

Research Article

Pre-Steady-State Kinetic Analysis of Truncated and Full-Length *Saccharomyces cerevisiae* DNA Polymerase Eta

Jessica A. Brown,¹ Likui Zhang,¹ Shanen M. Sherrer,¹ John-Stephen Taylor,²
Peter M. J. Burgers,³ and Zucui Suo¹

¹Department of Biochemistry, The Ohio State University, Columbus, OH 43210, USA

²Department of Chemistry, Washington University, St. Louis, MO 63130, USA

³Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

Correspondence should be addressed to Zucui Suo, suo.3@osu.edu

Received 11 March 2010; Accepted 30 April 2010

Academic Editor: Ashis Basu

Copyright © 2010 Jessica A. Brown et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Understanding polymerase fidelity is an important objective towards ascertaining the overall stability of an organism's genome. *Saccharomyces cerevisiae* DNA polymerase η (γ Pol η), a Y-family DNA polymerase, is known to efficiently bypass DNA lesions (e.g., pyrimidine dimers) in vivo. Using pre-steady-state kinetic methods, we examined both full-length and a truncated version of γ Pol η which contains only the polymerase domain. In the absence of γ Pol η 's C-terminal residues 514–632, the DNA binding affinity was weakened by 2-fold and the base substitution fidelity dropped by 3-fold. Thus, the C-terminus of γ Pol η may interact with DNA and slightly alter the conformation of the polymerase domain during catalysis. In general, γ Pol η discriminated between a correct and incorrect nucleotide more during the incorporation step (50-fold on average) than the ground-state binding step (18-fold on average). Blunt-end additions of dATP or pyrene nucleotide 5'-triphosphate revealed the importance of base stacking during the binding of incorrect incoming nucleotides.

1. Introduction

DNA polymerases are organized into seven families: A, B, C, D, X, Y, and reverse transcriptase [1, 2]. Among these families, DNA polymerases are involved in DNA replication, DNA repair, DNA lesion bypass, antibody generation, and sister chromatid cohesion [3]. Despite these diverse roles, DNA polymerases catalyze the nucleotidyl transfer reaction using a two divalent metal ion mechanism [4] with at least one positively charged residue [5] that functions as a general acid [6] at their active site, follow a similar minimal kinetic pathway [7], and share a similar structural architecture consisting of the fingers, palm, and thumb subdomains [8, 9]. Surprisingly, the polymerization fidelity of eukaryotic DNA polymerases spans a wide range: one error per one to one billion nucleotide incorporations (10^0 to 10^{-9}) [10].

The Y-family DNA polymerases are known for catalyzing nucleotide incorporation with low fidelity and poor processivity. These enzymes are specialized for translesion DNA synthesis which involves nucleotide incorporation opposite and downstream of a damaged DNA site. Lesion bypass can be either error-free or error-prone depending on the DNA polymerase and DNA lesion combination. To accommodate a distorted DNA substrate, Y-family DNA polymerases utilize several features: a solvent-accessible [11] and conformationally flexible active site [12], smaller fingers and thumb subdomains [11], an additional subdomain known as the little finger [11], the little finger and polymerase core domains move in opposite directions during a catalytic cycle [13], and a lack of 3' \rightarrow 5' exonuclease activity [14]. Unfortunately, these features, which facilitate lesion bypass, may also contribute to the low fidelity of a Y-family DNA polymerase during replication of a damaged or undamaged

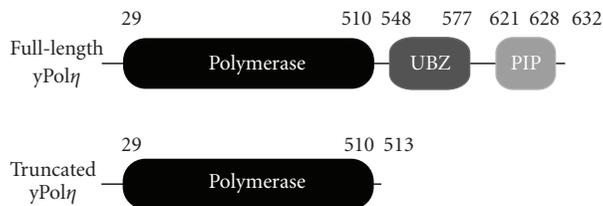


FIGURE 1: Schematic illustration of yPol η . The polymerase domain of yPol η is at the N-terminus while a ubiquitin-binding zinc finger (UBZ) domain and PCNA-interacting peptide (PIP) motif is at the C-terminus. Residue numbers are denoted above each region. For this study, the truncated construct contains only the polymerase domain.

DNA template. Thus, it is important to understand the mechanism and fidelity of the Y-family DNA polymerases.

Saccharomyces cerevisiae DNA polymerase η (yPol η), a Y-family DNA polymerase, is critical for the error-free bypass of UV-induced DNA damage such as a *cis-syn* thymine-thymine dimer [15–19]. To date, Pol η remains the only Y-family DNA polymerase with a confirmed biological function [20]. yPol η is organized into a polymerase domain, ubiquitin-binding zinc finger (UBZ) domain, and proliferating cell nuclear antigen- (PCNA) interacting peptide (PIP) motif (Figure 1). X-ray crystal structures of yPol η 's catalytic core have been solved alone [21] as well as in complex with a cisplatin-DNA adduct and an incoming nucleotide [22]. Due to a lack of structures for full-length yPol η , it is unclear if the C-terminal residues 514–632 interact with DNA and contribute to the polymerase function of yPol η . Using pre-steady-state kinetic techniques, we have measured the base-substitution fidelity of full-length and truncated yPol η (Figure 1) catalyzing nucleotide incorporation into undamaged DNA. In addition, we have determined the DNA binding affinity of both full-length and truncated yPol η . Our results show that the C-terminus of yPol η has a minor effect on the DNA binding affinity and the base substitution fidelity of this lesion bypass DNA polymerase.

