTMP incorporations, indicating that G → A mutations occurred. This observation was supported by steady-state kinetic studies. The miscoding specificities detected with these enzymes were consistent with mutagenic events induced by dG-N^2-TAM in a single-strand vector propagated in simian kidney (COS-7) cells (*24*).

Unlike other bulky DNA adducts (*38*, *39*, *54*), dG-N^2-TAM allowed pol α, β, or δ, in addition to pol η and κ, to extend past the adduct (*47*). Pol α promoted the misincorporation of dAMP and deletions at all of the diastereoisomers, accompanied by small amounts of dGMP incorporation. Pol β promoted deletions and misincorporation of dAMP and dGMP. With pol δ, the preferential incorporation of dCMP was observed: fr-1 of dG-N^2-TAM only promoted a small amount of dTMP incorporation. Some of these replicative DNA pols may also be involved in the miscoding events induced by TAM. Thus, the miscoding specificities and frequencies varied depending on the DNA polymerase used.

Among four diastereisomers (two trans forms (fr-1 and fr-2) and two cis forms (fr-3 and fr-4)), the primer extension reactions catalyzed by pol η and pol $\kappa\Delta$C were strongly retarded at fr-1; therefore, with pol η, the formation of dCMP and dAMP incorporations and deletions at fr-1 was much lower than that observed with other diastereoisomers. With pol $\kappa\Delta$C, small amounts of dAMP and dTMP incorporations and one-base deletion were observed opposite the fr-1, whereas only a small amount of dTMP incorporation was observed with other diastereoisomers. Thus, the miscoding specificity and frequency also varied depending on the diastereoisomer of the dG-N^2-TAM adduct. The confirmation of each diastereoisomer in the replication fork may affect the miscoding properties, as suggested by the 3D NMR studies (*55*).

As observed with COS-7 cells (*24*) and with pol η and κ in the present study, a high frequency of G → T and G → A mutations were detected at the second base in K-*ras* codon 12 in the endometrium of women treated with TAM (*23*). A phosphoramidite chemical synthesis established in our laboratory has made it possible to insert dG-N^2-TAM into the oligomers having any sequence context, such as the K-*ras* gene. The frequencies of dNTP insertion opposite the dG-N^2-TAM and the chain extension from the dN•dG-N^2-TAM pair were determined using the K-*ras* sequence and compared with those observed with nonspecific sequence. Because a trans form (fr-2) of dG-N^2-TAM was a major TAM−DNA adduct in rodents (*11*−*13*) and primates (*14*, *15*), this isomer was used for these kinetic studies. With pol η, the $F_{ins} \times F_{ext}$ for the dA•dG-N^2-TAM pair in the K-*ras* sequence was only 2.3 times lower than that of the dC•dG-N^2-TAM pair, whereas with the nonspecific sequence, the $F_{ins} \times F_{ext}$ for the dA•dG-N^2-TAM pair in the K-*ras* sequence was 62 times lower than that of the dC•dG-N^2-TAM pair (Table 1). This indicated that specific sequence context such as the K-*ras* gene may cause an increase in miscoding frequency, resulting in the construction of mutational hot spots.

Because G → A mutations were also observed in codon 12 of the K-*ras* gene in the endometrium of women treated with TAM (*23*), some DNA pols are expected to insert dTMP opposite the dG-N^2-TAM lesion. Only pol κ has this ability among the DNA pols examined. Although with pol $\kappa\Delta$C the $F_{ins} \times F_{ext}$ past the dT•dG-N^2-TAM was 50 times lower than that of the dC•dG-N^2-TAM pair, a low level of direct dTMP incorporation was detected during the translesion synthesis catalyzed by this enzyme (Figure 4). Since pol ι is highly

expressed in the reproductive organs (*44*, *56*) and tends to incorporate dTMP opposite the bulky DNA adducts (*27*), this enzyme may contribute to the mutagenic events of TAM−DNA adducts, resulting in the increase of G → A mutations.

