Translesion Synthesis past Acrolein-derived DNA Adduct, \(\gamma\)-Hydroxypropanodeoxyguanosine, by Yeast and Human DNA Polymerase \(\eta\)*

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\(\gamma\)-Hydroxy-1,\(N^2\)-propano-2’-deoxyguanosine (\(\gamma\)-HOPdG) is a major deoxyguanosine adduct derived from acrolein, a known mutagen. In vitro, this adduct has previously been shown to pose a severe block to translesion synthesis by a number of polymerases (pol). Here we show that both yeast and human pol \(\eta\) can incorporate a C opposite \(\gamma\)-HOPdG at \(-190\) and \(-100\)-fold lower efficiency relative to the control deoxyguanosine and extend from a C paired with the adduct at \(-8\) and \(-19\)-fold lower efficiency. Although DNA synthesis past \(\gamma\)-HOPdG by yeast pol \(\eta\) was relatively accurate, the human enzyme misincorporated nucleotides opposite the lesion with frequencies of \(-10^{-1}\) to \(-10^{-2}\). Because \(\gamma\)-HOPdG can adopt both ring closed and ring opened conformations, comparative replicative bypass studies were also performed with two model adducts, propanodeoxyguanosine and reduced \(\gamma\)-HOPdG. For both yeast and human pol \(\eta\), the ring open reduced \(\gamma\)-HOPdG adduct was less blocking than \(\gamma\)-HOPdG, whereas the ring closed propanodeoxyguanosine adduct was a very strong block. Replication of DNAs containing \(\gamma\)-HOPdG in wild type and xeroderma pigmentosum variant cells revealed a somewhat decreased mutation frequency in xeroderma pigmentosum variant cells. Collectively, the data suggest that pol \(\eta\) might potentially contribute to both error-free and mutagenic bypass of \(\gamma\)-HOPdG.

Acrolein (Fig. 1), the simplest \(\alpha,\beta\)-unsaturated aldehyde, is an environmental contaminant and a product of inborn metabolism. In organisms, acrolein is generated via a number of pathways, such as the oxidation of polyamines and lipid peroxidation (1, 2). Like many other bifunctional aldehydes, acrolein reacts with DNA bases to form several DNA adducts, among which the \(\gamma\)-hydroxy-1,\(N^2\)-propano-2’-deoxyguanosine (\(\gamma\)-HOPdG)\(^1\) was identified as a major deoxyguanosine (dG) derivative (3, 4). Importantly, \(\gamma\)-HOPdG has been detected in DNA from mammalian tissues (5–7), suggesting that this adduct is generated in vivo. The \(\gamma\)-HOPdG adduct is formed by conjugate addition of acrolein to \(N^2\) of dG to produce \(N^2\)-(3-oxopropyl)dG. Ring closure at N1 leads to the formation of the cyclic adduct (Fig. 1). In the nucleoside and presumably in single-stranded DNA, \(\gamma\)-HOPdG predominantly exists in the cyclic form, such that at physiological pH, the ring open species cannot be detected spectrophotometrically (8). However, in the presence of a reducing agent, the acyclic form can be trapped as the \(N^2\)-(3-hydroxypropyl) adduct (Fig. 1).

Another dG derivative, \(1,\(N^2\)-propanodeoxyguanosine (PdG) (Fig. 1), whose structure is similar to that of the ring closed \(\gamma\)-HOPdG, has been extensively exploited as a model compound for the \(\gamma\)-HOPdG and other exocyclic dG adducts in both structural and biological studies. NMR spectroscopy of the PdG-adducted oligodeoxynucleotides has revealed that when placed opposite dC, PdG adopts a \(\alpha\) syn orientation within the duplex and introduces a localized structural perturbation that is pH- and sequence-dependent (9, 10). The inability of PdG to form normal Watson-Crick hydrogen bonds severely blocks DNA synthesis both in vitro (11, 12) and in vivo (13–16), and the replication that does occur results in mutations (13–16). Specifically, PdG-induced base substitutions occurred at an overall frequency of \(7.8 \times 10^{-2}\) and \(7.5 \times 10^{-3}\)translesion synthesis in the COS-7 (14) and in the nucleotide excision repair-deficient human cells (16), respectively. In both strains, G to T transversions predominated.