2. Materials and Methods

2.1. Materials. Materials were purchased from the following companies: [γ - 32 P] ATP, MP Biomedicals (Solon, OH); Biospin columns, Bio-Rad Laboratories (Herclues, CA); dNTPs, GE Healthcare (Piscataway, NJ); oligodeoxyribonucleotides, Integrated DNA Technologies, Inc. (Coralville, IA); and OptiKinase, USB (Cleveland, OH).

2.2. Preparation of Substrates and Enzymes. The synthetic oligodeoxyribonucleotides listed in Table 1 were purified as described previously [23]. The primer strand 21-mer or blunt-end 16-mer was 5'-radiolabeled with [γ - 32 P]ATP and OptiKinase. Then, the 21-mer was annealed to the appropriate 41 mer template (Table 1) and the palindromic blunt-end substrates were annealed as described previously [23]. The catalytic core of yPol η (1–513) containing an N-terminal

TABLE 1: Sequences of DNA substrates^a.

D-1	5'–CGCAGCCGTCCAACCAACTCA–3' 3'–GCGTCGGCAGGTTGGTTGAGTAGCAGCTAGGTTACGGCAGG–5'
D-6	5'–CGCAGCCGTCCAACCAACTCA–3' 3'–GCGTCGGCAGGTTGGTTGAGTGCCAGCTAGGTTACGGCAGG–5'
D-7	5'–CGCAGCCGTCCAACCAACTCA–3' 3'–GCGTCGGCAGGTTGGTTGAGTTGCAGCTAGGTTACGGCAGG–5'
D-8	5'–CGCAGCCGTCCAACCAACTCA–3' 3'–GCGTCGGCAGGTTGGTTGAGTCGCAGCTAGGTTACGGCAGG–5'
F-8	5'–CGCAGCCGTCCAACCAACTCA–3' 3'–GCGTCGGCAGGTTGGTTGAGTCXCAGCTAGGTTACGGCAGG–5'
BE1	5'–ATGAGTTGCAACTCAT–3' 3'–TACTCAACGTTGAGTA–5'
BE2	5'–TTGAGTTGCAACTCAA–3' 3'–AACTCAACGTTGAGTT–5'
BE3	5'–CTGAGTTGCAACTCAG–3' 3'–GACTCAACGTTGAGTC–5'
BE4	5'–GTGAGTTGCAACTCAC–3' 3'–CACTCAACGTTGAGTG–5'

^aThe template base highlighted in bold is unique to each strand and X denotes 2-aminopurine.

MGSSH₆SSGLVPRGSH tag was purified as described previously [24]. The full-length yPol η (1–632) was expressed and purified from yeast [25]. Pyrene 5'-triphosphate (dPTP) was synthesized as described previously [26].

2.3. Pre-Steady-State Kinetic Assays. All experiments were performed in reaction buffer A which contained 40 mM Tris-HCl pH 7.5 at 23°C, 5 mM MgCl₂, 1 mM DTT, 10 μ g/mL BSA, and 10% glycerol. A rapid chemical-quench flow apparatus (KinTek, PA, USA) was used for fast reactions. For burst assays, a preincubated solution of yPol η (320 nM) and 5'-[32 P]-labeled D-1 DNA (480 nM) was mixed with dTTP·Mg²⁺ (100 μ M). To measure the dissociation rate of the yPol η ·DNA binary complex, a preincubated solution of yPol η (50 nM) and 5'-[32 P]-labeled D-1 DNA (100 nM) was mixed with a molar excess of unlabeled D-1 DNA (2.5 μ M) for various time intervals prior to initiating the polymerization reaction with dTTP·Mg²⁺ (150 and 400 μ M for truncated and full-length yPol η , resp.) for 15 s. For single-turnover kinetic assays, a preincubated solution of yPol η (150 nM) and 5'-[32 P]-labeled DNA (30 nM) was mixed with an incoming dNTP·Mg²⁺ (0.4–800 μ M). Reactions were quenched at the designated time by adding 0.37 M EDTA. Reaction products were analyzed by sequencing gel electrophoresis (17% acrylamide, 8 M urea, 1 \times TBE running buffer), visualized using a Typhoon TRIO (GE Healthcare), and quantitated with ImageQuant software (Molecular Dynamics).

2.4. DNA Binding Assays. The equilibrium dissociation constant (K_d^{DNA}) of the yPol η ·DNA binary complex was determined using two techniques. First, an electrophoretic mobility shift assay (EMSA) was employed by adding increasing concentrations of yPol η (10–450 nM) into a fixed concentration of 5'-[32 P]-labeled D-1 DNA (10 nM) in

buffer A. The solution established equilibrium during a 20-minute incubation period. Then, the binary complex was separated from unbound DNA using a 4.5% native polyacrylamide gel and running buffer as previously described except the final concentration of Tris was adjusted to 40 mM [27]. Second, a fluorescence titration assay was used. Increasing concentrations of γ Pol η (2–300 nM) were titrated into a fixed concentration of F-8 DNA (25 nM) in buffer A (devoid of BSA). The F-8 DNA substrate (Table 1) was excited at a wavelength of 312 nm with emission and excitation slit widths of 5 nm. The emission spectra were collected at 1 nm intervals from 320 to 500 nm using a Fluoromax-4 (Jobin Jvon Horiba). Emission background from the buffer and intrinsic protein fluorescence were subtracted from each spectrum.

