Human DNA Polymerase κ Bypasses and Extends beyond Thymine Glycols during Translesion Synthesis in Vitro, Preferentially Incorporating Correct Nucleotides*

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From the †Laboratory of Molecular Pathology, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-0672 and the §Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, University of Vermont, Burlington, Vermont 05405-0068

Human polymerase κ (polκ), the product of the human POLK (DINB1) gene, is a member of the Y superfamily of DNA polymerases that support replicative bypass of chemically modified DNA bases (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) Mol. Cell 8, 7–8; Gerlach, V. L., Aravind, L., Gotway, G., Schultz, R. A., Koonin, E. V., and Friedberg, E. C. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11922–11927). Polκ is shown here to bypass 5,6-dihydroxythymine (thymine glycol) generated in two different DNA substrate preparations. Polκ inserts the correct base adenine opposite thymine glycol in preference to the other three bases. Additionally, the enzyme correctly extends beyond the site of the thymine glycol lesion when presented with adenine opposite thymine glycol at the primer terminus. However, steady state kinetic analysis of nucleotides incorporated opposite thymine glycol demonstrates different misincorporation rates for guanine with each of the two DNA substrates. The two substrates differ only in the relative stereoisoform expressions that can result in the stalling or complete arrest of DNA synthesis during DNA replication (3, 4). However, the potential for cell death attendant on arrested DNA replication can be mitigated by a mechanism called translesion DNA synthesis (TLS) (5–7). This process effects the replicative bypass of sites of base damage, allowing high fidelity semiconservative DNA synthesis to continue. Important new insights into the biochemical mechanism of TLS have recently been gained by the discovery of a number of new DNA polymerases, all of which share the properties of limited fidelity and processivity when copying undamaged DNA, as well as a lack of 3′ → 5′ proofreading exonuclease activity (1, 5–9). Multiple DNA polymerases of this class have been shown to support TLS of one or more types of base damage in vitro. In some instances, this role is supported by genetic or other biological evidence. Hence, a general theme is beginning to emerge that the redundancy for error-prone DNA polymerases in prokaryotic and especially in eukaryotic cells reflects a requirement for the bypass of multiple types of base damage that can arrest normal DNA replication (5). Recent structural studies on a number of these polymerases suggest that translesion synthesis is effected by a less constrictive, more solvent-accessible active site, which allows for productive interactions with a wider range of template structures, including chemically modified bases (10–12). The increased error rates observed when copying undamaged DNA in vitro (1, 8, 9, 13) are presumably a direct reflection of this relaxed fidelity for nucleotide incorporation.

Among the many recently discovered specialized DNA polymerases is one called DNA polymerase κ (polκ)† from human cells, a highly conserved structural ortholog of a bacterial polymerase called DNA polymerase IV (2). Polκ is encoded by the POLK (DINB1) gene and has a predicted molecular mass of ~100 kDa (2). In previous studies, polκ was fused to glutathione S-transferase and expressed in insect cells (14). The purified fusion protein was shown to be a template-directed DNA polymerase with limited processivity and fidelity (15). GST-polκ protein lacks detectable 3′ → 5′ proofreading exonuclease activity and is not stimulated by recombinant human proliferating cell nuclear antigen (PCNA) in vitro (14). However, in the presence of the three replicative accessory factors, PCNA, replication factor C, and replication factor A, polκ exhibits a 50–200-fold stimulation in efficiency but no increase in processivity (16). Additionally, polκ interacts physicochemically with PCNA (16). Human polκ has optimal activity at 37 °C over the pH range 6.5–7.5 and is insensitive to inhibition by aphidicolin or dideoxynucleotides.

Many types of base damage in DNA cause structural modifications that can result in the stalling or complete arrest of DNA synthesis during DNA replication (3, 4). The abbreviations used are: polκ, -κ, -Δκ, -ηκ, and -δκ, polymerase κ, 1, λ, η, and δ, respectively; PCNA, proliferating cell nuclear antigen; Tg, thymine glycol; DPAGE, denaturing polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; TLS, translesion DNA synthesis.