Recently, the structure of the \(\gamma\)-HOPdG-containing oligodeoxynucleotide was solved by NMR spectroscopy (17). These data have indicated that within the duplex, \(\gamma\)-HOPdG exists primarily in the ring open form. In such a conformation, the modified base participates in standard Watson-Crick base pairing by adopting a regular anti orientation around the glycosidic torsion angle, with the \(N^2\)-propyl chain in the minor groove pointing toward the solvent (17). The structural differences between PdG and \(\gamma\)-HOPdG within the duplex have led to the hypothesis that the latter lesion would be less blocking for replication and less mutagenic than the former.

Biological studies aimed to test the cytotoxic and mutagenic effects of acrolein-modified DNAs and of site-specific \(\gamma\)-HOPdG adduct have generated conflicting results. It is known that acrolein itself causes mutations in both bacterial (18) and mammalian (19) systems and has tumor-initiating activity (20). When a DNA vector was treated with acrolein and propagated in human cells, the majority of mutations were single, tandem, and multiple base substitutions that predominantly occurred in G:C base pairs (21). However in bacteria, \(\gamma\)-HOPdG, the major acrolein-derived dG adduct, is not a strong block for DNA synthesis nor a miscoding lesion (22–24). Analyses of mutations caused by \(\gamma\)-HOPdG in wild type Escherichia coli and in polB, dinB, and umuCDC deficient strains

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‡The abbreviations used are: \(\gamma\)-HOPdG, \(\gamma\)-hydroxy-1,\(N^2\)-propano-2’-deoxyguanosine; dG, deoxyguanosine; PdG, \(1,\(N^2\)-propanodeoxyguanosine; pol, DNA polymerase; XPD, xeroderma pigmentosum variant.

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FIG. 1. Structures of the aerolein and related deoxyguanosine adducts.

revealed that in the absence of these "SOS" polymerases, the efficiency and accuracy of the translesion synthesis were not significantly affected (22). In contrast to the prokaryotic data, γ-HOPdG caused mutations at an overall frequency of 7.4 × 10⁻²/translation synthesis when a single-stranded, site-specifically modified vector was propagated in COS-7 cells (24). Interestingly, both the frequencies and types of mutations were remarkably similar to those reported for the PdG adduct (14, 16). However, γ-HOPdG was shown to be only marginally miscoding (≤1% base substitution) when double-stranded vector was utilized (16). In this investigation, a number of cell lines including HeLa, a nucleotide excision repair-deficient xeroderma pigmentosum group A, and polymerase η-deficient xeroderma pigmentosum variant were examined.

Although replication across γ-HOPdG in vivo was predominantly error-free (from 93 to 100% of the translesional events), the adduct was shown to be a severe block and a miscoding error-free (from 93 to 100% of the translesional events), and yeast (35, 40) pol substrates for human (35, oxoguanine, and do so in a predominantly error-free manner promoting replication across translesion DNA synthesis polymerases (25, 26) are involved in achieved, and it appeared to be highly mutagenic (24). We subsequently modified vector was propagated in COS-7 cells (24). In this investigation, a number of cell lines including HeLa, a nucleotide excision repair-deficient xeroderma pigmentosum group A, and polymerase η-deficient xeroderma pigmentosum variant were examined.