2.5. Data Analysis. For the pre-steady-state burst assay, the product concentration was graphed as a function of time (t) and the data were fit to the burst equation (1) using the nonlinear regression program, KaleidaGraph (Synergy Software):

$$[\text{Product}] = A[1 - \exp(-k_1 t) + k_2 t]. \quad (1)$$

A represents the fraction of active enzyme, k_1 represents the observed burst rate constant, and k_2 represents the observed steady-state rate constant.

Data for the EMSA were graphed by plotting the concentration of the binary complex as a function of enzyme concentration (E_0) and fitting it to a quadratic equation (2):

$$[\text{E} \cdot \text{DNA}] = 0.5 \left(K_d^{\text{DNA}} + E_0 + D_0 \right) - 0.5 \left[\left(K_d^{\text{DNA}} + E_0 + D_0 \right)^2 - 4E_0 D_0 \right]^{1/2}. \quad (2)$$

D_0 is the DNA concentration.

For the fluorescence titration experiments, a modified quadratic equation (3) was applied to a plot of the fluorescence intensity (F) measured at 370 nm versus enzyme concentration:

$$[F] = F_{\max} + \left[\frac{F_{\min} - F_{\max}}{2D_0} \right] \times \left\{ \left(K_d^{\text{DNA}} + E_0 + D_0 \right) - \left[\left(K_d^{\text{DNA}} + E_0 + D_0 \right)^2 - 4E_0 D_0 \right]^{1/2} \right\}. \quad (3)$$

F_{\max} and F_{\min} represent the maximum and minimum fluorescence intensity, respectively.

For the rate of DNA dissociation from the binary complex, a single-exponential equation (4) was applied to a plot of product concentration versus time:

$$[\text{Product}] = A[\exp(-k_{\text{off}} t)] + C. \quad (4)$$

A represents the reaction amplitude, k_{off} is the observed rate constant of DNA dissociation, and C is the concentration of

the radiolabeled DNA product in the presence of a DNA trap for unlimited time.

For the single-turnover kinetic assays, a plot of product concentration versus time was fit to a single-exponential equation (5) to extract the observed rate constant of nucleotide incorporation (k_{obs}):

$$[\text{Product}] = A[1 - \exp(-k_{\text{obs}} t)]. \quad (5)$$

To measure the maximum rate constant of incorporation (k_p) and the apparent equilibrium dissociation constant (K_d) of an incoming nucleotide, the extracted k_{obs} values were plotted as a function of nucleotide concentration and fit to a hyperbolic equation (6):

$$[k_{\text{obs}}] = \frac{k_p [\text{dNTP}]}{(K_d + [\text{dNTP}])}. \quad (6)$$

The free energy change ($\Delta\Delta G$) for a correct and incorrect nucleotide substrate dissociating from the E·DNA·dNTP complex was calculated according to (7).

$$\Delta\Delta G = RT \ln \left[\frac{(K_d)_{\text{incorrect}}}{(K_d)_{\text{correct}}} \right]. \quad (7)$$

Here, R is the universal gas constant and T is the reaction temperature in Kelvin.

3. Results and Discussion

3.1. Truncated and Full-Length γ Pol η Display Biphasic Kinetics. Previously, transient state kinetic techniques have been used to characterize full-length γ Pol η at 30°C [28]. Therefore, we first performed a burst assay (see Section 2) to ensure that our purified proteins, truncated and full-length γ Pol η (Figure 1), behaved in a similar manner at 23°C. Compared to wild-type γ Pol η , the truncated construct contains only the polymerase domain (Figure 1). A preincubated solution of γ Pol η (320 nM) and 5'-[³²P]-labeled 21/41 mer D-1 DNA (480 nM) was mixed with dTTP·Mg²⁺ (100 μ M) and quenched with EDTA at various times. Product concentration was plotted as a function of time and was fit to (1), since there were two distinct kinetic phases: a rapid, exponential phase and a slow, linear phase (data not shown). These burst results were similar to those previously published [28]. Biphasic kinetics of nucleotide incorporation indicated that the first turnover rate was the rate of nucleotide incorporation occurring at the enzyme's active site while subsequent turnovers (i.e., linear phase) were likely limited by the DNA product release step as demonstrated by full-length γ Pol η at 30°C [28] and other DNA polymerases [23, 29, 30].