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or by NaCl up to 50 mM. Either Mg\textsuperscript{2+} or Mn\textsuperscript{2+} can satisfy a metal cofactor requirement for polk activity (14). In vitro polk extends DNA oligonucleotide primers to a position one base short of the end of the DNA template (14).

Full-length purified polk fusion protein is unable to extend a DNA primer past bulky base adducts such as thymine-thymine dimers or [6-4]-pyrimidine-pyrimidone photoproducts generated by exposure of cells or DNA to UV radiation (17). Similarly, the enzyme does not support TLS past cisplatin intrastrand cross-links in template DNA (14). In contrast, the enzyme can support TLS past acetylaminofluorene-guanine, any of the four stereoisomers adducts resulting from reaction of benzo[a]pyrene-7,8-diol 9,10-epoxide at C-10 with the exocyclic N\textsuperscript{2} of guanine (BPDE-G) in template DNA, 1,N\textsuperscript{6}-ethenodeoxyadenosine, and sites of base loss (abasic sites) (14, 17–19). At present, the biological significance of these observations is not clear. Evidence has also been presented that polk can perform efficient extension synthesis following nucleotide incorporation directly opposite DNA lesions (18). Polk additionally supports extension synthesis on primer-template substrates terminating in a 3’-mispaired base, incorporating nucleotides with a high error rate (20).

**POLK mRNA and polk protein are highly expressed in the adrenal cortex of adult mice, beginning in early embryonic life (2).** Indeed, at embryonic day 15.5 this is the only tissue in which POLK expression can be detected by in vitro hybridization. Furthermore, this expression pattern appears to be relatively specific, since genes encoding two other recently discovered specialized DNA polymerases, polk and poln are not uniquely or highly expressed in the adrenal cortex. Steroid biosynthesis in the adrenal cortex is known to involve the generation of large amounts of reactive oxygen species, which may result in an unusual burden of oxidative DNA damage in adrenal cortical cells (23, 24). Consistent with a possible role in the replicative bypass of oxidative base damage to DNA, the Polk (DinB1) gene is up-regulated in mouse embryo fibroblasts following exposure of cells to either doxorubicin or UV radiation. Both of these agents are known to generate reactive oxygen species that can damage DNA (25–28). Furthermore, mouse embryo fibroblasts from a mutant mouse strain defective in polk activity manifest increased sensitivity to killing following exposure to UV radiation (29).

In the present studies, we have investigated the ability of human polk to support primer extension in vitro past thymine glycol (Tg) residues in DNA, a biologically important form of oxidative base damage that potently inhibits DNA replication by many high fidelity polymerases (30). Recently, another Y-family DNA polymerase, human polk, was reported to bypass Tg lesions in vitro (31). Polk synthesizes DNA past Tg with an efficiency nearly equal to that of undamaged DNA but with an extremely high rate of error (31). Additionally, polk exhibits a stereoechemical preference for the R stereoisomer at C-5 of Tg (31). For the present studies, we employed primer-template substrates in which a single Tg residue in the template DNA strand was generated by two different methods (Fig. 1A). Both procedures result in a mixture of the four possible stereoisomers of Tg in different relative proportions (Fig. 1B) (32, 33).

We show by both qualitative and quantitative steady-state kinetic analysis that polk supports TLS across both of these substrates. During this replicative bypass, the base A is preferentially incorporated opposite Tg. Additionally, polk is able to extend the primer template beyond the lesion, preferentially incorporating the correct next base. However, differences are observed both in the efficiency of A incorporation and in the misincorporation rate of G opposite Tg when comparing the two templates. TLS appears to be more efficient and specific opposite the substrate putatively containing a larger proportion of 5S stereoisomers.