To compare the behavior of pol η or pol κ past the dG-N^2-TAM adduct with that past several bulky dG-N^2 adducts, such as dG-N^2-3MeE, (+)-*trans*-dG-N^2-BPDE, or dG-N^2-AAF, these adducts were embedded in a similar sequence context (*39*, *54*, *57*) (Table 2). With pol η, like the dC•dG-N^2-AAF and dC•dG-N^2-3MeE pairs, the $F_{ins} \times F_{ext}$ for the dC•dG-N^2-TAM pair was 1 to 4 orders of magnitude higher than that of any of the nucleotides paired with dG-N^2-TAM. Interestingly, the $F_{ins} \times F_{ext}$ ratio for dC•dG-N^2-TAM/dA•dG-N^2-TAM pair was 62, and this number was higher than that for the dC•dG-N^2-3MeE/dA•dG-N^2-3MeE (310) or dC•dG-N^2-AAF/dA•dG-N^2-AAF (165) pairs, indicating that the dG-N^2-TAM adduct may have a higher miscoding potential than those of dG-N^2-3MeE or dG-N^2-AAF. Pol κ bypassed the dG-N^2-TAM adduct more efficiently by incorporating dCMP opposite the lesion, as observed with dG-N^2-3MeE, (+)-*trans*-dG-N^2-BPDE, or dG-N^2-AAF lesion (Table 2). However, the $F_{ins} \times F_{ext}$ ratio for the dC•dG-N^2-TAM/dT•dG-N^2-TAM pair was 50, and this number was 1 to 3 orders of magnitude higher than that for the dC•dG-N^2-3MeE/dT•dG-N^2-3MeE (5400) or dC•(+)-*trans*-dG-N^2-BPDE/dT•(+)-*trans*-dG-N^2-BPDE (21000), or dC•dG-N^2-AAF/dT•dG-N^2-AAF (210) pair, indicating that the dG-N^2-TAM adduct may also have a higher miscoding potential than other bulky DNA adducts. Pol κ and pol η may have evolved to bypass dG-N^2-TAM.

In conclusion, human DNA pols η and κ expressed highly in reproductive organs are more likely to be associated with miscoding events generated by TAM. The high frequency of mutations observed at the K-*ras* sequence may reflect the sequence context effect. Thus, dG-N^2-TAM adducts generate mutations and pose a potential risk to women treated with TAM. This result raises concerns about the use of TAM as a chemopreventive agent for healthy women as well as its use in the treatment of breast cancer.

ACKNOWLEDGMENT

We thank Drs. F. Hanaoka and H. Ohmori for providing DNA pol η and pol $\kappa\Delta$C, respectively.