3.2. The C-Terminal 119 Residues Slightly Enhance DNA Binding Affinity of γ Pol η . The equilibrium dissociation constant for the binary complex of γ Pol η ·DNA (K_d^{DNA}) was measured to determine if the C-terminus of γ Pol η affects DNA binding affinity (Scheme 1). First, the K_d^{DNA} was estimated using the EMSA (see Section 2). For example, varying concentrations

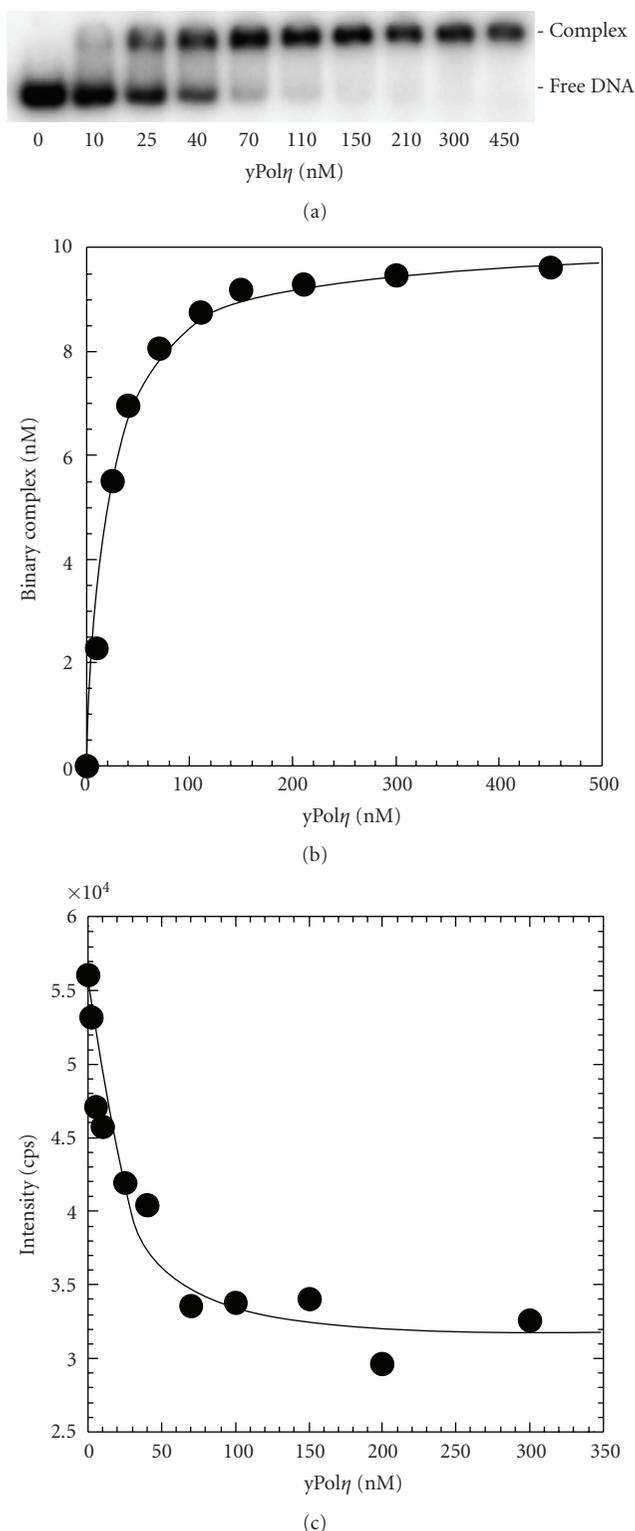


FIGURE 2: Equilibrium dissociation constant for full-length yPol η . (a) Gel image showing binary complex formation at various concentrations of full-length yPol η (10–450 nM) in the presence of 5′-[³²P]-labeled D-1 DNA (10 nM). (b) The concentration of the binary complex was plotted as a function of full-length yPol η concentration and fit to (2) to yield a $K_d^{\text{DNA}} = 16 \pm 1$ nM. (c) For the fluorescence titration assay, a plot of fluorescence intensity versus full-length yPol η concentration was fit to (3) which resolved a $K_d^{\text{DNA}} = 7 \pm 4$ nM.

various times (see Section 2). A plot of product concentration versus time was fit to (5) to extract the observed rate constant (k_{obs}) for dATP incorporation (Figure 3(a)). Then, the k_{obs} values were plotted as a function of dATP concentration and fit to a hyperbolic equation (6) which resolved a k_p of 6.9 ± 0.4 s⁻¹ and an apparent K_d of 17 ± 3 μ M (Figure 3(b)). The pre-steady-state kinetic parameters for the remaining 15 possible dNTP:dN base pair combinations were determined under single-turnover conditions and were used to calculate the substrate specificity constant (k_p/K_d), discrimination factor ($(k_p/K_d)_{\text{correct}}/(k_p/K_d)_{\text{incorrect}}$), and fidelity ($(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$) of truncated yPol η (Table 3).

Overall, the base substitution fidelity of truncated yPol η was in the range of 10^{-2} to 10^{-4} which translates into 1 misincorporation per 100 to 10,000 nucleotide incorporations (Table 3). Depending on the mispair, truncated yPol η catalyzed a misincorporation with 30- to 2,700-fold (640-fold on average) lower efficiency than the corresponding correct base pair. To better understand the mechanistic basis of truncated yPol η 's fidelity, the equation for polymerase fidelity can be simplified as follows:

$$\begin{aligned} \text{Fidelity} &= \frac{(k_p/K_d)_{\text{incorrect}}}{[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]} \\ &\approx \frac{(k_p/K_d)_{\text{incorrect}}}{(k_p/K_d)_{\text{correct}}} \\ &= \left[\frac{(k_p)_{\text{incorrect}}}{(k_p)_{\text{correct}}} \right]^{-1} \left[\frac{(K_d)_{\text{correct}}}{(K_d)_{\text{incorrect}}} \right]^{-1} \\ &= (\text{rate difference})^{-1} (\text{binding affinity difference})^{-1}. \end{aligned} \quad (8)$$

Thus, fidelity is inversely proportional to the rate difference and apparent binding affinity difference between correct and incorrect nucleotide incorporation. In general, the mechanistic basis of yPol η 's discrimination was due to a 3- to 68-fold (18-fold on average) weaker apparent binding affinity ($1/K_d$) and 5- to 220-fold (50-fold on average) slower rate constant of incorporation for a mismatched dNTP.