**EXPERIMENTAL PROCEDURES**

**Biochemical Reagents—Terminal deoxynucleotransferase and T4 DNA polymerase were obtained from Invitrogen. The Klenow fragment of E. coli DNA polymerase I (exo-) was obtained from New England Biolabs. T4 DNA polymerase was obtained from U.S. Biochemical Corp. Deoxynucleoside triphosphates were from Promega. Osmium tetroxide (OsO\textsubscript{4}) was purchased from Aldrich.**

**Expression and Purification of GST-Polk—GST-polk fusion protein was purified as previously described (14).**

**DNA Substrates**—The primer used for running start experiments was P4-OX-RS (5’-dGAATTCTCGACCCAGGAGGATCGACTGGTCC). The primer for steady-state extension experiments (k\textsubscript{eq}) was P5-SS-AS (5’-dGAATTCTCTGCACCCAGGAGGATCGACTGGTCC). The primer for steady-state extension experiments was the sequence 5’-dATTCGACAGCTGTCATGATAACACCGTGGGACCGAGTCACTGGTCCA). The DNA template for these experiments was the sequence 5’-dATTCGACAGCTGTCATGATAACACCGTGGGACCGAGTCACTGGTCCA. The site of thymine glycol modification is underlined. The control template was of the same sequence, except the underlined base was simply thymine. DNA oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis (DPAGE). Five pmol of each primer was 5’-end-labeled with T4 polynucleotide kinase (Invitrogen) in the presence of [γ-\textsuperscript{32}P]ATP, and the unincorporated radiolabel was removed using a Sephadex G-25 spin column equilibrated with STE (100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM Na\textsubscript{2}EDTA). Primers were annealed to template strands in a stoichiometric ratio of 1:1.5 (primer-template) by heating (90°C, 5 min, 1× STE buffer) and cooling on the bench top (15 min). Native polyacrylamide gels showed altered gel mobility for the labeled primers under these conditions when compared with single-stranded primers, indicating duplex DNA character for the template-annexed primers under these conditions.

**Thymine Glycol Template 1 (TgBr2) Has a Lower Proportion of 5R Stereoisomers**—5,6-Dihydroxy-5,6-dihydrothymidine-5’-triphosphate (thymidine glycol-5’-triphosphate) was synthesized according to a published protocol (34) and characterized by HPLC (one major peak, 32P-PITR, three lines, 1H NMR, and ESI mass spectrometry ((M-H\textsuperscript{+})\textsuperscript{1} = 515 atomic mass units). Additionally, the stereochemical composition of this sample was assayed by digestion of the deoxynucleoside triphosphate with alkaline phosphatase and separation of the deoxynucleosides by HPLC according to a previously published method (35). The data revealed that: 1) trans (5S,5R), and 3) the cis isomers, which elute together (5S,6R) and (5S,6S), with nearly identical retention times and peak area ratios, as had previously been reported for oxidation of thymidine deoxynucleoside (data not shown). Hence, stereochemical composition is identical to previous studies up to the point of incorporation of the nucleoside triphosphate.

The product nucleotide triphosphate was reacted with the 5’-end of a DNA oligonucleotide enzymatically, 5’-dATTCGACAGCTGACATTTAA(CCGGGCTGCAGCCCAGGATCGACTGGTCC). By incubation with terminal deoxynucleotransferase (DNA oligonucleotide) = 1 mM, [thymine glycol triphosphate] = 100 mM, [terminal deoxynucleotransferase] = 60 nM, 100 mM sodium cacodylate, 2 mM CoCl\textsubscript{2}, 0.2 mM dithiothreitol, pH 7.0, 1 h, 30°C). The product containing the addition of a single 3’-thymidine glycol nucleotide was purified by HPLC and gel electrophoresis, desalted by SepPak C\textsubscript{18} chromatography, and characterized by ESI mass spectrometry, which indicated the addition of a single thymidine glycol nucleotide residue to the unreacted oligonucleotide. Ligation to a 3’-flanker oligonucleotide was accomplished by annealing the thymine glycol oligonucleotide to the primer strand, 5’-phosphoryl-d(GGACGACTGCTTCGCTGGGGTGACAGTCACTGGTCC). As a result of annealing, a single thymine glycol nucleotide residue to the unreacted oligonucleotide. Ligation to a 3’-flanker oligonucleotide was accomplished by annealing the thymine glycol oligonucleotide to the primer strand, 5’-phosphoryl-d(GGAACGGCTGCTTCGCTGGGGTGACAGTCACTGGTCC). As a result of annealing, a single 3’-thymidine glycol nucleotide was purified by HPLC and gel electrophoresis, desalted by SepPak C\textsubscript{18} chromatography, and characterized by ESI mass spectrometry, which indicated the addition of a single thymidine glycol nucleotide residue to the unreacted oligonucleotide.