Among DNA polymerases proficient in translesion synthesis, yeast polymerase η (a product of the RAD30 gene) (27) and its human counterpart (a product of the RAD30A (XPV, POLH) gene) (28, 29) both possess a unique ability to replicate efficiently and accurately past a cis-syn cyclobutane pyrimidine dimer (30, 31), the predominant DNA lesion caused by ultraviolet irradiation. In the yeast Saccharomyces cerevisiae, deletion of RAD30 confers moderate sensitivity to UV irradiation and leads to increased UV-induced mutagenesis (32). Mutations in the human RAD30A gene cause the variant form of xeroderma pigmentosum (XPV), suggesting that predisposition of XPV individuals to sunlight-induced skin cancer is due to the lack of accurate translesion DNA synthesis across UV-induced DNA lesions (28, 29, 33). Yeast and human pol η also efficiently bypass a product of oxidative DNA damage, the 7,8-dihydro-8-oxoguanine, and do so in a predominantly error-free manner (34). In addition, several other DNA lesions were reported to be substrates for human (35–39) and yeast (35, 40) pol η.

In the present study, the ability of yeast and human pol η to perform translesion DNA synthesis across γ-HOPdG has been examined, and the efficiency and fidelity of synthesis have been tested using steady-state kinetic analyses. To further explore the bypass mechanism, comparative studies were also performed with two model DNA adducts: PdG, which mimics the cyclic form of γ-HOPdG, and N²-(3-hydroxypropyl)dG, which is similar to γ-HOPdG in its ring open form. In addition, the mutagenic potential of γ-HOPdG was tested in vivo in both human fibroblasts and pol-γ-deficient XPV cells utilizing a site-specifically modified single-stranded pMS2 vector.

EXPERIMENTAL PROCEDURES

Materials—T4 DNA ligase, T4 polynucleotide kinase, and ECoR were obtained from New England BioLabs (Beverly, MA). S1 nuclease and proteinase K were purchased from Invitrogen. [γ⁻²⁵P]ATP was purchased from PerkinElmer Life Sciences. Bio-Spin columns were purchased from Bio-Rad. Centricon 100 concentrators were obtained from Amicon Inc. (Beverly, CA). Slide-A-Lyzer Dialysis Cassettes were obtained from Pierce.

Strains and Vectors—Single-stranded pMS2 DNA was a generous gift from Dr. M. Moriya (State University of New York, Stony Brook, NY). SV40-transformed DH5α cells were purchased for XPD/E cell and SV80 normal human fibroblasts were obtained from Dr. Marla Cordeiro-Scott (University of North Carolina, Chapel Hill, NC). The E. coli DH10B cells used for amplification of transformed DNA isolated from mammalian cells were purchased from Invitrogen.

Oligodeoxynucleotides—12-mer oligodeoxynucleotide modified with γ-HOPdG, 5'-GCTAGCγ-ΗΟΡDΓAGTC-3', was kindly provided by Dr. T. M. Harris and Dr. C. M. Harris (Vanderbilt University, Nashville, TN), and it was prepared by a previously described procedure (8). The 24-mer oligodeoxynucleotide, 5'-GCAGATCGCCG- (PdG)GGGATGAGCT-3', adducted with PdG was synthesized as described (41) and was a generous gift from Dr. L. J. Marnett (Vanderbilt University, Nashville, TN). Nondamaged 12- and 24-mer with a γg in place of γ-HOPdG or PdG, respectively, were purchased from Midland Certified Reagents Co. (Midland, TX). All of the other oligodeoxynucleotides were synthesized by the Molecular Biology Core Laboratory of the National Institute of Environmental Health Sciences Toxicology Center at the University of Texas Medical Branch (Galveston, TX) and purified by electrophoresis through a 15% denaturing PAGE (in the presence of 7% urea).