3.4. Kinetic Significance of Base Stacking Contributing to the Binding Affinity of an Incoming Nucleotide. Although all four correct dNTPs were bound with similarly high affinity (Table 3), mismatched purine deoxyribonucleotides have 2- to 6-fold lower apparent K_d values than mismatched pyrimidine deoxyribonucleotides. Because 5′-protruding purines have been found to have stronger stacking interactions with a terminal DNA base pair than 5′-protruding pyrimidines [33], the difference in apparent K_d values suggests that base-stacking interactions between an incorrect dNTP and the terminal primer/template base pair dA:dT (Table 1) play a role on the binding of dNTP by truncated yPol η . Interestingly, we have previously demonstrated that the preferred nucleotide for template-independent nucleotide

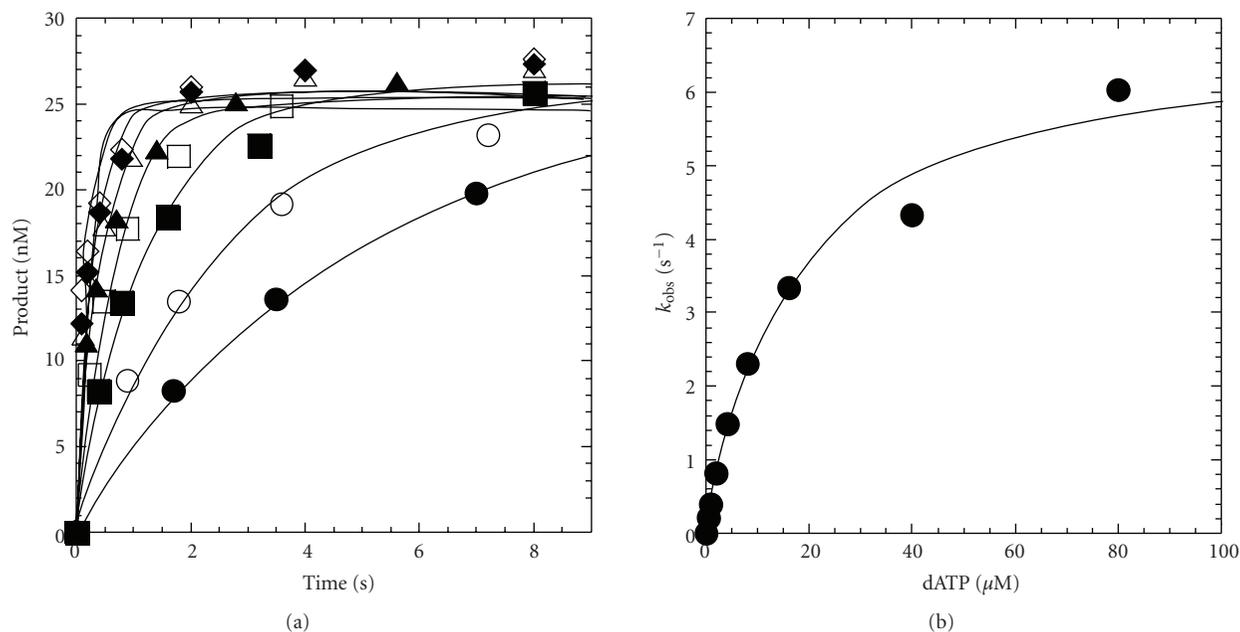


FIGURE 3: Concentration dependence on the pre-steady-state rate constant of nucleotide incorporation catalyzed by truncated yPol η . (a) A preincubated solution of truncated yPol η (150 nM) and 5'-[³²P]-labeled D-7 DNA (30 nM) was mixed with dATP·Mg²⁺ (0.4 μM, ●; 0.8 μM, ○; 2 μM, ■; 4 μM, □; 8 μM, ▲; 16 μM, △; 40 μM, ◆; 80 μM, ◇) and quenched with EDTA at various time intervals. The solid lines are the best fits to a single-exponential equation which determined the observed rate constant, k_{obs} . (b) The k_{obs} values were plotted as a function of dATP concentration. The data (●) were then fit to a hyperbolic equation, yielding a k_p of $6.9 \pm 0.4 \text{ s}^{-1}$ and a K_d of $17 \pm 3 \mu\text{M}$.