REFERENCES

1. Seoud, M. A.-F., Johnson, J., and Weed, J. C. (1993) Gynecologic tumors in tamoxifen-treated women with breast cancer, *Obstet. Gynecol. (N.Y.) 82*, 165−169.
2. van Leeuwen, F. E., Benraadt, J., Coebergh, J. W. W., Kiemeney, L. A. L. M., Diepenhorst, F. W., van den Belt-Dusebout, A. W., and van Tinteren, H. (1994) Risk of endometrial cancer after tamoxifen treatment of breast cancer, *Lancet 343*, 448−452.
3. Killackey, M., Hakes, T. B., and Pierce, V. K. (1985) Endometrial adenocarcinoma in breast cancer patients receiving antiestrogens, *Cancer Treat. Rep. 69*, 237−238.
4. Bernstein, L., Deapen, D., Cerhan, J. R., Schwartz, S. M., Liff, J., McGann-Maloney, E., Perlman, J. A., and Ford, L. (1999) Tamoxifen therapy for breast cancer and endometrial cancer risk, *J. Natl. Cancer Inst. 91*, 1654−1662.
5. Bernstein, L., Deapen, D., Cerhan, J. R., Schwartz, S. M., Liff, J., McGann-Maloney, E., Perlman, J. A., and Ford, L. (1999) Tamoxifen therapy for breast cancer and endometrial cancer risk, *J. Natl. Cancer Inst. 91*, 1654−1662.
6. Han, X., and Liehr, J. G. (1992) Induction of covalent DNA adducts in rodents by tamoxifen, *Cancer Res. 52*, 1360−1363.
7. Osborne, M. R., Hewer, A., Hardcastle, I. R., Carmichael, P. L., and Phillips, D. H. (1996) Identification of the major tamoxifen-deoxyguanosine adduct formed in the liver DNA of rats treated with tamoxifen, *Cancer Res. 56*, 66−71.
8. Greaves, P., Goonetilleke, R., Nunn, G., Topham, J., and Orton, T. (1993) Two-year carcinogenicity study of tamoxifen in Alderley Park Wister-derived rats,*Cancer Res. 53*, 3919−3924.
9. Hard, G. C., Iatropoulos, M. J., Jordan, K., Radi, L., Kaltenberg, O. P., Imondi, A. R., and Williams, G. M. (1993) Major difference in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats, *Cancer Res. 53*, 4534−4541.
10. Kim, S. Y., Suzuki, N., Laxmi, Y. R. S., and Shibutani, S. (2004) Genotoxic mechanism of tamoxifen in developing endometrial cancer, *Drug Metab. Rev. 36*, 199−218.
11. Rajaniemi, H., Rasanen, I., Koivisto, P., Peltonen, K., and Hemminki, K. (1999) Identification of the major tamoxifen-DNA adducts in rats liver by mass spectroscopy, *Carcinogenesis 20*, 305−309.
12. Umemoto, A., Monden, Y., Suwa, M., Kanno, Y., Suzuki, M., Lin, C.-X., Ueyama, Y., Momen, M. A., Ravindernath, A., Shibutani, S., and Komaki, K. (2000) Identification of hepatic tamoxifen-DNA adducts in mice: α-(N^2-deoxyguanosinyl)tamoxifen and α-(N^2-deoxyguanosinyl)tamoxifen *N*-oxide, *Carcinogenesis 21*, 1737−1744.
13. Umemoto, A., Komaki, K., Monden, Y., Suwa, M., Kanno, Y., Kitagawa, M., Suzuki, M., Lin, C.-X., Ueyama, Y., Momen, M. A., Ravindernath, A., and Shibutani, S. (2001) Identification and quantification of tamoxifen-DNA adducts in the liver of rats and mice, *Chem. Res. Toxicol. 14*, 1006−1013.