Construction of site-specifically modified linear templates for in vitro replication assays was done according to the previously described procedure (24). Sequences of the resulting oligodeoxynucleotides were identical: 5'-GCTAGCGATCCAGCCAGTTGCAGCCTGAGCTC-3', where the underlined G is either γ-HOPdG or nonadducted dG and 5'-GCTAGCGATCCAGCCAGTTGCAGCCTGAGCTGGC- (PdG)GGGATGAGCTGAGCTC-3', where the underlined G is either PdG or nonadducted dG. To obtain the N²-(3-hydroxypropyl)dG-containing DNA substrate, 10 μl of 1 μ NaBH₄ dissolved in 1 ml Hepes buffer (pH 7.4) were added twice to 200 μl of the γ-HOPdG-adducted 38-mer oligodeoxynucleotide (1–2 μM). Each addition of the reducing agent was followed by incubation at room temperature for 4 h. DNA was then dialyzed against 10 mM Tris-HCl (pH 7.0), 1 mM EDTA overnight using Slide-A-Lyzer Dialysis Cassettes (3,500 molecular weight cut off). To confirm the completeness of reduction, the polypeptide trapping technique was utilized (42) modified by A. J. Kurtz for γ-HOPdG-containing DNAs. Briefly, probes of both γ-HOPdG- and reduced γ-HOPdG-adducted oligodeoxynucleotides (50 nt) were incubated with 50 mM lysine-tRNA-synthetase-lysine in the presence of 25 mM NaCNBH₄ and 100 mM Hepes (pH 7.4) for 5 h. The reactions were terminated by the addition of an equal volume of 95% (v/v) formamide, 20 mM EDTA, 0.02% (w/v) xylene cyanol, and 0.02% (w/v) bromophenol blue and heating at 90 °C for 2 min. Next, DNAs were resolved through a 15% denaturing PAGE and visualized with PhosphorImager Screen. Under these conditions, no trapping was detected in reactions with γ-HOPdG-containing oligodeoxynucleotide, whereas the γ-HOPdG-containing DNA was completely complexed with the polypeptide.

Pol η Purification—Purifications of yeast pol η and human pol η were done as described in Refs. 27 and 31, respectively.

DNA Polymerase Reaction—The 21-mer oligodeoxynucleotides were used as primers for in vitro polymerase reactions. Their sequences were: 5'-CTCTGTCCAGCCAAAAGTGGC-3', which is complementary to the 38-mer γ-HOPdG-containing template DNAs from positions −9 to −29 relative to the site of lesion (−9 primer) as well as complementary to the PdG-adducted 50-mer from positions −15 to −35 (−15
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RESULTS

In Vitro Lesion Bypass with Yeast DNA Polymerase η—To examine whether yeast pol η was able to replicate past a γ-HOPdG adduct, running start primer extension experiments were performed (Fig. 2A). A 21-mer primer was annealed to the template DNA so that it allowed the addition of 9 nucleotides before encountering the adduct (−9 primer). On the nondamaged DNA substrate, primers were efficiently extended by yeast pol η (Fig. 2A, lanes 1–4). On the γ-HOPdG-containing substrate (Fig. 2A, lanes 5–8), yeast pol η appeared to be capable of bypassing the lesion and forming full-length products. However, DNA synthesis was partially inhibited right before the lesion DNA and opposite from it.

To understand better the importance of ring opening during replication, primer extension experiments were carried out using two model DNA substrates: the PdG adduct, which is an analogue of the ring closed form of the γ-HOPdG, and the reduced γ-HOPdG, which is similar to the ring open form of the natural adduct. In the case of the 50-mer DNA-containing substrate, 21-mer primer was positioned on the template so that the incorporation of 15 nucleotides was needed before reaching the lesion (−15 primer). Because both efficiency and accuracy of the DNA synthesis are known to be sequence-dependent (43, 45), an additional nondamaged control 50-mer DNA template was utilized that had the same sequence as the PdG-adducted template. These data revealed that the PdG adduct was a much stronger block for replication by yeast pol η than by γ-HOPdG. Under conditions that allowed an efficient replication of the nondamaged DNA template (Fig. 2A, lanes 13–16), DNA synthesis on the PdG-adducted template was greatly inhibited one nucleotide before the lesion, and synthesis was completely aborted after incorporating a nucleotide opposite the lesion (Fig. 2A, lanes 17–20). However, replication by yeast pol η beyond the PdG can be achieved but at much higher concentrations of enzyme (data not shown). With the reduced γ-HOPdG-adducted template (Fig. 2A, lanes 9–12), the bypass efficiency by yeast pol η seemed to be comparable with that on the γ-HOPdG-adducted template.

