Efficient and Erroneous Incorporation of Oxidized DNA Precursors by Human DNA Polymerase η[†]

Masatomi Shimizu,[‡,§] Petr Gruz,[‡] Hiroyuki Kamiya,[||] Chikahide Masutani,[⊥] Yan Xu,[#] Yukio Usui,[▽] Hiroshi Sugiyama,[#,○] Hideyoshi Harashima,[||] Fumio Hanaoka,[⊥] and Takehiko Nohmi*[,‡]

Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan, Graduate School of Frontier Biosciences, Osaka University and SORST, JST, Osaka, Japan, Institute of Biomedical and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan, and Division of Medical Nutrition, Tokyo Healthcare University, Tokyo, Japan

Received October 30, 2006; Revised Manuscript Received February 4, 2007

ABSTRACT: Altered oxidative metabolism is a property of many tumor cells. Oxidation of DNA precursors, i.e., dNTP pool, as well as DNA is a major source of mutagenesis and carcinogenesis. Here, we report the remarkable nature of human DNA polymerase η that incorporates oxidized dNTPs into a nascent DNA strand in an efficient and erroneous manner. The polymerase almost exclusively incorporated 8-hydroxy-dGTP (8-OH-dGTP) opposite template adenine (A) at 60% efficiency of normal dTTP incorporation, and incorporated 2-hydroxy-dATP (2-OH-dATP) opposite template thymine (T), guanine (G), or cytosine (C) at substantial rates. The synthetic primers having 8-hydroxy-G paired with template A or 2-hydroxy-A paired with template T, G, or C at the termini were efficiently extended. In contrast, human DNA polymerase ι incorporated 8-OH-dGTP opposite template A with much lower efficiency and did not incorporate 2-OH-dATP opposite any of the template bases. It did not extend the primers having the oxidized bases at the termini either. We propose that human DNA polymerase η may participate in oxidative mutagenesis through the efficient and erroneous incorporation of oxidized dNTPs during DNA synthesis.

Reactive oxygen species (ROS[1]), such as superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, are produced through normal cellular metabolism, and formation of such radicals is further enhanced by irradiation or chemical exposure (*1*, *2*). ROS generates a variety of altered purines and pyrimidines in DNA (*3*, *4*), and oxidation of DNA plays important roles in mutagenesis, carcinogenesis, and aging (*5*, *6*). Several lines of evidence indicate, however, that oxidation of DNA precursors in the nucleotide pool, i.e., dNTPs, is another important cause of genome instability (*7*−*9*). Indeed, the frequency of A:T-to-C:G transversion mutations increases more than 1000-fold over the wild-type level in *Escherichia coli mutT* mutants, which are deficient in the ability to hydrolyze oxidized dGTP, i.e., 7,8-dihydro-8-oxo-dGTP (8-hydroxy-dGTP, 8-OH-dGTP, Figure 1A) (*10*, *11*). 8-OH-dGTP leads to A:T-to-C:G mutations when it is incorporated opposite adenine (A) in the template DNA because the incorporated 8-OH-G in DNA can pair with incoming dCMP in the next round of DNA replication (*8*, *12*). The high spontaneous A:T-to-C:G mutations in the *mutT* strain are almost completely suppressed when the *mutT* cells are cultured in anaerobic conditions, indicating the essential role of oxygen in the mutagenesis (*13*). Another oxidized nucleotide, i.e., 1,2-dihydro-2-oxo-dATP (2-hydroxy-dATP, 2-OH-dATP, Figure 1B), can induce G:C-to-T:A transversions when it is incorporated opposite guanine (G) in the template (*14*, *15*). The sanitizing enzyme, i.e., Orf135, in *E. coli* degrades 2-OH-dATP, and G:C-to-T:A mutations occur in the *orf135*-deficient strain more frequently than in the wild-type strain (*16*, *17*).

Oxidized dNTPs also cause genome instability in mammalian cells. Spontaneous tumorigenesis in mice deficient in *Mth1*, a mammalian counterpart of *mutT*, is much enhanced in lung, liver, and stomach, and the MTH1 protein hydrolyzes both 8-OH-dGTP and 2-OH-dATP (*18*, *19*). Recent studies with mismatch repair defective cells suggest that the majority of mutations in human cells that are deficient in mismatch repair functions do not arise from spontaneous replication errors, but from the incorporation of oxidized dNTPs (*20*, *21*). Thus, it is of great interest in

[†] The work was supported by grants-in-aid for Basic Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to T.N. and M.S.) and from the Ministry of Health, Labour and Welfare, Japan, for Cancer Research from the Ministry of Health, Labour and Welfare, Japan and for International Collaborative Research from the Japan Health Science Foundation (SH34407).

* To whom correspondence should be addressed. Phone:+81-3-3700-9873. Fax: +81-3-3707-6950. E-mail: nohmi@nihs.go.jp.

[‡] National Institute of Health Sciences.

[§] Present address: Tokyo Healthcare University.

[||] Hokkaido University.

[⊥] Osaka University.

[#] Tokyo Medical and Dental University.

[▽] Tokyo Healthcare University.

[○] Present address: Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan.

[1] Abbreviations: ROS, reactive oxygen species; 8-OH-dGTP, 7,8-dihydro-8-oxo-dGTP; A, adenine; 2-OH-dATP, 1,2-dihydro-2-oxo-dATP; G, guanine; Pols, DNA polymerases; C, cytosine; TLS, translesion DNA synthesis; T, thymine; Polη, DNA polymerase η; Polι, DNA polymerase ι; HPLC, high performance liquid chromatography.

10.1021/bi062238r CCC: $37.00
Published on Web 04/18/2007

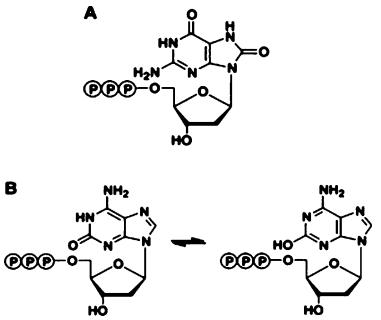

FIGURE 1: Structures of 8-OH-dGTP (A) and 2-OH-dATP (B). P represents a phosphate group.

the mechanisms as to how these oxidized dNTPs induce genome instability and oxidative carcinogenesis.