TABLE 3: Kinetic parameters of nucleotide incorporation into D-DNA catalyzed by truncated yPol η at 23°C.

dNTP	k_p (s ⁻¹)	K_d (μM)	k_p/K_d (μM ⁻¹ s ⁻¹)	Discrimination Factor ^a	Fidelity ^b
<i>Template dA (D-1)</i>					
dTTP	3.9 ± 0.2	15 ± 2	2.6×10^{-1}		
dATP	0.089 ± 0.005	80 ± 20	1.1×10^{-3}	230	4.3×10^{-3}
dCTP	0.43 ± 0.06	210 ± 60	2.0×10^{-3}	130	7.8×10^{-3}
dGTP	0.15 ± 0.01	80 ± 20	1.9×10^{-3}	140	7.2×10^{-3}
<i>Template dG (D-6)</i>					
dCTP	15.6 ± 0.3	11.2 ± 0.8	1.4		
dATP	0.071 ± 0.002	138 ± 9	5.1×10^{-4}	2700	3.7×10^{-4}
dGTP	0.116 ± 0.006	80 ± 10	1.5×10^{-3}	960	1.0×10^{-3}
dTTP	0.92 ± 0.07	330 ± 40	2.8×10^{-3}	500	2.0×10^{-3}
<i>Template dT (D-7)</i>					
dATP	6.9 ± 0.4	17 ± 3	4.1×10^{-1}		
dCTP	1.00 ± 0.04	210 ± 20	4.8×10^{-3}	85	1.2×10^{-2}
dGTP	0.55 ± 0.01	46 ± 3	1.2×10^{-2}	30	2.9×10^{-2}
dTTP	0.62 ± 0.02	280 ± 20	2.2×10^{-3}	180	5.4×10^{-3}
<i>Template dC (D-8)</i>					
dGTP	6.3 ± 0.1	6.8 ± 0.4	9.3×10^{-1}		
dATP	0.087 ± 0.003	90 ± 10	9.7×10^{-4}	960	1.0×10^{-3}
dCTP	0.127 ± 0.007	200 ± 30	6.4×10^{-4}	1500	6.9×10^{-4}
dTTP	1.39 ± 0.06	460 ± 40	3.0×10^{-3}	310	3.3×10^{-3}

^a Calculated as $(k_p/K_d)_{\text{correct}} / (k_p/K_d)_{\text{incorrect}}$.

^b Calculated as $(k_p/K_d)_{\text{incorrect}} / [(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

TABLE 4: Kinetic parameters for nucleotide incorporation onto blunt-end DNA catalyzed by truncated yeast Pol η at 23°C.

DNA (Terminal base pair)	dNTP	k_p (s $^{-1}$)	K_d (μ M)	k_p/K_d (μ M $^{-1}$ s $^{-1}$)	Efficiency Ratio ^a
BE1 (dT:dA)	dATP	0.026 \pm 0.002	1200 \pm 200	2.2 \times 10 $^{-5}$	—
	dPTP	1.27 \pm 0.08	60 \pm 10	2.1 \times 10 $^{-2}$	980
BE2 (dA:dT)	dATP	0.036 \pm 0.002	220 \pm 30	1.6 \times 10 $^{-4}$	—
	dPTP	0.68 \pm 0.03	23 \pm 3	3.0 \times 10 $^{-2}$	180
BE3 (dG:dC)	dATP	0.0087 \pm 0.0003	360 \pm 30	2.4 \times 10 $^{-5}$	—
	dPTP	0.22 \pm 0.01	9 \pm 2	2.4 \times 10 $^{-2}$	1000
BE4 (dC:dG)	dATP	0.032 \pm 0.001	930 \pm 70	3.4 \times 10 $^{-5}$	—
	dPTP	0.74 \pm 0.03	12 \pm 2	6.2 \times 10 $^{-2}$	1800

^aCalculated as $(k_p/K_d)_{\text{dPTP}}/(k_p/K_d)_{\text{dATP}}$.

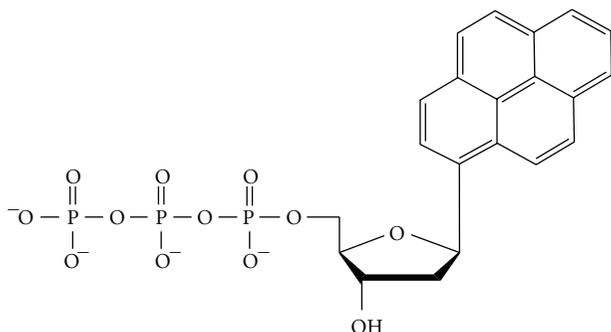


FIGURE 4: Chemical structure of a nonnatural nucleotide analog, dPTP.

incorporation catalyzed by Dpo4, another Y-family DNA polymerase, is dATP mainly due to its strong intrahelical base-stacking ability [26]. To further evaluate the role of base stacking, we first examined if truncated γ Pol η can catalyze template-independent nucleotide incorporation of dATP or dPTP (Figure 4) onto four palindromic, blunt-end DNA substrates (BE1, BE2, BE3, and BE4 in Table 1). The base of dPTP, a dNTP analog, has four conjugated benzene rings but possesses no hydrogen-bonding abilities. The DNA substrates possess all four possible terminal base pairs and each molecule of them can be bound by a single polymerase molecule. Our radioactive experiments showed that truncated γ Pol η was able to incorporate dATP and dPTP (data not shown). Then, we individually measured the kinetic parameters for dATP and dPTP incorporation under single-turnover reaction conditions (Table 4). Interestingly, the apparent K_d values of dATP were 3- to 5-fold smaller with a purine than those with a pyrimidine on the primer's 3'-base, indicating that base stacking is also important for the binding of dATP to the binary complex of γ Pol η -blunt-end DNA. This base-stacking effect is more dramatic for dPTP incorporation onto blunt-end DNA because the apparent K_d values of dPTP are 10- to 80-fold tighter than dATP incorporation onto the same blunt-end DNA substrate (Table 4). Thus, the binding free energy difference between dATP and dPTP is 1.4 to 2.6 kcal/mol. Previously, we have obtained a comparable binding free energy difference of 2.3 kcal/mol for similar blunt-end dATP and dPTP incorporation at 37°C