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Fig. 1. A, schematic showing the primers and template DNAs prepared for this study. The two methods of thymine oxidation are indicated as well as the position and sequence context of the lesion. B, literature values for the putative stereochemical make-up of each thymine glycol sample. Note that in the TgOsO4 template, the 5R stereoisomers are in greater relative abundance.

### RESULTS

Preparation of Two Substrates Containing Different Relative Amounts of Thymine Glycol Stereoisomers—We generated a single Tg lesion at a defined position in a DNA oligonucleotide template using two different procedures. One substrate (TgBr2) was generated by incorporation of Tg from thymidine glycol-5'-triphosphate prepared by bromination and oxidation as previously described (33). This procedure has been shown to yield a stereochimical mixture containing 64.3% of the two 5R stereoisomers, and 35.7% of the 5S stereoisomers (33). Following oxidation, the stereochemistry at C-5 is fixed, but epimerization occurs about the C-6 center to yield 87% cis oxidation, the stereochemistry at C-5 is fixed, but epimerization occurs about the C-6 center to yield 87% cis, 13% trans for the 5R stereoisomer and 80% cis, 20% trans for the 5S stereoisomer (33). Epimerization equilibrates at room temperature within a few hours (33), yielding an equilibrium distribution of 55.9% (5R,6S), 8.4% (5R,6R), 7.1% (5S,6R) (Fig. 1B). When the thymidine glycol triphosphate used to prepare this substrate was digested to the nucleoside with alkaline phosphatase and analyzed by HPLC under conditions previously described (33), the same ratios of the two trans and cis isomers were obtained as reported in the earlier study (data not shown). A second substrate of identical nucleotide se-

- It should be noted that, following preparation of the thymidine glycol triphosphate, the glycol nucleotide was incorporated enzymatically to create the site-specifically modified substrate, a process that could in principle proceed with stereochemical selection. Hence, although there is no reason to believe otherwise, we cannot be certain that the stereochemical composition of the final substrate is identical to that in the triphosphate.
sequence (T<sub>Br2</sub>) was generated by direct oxidation of the single T residue in the template DNA using osmium tetroxide (OsO<sub>4</sub>). With this procedure, the 5R and 5S stereoisomers are formed in a ratio of 6:1 (32), and following epimerization the relative percentages of the four stereoisomers are expected to be 74.7% (5R,6S), 11.3% (5R,6R), 11.1% (5S,6R), and 2.9% (5S,6R) (Fig. 1B). Thus, in the T<sub>Br2</sub> template, the 5R stereoisomers are expected to be in slightly greater relative abundance than in the T<sub>OsO4</sub> template (Fig. 1B).

**TLS across Thymine Glycol by PolA**—We previously reported that the GST-pol fusion protein used in the present studies does not support TLS across cisplatin adducts in DNA (14). In contrast, incubation of a primer-T<sub>Br2</sub> template with purified GST-pol fusion protein revealed progressively more efficient TLS past Tg as a function of increasing enzyme concentration (Fig. 2). The high fidelity replicative enzyme DNA polymerase δ from calf thymus did not support bypass across the Tg lesion at the enzyme concentrations tested, although it exhibited primer extension of the undamaged control template DNA that was characteristically stimulated by the accessory factor PCNA (Fig. 2, lanes 10–14). Like all of the replicative polymerases, polδ exhibits a 3′→5′ exonuclease activity, which yielded faint primer degradation bands in lanes 10–14 below the portion of the gel shown in Fig. 2 (data not shown). Consistent with the results of previous studies (37), comparable levels of the Klenow exo<sup>−</sup> form (devoid of 3′→5′ exonuclease activity) of E. coli DNA polymerase I also bypassed Tg in this substrate (Fig. 2). Essentially identical results were observed with the primer-T<sub>OsO4</sub> template (Fig. 3A). Once again, bypass was observed in the presence of *Escherichia coli* Klenow exo<sup>−</sup> fragment (Fig. 3B). However, comparable amounts of the high fidelity replicative DNA polymerases from phage T4 (Fig. 3B) and phage T7 (data not shown) characteristically did not support bypass of Tg. Additionally, when we performed side-by-side standing start experiments in which the two templates were compared directly, we observed essentially identical levels of bypass by GST-pol (Fig. 3C). In all reactions with or without the presence of Tg primer, extension terminated one nucleotide short of the end, a previously described intrinsic property of pol<sup>−</sup> (14).