When the oxidized dNTPs escape from the sanitation by the enzymes, they will be incorporated into nascent DNA by DNA polymerases (Pols). However, oxidized dNTPs are in general poor substrates for Pols (*22*). For example, the efficiency of incorporation of 8-OH-dGTP by Polδ is more than 10^4-fold lower than that of incorporation of normal dGTP, and the enzyme prefers to incorporate 8-OH-dGTP opposite template cytosine (C) (*23*). 8-OH-dGTP is poorly incorporated into DNA by T7 Pol exo$^-$, HIV reverse transcriptase, *E. coli* Pol II, and Klenow exo$^-$ as well (*24*). An exception may be human Polβ, which incorporates 8-OH-dGTP into DNA with an efficiency of 10−20% of normal dGTP incorporation and favors its incorporation opposite template A (*25*). 2-OH-dATP is also a poor substrate for mammalian Pols. The efficiencies of incorporation of 2-OH-dATP opposite template T and C by Polα are more than 100-fold and 1000-fold, respectively, lower than those of incorporation of normal dATP and dGTP (*26*).

The Y-family Pols are recently recognized Pols that comprise proteins from different species, including members of Archaea, Bacteria, and Eukarya (*27*). The most distinct feature of this family of enzymes is their ability to bypass various lesions, such as ultraviolet light photoproducts, in template DNA (*28*−*30*). Some bypass reactions, i.e., translesion DNA synthesis (TLS), catalyzed by these enzymes are error prone while others are error free (*31*). Thus, this family of Pols seems to be involved in mutagenesis and DNA-damage tolerance (*32*). Interestingly, some of the Y-family Pols are shown to incorporate oxidized dNTPs into DNA in an erroneous manner. Archaeal Y-family Pols from *Sulfolobus* sp. and a bacterial Y-family Pol, i.e., DNA Pol IV (DinB) of *E. coli*, almost exclusively incorporate 8-OH-dGTP opposite template A, and 2-OH-dATP opposite template G and thymine (T) (*33*, *34*). Further genetic analysis with *E. coli sod/fur* mutants suggests that both Y-family Pols of *E. coli*, i.e., Pol IV and Pol V (UmuD′C), are involved in oxidative mutagenesis caused by oxidized dNTPs (*34*). The enzymes may participate in sequential biochemical steps, such as incorporation of oxidized dNTPs into DNA and extension of primers having oxidized bases at the termini. Pol IV is also shown to be involved in induction of G:C-to-T:A mutations when 2-OH-dATP is directly introduced into *E. coli* cells by $CaCl_2$ treatment (*35*). Collectively, these results led us to postulate that certain human Y-family Pols might be involved in oxidative mutagenesis through incorrect and efficient incorporation of oxidized dNTPs into nascent DNA. In fact, human Polη incorporates oxidized dNTPs into DNA in an erroneous manner like the archaeal and bacterial Y-family Pols (*33*).

In this study, we quantitatively compared two human Y-family Pols, i.e., Polη and Polι, in the specificity and efficiency of incorporation of 8-OH-dGTP and 2-OH-dATP into a nascent DNA strand. Surprisingly, Polη incorporated 8-OH-dGTP opposite template A at almost the same efficiency as incorporation of normal dTTP, and incorporated 2-OH-dATP opposite template T, G, and C at substantial rates. In contrast, Polι poorly incorporated 8-OH-dGTP opposite template A and did not incorporate 2-OH-dATP opposite any of the bases in template DNA. Since Polη is constitutively associated with replication factories (*36*, *37*) and involved in multiple DNA transactions (*38*−*40*), we propose that this enzyme may be involved in mutagenesis through the efficient and erroneous incorporation of oxidized dNTPs into DNA strand.

EXPERIMENTAL PROCEDURES

Materials. Human Polη and Polι were purified as described (*41*, *42*). 8-OH-dGTP and 2-OH-dATP were prepared as described (*26*, *43*), and no discernible impurities were detected by high performance liquid chromatography (HPLC). dNTPs (ultrapure grade) were purchased from GE Healthcare Bio-Sciences KK (Tokyo).

DNA Substrates. Oligonucleotides containing 8-OH-G and 2-OH-A were prepared by the phosphoramidite method on controlled pore glass supports (1 μmol) by using a Beckman OLIGO1000 DNA synthesizer. After automated synthesis, the oligomers were detached from the support, deprotected, and purified by HPLC. The oligomers were identified by complete digestion of the oligomers with alkaline phosphatase and P1 nuclease to 2′-deoxymononucleosides. Other oligonucleotides were purchased from BEX Corp. (Tokyo). A Cy3-labeled 18-mer primer (5′-CGCGCGAAGACCG-GTTAC-3′) or Cy3-labeled 19-mer primers (5′-CGCGC-GAAGACCGGTTACN-3′ where N represents either 8-OH-G or 2-OH-A) were annealed to 36-mer templates (5′-GAAGGGATCCTTAAGACXGTAACCGGTCTTCGCGCG-3′ where X represents A, C, G, or T) at a molar ratio of 1:1 to generate substrates. The 5′-Cy3-primer/templates generated by annealing the 18-mer primer to the 36-mer template were named substrate 1, and those generated by annealing the 19-mer primer having 8-OH-G or 2-OH-A to the 36-mer templates were named substrate 2 or 3, respectively. In control reactions, Cy3-labeled 19-mer primers (5′-CGCGC-GAAGACCGGTTACN-3′ where N represents either G or A) were annealed to the 36-mer templates. The 5′-Cy3-primer/templates generated by annealing the 19-mer primer having G or A to the 36-mer templates were named substrate 4 or 5, respectively.

Steady-State Kinetic Analyses. The standard reaction mixtures (10 μL) contained 40 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 10 mM dithiothreitol, 250 μg/mL bovine serum albumin, 60 mM KCl, 2.5% glycerol, 1 nM Polη or Polι, 100 nM primer/template DNA (substrate 1, 2, or 3), and a single oxidized dNTP (8-OH-dGTP or 2-OH-dATP) or normal dNTP (dATP, dTTP, dGTP, or dCTP). The activity of Polι was also measured in the same reaction mixtures but without KCl (*44*). In that case, the concentration of $MgCl_2$ was reduced to 1 mM instead of 5 mM. Six different

concentrations of oxidized dNTP or normal dNTP were used to determine the kinetic parameters, i.e., k_{cat} and K_m (see below). The mixtures were incubated at 37 °C, and the reactions were terminated by adding 10 μL of stop solution (98% formamide, 10 mM EDTA, 10 mg/mL Blue Dextran). After heat denaturation for 10 min at 100 °C, the samples were separated by electrophoresis with 15% denaturing polyacrylamide gel containing 8 M urea and visualized by the Molecular Imager FX Pro System (Bio-Rad Laboratories, CA).

