

## Evidence for a Watson-Crick Hydrogen Bonding Requirement in DNA Synthesis by Human DNA Polymerase $\kappa$

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**The efficiency and fidelity of nucleotide incorporation by high-fidelity replicative DNA polymerases (Pols) are governed by the geometric constraints imposed upon the nascent base pair by the active site. Consequently, these polymerases can efficiently and accurately replicate through the template bases which are isosteric to natural DNA bases but which lack the ability to engage in Watson-Crick (W-C) hydrogen bonding. DNA synthesis by Pol $\eta$ , a low-fidelity polymerase able to replicate through DNA lesions, however, is inhibited in the presence of such an analog, suggesting a dependence of this polymerase upon W-C hydrogen bonding. Here we examine whether human Pol $\kappa$ , which differs from Pol $\eta$  in having a higher fidelity and which, unlike Pol $\eta$ , is inhibited at inserting nucleotides opposite DNA lesions, shows less of a dependence upon W-C hydrogen bonding than does Pol $\eta$ . We find that an isosteric thymidine analog is replicated with low efficiency by Pol $\kappa$ , whereas a nucleobase analog lacking minor-groove H bonding potential is replicated with high efficiency. These observations suggest that both Pol $\eta$  and Pol $\kappa$  rely on W-C hydrogen bonding for localizing the nascent base pair in the active site for the polymerization reaction to occur, thus overcoming these enzymes' low geometric selectivity.**

Classical DNA polymerases (Pols) replicate DNA with a high fidelity and are unable to replicate through DNA-distorting lesions. From studies with nonpolar, isosteric analogs, which lack the ability to form canonical Watson-Crick (W-C) hydrogen bonds, it has been concluded that W-C H bonding is not the determining factor for the efficiency and accuracy of nucleotide incorporation in high-fidelity polymerases such as T7 and *Escherichia coli* Pol I (20, 23, 24); rather, it is apparently the geometric fit within the active site of the incoming nucleoside triphosphate with the templating nucleotide that governs polymerase efficiency and accuracy (3, 5, 13, 15).

Members of the Y family of DNA polymerases replicate DNA with a low fidelity, and unlike the classical polymerases, they are able to replicate through DNA lesions (28, 29). Humans have four Y family Pols:  $\eta$ ,  $\iota$ ,  $\kappa$ , and Rev1. Of these, Rev1 is a highly specialized polymerase which predominantly incorporates a C opposite template G and also opposite an abasic site (8, 26), and genetic studies of *Saccharomyces cerevisiae* have suggested that a major role of Rev1 is to act as an assembly factor in Pol $\zeta$ -dependent lesion bypass (28). Although not as extreme in its nucleotide insertion specificity as Rev1, Pol $\iota$  incorporates nucleotides opposite the four different template bases with very different efficiencies and fidelities, and it incorporates nucleotides opposite template purines with a much higher efficiency and fidelity than opposite template

pyrimidines (6, 11, 34, 39, 46). From the ternary crystal structure of Pol $\iota$ , with a templating A and an incoming dTTP, it has been determined that unlike all other DNA polymerases, which impose Watson-Crick base pairing in their active site, Pol $\iota$  uses Hoogsteen base pairing for DNA synthesis (25).

In contrast to the specificity of Rev1 and Pol $\iota$  for nucleotide incorporation opposite template bases, all other known DNA polymerases, including the high-fidelity replicative/repair DNA polymerases, as well as the two other members of the Y family, Pol $\eta$  and Pol $\kappa$  (10, 12, 41), form the four possible correct base pairs with nearly equivalent catalytic efficiencies. Pols  $\eta$  and  $\kappa$ , however, differ from the high-fidelity polymerases in their low fidelity of nucleotide incorporation and in their ability to replicate through DNA lesions. For example, both yeast and human Pol $\eta$  misincorporate nucleotides with a frequency of  $\sim 10^{-2}$  to  $10^{-3}$ , and both of these enzymes replicate through a *cis-syn* TT dimer by inserting two A's opposite the two T's of the dimer with the same efficiency and fidelity as opposite the undamaged T's (12, 40, 41, 43). The structure of yeast Pol $\eta$  modeled with a templating TT dimer and an incoming dATP has indicated that it can accommodate both of the residues of the dimer in its active site (35); in this respect, Pol $\eta$  differs from other polymerases, including the other Y family polymerases, as they can accommodate only the templating residue in their active site, while the next 5' template base and the rest of the unpaired template are pushed out of the active site at a 90° angle (28).