The specificity of nucleotide incorporation by yeast pol η opposite and beyond the lesions was also tested. To identify the nucleotide that is incorporated by this polymerase opposite the adducted base, single-nucleotide incorporation experiments were carried out using standing start DNA substrates in which 3′ terminus of the primer was located one nucleotide before the lesions (−1 primers) (Fig. 2B). On both nondamaged substrates, yeast pol η preferentially incorporated a C opposite G (Fig. 2B, lanes 3 and 18). Incorporation of a T and to a lesser extent an A and a G was also observed, especially on the 38-mer template. Interestingly, incorporation of a correct nucleotide (C) was predominant opposite each of the modified bases, namely the γ-HOPdG (Fig. 2B, lane 8), the reduced γ-HOPdG (Fig. 2B, lane 13), and the PdG (Fig. 2B, lane 23) adducts.

To test whether any misincorporation occurred past the le-
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In Vitro Lesion Bypass with Human DNA Polymerase η—Primer extension reactions and single-nucleotide incorporation experiments were carried out with human pol η (Fig. 3) using the same set of the primer/templates as with the yeast enzyme. Similar to the yeast pol η, human polymerase was able to replicate past the γ-HOPdG (Fig. 3A, lanes 5–8) and the reduced γ-HOPdG lesions (Fig. 3A, lanes 9–12). However, unlike yeast pol η, at higher enzyme concentrations human pol η appeared to bypass the PdG adduct (Fig. 3A, lanes 17–20).

Single-nucleotide incorporation experiments with human pol η revealed significant differences between the human and yeast enzymes in their discrimination abilities during nucleotide insertion opposite the γ-HOPdG adduct. Whereas yeast pol η preferentially incorporated the correct nucleotide (C) opposite the lesion, human polymerase extended the −1 primer almost equally well in the presence of A, C, and G (Fig. 3B, lanes 6–10). On the PdG-adducted template, the difference between these two polymerases was even more striking. In contrast to the yeast pol η that incorporated a C opposite PdG, human polymerase inserted A, G, and T better than the correct nucleotide (Fig. 3B, lanes 21–25). Interestingly, incorporation by human pol η is much more accurate opposite the reduced γ-HOPdG adduct (Fig. 3B, lanes 11–15) than opposite the non-reduced adduct (Fig. 3B, lanes 6–10).

Single-nucleotide incorporation experiments were carried also out using 0 primers with the C primed with the adducted base. Yielding data similar to that of the yeast pol η, human polymerase preferentially incorporated the correct nucleotide on all five substrates tested (data not shown).

**Efficiency of Nucleotide Incorporation and Extension**—To compare the efficiency of translesion synthesis by yeast and human pol η, steady-state kinetic parameters $k_{cat}$ and $K_m$ were first determined for the correct nucleotide (C) incorporation opposite dG in two different sequence contexts and also opposite γ-HOPdG, reduced γ-HOPdG, and PdG adducts. The reactions were performed using the same 21-mer −1 primers as in the single-nucleotide incorporation experiments. For yeast pol η, C is incorporated opposite the ring closed PdG adduct with a 1600-fold lower efficiency ($k_{cat}/K_m$) than C is incorporated opposite the unadducted dG (Table I). In contrast, yeast pol η