When the kinetic parameters for incorporation of oxidized or normal dNTPs into DNA were determined, the reaction mixtures contained substrate 1. The reaction time and the concentrations of oxidized dNTP and normal dNTP varied depending on the rates of reactions and the K_m values. For example, the incubation time was 1 min and the concentrations of 8-OH-dGTP varied from 0.05 to 10 μM for the analysis of incorporation of 8-OH-dGTP opposite template A by Polη. Identical reaction conditions were used for the analyses of incorporation of a single normal dNTP opposite a template canonical base by Polη. On the other hand, the incubation time was 10 min and the range of concentrations of 8-OH-dGTP was 5 to 100 μM to determine the parameters for incorporation of 8-OH-dGTP opposite template A by Polι. For the kinetic analyses of extension of primers having oxidized bases at the 3′-termini by Polη, the incubation time was 5 min and the concentrations of dGTP varied from 0.05 to 10 μM with the template/primer DNAs of substrate 2 or 3.

Data Analyses. Gel band intensities were measured using the Molecular Imager FX Pro System and Quantity One software (Bio-Rad Laboratories, CA), and nucleotide incorporation parameters were determined (*45*). Less than 20% of the primers were extended in these steady-state kinetic analyses, ensuring single-hit kinetics (*46*). For each DNA substrate, the rate of incorporation was plotted as a function of dNTP concentration, and the V_{max} and K_m values were determined by nonlinear regression fitting using the SigmaPlot software (Systat Software Inc., CA). k_{cat} was calculated by dividing V_{max} by the enzyme concentration. The fidelity of incorporation, i.e., F_{inc}, was calculated using the equation $F_{inc} = (k_{cat}/K_m)_{incorrect}/(k_{cat}/K_m)_{correct}$. All values are means ± standard errors from three experiments.

RESULTS

Efficient and Erroneous Incorporation of 8-OH-dGTP and 2-OH-dATP into DNA by Polη. Human Polη almost exclusively incorporates 8-OH-dGTP opposite template A and incorporates 2-OH-dATP opposite template T, G, and C (*33*). To compare two human Y-family Pols, we at first examined the specificity of human Polι incorporating 8-OH-dGTP and 2-OH-dATP into DNA. To this end, we used substrate 1 having four different bases in the N position in the template and incubated it with Polι in the presence of either 8-OH-dGTP or 2-OH-dATP. For the incorporation of 8-OH-dGTP, the primer annealed to the template strand having A in the N position was elongated, and no other primers annealed to templates having C, G, or T in the N position were extended (Figure 2A). These results indicate that Polι almost exclusively incorporates 8-OH-dGTP opposite template A as in the case of Polη. For the incorporation of 2-OH-dATP, no primers were elongated regardless of the bases in the template strand (Figure 2B). The activity of Polι is enhanced by omission of KCl from the reaction mixtures (*44*). We conducted, therefore, the experiments in the absence of KCl. Although the activity was enhanced several times (Table 1), the specificity was unchanged. 8-OH-dGTP was almost exclusively incorporated opposite template A, and no 2-OH-dATP was incorporated into DNA regardless of the template bases (data not shown). In control reactions, Polι incorporated normal dGTP opposite template C and T and incorporated normal dATP opposite template T and slightly opposite template C (Figure 2C,D). Thus, we conclude that, although the incorporation of 8-OH-dGTP opposite template A is a common feature to Y-family Pols, the specificity incorporating 2-OH-dATP into DNA is markedly distinct even within two human homologues.

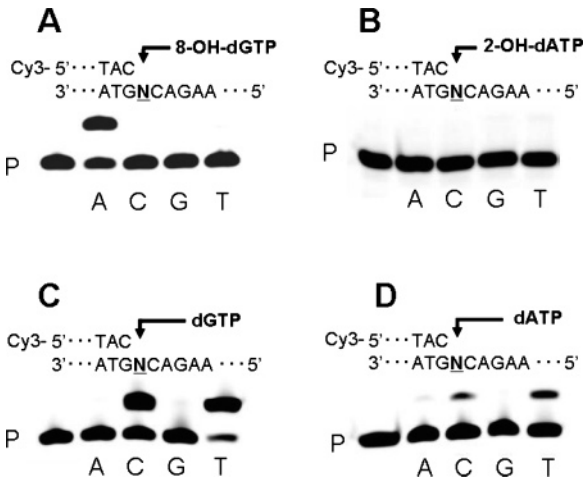

FIGURE 2: Incorporation of 8-OH-dGTP (A), 2-OH-dATP (B), dGTP (C), and dATP (D) into DNA by Polι. For analysis of incorporation of oxidized dNTPs (A and B), the reaction mixtures described in Experimental Procedures contained 1 nM Polι, 50 μM either 8-OH-dGTP or 2-OH-dATP, and 100 nM substrate 1. No normal dNTPs were included. For analysis of incorporation of normal dNTPs (C and D), the reaction mixtures contained 5 nM Polι, 50 μM either dGTP or dATP, and 100 nM substrate 1. In the reaction, KCl was omitted from the reaction mixtures and the concentration of $MgCl_2$ was 1 mM. The mixtures were incubated for 15 min at 37 °C, and the samples were processed as described in Experimental Procedures. The N in the template strand represents A, C, G, or T.

Table 1: Kinetic Parameters for Incorporation of 8-OH-dGTP and 2-OH-dATP by Polη and Polι

Pol	template base/ dNTP	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})	F_{inc}
Polη	A/dTTP	3.8 ± 0.52	15.4 ± 0.82	4.1	1
	A/8-OH-dGTP	4.2 ± 0.35	10.1 ± 0.35	2.4	0.59
	C/dGTP	1.2 ± 0.22	1.9 ± 0.1	1.6	1
	C/8-OH-dGTP	22.5 ± 8.3	0.3 ± 0.05	0.013	0.008
	C/dGTP	1.2 ± 0.22	1.9 ± 0.1	1.6	1
	C/2-OH-dATP	1.7 ± 0.55	0.1 ± 0.007	0.06	0.038
	G/dCTP	1.6 ± 0.12	9.5 ± 0.22	5.9	1
	G/2-OH-dATP	3.6 ± 0.6	0.35 ± 0.02	0.1	0.017
	T/dATP	5.2 ± 0.47	15 ± 0.61	2.9	1
	T/2-OH-dATP	3.0 ± 0.52	0.55 ± 0.03	0.18	0.062
Polι	A/dTTP	14.4 ± 3.0	5.1 ± 0.67	0.35	1
	A/8-OH-dGTP	132 ± 45.4	1.4 ± 0.31	0.011	0.031
	A/dTTP[a]	0.86 ± 0.14[a]	2.24 ± 0.08[a]	2.6[a]	1[a]
	A/8-OH-dGTP[a]	48.5 ± 7.37[a]	2.17 ± 0.15[a]	0.045[a]	0.017[a]

[a] The activities were measured without KCl in the reaction mixtures.