One possible consequence of the proficient ability of Pol $\eta$  to hold two templating residues in its active site instead of one is that its active site may not be as closely juxtaposed to the templating base and the incoming deoxynucleoside triphosphate (dNTP) as occurs in the high-fidelity polymerases, which exercise a high degree of geometric selection because of the

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tight fit of the correct base pair in their active sites (3, 5, 14, 15). We have shown previously that Pol $\eta$  differs from high-fidelity replication/repair polymerases in its inability to replicate non-hydrogen-bonding nucleotide analogs. We hypothesized that this enzyme, lacking a tight geometric constraint, may rely instead upon Watson-Crick hydrogen bonding for the efficiency and fidelity of DNA synthesis (37).

Polk differs from Pol $\eta$  in several respects, including its higher fidelity and its inability to incorporate nucleotides opposite DNA lesions. Polk exhibits the highest fidelity among the Y family polymerases, as it misincorporates nucleotides with a frequency of  $\sim 10^{-3}$  to  $10^{-4}$  opposite all four template bases (10). Also, in contrast to Pol $\eta$ , Polk is unable to replicate through a *cis-syn* TT dimer, which results primarily from its inability to incorporate nucleotides opposite the 3' T of the dimer (10, 38). Polk is also highly inefficient at incorporating nucleotides opposite various other lesions, such as a (6-4) TT photoproduct, an *N*<sup>2</sup>-acetyl-aminofluorene adduct, and others (9, 10, 17, 33, 38). For some DNA lesions, however, Polk can promote the extension reaction wherein, following the incorporation of a nucleotide opposite the lesion site by another DNA polymerase, it performs the further extension of the primer terminus (7, 38, 42).

The higher fidelity and the inability to incorporate nucleotides opposite DNA lesions could result if the Polk active site were more geometrically constrained than that of Pol $\eta$ . The structure of the Polk apoenzyme and the modeling of Polk in a ternary complex with DNA and an incoming nucleotide have suggested this to be the case, as Polk's active site appears to be more constrained in the vicinity of the templating base than that of Pol $\eta$  (36). Since the more enclosed active site of Polk could provide for a higher degree of geometric selection than would be possible for a polymerase with a more open active site, the dependence of Polk upon other factors such as Watson-Crick or minor-groove hydrogen bonding may be lessened.

Here we examine the effects of difluorotoluene (F), which is identical in shape, size, and conformation to thymine but lacks the ability to form W-C hydrogen bonds with adenine (A) (30, 31), on DNA synthesis by human Polk (hPolk). Interestingly, we find that F is highly inhibitory to DNA synthesis by Polk, regardless of whether it is used as a templating residue or as an incoming nucleotide. This is consistent with the notion that W-C hydrogen bonding makes a paramount contribution to DNA synthesis by Polk. The implications of this observation are discussed.

#### MATERIALS AND METHODS

**Purification of DNA polymerases.** *S. cerevisiae* strain BJ5464 was transformed with plasmid pPol42, which carries the gene encoding wild-type hPolk fused in frame with glutathione *S*-transferase. Cells were grown and hPolk was purified as described previously (10). Cleavage of the glutathione *S*-transferase tag from Polk resulted in a seven-amino-acid peptide leader sequence attached to the N terminus of the polymerase. The protein concentration was determined by a Bio-Rad protein assay (Bio-Rad) and by UV absorbance at 280 nm under denaturing conditions (8 M urea), using the molar extinction coefficient calculated from the amino acid composition. The purified hPolk was stored in 5- $\mu$ l aliquots at  $-80^{\circ}\text{C}$ . *Taq* was purchased from Gene Choice, Inc.

**Nucleotides and DNA substrates.** 5'-dNTPs (100 mM) were purchased from Roche Diagnostics and stored in aliquots at  $-20^{\circ}\text{C}$ . Synthetic oligodeoxynucleotide template and primers were used to prepare the nondamaged DNA substrates. The 3-deazaguanine (3DG)- and F-containing DNA substrates were synthesized and purified as described previously (37, 45). For the 3DG-containing substrates, the following template/primer pairs were used. The incoming