catalyzed by Dpo4 [26]. Although neither dATP nor dPTP forms any hydrogen bonds with a template base when bound by γ Pol η -blunt-end DNA, the bases of these two nucleotides should have different base-stacking interactions with a terminal base pair of a blunt-end DNA substrate considering that a dangling pyrene base (1.7 kcal/mol) has previously been found to possess a higher base-stacking free energy than a dangling adenosine (1.0 kcal/mol) [33]. However, the base-stacking free energy difference (0.7 kcal/mol) between pyrene and adenosine is smaller than the aforementioned binding free energy difference (1.4–2.6 kcal/mol) between dPTP and dATP. Thus, other sources likely contribute to the tighter binding of dPTP over dATP. One possible source is favorable van der Waals interactions between pyrene and active site residues of truncated γ Pol η . In addition, the base-stacking effect and van der Waals interactions may stabilize the ternary complex of γ Pol η -blunt-end DNA-nucleotide and facilitate catalysis, leading to much higher k_p values with dPTP than those with dATP (Table 4). Due to the differences in k_p and apparent K_d , the substrate specificity values of dPTP are 100- to 1,000-fold higher than those of dATP with blunt-end DNA (Table 4) and 10- to 100-fold higher than mismatched dATP with regular DNA (Table 3).

3.5. Base Substitution Fidelity of Full-Length γ Pol η . The base substitution fidelities of full-length and truncated γ Pol η may differ because the C-terminal, nonenzymatic regions may alter the polymerization fidelity. For example, the proline-rich domain of human DNA polymerase λ has been shown to upregulate the polymerase fidelity up to 100-fold [34]. To determine if the C-terminus of γ Pol η influences polymerization fidelity, we measured the pre-steady-state kinetic parameters for dNTP incorporation into D-1 DNA (template dA) catalyzed by full-length γ Pol η (Table 5). The fidelity was calculated to be in the range of $(1.4 \text{ to } 2.6) \times 10^{-3}$ for full-length γ Pol η (Table 5). Relative to the fidelity of truncated γ Pol η with D-1 (Table 3), full-length γ Pol η has a 3-fold higher fidelity. Therefore, the C-terminus of γ Pol η slightly affects the base substitution fidelity. Moreover, truncated γ Pol η discriminated between a correct and incorrect dNTP by \sim 30-fold on average based on the k_p difference while the discrimination for full-length γ Pol η was \sim 170-fold on average for incorporation into D-1 DNA (Tables 3 and 5). The incorporation rate constant for correct dTTP was

TABLE 5: Kinetic parameters of nucleotide incorporation into D-1 DNA catalyzed by full-length γ Pol η at 23°C.

dNTP	k_p (s ⁻¹)	K_d (μ M)	k_p/K_d (μ M ⁻¹ s ⁻¹)	Discrimination Factor ^a	Fidelity ^b
<i>Template dA (D-1)</i>					
dTTP	4.2 \pm 0.5	40 \pm 10	1.1 \times 10 ⁻¹		
dATP	0.0235 \pm 0.0003	156 \pm 7	1.5 \times 10 ⁻⁴	700	1.4 \times 10 ⁻³
dCTP	0.019 \pm 0.001	70 \pm 10	2.7 \times 10 ⁻⁴	390	2.6 \times 10 ⁻³
dGTP	0.043 \pm 0.003	170 \pm 40	2.5 \times 10 ⁻⁴	420	2.4 \times 10 ⁻³

^aCalculated as $(k_p/K_d)_{\text{correct}}/(k_p/K_d)_{\text{incorrect}}$.

^bCalculated as $(k_p/K_d)_{\text{incorrect}}/[(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$.

~ 4 s⁻¹ for both γ Pol η enzymes, but the misincorporation rate was 3- to 23-fold faster for truncated γ Pol η . This rate enhancement for truncated γ Pol η is partially offset by a greater discrimination at the apparent ground-state binding level so that the fidelity of truncated γ Pol η was only 3-fold lower than that of full-length γ Pol η .

3.6. Effect of the Nonenzymatic C-Terminus of γ Pol η on Its Polymerase Activity. Our above studies demonstrated that the C-terminus of γ Pol η enhances this enzyme's DNA binding affinity and base substitution fidelity by 2- and 3-fold, respectively. These results suggest that the nonenzymatic, C-terminal region of γ Pol η (Figure 1) has a mild impact on the N-terminal polymerase domain and its activity. This conclusion is inconsistent with previous studies which have qualitatively demonstrated that mutations or deletions in the UBZ domain or PIP motif do not affect polymerase activity [35–37]. However, these reported qualitative assays are not sufficiently sensitive to detect the small perturbation on polymerase activity as described in this paper. The presence of the C-terminal 119 residues of γ Pol η may either interact with DNA, slightly alter the conformation of the polymerase domain, or both (see above discussion), thereby enhancing its DNA binding affinity and polymerase fidelity.