To compare the efficiency of two Pols for the incorporation of oxidized dNTPs quantitatively, we determined the steady-state kinetic parameters, i.e., K_m and k_{cat} (Table 1). The most remarkable feature of the kinetic data is the high efficiency of Polη incorporating 8-OH-dGTP opposite template A. The K_m and k_{cat} values (4.2 μM and 10.1 min^{-1}, respectively) were similar to those of incorporation of normal dTTP opposite template A (3.8 μM and 15.4 min^{-1}, respectively). Thus, the F_{inc}, i.e., $(k_{cat}/K_m)_{8-OH-dGTP}/(k_{cat}/K_m)_{dTTP}$, was about 0.6 (=2.4/4.1), which suggests that Polη incorporates 8-OH-dGTP opposite template A with the efficiency of about 60% of that of incorporation of normal dTTP. In addition, the incorporation of 8-OH-dGTP by Polη was strongly biased opposite template A compared to template C. The k_{cat}/K_m value of incorporation of 8-OH-dGTP opposite template A was more than 180 times greater than that of incorporation of 8-OH-dGTP opposite template C (2.4 versus 0.013). In contrast, Polι incorporates 8-OH-dGTP opposite template A with much lower efficiency. The k_{cat}/K_m value of incorporation of 8-OH-dGTP opposite template A was more than 200 and 30 times, respectively, less than that of incorporation of 8-OH-dGTP opposite template A by Polη (0.011 versus 2.4) and normal dTTP by Polι (0.011 versus 0.35). The k_{cat}/K_m value of incorporation of normal dTTP and 8-OH-dGTP opposite template A by Polι was enhanced several times by omission of KCl from the reaction mixtures (0.35 versus 2.6 and 0.011 versus 0.045). Nevertheless, the k_{cat}/K_m value of incorporation of 8-OH-dGTP opposite template A was more than 50 times less than that of incorporation of 8-OH-dGTP opposite template A by Polη (0.045 versus 2.4) and normal dTTP by Polι (0.045 versus 2.6).

We then determined the kinetic parameters of incorporation of 2-OH-dATP opposite template C, G, or T by Polη (Table 1). We did not determine the parameters for Polι because the enzyme did not incorporate 2-OH-dATP into DNA (Figure 2B). The K_m values for the incorporation of 2-OH-dATP by Polη were indistinguishable from those for the incorporation of normal dNTPs opposite template C, G, or T. However, the k_{cat} values for the incorporation of 2-OH-dATP were 20 to 30 times less than those of incorporation of normal dNTPs. Nevertheless, the F_{inc} values, i.e., $(k_{cat}/K_m)_{2-OH-dATP}/(k_{cat}/K_m)_{dNTP}$, opposite template C, G, and T were 0.038, 0.017, and 0.062, respectively, suggesting that Polη incorporates 2-OH-dATP opposite multiple template bases (except for A) with efficiency of 2 to 6% of that of incorporation of normal dNTPs. The order of k_{cat}/K_m values of incorporation of 2-OH-dATP opposite template bases was T (0.18) > G (0.10) > C (0.06).

Efficient Extension of Primers Having 8-OH-G or 2-OH-A at the 3′-Termini by Polη. To investigate whether Polη and Polι can extend the primers upon incorporation of oxidized dNTPs during DNA synthesis, we examined whether these Pols extend primers having either 8-OH-G or 2-OH-A at the 3′-termini. We annealed the primers to the template strands where the terminal oxidized base was paired with template A, C, G, or T and incubated the primer/templates, i.e., substrate 2 or 3, with Polη or Polι in the presence of four normal dNTPs. Polη extended the primer having 8-OH-G when the terminal oxidized base was paired with template A or C (Figure 3A). Virtually no extension was observed, however, when the terminal 8-OH-G was paired with template G or T. Polη could also extend primers having

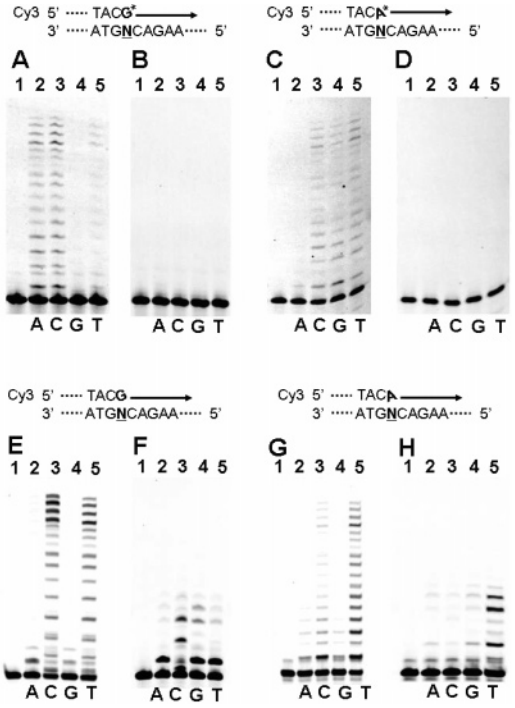

FIGURE 3: Extension of primers having 8-OH-G or 2-OH-A at the termini by Polη and Polι. Primers having 8-OH-G were extended by Polη (A) or Polι (B), and those having 2-OH-A were extended by Polη (C) or Polι (D). As control reactions, primers having G were extended by Polη (E) or Polι (F) and those having A were extended by Polη (G) or Polι (H). The reaction mixtures described in Experimental Procedures contained 1 nM Polη (A and C) or Polι (B and D), 200 μM four normal dNTPs (dATP, dCTP, dGTP, and dTTP), and 100 nM substrate 2 (A and B) or substrate 3 (C and D). The reaction mixtures were incubated for 5 min at 37 °C. In control reactions, the reaction mixtures contained 10 nM Polη (E and G) or Polι (F and H), 200 μM four normal dNTPs, and 100 nM substrate 4 (E and F) or substrate 5 (G and H). In control reactions with Polι (F and H), KCl was omitted from the reaction mixtures and the concentration of $MgCl_2$ was 1 mM. The reaction mixtures were incubated for 5 min (E and G) or 15 min (F and H) at 37 °C. The samples were processed as described in Experimental Procedures. Lane 1 (the leftmost lane) represents the position of the primers alone. The N in the template strands represents A (lane 2), C (lane 3), G (lane 4), and T (lane 5).