nucleotide ( $P_0$ ) template/primer was 5'-CTGCGACTGCTGCGTCTGCGGTG C-3'/5'-GCACCGCAGACGAGCA. For the  $P_0$  substrate, the bold and underlined nucleotide is the templating base, and either a dGTP or a 3DG nucleoside triphosphate (d3DGTP) was used as the incoming dNTP. The templating residue ( $T_0$ ) template/primer was 5'-CTGCGACTXCTGCGTCTGCGGTGC-3'/5'-GCACCGCAGACGAGCA. For the  $T_0$  substrate, the bold and underlined nucleotide represents a G or a 3DG. The 2,4-difluorotoluene-containing template sequence was 5'-ACTGXCTCCCTATAGTGAGTCGTATTA-3', where the bold and underlined X represents either an A, a T, or an F. The standing-start and running-start primer sequences were 5'-TAATACGACTCACTATAGGGAG A-3' and 5'-TAATACGACTCACTATAG-3', respectively. For the polymerase assays, <sup>32</sup>P-5'-end-labeled primers (1.0  $\mu$ M) were annealed to templates (1.5  $\mu$ M) in 50 mM Tris HCl (pH 7.5) and 100 mM NaCl by being heated to  $95^{\circ}\text{C}$  for 2 min, followed by slow cooling to room temperature. The resulting DNA substrates were used to perform DNA synthesis reactions and to determine the steady-state kinetics parameters.

**DNA polymerase assays.** The standard DNA polymerase reaction mixture contained 25 mM Tris HCl (pH 7.5), 5 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 5 mM MgCl<sub>2</sub>, 10 nM DNA, 1 nM polymerase, and dNTPs. Reactions were carried out at  $23^{\circ}\text{C}$  for 3DG or at  $30^{\circ}\text{C}$  for F, and reaction mixtures were subsequently quenched with 95% formamide loading buffer. Quenched reaction products were heat denatured at  $95^{\circ}\text{C}$  for 3 min, and product formation was determined by 15% polyacrylamide gel electrophoresis (8 M urea) and quantified by PhosphorImager analysis (Molecular Dynamics).

For the steady-state kinetic analyses of the 3DG-containing substrates, standard DNA polymerase reaction conditions were used, with the addition of a single dNTP complementary to the templating base (e.g., dCTP opposite G and 3DG, dATP opposite T, etc.) in a concentration range appropriate for  $K_m$  determinations. Deoxynucleotide incorporation was measured at multiple dNTP concentrations for a single time point, and the steady-state kinetic parameters  $k_{\text{cat}}$  and  $K_m$  were determined by the best fit to the Michaelis-Menten equation (Sigma Plot 7.0).

For the steady-state kinetic analyses of the 2,4-difluorotoluene-containing substrates, standard DNA polymerase reaction conditions were used, with the addition of a single dNTP complementary to the templating base (e.g., dATP opposite T and F, dFTTP opposite A, or dTTTP opposite A) in a concentration range appropriate for  $K_m$  determinations. Deoxynucleotide incorporation was measured at multiple time points for each respective dNTP concentration, and the observed rate of nucleotide incorporation was determined by linear regression. Values of the steady-state parameters  $k_{\text{cat}}$  and  $K_m$  for nucleotide incorporation were then determined by the best fit to the Michaelis-Menten equation (Sigma Plot 7.0).

#### RESULTS

**DNA synthesis past a template F residue by Polk.** To determine whether human Polk could synthesize DNA in the absence of Watson-Crick hydrogen bonds, we examined its ability to polymerize nucleotides past a template F, which is a nonpolar, isosteric analog of thymine. As F lacks the Watson-Crick O<sup>2</sup> and O<sup>4</sup> hydrogen bond acceptors and N-H<sup>3</sup> donor of thymine (Fig. 1A), it is unable to participate in W-C hydrogen bonding (31). Since W-C hydrogen bonding is not needed for nucleotide incorporation by the *Thermus aquaticus* (*Taq*) DNA polymerase (23), we compared the abilities of *Taq* and Polk to synthesize DNA on substrates containing an F residue at either the sixth or the first templating position of the DNA substrate (Fig. 1B). As expected, *Taq* was able to incorporate a nucleotide opposite the template F residue (Fig. 1C, left panel, lanes 3 and 6) and could synthesize DNA past it, but it was somewhat hindered in extending from the nucleotide incorporated opposite F, as evidenced by the presence of a stall site opposite the lesion. Polk, on the other hand, was unable to synthesize DNA when F was present at either of the template positions (Fig. 1C, right panel, lanes 3 and 6). Also, in the running-start assay, a strong stall site was observed immediately before the F residue, indicating that Polk is strongly inhibited at incorporating a nucleotide opposite the F residue.