14. Shibutani, S., Suzuki, N., Laxmi, Y. R., Schild, L. J., Divi, L. J., Divi, R. L., Grollman, A. P., and Poirier, M. C. (2003) Identification of tamoxifen-DNA adducts in monkeys treated with tamoxifen, *Cancer Res. 63*, 4402−4406.
15. Schild, L. J., Divi, R. L., Beland, F. A., Churchwell, M. I., Doerge, D. R., Gamboa da Costa, G., Marques, M. M., and Poirier, M. C. (2003) Formation of tamoxifen-DNA adducts in multiple organs of adult female cynomolgus monkeys dosed with tamoxifen for 30 days, *Cancer Res. 63*, 5999−6003.
16. Shibutani, S., Ravindernath, A., Suzuki, N., Terashima, I., Sugarman, S. M., Grollman, A. P., and Pearl, M. L. (2000) Identification of tamoxifen-DNA adducts in the endometrium of women treated with tamoxifen, *Carcinogenesis 21*, 1461−1467.
17. Martin, E. A., Brown, K., Gaskell, M., Al-Azzawi, F., Garner, R. C., Boocock, D. J., Mattock, E., Pring, D. W., Dingley, K., Turteltaub, K. W., Smith, L. L., and White, I. N. (2003) Tamoxifen DNA damage detected in human endometrium using accelerator mass spectrometry, *Cancer Res. 63*, 8461−8465.
18. Shibutani, S. (2005) Tamoxifen is a genotoxic carcinogen, *Chem. Res. Toxicol. 18*, 1509−1511.
19. Carmichael, P. L., Ugwumadu, A. H., Neven, P., Hewer, A. J., Poon, G. K., and Phillips, D. H. (1996) Lack of genotoxicity of tamoxifen in human endometrium, *Cancer Res. 56*, 1475−1479.
20. Carmichael, P. L., Sardar, S., Crooks, N., Neven, P., Van Hoof, I., Ugwumadu, A., Bourne, T., Tomas, E., Hellberg, P., Hewer, A. J., and Phillips, D. H. (1999) Lack of evidence from HPLC ^{32}P-post-labeling for tamoxifen-DNA adducts in the human endometrium, *Carcinogenesis 20*, 339−342.
21. Beland, F. A., Churchwell, M. I., Doerge, D. R., Parkin, D. R., Malejka-Giganti, D., Hewer, A., Phillips, D. H., Carmichael, P. L., Gamboa da Costa, G., and Marques, M. M. (2004) Electrospray ionization-tandem mass spectrometry and ^{32}P-postlabeling analyses of tamoxifen-DNA adducts in humans, *J. Natl. Cancer Inst. 96*, 1099−1104.
22. Beland, F. A., Marques, M. M., Gamboa da Costa, G., Phillips, D. H. (2005) Tamoxifen-DNA adduct formation in human endometrium, *Chem. Res. Toxicol. 18*, 1507−1509.
23. Hachisuga, T., Tsujioka, H., Horiuchi, S., Udou, T., Emoto, M., and Kawarabayashi, T. (2005) K-ras mutation in the endometrium of tamoxifen-treated breast cancer patients, with a comparison of tamoxifen and toremifene, *Br. J. Cancer 92*, 1098−1103.
24. Shibutani, S., and Dasaradhi, L. (1997) Miscoding potential of tamoxifen-derived DNA adducts: α-(N^2-deoxyguanosinyl)tamoxifen, *Biochemistry 56*, 13010−13017.
25. Stygar, D., Muravitskaya, N., Eriksson, B., Eriksson, H., and Sahlin, L. (2003) Effects of SERM (selective estrogen receptor