2-OH-A when the terminal oxidized base was paired with template C, G, or T (Figure 3C). In contrast, Polι was unable to extend any primers containing either 8-OH-G or 2-OH-A at the termini regardless of the pairing template bases (Figure 3B,D). In control reactions, Polη extended the primers having G at the termini when the terminal base was paired with template C or T (Figure 3E) and extended the primers having A at the termini when the terminal base was paired with template T (Figure 3G). Weak extension was observed when the terminal A was paired with template C. Polι was a poor extender even without oxidized bases at the termini of primers. It weakly extended primers when the terminal G or A was paired with canonical template bases (Figure 3F,H). These results suggest that Polη extends the primers upon incorporation of 8-OH-dGTP opposite template A or C, and extends the primers upon incorporation of 2-OH-dATP opposite template C, G, or T. Two Y-family Pols are distinct for their ability to extend the primers having oxidized bases at the termini.

We then determined the steady-state kinetic parameters for the extension, i.e., incorporation of next dGTP opposite

Table 2: Kinetic Parameters for Incorporation of dGTP with Primers Having Terminal 8-OH-G or 2-OH-A Pairing with Multiple Template Bases by Polη[a]

Template base/dNTP	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (μM^{-1}min^{-1})
dGTP G* / A C	0.98±0.11	2.4±0.08	2.4
dGTP G* / C C	2.5±0.38	2.6±0.14	1.0
dGTP A* / G C	7.3±0.89	9.9±0.61	1.4
dGTP A* / G C	6.9±1.3	7.3±0.7	1.1
dGTP A* / T C	2.8±0.25	8.9±0.28	3.2

[a] G* = 8-OH-G; A* = 2-OH-A.

template C, by Polη from the primers having either 8-OH-G or 2-OH-A at the 3′-termini (Table 2). We did not determine the parameters for Polι because it did not extend the primers having 8-OH-G or 2-OH-A (Figure 3B,D).The terminal 8-OH-G in the primer was paired with template A or C while the terminal 2-OH-A was paired with template C, G, or T. The primer/templates, i.e., substrates 2 or 3, were incubated with Polη in the presence of dGTP without other dNTPs. For the extension from the primer having 8-OH-G at the termini, Polη extended the primer more efficiently when the terminal oxidized base was paired with template A than template C. The K_m value for the extension from the primer having 8-OH-G paired with template A was 2.5 times less than that for the extension from the primer having the oxidized base paired with template C (0.98 versus 2.5 μM) although the k_{cat} values were very similar between the two substrates (2.4 versus 2.6 min^{-1}). Thus, the ratio, i.e., $(k_{cat}/K_m)_{8-OH-G:A}/(k_{cat}/K_m)_{8-OH-G:C}$, was calculated to be 2.4 (=2.4/1.0), suggesting that Polη more than two times more efficiently extends the primer having 8-OH-G paired with template A compared to that having 8-OH-G paired with template C. For the extension from the primer having 2-OH-A at the 3′-temini, Polη extended the primer most efficiently when the oxidized base was paired with template T followed by template C and template G. The K_m value for the extension from the primer having 2-OH-A paired with template T (2.8 μM) was 2 to 3 times less than those for the extension from the primers having 2-OH-A paired with template G or template C (6.9 or 7.3 μM), and the k_{cat} values were not very different among the three substrates (8.9, 7.3, or 9.9 min^{-1}). Thus, the ratio of k_{cat}/K_m values, i.e., $(k_{cat}/K_m)_{2-OH-A:T}$:$(k_{cat}/K_m)_{2-OH-A:G}$:$(k_{cat}/K_m)_{2-OH-A:C}$, was 1: 0.34: 0.44, which suggests that Polη extends the primers having terminal 2-OH-A paired with template G or C at rates of 30 to 40% of that of extension from the primer having 2-OH-A paired with template T.

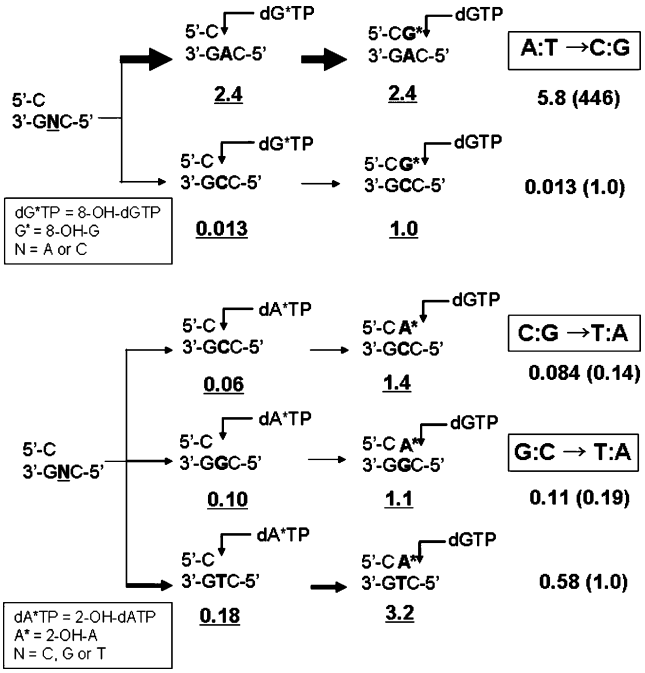

FIGURE 4: Total catalytic efficiency of incorporation of 8-OH-dGTP and 2-OH-dATP into DNA and the following primer extension by Polη. The total efficiency of incorporation and extension was calculated as the product of the individual k_{cat}/K_m values, e.g., 2.4 × 2.4 = 5.8 for the incorporation of 8-OH-dGTP opposite template A and the extension by incorporation of normal dGTP opposite template C. The individual k_{cat}/K_m value for each step is underlined. Relative efficiency is presented in parentheses. The relative values were calculated by setting the efficiency of incorporation of 8-OH-dGTP opposite template C and the following extension or incorporation of 2-OH-dATP opposite template T and the following extension as 1.0.