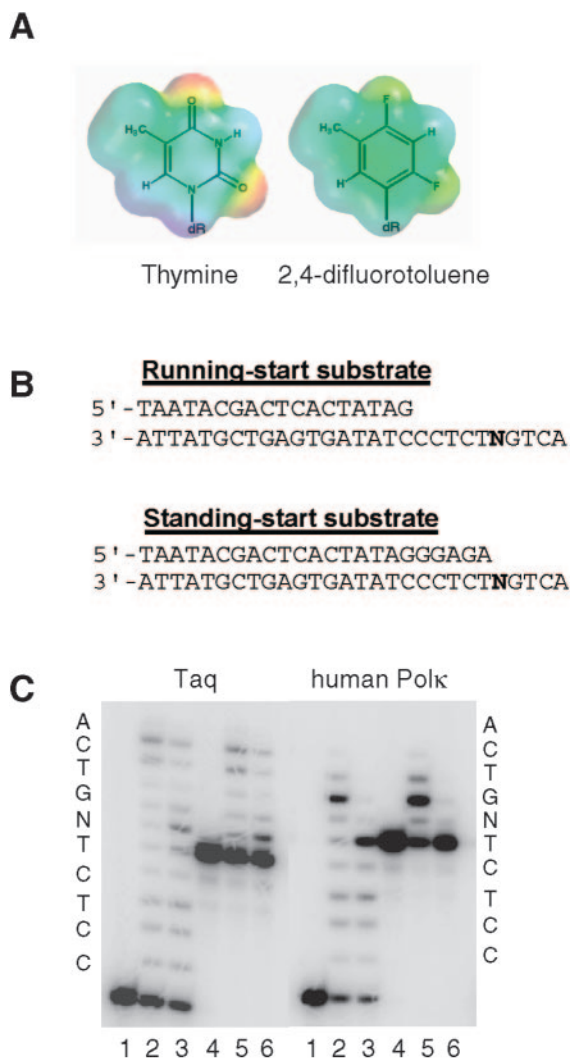


FIG. 1. (A) Overlay of the electrostatic potential and chemical structure of thymine (left) and the nonpolar, isosteric nucleobase analog, 2,4-difluorotoluene (right). (B) Running-start and standing-start DNA substrates. The 28-mer templates have either a T or an F at position 24, shown as a bold N. (C) Running-start and standing-start assays with *Taq* and human Polk. Lanes 1 to 3 are running-start experiments, and lanes 4 to 6 are standing-start experiments. Lanes 1 and 4 contain no protein, lanes 2 and 5 contain a template T residue, and lanes 3 and 6 contain a template F residue. The stalling of synthesis by Polk 1 to 2 nucleotides prior to the end of the DNA template is a feature that is characteristic of this polymerase (10).

#### Steady-state kinetics of nucleotide incorporation by Polk.

Next, we quantified the effects of an F residue on the efficiency and accuracy of nucleotide incorporation by Polk, by using steady-state kinetic assays on DNA substrates that contained an F residue at the position of the incoming nucleotide or the templating base. From the rate of nucleotide incorporation, graphed as a function of [dNTP], and the subsequent best fit to the Michaelis-Menten equation, the steady-state kinetic parameters  $k_{\text{cat}}$  and  $K_m$  and the efficiency ( $k_{\text{cat}}/K_m$ ) of nucleotide incorporation with respect to F substitution were determined. When F was used as the incoming dNTP opposite template A (i.e., a dFTP · A base pair), nucleotide incorporation was below

detectable limits (Fig. 2A). Based upon the estimated minimal detectable levels of incorporation, the dFTP incorporation efficiencies were determined to be  $\leq 1 \times 10^{-5}$ . For comparison, the misincorporation of an A opposite template A occurred with an efficiency of  $4 \times 10^{-3}$  (Table 1). We then determined the efficiency of nucleotide incorporation when F is the templating base (Fig. 2B). Polk incorporates an A opposite template T with an efficiency of 1.7 (Fig. 2C, left panel; Table 1), and it misincorporates a T opposite a template T with an efficiency of  $3.4 \times 10^{-4}$  (Table 1). When F occupies the position of the templating base, dATP is incorporated with an efficiency of  $4.5 \times 10^{-3}$  (Fig. 2C, right panel; Table 1), suggesting that disrupting the Watson-Crick hydrogen bond interactions with the incoming dNTP results in a block which is almost as severe as the block for mispair formation. Thus, the substitution of an F for a T, whether at the templating position or the incoming nucleotide position, elicits a large reduction in the efficiency and fidelity of nucleotide incorporation by Polk.