**Fidelity of Nucleotide Incorporation Opposite Tg by GST-pol**—To determine the fidelity of TLS across Tg by the GST-pol fusion protein, we performed standing start primer extension reactions in the presence of each of the four individual deoxynucleoside triphosphates. As shown in Fig. 4, the correct complementary base A is qualitatively preferentially incorpo-
rated opposite Tg. However, the incorrect bases C, T, and especially G are also misincorporated. To compare the efficiency and fidelity of nucleotide incorporation in undamaged template DNA and that of DNA containing a single Tg residue at the identical position but prepared using two different protocols, we performed experiments under steady state conditions (Fig. 5 and Table I).

A comparison of the parameter $k_{\text{ext}}/K_m$ for the incorporation of A opposite T and Tg shows a 20-fold reduced efficiency for the Tg Br2 template and a 50-fold reduced efficiency for the Tg OsO4 template relative to T (Table I and Fig. 6A). When copying the undamaged template, the GST-polk fusion protein misincorporates nucleotides opposite the T residue with frequencies of $2.7 \times 10^{-3}$ (G), $9.6 \times 10^{-4}$ (C), and $2.7 \times 10^{-4}$ (T) relative to the correct nucleotide A (Table I). The same comparison for nucleotide incorporation opposite Tg in either substrate (i.e. the frequency of misincorporation of G, C, and T relative to the correct incorporation of A) reveals $\sim1.2-3$-fold reduced discrimination between the correct and incorrect nucleotide in all cases except G misincorporation opposite the Tg OsO4 template lesion (Table I and Fig. 6B). Nonetheless, the preference for A opposite Tg is 2–3 orders of magnitude greater than for any other nucleotide. After A, the base most frequently incorporated opposite either T and Tg is G.

Whereas the relative preference for incorporating G compared with the other bases opposite T in the undamaged template or Tg in the Tg Br2 template is similar (Table I and Fig. 6B), this preference is increased $\sim5-20$-fold in the Tg OsO4 template (Table I and Fig. 6B). This may relate to the fact that the Tg OsO4 template is expected to contain a greater proportion of the 5R stereoisomers. This stereochemically based difference presumably interferes with discrimination of the purine versus pyrimidine character of the incoming nucleotide. A myriad of models are tenable to explain this phenomenon, especially since the site of hydrogen bonding is on the opposite end of the template base. It is important to note that the relative percentage of each of the stereoisomers is not anticipated to be greatly different between the two templates tested. The 5R stereoisomers are expected to be only $\sim25\%$ more abundant in the Tg OsO4 template. Hence, one might predict even greater stereochemical effects on TLS by polk in a stereochemically pure 5R or 5S sample. Accordingly, in vivo the 5S stereoisomers may be the preferred substrate for this polymerase.

Having established that GST-polk inserts a base opposite Tg in a largely correct fashion, we examined the fidelity of extension beyond the lesion. Recent reports have documented the ability of polk to extend mispaired primer-termini promiscuously (20). Additionally, extension of the Tg-A base pair is believed to be the arresting substrate for most of the polymerases arrested by Tg, since a number of them insert A correctly opposite Tg but are unable to incorporate the next base (38–43). We determined steady state values for the extension of a primer terminating with correctly base-paired deoxyadenosine (P5-ox-ss-A) opposite the Tg lesion (in Tg OsO4), using each of the four deoxyribonucleoside triphosphates. The next correct base, C, was incorporated in clear preference to the other bases (Table I).