### Table I

| Substrate | dNTP  | $k_{cat}$ | $K_m$ | $k_{cat}/K_m$ | Fold reduction in efficiency | Fidelity of incorporation |
|-----------|-------|-----------|-------|--------------|-----------------------------|--------------------------|
| −1 primer | dG (38-mer) | dATP | 0.90 ± 0.15 | 200 ± 70 | 4.5 x 10^{-3} | 1.7 x 10^{-4} |
|           |       | dCTP    | 4.9 ± 0.2 | 0.19 ± 0.02 | 26 |
|           |       | dGTP    | 0.69 ± 0.04 | 81 ± 14 | 8.5 x 10^{-3} |
|           |       | dTTP    | 0.39 ± 0.04 | 8.7 ± 4.0 | 4.5 x 10^{-2} |
|           | γ-HOPdG | dATP | 0.28 ± 0.01 | 210 ± 30 | 1.3 x 10^{-3} |
|           |       | dCTP    | 0.28 ± 0.02 | 2.0 ± 0.5 | 1.4 x 10^{-1} |
|           |       | dGTP    | 0.17 ± 0.003 | 88 ± 5 | 1.9 x 10^{-3} |
|           |       | dTTP    | 0.098 ± 0.014 | 190 ± 80 | 5.2 x 10^{-4} |
| Reduced   | dCTP | 0.35 ± 0.009 | 0.16 ± 0.01 | 22 |
| γ-HOPdG   | dG (50-mer) | dCTP | 3.2 ± 0.08 | 0.13 ± 0.01 | 25 |
|           |       | dCTP | 0.35 ± 0.01 | 22 ± 2 | 1.6 x 10^{-2} |
| 0 primer  | dG (38-mer) | dCTP | 2.3 ± 0.01 | 0.19 ± 0.03 | 12 |
|           | γ-HOPdG | dGTP | 0.94 ± 0.003 | 0.57 ± 0.09 | 1.6 |
| Reduced   | γ-HOPdG | dGTP | 0.89 ± 0.03 | 0.35 ± 0.06 | 2.5 |
| PdG       | dGTP | 2.2 ± 0.1 | 0.27 ± 0.05 | 8.1 |

*For nucleotide incorporation opposite a given adduct, the fold reduction in efficiency was calculated as $(k_{cat}/K_m)_{normal} / (k_{cat}/K_m)_{adduct}$. Similarly, the fold reduction in the efficiencies for extension were calculated as $(k_{cat}/K_m)_{normal} / (k_{cat}/K_m)_{adduct}$. The fidelity of incorporation was calculated as $(k_{cat}/K_m)_{correct} / (k_{cat}/K_m)_{incorrect}$.  

**ND, not determined.**
incorporates a C opposite the ring open reduced \( \gamma \text{-HOPdG} \) with only a 12-fold lower efficiency than opposite the unadducted dG. The efficiency of incorporation opposite the \( \gamma \text{-HOPdG} \) adduct is in between these two extremes with a 190-fold reduction relative to the unadducted dG. The same trends were also observed with human pol \( \eta \) (Table II).

Next, the steady-state kinetic parameters were determined for the extension from a C residue paired with the modified bases and were used to determine the efficiency of extending from each adduct relative to the extension from an unadducted dG (Tables I and II). For both yeast and human pol \( \eta \), the efficiencies of extensions from the \( \gamma \text{-HOPdG} \) and the reduced \( \gamma \text{-HOPdG} \) were reduced \( \sim 5 \)–30-fold relative to the unadducted dG. In contrast, the extension from the PdG was blocked to a much greater extent, especially in the case of the yeast enzyme (6800-fold; Table I).

**Fidelity of Nucleotide Incorporation by Yeast and Human Pol \( \eta \) Opposite \( \gamma \text{-HOPdG} \)**—In the single-nucleotide incorporation experiments, yeast and human pol \( \eta \) displayed different accuracies of replication across the \( \gamma \text{-HOPdG} \) adduct. To further evaluate the accuracy of nucleotide incorporation opposite the lesion, kinetic analyses were carried out using \( -1 \) primer, and the frequencies of misincorporation were calculated as the ratio of \( k_{\text{inc}}/K_m \) of the incorrect nucleotide to the correct nucleotide (43). These data showed that yeast pol \( \eta \) synthesizes past \( \gamma \text{-HOPdG} \) relatively accurately with efficiency of incorporation of a C \( \sim 75 \) times higher than that of the next most preferred nucleotide (G) (Table I). In contrast, human pol \( \eta \) discriminated poorly between the correct and wrong nucleotides incorporating opposite \( \gamma \text{-HOPdG} \). Particularly, high misincorporation frequencies were observed for A and G (Table II).