**DNA synthesis by Polk on substrates containing a 3DG substitution.** In addition to the geometric constraints imposed by a tight active site, DNA polymerases can check for the correctness of a base pair by participating in specific hydrogen bonding interactions with the minor-groove hydrogen bond acceptors, N3 for purines and O<sup>2</sup> for pyrimidines, in DNA (2, 4, 18, 19, 21, 22, 32, 45). Because of the absence of the O<sup>2</sup> hydrogen bond acceptor in F, we considered the possibility that the reduction in the efficiency and fidelity of nucleotide incorporation that occurs when F is used as a templating residue or as an incoming nucleotide originates from a possible dependence of Polk on minor-groove hydrogen bonding interactions with both the templating base and the incoming nucleotide. To probe for such effects, we used 3DG, which is a base analog of guanine (G) but differs from G in having a carbon instead of a nitrogen at position 3 of the base (Fig. 3A). Because of the absence of an N3 hydrogen bond acceptor, the 3DG analog cannot participate in minor-groove hydrogen bonding interactions with the polymerase.

We examined the effects of a 3DG substitution for G at the position of the incoming nucleotide, referred to as P<sub>0</sub>, or at the templating position, referred to as T<sub>0</sub>. Compared to the efficiency of G incorporation opposite template C, Polk incorporated 3DG with an ~15-fold reduction in efficiency (Fig. 3B, C, and D; Table 2). By contrast, Polk misincorporated a C opposite template C with an ~5,000-fold reduction in efficiency (Table 2; Fig. 3D). The substitution of 3DG for G at the templating position led to only an approximately fivefold reduction in the efficiency of C incorporation, while the efficiency of G misincorporation opposite template G was reduced by ~2,500-fold (Table 2; Fig. 3D). Thus, the ablation of any possible minor-groove hydrogen bonding interactions with the P<sub>0</sub> and T<sub>0</sub> positions in DNA confers only a small impairment in the efficiency of correct nucleotide incorporation by Polk. The results therefore indicate strongly that the large reduction in the efficiency and fidelity of nucleotide incorporation that occurs with analog F must derive from a different molecular origin.

#### DISCUSSION

Based upon thermal denaturation studies of DNA alone, the base pairing free energy differences ( $\Delta\Delta G^\circ$ ) between correct