DISCUSSION

Oxidation is a major cause of spontaneous DNA damage that may contribute to mutagenesis, carcinogenesis, and aging (*5*, *6*). Oxidative damage in DNA is caused not only by direct oxidation of bases in DNA but also by incorporation of oxidized dNTPs in the nucleotide pool into DNA during chromosome replication (*7*, *47*). In this study, we have revealed the remarkable nature of Polη that incorporates 8-OH-dGTP opposite template A almost as efficiently as the incorporation of normal dTTP (Table 1). In addition, Polη extends the primer having 8-OH-G paired with template A more than two times more efficiently than the same primer having 8-OH-G paired with template C (Table 2). Calculation of the total efficiency by multiplying the individual k_{cat}/K_m values suggests that Polη prefers template A more than 400 times (446 = 5.8/0.013) to template C for the incorporation and extension (Figure 4). The efficient and erroneous incorporation of 8-OH-dGTP and the following extension may lead to A:T-to-C:G transversions because the incorporated 8-OH-G opposite template A may pair with dCMP in the next round of DNA replication (*8*, *43*, *48*). Besides 8-OH-dGTP, Polη incorporates 2-OH-dATP opposite template T, G, and C with efficiency of 2 to 6% of that of incorporation of normal dNTPs (Table 1). The F_{inc} values of incorporation of 2-OH-dATP opposite template T and C are about 20 and 50 times higher than the corresponding values by calf thymus Polα (*26*). Moreover, Polη can extend the primers having terminal 2-OH-A paired with template T, G, and C

(Figure 3, Table 2). The relative preference of Polη to template T:G:C for incorporation and extension is calculated as 1:0.19:0.14 (Figure 4). Incorporation of 2-OH-dATP opposite template G or C and the following extension may lead to G:C-to-T:A and C:G-to-T:A mutations since the incorporated 2-OH-A can pair with dTMP during the next round of DNA replication (*14*, *49*). Thus, we suggest that Polη has a potential to enhance mutagenesis through the efficient and erroneous incorporation of 8-OH-dGTP and 2-OH-dATP and the following extension during DNA replication (see below). It is possible, however, that other Pols may be involved in extension steps once oxidized dNTPs are inserted into nascent DNA by Polη.

Although Polη was initially identified as an error-free bypass Pol across a *cis-syn* thymine−thymine dimer in DNA (*28*, *29*), it appears to play important roles in several DNA transactions besides TLS (*50*). Polη interacts with RAD51 and has an activity to extend D loop, which is an intermediate of homologous recombination (*38*, *39*). It also participates in mutagenesis at A:T base pairs in immunoglobulin genes during somatic hypermutation (*40*). In addition, Polη is identified in replication factories in cells not deliberately exposed to DNA damaging agents (*36*, *37*), suggesting that it might constitutively gain access to the genomic DNA and contribute to mutagenesis and/or damage avoidance even without external DNA damage. Interestingly, *E. coli* Y-family Pols, i.e., Pol IV (DinB) and variants of Pol V (UmuD′C), are shown to be involved in the chromosome replication when the cells are treated with hydroxyurea, which does not induce DNA damage but depletes the nucleotide pool (*51*). In this case, the Y-family Pols appear to rescue the stalled replication by efficient incorporation of dNTPs in the depleted nucleotide pool at the expense of increased mutations. By analogy, we envisage that Polη might be localized in replication factories even without external DNA damage and contribute to rescue the stalled replication by incorporation of oxidized dNTPs when the nucleotide pool is heavily oxidized and/or the ratio of oxidized versus normal dNTPs is substantially enhanced. The replicative Pols might have difficulty to extend the primers without Polη in the situation since the oxidized dNTPs are generally difficult substrates for replicative Pols. However, the incorporation by Polη is erroneous and thus may induce mutations as described above. In fact, the mutagenicity of 8-OH-dGTP directly incorporated into human cells is reduced significantly when the expression of Polη is suppressed by RNAi technique (Kamiya et al., unpublished results). It is demonstrated that both 8-OH-dGTP and 2-OH-dATP are significant contributors to mutations in mismatch-repair-defective cells and incorporation of oxidized DNA precursors is a significant influence on microsatellite instability in repair-defective human tumor cells (*21*). We suggest, therefore, that Polη may be involved in mutagenesis by the incorporation of oxidized dNTPs in various DNA transactions when the nucleotide pool is oxidized and imbalanced.

Unlike Polη, Polι has a limited ability to incorporate oxidized dNTPs and extend the primers having the oxidized bases at the termini. It could not incorporate 2-OH-dATP into DNA and extend the primers having 8-OH-G or 2-OH-A regardless of the template bases under the experimental conditions (Figure 2B, Figure 3B,D). The only activity we could detect in this study was the incorporation of 8-OH-dGTP opposite template A (Figure 2A, Table 1). Polι is known to form Hoogsteen base pairing in the active site where the template A is rotated to the *syn* conformation while the incoming dTTP is in the *anti* conformation (*52*). Thus, the template A may be driven to the *syn* conformation when 8-OH-dGTP is incorporated. The Hoogsteen base pairing, i.e., template A (*syn*):8-OH-dGTP (*syn* or *anti*), may be the basis for the limited catalysis of incorporation of 8-OH-dGTP opposite template A. Nevertheless, Polι could not extend the primers having 8-OH-G at the termini. This may resemble the situation where Polι fails to further elongate the primers upon incorporation of C or T opposite 1,N^2-propano-2′-deoxyguanosine, which forms a *syn* conformation in the template DNA strand (*53*). Although it is possible that other Pols such as Polκ may help the extension step upon incorporation of 8-OH-G opposite template A by Polι, it seems unlikely that Polι is heavily involved in oxidative mutagenesis through the incorporation of oxidized dNTPs into nascent DNA.

In this study, we have revealed the remarkable nature of Polη that incorporates 8-OH-dGTP and 2-OH-dATP into DNA in an efficient and erroneous manner. We assume that the incoming 8-OH-dGTP adopts the *syn* conformation in the active site of Polη, which allows the formation of Hoogsteen base pairing with the *anti* form of template A. The active site large enough to accommodate bulky DNA adducts may permit the *syn* conformation of incoming 8-OH-dGTP, which is favored when it pairs with A in a DNA duplex (*54*). The Hoogsteen base pairing, i.e., 8-OH-dGTP (*syn*) and template A (*anti*), which might be opposite to the conformation of pairing bases in the active site of Polι, could be the basis for the efficient and erroneous incorporation of 8-OH-dGTP by Polη. Interestingly, yeast and human Polη can bypass 8-OH-G in DNA in an efficient but error-free manner by insertion of dCMP opposite the oxidized base in the template strand (*55*). In these cases, the large active site of Polη is thought to allow the *anti* conformation of 8-OH-G in the template strand, which introduces barriers for other Pols (*56*). Since oxidative damage in the nucleotide pool as well as DNA is a major source of spontaneous mutagenesis and carcinogenesis, the present study warrants the validity of further research on the roles of Polη in the oxidative mutagenesis in human cells.