modulator) treatment on growth and proliferation in the rat uterus, *Reprod. Biol. Endcrinol. 1*, 1−8.
26. Goodman, M. F., and Tippin, B. (2000) The expanding polymerase universe, *Nat. Rev. Mol. Cell Biol. 1*, 101−109.
27. Kunkel, T. A., Pavlov, Y. I., and Bebenek, K. (2003) Functions of human DNA polymerases *η*, *κ* and *ι* suggested by their properties, including fidelity with undamaged DNA templates, *DNA Repair 3*, 135−149.
28. Ohashi, E., Bebenek, K., Matsuda, T., Feaver, W. J., Gerlach, V. L., Friedberg, E. C., Ohmori, H., and Kunkel, T. A. (2000) Fidelity and processivity of DNA synthesis by DNA polymerase kappa, the product of the human DINB1 gene, *J. Biol. Chem. 275*, 39678−39684.
29. Zhang, Y., Yuan, F., Xin, H., Wu, X., Rajpal, D. K., Yang, D., and Wang, Z. (2000) Human DNA polymerase *κ* synthesizes DNA with extraordinarily low fidelity, *Nucleic Acids Res. 28*, 4147−4156.
30. Masutani, C., Araki, M., Yamada, A., Kusumoto, R., Nogimori, T., Maekawa, T., Iwai, S., and Hanaoka, F. (1999) Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity, *EMBO J. 18*, 3491−3501.
31. Johnson, R. E., Prakash, S., and Prakash, L. (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, pol *η*, *Science 283*, 1001−1004.
32. Haracska, L., Yu, S. L., Johnson, R. E., Prakash, L., and Prakash, S. (2000) Efficient and accurate replication in the presence of 7, 8-dihydro-8-oxoguanine by DNA polymerase *η*, *Nat. Genet. 25*, 458−461.
33. Masutani, C., Kusumoto, R., Iwai, S., and Hanaoka, F. (2000) Mechanisms of accurate translesion synthesis by human DNA polymerase *η*, *EMBO J. 19*, 3100−3109.
34. Zhang, Y., Yuan, F., Wu, X., Rechkoblit, O., Taylor, J. S., Geacintov, N. E., and Wang, Z. (2000) Error-prone lesion bypass by human DNA polymerase *η*, *Nucleic Acids Res. 28*, 4717−4724.
35. Ohashi, E., Ogi, T., Kusumoto, R., Iwai, S., Masutani, C., Hanaoka, F., and Ohmori, H. (2000) Error-prone bypass of certain DNA lesions by the human DNA polymerase *κ*, *Genes Dev. 14*, 1589−1594.
36. Zhang, Y., Yuan, F., Wu, X., Wang, M., Rechkoblit, O., Taylor, J.-S., Geacintov, N. E., and Wang, Z. (2000) Error-free and error-prone lesion bypass by human DNA polymerase *κ* in vitro, *Nucleic Acids Res. 28*, 4138−4146.
37. Gerlach, V. L., Feaver, W. J., Fischhaber, P. L., and Friedberg, E. C. (2001) Purification and characterization of pol *κ*, a DNA polymerase encoded by the human *DINB1* gene, *J. Biol. Chem. 276*, 92−98.
38. Suzuki, N., Ohashi, E., Hayashi, K., Ohmori, H., Grollman, A. P., and Shibutani, S. (2001) Translesional synthesis past acetylaminofluorene-derived DNA adducts catalyzed by human DNA polymerase *κ* and *Escherichia coli* DNA polymerase IV, *Biochemistry 40*, 15176−15183.
39. Suzuki, N., Ohashi, E., Kolbanovskiy, A., Geacintov, N. E., Grollman, A. P., Ohmori, H., and Shibutani, S. (2002) Translesion synthesis by human DNA polymerase *κ* on a DNA template containing a single stereoisomer of dG-(+)- or dG-(-)-anti-N^2-BPDE (7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrene), *Biochemistry 41*, 6100−6106.
40. Gerlach, V. L., Aravind, L., Gotway, G., Schultz, R. A., Koonin, E. V., and Friedberg, E. C. (1999) Human and mouse homologs of *Escherichia coli* DinB (DNA polymerase IV), members of the UmuC/DinB superfamily, *Proc. Natl. Acad. Sci. U.S.A. 96*, 11922−11927.
41. Ogi, T., Kato, Jr., T., Kato, T., and Ohmori, H. (1999) Mutation enhancement by DINB1, a mammalian homologue of the *Escherichia coli* mutagenesis protein dinB, *Genes Cells 4*, 607−618.