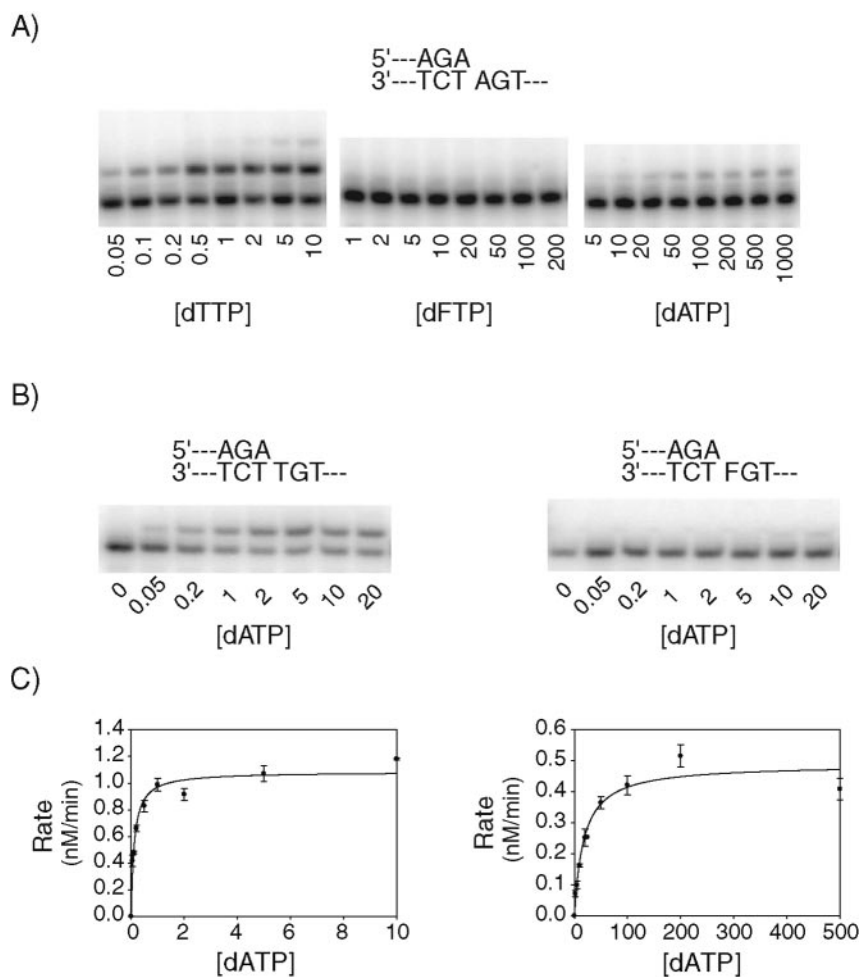


FIG. 2. Effects of F on DNA synthesis by hPol $\kappa$ . (A) dTTP, dFTP, or dATP incorporation opposite template A. (B) Incorporation of dATP opposite a template T or F. (C) Rates of dATP incorporation opposite a template T (left) and a template F (right), graphed as a function of [dATP]. The solid line represents the best fit to the Michaelis-Menten equation. Steady-state kinetic parameters are listed in Table 1. A portion of the DNA substrates used is shown in panel B.

Watson-Crick base pairs and mismatched base pairs are as high as 4.0 kcal/mol (1, 16). At the terminus of DNA, where DNA synthesis occurs, however, selectivities are considerably smaller (27). Thus, these free energy differences are far too small to account for the millionfold stringency manifested by replicative DNA polymerases. Furthermore, the replacement of normal nucleotides with the nucleotide analogs which im-

pair Watson-Crick hydrogen bonding has very little effect on the efficiency and fidelity of nucleotide incorporation by DNA polymerases such as *E. coli* Klenow fragment and T7 (20, 23, 24). From such analyses, it has been inferred that W-C hydrogen bonding is not needed for base pair synthesis by the high-fidelity polymerases.

In the high-fidelity DNA polymerases, the long  $\alpha$  helices of

TABLE 1. Steady-state kinetic parameters for nucleotide incorporation by hPol $\kappa$  with 2,4-difluorotoluene-containing DNA substrates

DNA substrate	dNTP	$k_{cat}$ ( $\text{min}^{-1}$ )	$K_m$ ( $\mu\text{M}$ )	$k_{cat}/K_m$ ( $\mu\text{M}^{-1} \text{min}^{-1}$ )	Relative efficiency
5'-AGA 3'-TCTA	dTTP	$0.19 \pm 0.008$	$0.16 \pm 0.03$	1.2	1
	dFTP	ND <sup>a</sup>	ND	$\leq 1 \times 10^{-5}$	$\leq 8 \times 10^{-6}$
	dATP	$0.036 \pm 0.002$	$8.6 \pm 3$	$4.2 \times 10^{-3}$	$4 \times 10^{-3}$
5'-AGA 3'-TCTT	dATP	$0.20 \pm 0.006$	$0.12 \pm 0.02$	1.7	1
	dTTP	$0.086 \pm 0.004$	$250 \pm 30$	$3.4 \times 10^{-4}$	$2 \times 10^{-4}$
5'-AGA 3'-TCTF	dATP	$0.095 \pm 0.005$	$21 \pm 4$	$4.5 \times 10^{-3}$	$3 \times 10^{-3}$
	dTTP	$0.11 \pm 0.01$	$630 \pm 140$	$1.7 \times 10^{-4}$	$1 \times 10^{-4}$

<sup>a</sup> ND, no nucleotide incorporation detected. Nucleotide incorporation efficiencies are based upon the estimated minimal detectable levels of incorporation.