**Mutagenicity of \( \gamma \text{-HOPdG} \)-modified Single-stranded pMS2 Vectors in Normal Human Fibroblasts and XPV Cells**—Table III shows the outcomes of *in vivo* replication of pMS2 (dG) and pMS2 (\( \gamma \text{-HOPdG} \)) in SV80 and XPV cells. The data presented for XPV cells were obtained from five independent experiments. All of the 1104 *E. coli* transformants resulting from replication of modified pMS2 (\( \gamma \text{-HOPdG} \)) in XPV cells were picked and grown in 96-well plates. Hybridization analysis revealed that 767 colonies hybridized with either one of the four probes, whereas 337 colonies did not hybridize with any of the four probes. Of those transformants that did not hybridize with any sequence-specific probe, none of those hybridized to sequences immediately upstream of the oligodeoxynucleotide ligation site, suggesting that this deletion was not caused by the adduct. Although 96% of the hybridized transformants did not contain any targeted mutations (Table III), 1.3% (10/767) were G to A transitions, 0.5% (4/767) were G to C transversions, and 2.1% (18/767) were G to T transversions. Sequencing of plasmid DNA prepared from these colonies confirmed the presence of T, C, or A, respectively, opposite the site of the adducted guanine. No mutations were observed when 192 colonies were screened out of transformants obtained from nonadducted pMS2(dG).

When these experiments were repeated in SV80 normal human fibroblasts, all of the 288 transformed colonies subsequently obtained from two experiments were analyzed for mutations by differential hybridization strategy. Although only 92 colonies hybridized with either one of the four probes, 89% (82/92) contained the correct base opposite the adducted guanine,
whereas 8.6% (8/92) were G to T transversions, and 1.1% (1/92) were G to C and G to A mutations. None of the colonies from the control pMS2(dG) transformants showed any mutation. Thus, XPV cells appeared to have a lower mutation frequency (3.9%) when compared with normal human fibroblast cells (11%).

**DISCUSSION**

The γ-HOPdG adduct was not a significant block for replication when site-specifically modified vectors were propagated in *E. coli* (22–24) or in mammalian cells (16, 24). In *E. coli*, the adduct appeared not to be miscoding (22–24). Depending on the cell type and vector used, 93–100% of the translesion events were mutagenic during in *vivo* replication in mammalian cells (16, 24). Thus, in both prokaryotic and eukaryotic systems, DNA polymerases exist that are able to synthesize past γ-HOPdG efficiently and in a predominantly error-free manner. On the other hand, none of the polymerases examined *in vitro* so far, namely, Klconex fragment of *E. coli* pol I (22, 23), calf thymus pol δ (24), and human pol ε (24), were able to incorporate the correct nucleotide opposite this adduct. In the synthesis past γ-HOPdG adduct efficiently and in a predominantly error-free manner. On the other hand, none of the polymerases examined *in vitro* so far, namely, Klconex fragment of *E. coli* pol I (22, 23), calf thymus pol δ (24), and human pol ε (24), were able to incorporate the correct nucleotide opposite this adduct. In the present study, yeast pol η has been identified as the first polymerase that possesses an ability to replicate across the γ-HOPdG adduct relatively accurately. Comparable efficiency of DNA syntheses past γ-HOPdG was also observed for human pol η, but this polymerase displayed a much higher propensity for misincorporation. Single-nucleotide experiments as well as steady-state analyses showed that human pol η frequently incorporates an A or a G opposite γ-HOPdG and therefore is likely to introduce G to T and G to C transversions.