42. Velasco-Miguel, S., Richardson, J. A., Gerlach, V. L., Lai, W. C., Gao, T., Russell, L. D., Hladik, C. L., White, C. L., and Friedberg, E. C. (2003) Constitutive and regulated expression of the mouse Dinb (Pol *κ*) gene encoding DNA polymerase *κ*, *DNA Repair 2*, 91−106.
43. Yamada, A., Masutani, C., Iwai, S., and Hanaoka, F. (2000) Complementation of defective translesion synthesis and UV light sensitivity in xeroderma pigmentosum variant cells by human and mouse DNA polymerase *η*, *Nucleic Acids Res. 28*, 2473−2480.
44. Kawamura, K., Bahar, R., Seimiya, M., Chiyo, M., Wada, A., Okada, S., Hatano, M., Tokuhisa, T., Kimura, H., Watanabe, S., Honda, I., Sakiyama, S., Tagawa, M., and O-Wang, J. (2004) DNA polymerase *θ* is preferentially expressed in lymphoid tissues and upregulated in human cancers, *Int. J. Cancer 109*, 9−16.
45. Shibutani, S., Suzuki, N., Matsumoto, Y., and Grollman, A. P. (1996) Miscoding properties of 3,N^4-etheno-2′-deoxycytidine in reactions catalyzed by mammalian DNA polymerases, *Biochemistry 35*, 14992−14998.
46. Laxmi, Y. R. S., Suzuki, N., Dasaradhi, L., Johnson, F., and Shibutani, S. (2002) Preparation of oligodeoxynucleotides containing a diastereoisomer of α-(N^2-2′-deoxyguanosinyl)tamoxifen by phosphoramidite chemical synthesis, *Chem. Res. Toxicol. 15*, 218−225.
47. Terashima, I., Suzuki, N., and Shibutani, S. (1999) Mutagenic potential of α-(N^2-deoxyguanosinyl)tamoxifen lesions, the major DNA adducts detected in endometrial tissues of patients treated with tamoxifen, *Cancer Res. 59*, 2091−2095.
48. Shibutani, S. (1993) Quantitation of base substitutions and deletions induced by chemical mutagens during DNA synthesis in vitro, *Chem. Res. Toxicol. 6*, 625−629.
49. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
50. Mendelman, L. V., Boosalis, M. S., Petruska, J., and Goodman, M. F. (1989) Nearest neighbor influences on DNA polymerase insertion fidelity, *J. Biol. Chem. 264*, 14415−14423.
51. Mendelman, L. V., Petruska, J., and Goodman, M. F. (1990) Base mispair extension kinetics, *J. Biol. Chem. 265*, 2338−2346.
52. Clark, J. M., Joyce, C. M., and Beardsley, G. P. (1987) Novel blunt-end addition reactions catalyzed by DNA polymerase I of *Escherichia coli*, *J. Mol. Biol. 198*, 123−127.
53. Terashima, I., Suzuki, N., Dasaradhi, L., Tan, C. K., Downey, K. M., and Shibutani, S. (1998) Translesional synthesis on DNA templates containing an estrogen quinone-derived adduct: N^2-(2-hydroxyestron-6-yl)-2′-deoxyguanosine and N^6-(2-hydroxyestron-6-yl)-2′-deoxyadenosine, *Biochemistry 37*, 13807−13815.
54. Suzuki, N., Itoh, S., Poon, K., Masutani, C., Hanaoka, F., Ohmori, H., Yoshizawa, I., and Shibutani, S. (2004) Translesion synthesis past estrogen-derived DNA adducts by human DNA polymerases *η* and *κ*, *Biochemistry 43*, 6304−6311.
55. Shimotakahara, S., Gorin, A., Kolbanovskiy, A., Kettani, A., Hingerty, B. E., Amin, S., Broyde, S., Geacintov, N., and Patel, D. J. (2000) Accomodation of S-cis-tamoxifen-N^2-guanine adduct within a bent and widened DNA minor groove, *J. Mol. Biol. 302*, 377−393.
56. Frank, E. G., Tissier, A., McDonald, J. P., Rapić-Otrin, V., Zeng, X., Gearhart, P. J., and Woodgate, R. (2001) Altered nucleotide misinsertion fidelity associated with poliota-dependent replication at the end of a DNA template, *EMBO J. 20*, 2914−2922.
57. Yasui, M., Dong, H., Bonala, R. R., Suzuki, N., Ohmori, H., Hanaoka, F., Johnson, F., Grollman, A. P., and Shibutani, S. Mutagenic properties of 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene, a persistent acetylaminofluorene-derived DNA adduct in mammalian cells, *Biochemistry 43*, 15005−15013.

BI0608461