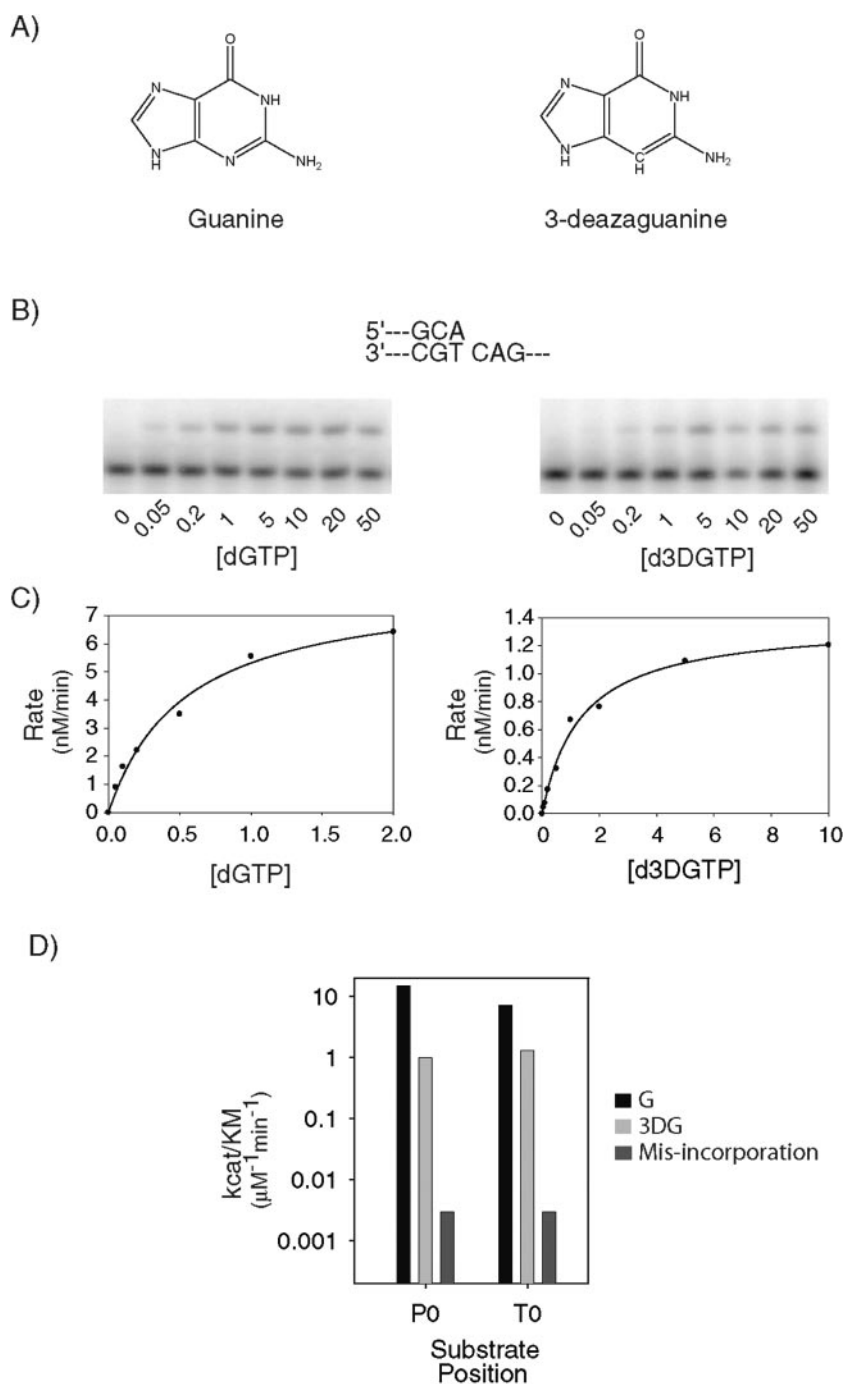


FIG. 3. Effects of 3DG on DNA synthesis by hPolk. (A) Chemical structures of guanine (left) and 3DG (right). (B) Incorporation of dGTP or d3DGTP opposite template C. A portion of the DNA substrate used is shown (top). (C) Rates of dGTP and d3DGTP incorporation opposite a template C, graphed as a function of [dNTP]. The solid line represents the best fit to the Michaelis-Menten equation. Steady-state kinetic parameters are listed in Table 2. (D) Bar graph showing the effect of 3DG substitution on the efficiency ( $k_{cat}/K_m$ ) of correct nucleotide incorporation when a G (black) or 3DG (light gray) is used as the incoming nucleotide ( $P_0$ ) opposite template C or as the templating nucleotide ( $T_0$ ) for the incoming C. For comparison, the efficiencies of misincorporation of a C ( $P_0$ ) opposite a template C and of a G opposite template G ( $T_0$ ) are shown (dark gray).

the fingers domain close tightly on the incoming nucleotide and the templating residue, and the resulting snug active site may provide for a high degree of geometric selectivity that these enzymes apparently possess (2, 4, 18, 19). Consequently,

any need for W-C hydrogen bonding is minimized. By contrast, the active site of Y family polymerases is much more open and sterically less constrained around the nascent base pair, and this is primarily due to the fingers domain being very small and

TABLE 2. Steady-state kinetic parameters for nucleotide incorporation by hPolk with 3DG-containing DNA substrates

DNA substrate <sup>a</sup>	dNTP/template	$k_{\text{cat}}$ (min <sup>-1</sup> )	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1} \text{min}^{-1}$ )	Relative efficiency
P <sub>0</sub> -G	G/C	8.2 ± 0.6	0.54 ± 0.09	15	1
	C/C	0.52 ± 0.01	150 ± 10	3.4 × 10 <sup>-3</sup>	2 × 10 <sup>-4</sup>
P <sub>0</sub> -3DG	3DG/C	1.4 ± 0.05	1.4 ± 0.2	1.0	0.07
T <sub>0</sub> -G	C/G	4.1 ± 0.4	0.57 ± 0.2	7.2	1
	G/G	1.2 ± 0.07	400 ± 60	3.0 × 10 <sup>-3</sup>	4 × 10 <sup>-4</sup>
T <sub>0</sub> -3DG	C/3DG	2.3 ± 0.14	1.7 ± 0.2	1.3	0.18
	G/3DG	0.35 ± 0.008	150 ± 10	2.3 × 10 <sup>-3</sup>	3 × 10 <sup>-4</sup>