We also measured $k_{\text{ext}}$ for C incorporation using the Tg Br2 template. This returned $k_{\text{ext}}$ and $K_m$ values very similar to those observed with Tg OsO4, suggesting little or no stereochem-

![Fig. 4. GST-polk preferentially incorporates adenine across from thymine glycol. Radiolabeled primer-templates (5 nM) were incubated with the indicated deoxynucleotide triphosphate(s) (100 mM total [dNTP]) and GST-polk (5 nM) and the resulting primer extension products were resolved by PAGE. Lanes 1–6 contained undamaged template DNA; lanes 7–12 contained Tg Br2 template. Lanes 1 and 7, control experiments with no enzyme added. Lanes 2 and 8, only dATP; lanes 3 and 9, only dCTP; lanes 4 and 10, only dGTP; lanes 5 and 11, only TTP. Lanes 6 and 12 contained a mixture of all four dNTPs.](image)

![Fig. 5. Representative results for steady state kinetics analysis for incorporation of the four dNTPs opposite thymine glycol by GST-polk. The nucleotide incorporated is shown immediately below each gel, and the micromolar dNTP concentration incubated in each reaction is shown immediately below the corresponding lane of each gel. Experiments were performed using the undamaged template (A), the Tg Br2 template (B), or the Tg OsO4 template (C). Raw data were analyzed as described under “Experimental Procedures,” and the resulting steady state kinetic parameters are reported in Table I. Note that most of the panels shown represent initial conditions with a broad dNTP range. dCTP and TTP gels in B show representative results from more narrowly focused working ranges used in subsequent experiments.](image)
Plotted to the other bases in all three of the templates and that GST-pol glycol in the TgOsO4 template (TgOsO4 template is defined as ((TgOsO4 experiments.

between the purines during incorporation opposite the lesion. 

frequency of G increases in template Tg OsO4, suggesting that thymine glycol stereochemistry influences the ability of GST-pol glycols.

B

right graph

Graphical representation of select kinetic parameters from Table I. A, kcat/Km values for nucleotide insertion opposite thymine glycol in the TgBr2 template (black bars), the TgOsO4 template (blue bars), and the undamaged template (yellow bars). The left graph shows directly plotted kcat/Km values obtained for incorporation of the bases A, C, G, or T, emphasizing the difference in incorporation efficiency of A between the control and Tg templates. The right graph is a y axis blown up version of the same graph to emphasize that A is incorporated in great preference to the other bases in all three of the templates and that GST-pol may exhibit stereochemical preference during insertion of A opposite of thymine glycols. B, comparison of the finc values obtained for each template, measuring the degree of preference for incorporation of the correct base. finc is defined as (kcat/Km) incorrect/(kcat/Km) correct. The left graph is a direct plot of the finc values for nucleotide insertion opposite thymine glycol in the TgOsO4 template (black bars), the TgBr2 template (blue bars), and the undamaged template (yellow bars), emphasizing the high degree of preference for A incorporation in each template. The graph on the right is a y axis blown up version of the same graph to emphasize that the misincorporation frequency of G increases in template TgOsO4, suggesting that thymine glycol stereochemistry influences the ability of GST-pol to discriminate between the purines during incorporation opposite the lesion.

a finc is defined as (kcat/Km) incorrect/(kcat/Km) correct.
b Obtained by comparison of indicated dCTP or TTP kcat/Km values with the dATP kcat/Km value obtained by averaging results from both TgBr2 and TgOsO4 experiments.
Polk Bypasses and Extends beyond Thymine Glycols

Fig. 7. Schematic drawing of the most important conformers expected from the most abundant stereoisomers of thymine glycol. The 5R,6S form (left drawings, putatively in greater abundance in TgOsO4) may give rise to G-T wobble base-pairing in the GST-pol active site, resulting in higher levels of misincorporation of G. The 5S,6R isomer (right drawings) may adopt a much different conformation. Dr, deoxyribose. As a result of different half-chair conformations, the stereoisomers may each present the hydrogen bonding surface of the base at different angles, giving rise to the observed differences for misincorporation of G between the TgBr2 and TgOsO4 templates.