We note that the observed *kcat* for C incorporation opposite the undamaged G template (−5 min⁻¹; Table I) is slower than the rate of nucleotide incorporation measured during processive synthesis (−80 min⁻¹; Ref. 46) for yeast pol η. This suggests that *kcat* reflects the rate of DNA release and thus is an underestimate of the actual rate of nucleotide incorporation. Nevertheless, because the observed *Km* is expected to be decreased with the *kcat* in a compensatory manner, the efficiencies of nucleotide incorporation (*kcat*Km) determined under steady-state conditions provide a measure of catalytic efficiencies of the enzyme. More detailed kinetic studies are needed, however, to more accurately define the mechanisms controlling the fidelity of pol η opposite these DNA adducts.

The nucleotide incorporation data for pol η are in agreement with results of the *in vitro* replication assays when site-specifically modified single-stranded pMS2 vector was propagated in XPV cells. Overall mutagenic frequency determined in the XPV cells (3.9 × 10⁻²/translation synthesis) was about two and three times less than that in COS-7 (24) and normal human cells, respectively. Importantly, lower frequencies of transversions (particularly G to T) in XPV cells, but not G to A transitions, accounted for the observed difference between two types of cells. Thus, pol η might potentially contribute to the bypass of the γ-HOPdG adduct in mammalian cells being responsible for both error-free and error-prone replicative events.

Based on the NMR spectroscopy data, a model of error-free bypass of γ-HOPdG has been proposed in which the incoming dCTP triggers a structural rearrangement of the adduct from the ring closed to the ring open form. This change allows the formation of the standard Watson-Crick hydrogen bonds, stabilizes the structure, and facilitates the subsequent extension reaction (17). To examine the role of ring opening during replication by pol η, we compared the efficiency of incorporation opposite γ-HOPdG to the incorporation opposite the two model adducts: PdG and reduced γ-HOPdG. For both yeast and human pol η, cyclic PdG was a very strong block for the incorporation of a C relative to the acyclic reduced γ-HOPdG. For incorporation opposite γ-HOPdG, both polymerases had an intermediate incorporation efficiency. Ring opening was also important for the extension from a C paired with the adduct. For both yeast and human pol η, relative efficiencies of extension were similar when γ-HOPdG- and reduced γ-HOPdG-modified DNA substrates were used. By contrast, the cyclic PdG adduct is a very strong block for extension by these polymerases, especially for the yeast enzyme. Overall, these data are consistent with the proposed model of de los Santos (17), such that ring opening of γ-HOPdG is essential not only for efficient incorporation opposite the lesion by yeast and human pol η but also for efficient extension. However, from these data it cannot be concluded whether the incoming nucleotide causes the transformation of the adduct from the ring closed to the ring open form or whether the equilibrium is shifted toward ring open conformation by protein-DNA interactions in the polymerase active site.

The steady-state kinetic analyses and single-nucleotide incorporation experiments have revealed significant differences between yeast and human pol η with respect to their accuracies of replication across modified bases. For the human enzyme, frequencies of misincorporation opposite γ-HOPdG were on average, 1 order of magnitude higher than for the yeast enzyme. In addition, the incorporation by human pol η opposite PdG was extremely error-prone, whereas yeast pol η inserted the correct nucleotide preferentially.

The proficient ability of yeast and human pol η to replicate across the ring open form of γ-HOPdG strongly indicates that in spite of the fact that it is located in the minor groove, the presence of this adduct on the templating residue poses no significant hindrance to these polymerases. This suggests the lack of any specific contact of these enzymes with the minor groove of the templating residue, which would permit pol η to replicate across DNA adducts, which protrude into the minor groove.

Although DNA synthesis past γ-HOPdG by pol η is very efficient when the adduct exists in its ring open form, in *vivo* replication data (16, this report) clearly show that pol η is not solely responsible for bypass of this lesion in humans. Thus, another polymerase is likely involved in translesion synthesis past γ-HOPdG. The yet unidentified polymerase may be able to efficiently bypass the ring closed form of γ-HOPdG and perhaps other exocyclic dG adducts (1, 2), in which N1 modifications prevent Watson-Crick pairing.

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Translesion Synthesis past Acrolein-derived DNA Adduct, $\gamma$-Hydroxypropanodeoxyguanosine, by Yeast and Human DNA Polymerase $\eta$

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