<sup>a</sup> P<sub>0</sub>-G and P<sub>0</sub>-3DG refer, respectively, to the incoming G and 3DG nucleotides opposite template C. T<sub>0</sub>-G and T<sub>0</sub>-3DG refer, respectively, to the templates G and 3DG.

stubby (28). As a consequence, the Y family polymerases are not as sensitive to the geometric distortions conferred upon DNA by the presence of lesions.

In a previous study, we showed that the substitution of an F for a T at the templating site or as an incoming nucleotide is highly inhibitory to DNA synthesis by yeast Pol $\eta$  (37). Here we provide evidence that DNA synthesis by Polk is severely impaired when F is used either as a templating residue or as an incoming nucleotide. From these and other observations reported here, we hypothesize that DNA synthesis by Polk is strongly dependent upon W-C hydrogen bonding, and in this respect, it closely resembles Pol $\eta$ . We suggest that, in a relatively roomy active site, the analog does not fill the available space and is therefore not fixed in the correct location for proper alignment of the triphosphate group. In contrast to this, thymine would be constrained by optimum hydrogen bonding geometry to a fixed location, thus aligning the reactive triphosphate for phosphodiester bond formation. This would also be consistent with the poor activity of mismatched pairs, which, although hydrogen bonded, would be fixed in an unfavorable position for base pair synthesis.

In making this hypothesis, we considered a number of other possible reasons for the poor activity of the analog F with Polk. First is the size of the F · A base pair, which is likely larger than the natural T · A pair by a small amount (approximately 0.3 Å), due to the lack of a hydrogen bonding contraction. However, since the enzyme can replicate through lesions and form mispairs with greater efficiency, this seems an unlikely explanation for the present findings. Second is the aforementioned lack of a minor-groove H bond acceptor in F (31); however, our data with 3DG show clearly that this lack has only a very small kinetic effect, both in the template and in the incoming nucleotide. Since the chief chemical difference between F and T is the strong electrostatic difference along the Watson-Crick edge, we arrived at the lack of Watson-Crick hydrogen bonding as the best available explanation.

The observation that Polk shows the same high degree of apparent dependence upon W-C hydrogen bonding as Pol $\eta$  may seem surprising, in view of the very considerable differences that exist in their fidelities and damage bypass abilities. Whereas Pol $\eta$  misincorporates nucleotides with a frequency of ~10<sup>-2</sup> to 10<sup>-3</sup> and is able to proficiently replicate through cyclobutane pyrimidine dimers as well as through many other sorts of DNA lesions, Polk displays a fidelity of ~10<sup>-3</sup> to 10<sup>-4</sup> and is unable to replicate through cyclobutane pyrimidine dimers and other DNA lesions, which primarily results from its

inability to incorporate nucleotides opposite the lesion site. As the lower fidelity and the more proficient ability of Pol $\eta$  to incorporate nucleotides opposite lesions such as cyclobutane pyrimidine dimers (and others) could be ascribed to this polymerase having a more open active site, for Polk, the higher fidelity and the inability to incorporate nucleotides opposite lesion sites could suggest a more constrained active site. Consequently, Polk's active site could have provided for a higher degree of geometric selection than that of Pol $\eta$ , thereby lessening the dependence of Polk upon W-C hydrogen bonding. Our finding that a loss of W-C hydrogen bonding is apparently as detrimental to DNA synthesis by Polk as it is to DNA synthesis by Pol $\eta$ , however, is more in line with the view that, overall, the active sites of both of these polymerases share common structural features which do not allow for a great deal of geometric selectivity. That, in turn, puts a strong premium upon W-C hydrogen bonding for the proper positioning of the nascent base pair in the active site, so that the subsequent induced fit conformational change (44) and the chemical reaction of phosphodiester bond formation may occur. We presume then that the different fidelities and damage bypass abilities of Pol $\eta$  and Polk arise from subtle structural differences in their active sites and not from a large global change that could have allowed for a significantly higher degree of geometric selectivity in one polymerase than in the other.

#### ACKNOWLEDGMENTS

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