The propensity for accurate TLS of Tg by GST-polK is manifested both with respect to the nucleotide inserted directly opposite the lesion and extension for at least 1–2 bases. GST-polK inserts the correct nucleotide with a specificity of >98% and extends correctly with ~97% accuracy. Taken together (i.e., the multiples of these yields), GST-polK would be expected to bypass a stereochromically mixed Tg environment with greater than 92% accuracy, inserting the correct nucleotide opposite and (at least) two nucleotides beyond the lesion. This contrasts with the reported kinetic parameters for human polη, which inserts A opposite Tg almost as efficiently as it does opposite T in undamaged DNA but with a level of overall misincorporation of ~7% (31). The authors of this study did not report misincorporation rates for extension, but even in the unlikely case that those were modest, the overall yield would decrease with each extension event, yielding an overall rate much lower than that exhibited by GST-polK.

Interestingly, like GST-polK, polη misincorporates G with the highest frequency relative to the other bases (31). Polη synthesizes DNA more rapidly than polκ, with kcat/Km values approximately an order magnitude higher, but is more error-prone when synthesizing past Tg. Most provocatively, however, polη apparently prefers the 5R Tg stereoisomers over the 5S forms. Steady state kinetics results were not reported for 5S; however, running start synthesis clearly demonstrated less robust bypass. In the present study, we observed the opposite behavior for GST-polK, which exhibits small, but reproducible differences in the efficiency of incorporation of A opposite Tg in templates differing only in stereochemical composition. The template that exhibits more efficient turnover and greater accuracy of incorporation by GST-polK may contain a greater relative abundance of the 5S stereoisomer. Whereas polη is approximately an order of magnitude more efficient than polκ in vitro, the role of accessory proteins may influence the kcat/Km values reported here and elsewhere. Additionally, up-regulation of the POLK gene in tissues with DNA oxidative damage-prone environments may promote higher levels of polκ.

Our observation that the misincorporation rate for G by GST-polK is increased in the template with a putatively greater proportion of 5R stereoisomers may reflect important features of the polκ active site. The conformation of the Tg base is calculated to be the “half-chair” in which the most abundant 5R stereoisomer, (5R,6S) has been calculated to reside largely in a conformation that places the methyl group of C-5 pseudoaxial and the hydroxyl group of C-6 pseudoequatorial (49). It has been proposed that such a structure could generate a G-T wobble base pair (49). Perhaps the Tg base preference for the “half-chair” down conformation in 5R,6S adjusts the plane of the opposite edge of the base (the hydrogen bonding surface), allowing for a G-T wobble base pair, whereas in the opposite stereoisomer (5S,6R) a half-chair “up” conformer would predominate, which may leave the hydrogen bonding edge of the
base more closely aligned with that of a normal thymine template base (Fig. 7). Interestingly, human Rev1 polymerase, another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C opposite a number of DNA lesions but incorporates T next most frequently (50). Thus, while Polδ and Polκ apparently prefer incorporating purines opposite template lesions, Rev1 inserts pyrimidines preferentially. This contrast suggests that some Y family polymerases may be more specialized for purine-based lesions and others for pyrimidine-based lesions.

Importantly, GST-polκ is able to extend beyond sites of Tg damage. The enzyme exhibits an increase in overall misincorporation at sites beyond the base damage. However, GST-polκ manifests a similar preference for the correct base, incorporating C greater than 97% of the time immediately beyond the site of the Tg lesion. This observation is significant, because Tg lesions are typically arresting to polymerases favoring C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of the Tg lesion. This observation is significant, because Tg lesions are typically arresting to polymerases favoring C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of the Tg lesion. This observation is significant, because Tg lesions are typically arresting to polymerases favoring C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that preferentially incorporates C greater than 97% of the time immediately beyond the site of another Y family member, has been shown to be a dCMP nucleotidyl transferase that prefers...