ACKNOWLEDGMENT

We thank Dr. Roger Woodgate at National Institutes of Health (Bethesda, MD) for a generous gift of Polι and for helpful discussion. The work is supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan, the Ministry of Health, Labour and Welfare, Japan, and the Japan Health Science Foundation.

REFERENCES

1. Ames, B. N., and Gold, L. S. (1991) Endogenous mutagens and the causes of aging and cancer, *Mutat. Res. 250*, 3−16.
2. Henle, E. S., and Linn, S. (1997) Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide, *J. Biol. Chem. 272*, 19095−19098.
3. Bjelland, S., and Seeberg, E. (2003) Mutagenicity, toxicity and repair of DNA base damage induced by oxidation, *Mutat. Res. 531*, 37−80.
4. Kamiya, H. (2003) Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: approaches using

synthetic oligonucleotides and nucleotides: survey and summary, *Nucleic Acids Res. 31*, 517−531.
5. Ames, B. N. (1983) Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases, *Science 221*, 1256−1264.
6. Jackson, A. L., and Loeb, L. A. (2001) The contribution of endogenous sources of DNA damage to the multiple mutations in cancer, *Mutat. Res. 477*, 7−21.
7. Sekiguchi, M., and Tsuzuki, T. (2002) Oxidative nucleotide damage: consequences and prevention, *Oncogene 21*, 8895−8904.
8. Michaels, M. L., and Miller, J. H. (1992) The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine), *J. Bacteriol. 174*, 6321−6325.
9. Nakabeppu, Y., Sakumi, K., Sakamoto, K., Tsuchimoto, D., Tsuzuki, T., and Nakatsu, Y. (2006) Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids, *Biol. Chem. 387*, 373−379.
10. Maki, H., and Sekiguchi, M. (1992) MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis, *Nature 355*, 273−275.
11. Yanofsky, C., Cox, E. C., and Horn, V. (1966) The unusual mutagenic specificity of an *E. coli* mutator gene, *Proc. Natl. Acad. Sci. U.S.A. 55*, 274−281.
12. Kasai, H. (2002) Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis, *Free Radical Biol. Med. 33*, 450−456.
13. Sakai, A., Nakanishi, M., Yoshiyama, K., and Maki, H. (2006) Impact of reactive oxygen species on spontaneous mutagenesis in *Escherichia coli*, *Genes Cells 11*, 767−778.
14. Inoue, M., Kamiya, H., Fujikawa, K., Ootsuyama, Y., Murata-Kamiya, N., Osaki, T., Yasumoto, K., and Kasai, H. (1998) Induction of chromosomal gene mutations in *Escherichia coli* by direct incorporation of oxidatively damaged nucleotides. New evaluation method for mutagenesis by damaged DNA precursors in vivo, *J. Biol. Chem. 273*, 11069−11074.
15. Kamiya, H., and Kasai, H. (2000) 2-Hydroxy-dATP is incorporated opposite G by *Escherichia coli* DNA polymerase III resulting in high mutagenicity, *Nucleic Acids Res. 28*, 1640−1646.
16. Kamiya, H., Iida, E., Murata-Kamiya, N., Yamamoto, Y., Miki, T., and Harashima, H. (2003) Suppression of spontaneous and hydrogen peroxide-induced mutations by a MutT-type nucleotide pool sanitization enzyme, the *Escherichia coli* Orf135 protein, *Genes Cells 8*, 941−950.
17. Kamiya, H., Murata-Kamiya, N., Iida, E., and Harashima, H. (2001) Hydrolysis of oxidized nucleotides by the *Escherichia coli* Orf135 protein, *Biochem. Biophys. Res. Commun. 288*, 499−502.
18. Tsuzuki, T., Egashira, A., Igarashi, H., Iwakuma, T., Nakatsuru, Y., Tominaga, Y., Kawate, H., Nakao, K., Nakamura, K., Ide, F., Kura, S., Nakabeppu, Y., Katsuki, M., Ishikawa, T., and Sekiguchi, M. (2001) Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase, *Proc. Natl. Acad. Sci. U.S.A. 98*, 11456−11461.
19. Fujikawa, K., Kamiya, H., Yakushiji, H., Fujii, Y., Nakabeppu, Y., and Kasai, H. (1999) The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein, *J. Biol. Chem. 274*, 18201−18205.
20. Colussi, C., Parlanti, E., Degan, P., Aquilina, G., Barnes, D., Macpherson, P., Karran, P., Crescenzi, M., Dogliotti, E., and Bignami, M. (2002) The mammalian mismatch repair pathway removes DNA 8-oxodGMP incorporated from the oxidized dNTP pool, *Curr. Biol. 12*, 912−918.
21. Russo, M. T., Blasi, M. F., Chiera, F., Fortini, P., Degan, P., Macpherson, P., Furuichi, M., Nakabeppu, Y., Karran, P., Aquilina, G., and Bignami, M. (2004) The oxidized deoxynucleoside triphosphate pool is a significant contributor to genetic instability in mismatch repair-deficient cells, *Mol. Cell Biol. 24*, 465−474.
22. Nohmi, T., Kim, S. R., and Yamada, M. (2005) Modulation of oxidative mutagenesis and carcinogenesis by polymorphic forms of human DNA repair enzymes, *Mutat. Res. 591*, 60−73.
23. Einolf, H. J., and Guengerich, F. P. (2001) Fidelity of nucleotide insertion at 8-oxo-7,8-dihydroguanine by mammalian DNA polymerase δ. Steady-state and pre-steady-state kinetic analysis, *J. Biol. Chem. 276*, 3764−3771.
24. Einolf, H. J., Schnetz-Boutaud, N., and Guengerich, F. P. (1998) Steady-state and pre-steady-state kinetic analysis of 8-oxo-7,8-dihydroguanosine triphosphate incorporation and extension by replicative and repair DNA polymerases, *Biochemistry 37*, 13300−13312.
25. Miller, H., Prasad, R., Wilson, S. H., Johnson, F., and Grollman, A. P. (2000) 8-oxodGTP incorporation by DNA polymerase β is modified by active-site residue Asn279, *Biochemistry 39*, 1029−1033.
26. Kamiya, H., and Kasai, H. (1995) Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damaged nucleotide, and its incorporation by DNA polymerases. Steady-state kinetics of the incorporation, *J. Biol. Chem. 270*, 19446−19450.
27. Ohmori, H., Friedberg, E. C., Fuchs, R. P., Goodman, M. F., Hanaoka, F., Hinkle, D., Kunkel, T. A., Lawrence, C. W., Livneh, Z., Nohmi, T., Prakash, L., Prakash, S., Todo, T., Walker, G. C., Wang, Z., and Woodgate, R. (2001) The Y-family of DNA polymerases, *Mol. Cell 8*, 7−8.
28. Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K., and Hanaoka, F. (1999) The *XPV* (xeroderma pigmentosum variant) gene encodes human DNA polymerase η, *Nature 399*, 700−704.
29. Johnson, R. E., Prakash, S., and Prakash, L. (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Polη, *Science 283*, 1001−1004.
30. Prakash, S., Johnson, R. E., and Prakash, L. (2005) Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function, *Annu. Rev. Biochem. 74*, 317−353.
31. Friedberg, E. C. (2005) Suffering in silence: the tolerance of DNA damage, *Nat. Rev. Mol. Cell Biol. 6*, 943−953.
32. Nohmi, T. (2006) Environmental Stress and Lesion-Bypass DNA Polymerases, *Annu. Rev. Microbiol. 60*, 231−253.
33. Shimizu, M., Gruz, P., Kamiya, H., Kim, S. R., Pisani, F. M., Masutani, C., Kanke, Y., Harashima, H., Hanaoka, F., and Nohmi, T. (2003) Erroneous incorporation of oxidized DNA precursors by Y-family DNA polymerases, *EMBO Rep. 4*, 269−273.
34. Yamada, M., Nunoshiba, T., Shimizu, M., Gruz, P., Kamiya, H., Harashima, H., and Nohmi, T. (2006) Involvement of Y-family DNA polymerases in mutagenesis caused by oxidized nucleotides in *Escherichia coli*, *J. Bacteriol. 188*, 4992−4995.
35. Satou, K., Yamada, M., Nohmi, T., Harashima, H., and Kamiya, H. (2005) Mutagenesis induced by oxidized DNA precursors: roles of Y family DNA polymerases in *Escherichia coli*, *Chem. Res. Toxicol. 18*, 1271−1278.
36. Kannouche, P., Broughton, B. C., Volker, M., Hanaoka, F., Mullenders, L. H., and Lehmann, A. R. (2001) Domain structure, localization, and function of DNA polymerase η, defective in xeroderma pigmentosum variant cells, *Genes Dev. 15*, 158−172.
37. Tissier, A., Kannouche, P., Reck, M. P., Lehmann, A. R., Fuchs, R. P., and Cordonnier, A. (2004) Co-localization in replication foci and interaction of human Y-family members, DNA polymerase polη and REVl protein, *DNA Repair (Amsterdam) 3*, 1503−1514.
38. McIlwraith, M. J., Vaisman, A., Liu, Y., Fanning, E., Woodgate, R., and West, S. C. (2005) Human DNA polymerase η promotes DNA synthesis from strand invasion intermediates of homologous recombination, *Mol. Cell 20*, 783−792.
39. Kawamoto, T., Araki, K., Sonoda, E., Yamashita, Y. M., Harada, K., Kikuchi, K., Masutani, C., Hanaoka, F., Nozaki, K., Hashimoto, N., and Takeda, S. (2005) Dual roles for DNA polymerase η in homologous DNA recombination and translesion DNA synthesis, *Mol. Cell 20*, 793−799.
40. Mayorov, V. I., Rogozin, I. B., Adkison, L. R., and Gearhart, P. J. (2005) DNA polymerase η contributes to strand bias of mutations of A versus T in immunoglobulin genes, *J. Immunol. 174*, 7781−7786.
41. 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.
42. Tissier, A., McDonald, J. P., Frank, E. G., and Woodgate, R. (2000) polι, a remarkably error-prone human DNA polymerase, *Genes Dev. 14*, 1642−1650.
43. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G----T and A----C substitutions, *J. Biol. Chem. 267*, 166−172.
44. Vaisman, A., Frank, E. G., Iwai, S., Ohashi, E., Ohmori, H., Hanaoka, F., and Woodgate, R. (2003) Sequence context-dependent replication of DNA templates containing UV-induced lesions by human DNA polymerase ι, *DNA Repair (Amsterdam) 2*, 991−1006.

45. Creighton, S., Bloom, L. B., and Goodman, M. F. (1995) Gel fidelity assay measuring nucleotide misinsertion, exonucleolytic proofreading, and lesion bypass efficiencies, *Methods Enzymol. 262*, 232−256.
46. Goodman, M. F., Creighton, S., Bloom, L. B., and Petruska, J. (1993) Biochemical basis of DNA replication fidelity, *Crit. Rev. Biochem. Mol. Biol. 28*, 83−126.
47. Slupphaug, G., Kavli, B., and Krokan, H. E. (2003) The interacting pathways for prevention and repair of oxidative DNA damage, *Mutat. Res. 531*, 231−251.
48. Pavlov, Y. I., Minnick, D. T., Izuta, S., and Kunkel, T. A. (1994) DNA replication fidelity with 8-oxodeoxyguanosine triphosphate, *Biochemistry 33*, 4695−4701.
49. Satou, K., Harashima, H., and Kamiya, H. (2003) Mutagenic effects of 2-hydroxy-dATP on replication in a HeLa extract: induction of substitution and deletion mutations, *Nucleic Acids Res. 31*, 2570−2575.
50. Lehmann, A. R. (2006) New functions for Y family polymerases, *Mol. Cell 24*, 493−495.
51. Godoy, V. G., Jarosz, D. F., Walker, F. L., Simmons, L. A., and Walker, G. C. (2006) Y-family DNA polymerases respond to DNA damage-independent inhibition of replication fork progression, *EMBO J. 25*, 868−879.
52. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L., and Aggarwal, A. K. (2004) Replication by human DNA polymerase-iota occurs by Hoogsteen base-pairing, *Nature 430*, 377−380.
53. Wolfle, W. T., Johnson, R. E., Minko, I. G., Lloyd, R. S., Prakash, S., and Prakash, L. (2005) Human DNA polymerase *ι* promotes replication through a ring-closed minor-groove adduct that adopts a *syn* conformation in DNA, *Mol. Cell Biol. 25*, 8748−8754.
54. Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., and Patel, D. J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7H-dG(syn).dA(anti) alignment at lesion site, *Biochemistry 30*, 1403−1412.
55. 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.
56. Carlson, K. D., and Washington, M. T. (2005) Mechanism of efficient and accurate nucleotide incorporation opposite 7,8-dihydro-8-oxoguanine by *Saccharomyces cerevisiae* DNA polymerase *η*, *Mol. Cell Biol. 25*, 2169−2176.

BI062238R