3.7. Kinetic Comparison among Y-Family DNA Polymerases. The fidelity of several Y-family DNA polymerases synthesizing undamaged DNA has been determined by employing steady-state [38–48], pre-steady-state [28, 30, 49–53], or M13-based mutation assays [39, 41, 42, 45, 54, 55]. From these studies, the fidelity ranges from 10⁰ to 10⁻⁴. Under steady-state reaction conditions, the base substitution fidelity of γ Pol η and human Pol η has been measured to be in the range from 10⁻² to 10⁻⁴ and 10⁻² to 10⁻³, respectively [38, 40], which is similar to our pre-steady-state kinetic results. Consistently, Pol η displays the highest substrate specificity for the dCTP:dG base pair under both steady-state and pre-steady-state reaction conditions (Table 3 and unpublished data, Brown and Suo) [38, 40]. This may seem surprising, since Pol η participates in the efficient bypass of UV-induced DNA damage such as a *cis-syn* thymine-thymine dimer (i.e., a dATP:dT base pair) [15–20, 56, 57]. However, Pol η has also been shown to be efficient at bypassing guanine-specific damage such as 8-oxo-7,8-dihydro-dG [58, 59], 1,2-*cis*-diammineplatinum(II)-d(GpG) intrastrand cross-links [60–63], and various N2-dG lesions [64, 65].

Among the four eukaryotic Y-family DNA polymerases (i.e., Pol η , DNA polymerase κ , DNA polymerase ι (Pol ι), and Rev1), Rev1 exhibits low fidelity on undamaged DNA due to its strong preference for inserting dCTP [46, 52] while Pol ι has an unusual preference for dGTP:dT mispairs over dATP:dT due to Hoogsteen base pair formation [51, 69]. Interestingly, the lowest fidelity base pair for truncated γ Pol η was dGTP:dT (Table 3). This observation likely results from the formation of a wobble base pair. The two hydrogen bonds established in the wobble base pair may enhance the catalytic efficiency of γ Pol η since hydrogen bonding is important for the efficiency and accuracy of γ Pol η [70]. Also noteworthy, the truncated versions of eukaryotic Y-family DNA polymerases have been used for many biochemical studies in literature. Based on our quantitative kinetic analysis of γ Pol η , these results suggested the nonenzymatic regions of Y-family DNA polymerases do not alter the polymerase activity significantly.

3.8. Fidelity Comparison among Various DNA Polymerase Families. As a Y-family DNA polymerase, γ Pol η displays low fidelity on undamaged DNA (Tables 3 and 5) [38]. In contrast, replicative DNA polymerases in the A- and B-families have a polymerization fidelity that is 1–3 orders of magnitude greater than the Y-family DNA polymerases (Table 6). DNA polymerases with higher fidelity are more proficient at using the ground-state binding affinity to discriminate between a correct and incorrect dNTP. The Y-family DNA polymerases provide little to no discrimination based on the K_d difference while replicative DNA polymerases discriminate up to almost three orders of magnitude. This lack of selection in the ground state by the Y-family DNA polymerases may be due to the relatively loose and solvent-accessible active site which has minimal contacts with the nascent base pair [11, 21, 71]. Moreover, nucleotide selection by the Y-family DNA polymerases in the ground state may be mainly governed by Watson-Crick base pairing, since the calculated $\Delta\Delta G$ values (0.95–1.7 kcal/mol) are similar to the free energy differences between correct and incorrect base pairs (0.3–1.0 kcal/mol at 37°C) at the primer terminus based on DNA melting studies (Table 6) [72]. However, with $\Delta\Delta G$ values ≥ 3.0 kcal/mol, the replicative DNA polymerases harness the additional 2.0 kcal/mol of energy from other sources such as a tight active site or close contacts with the nascent base pair. One common fidelity checkpoint among DNA polymerases is the varying rate differences between a matched and mismatched

TABLE 6: Comparison of base substitution fidelity for various DNA polymerases.

Polymerase	Polymerase Family	Fidelity ^a	K_d Difference ^b	k_p Difference ^b	$\Delta\Delta G$ (kcal/mol) ^c
Truncated γ Pol η ^d	Y	3.7×10^{-4} to 2.9×10^{-2}	3 to 68	5 to 220	1.6
Dpo4 ^e	Y	1.5×10^{-4} to 3.2×10^{-3}	1 to 18	240 to 1700	0.95
rPol β ^f	X	1.1×10^{-5} to 5.9×10^{-4}	35 to 342	28 to 708	3.0
PolB1 exo- ^g	B	3.5×10^{-6} to 1.2×10^{-4}	109 to 918	4 to 589	3.7
hPol γ ^h	A	4.6×10^{-7} to 2.9×10^{-4}	42 to 900	39 to 12000	3.4

^aCalculated as $(k_p/K_d)_{\text{incorrect}} / [(k_p/K_d)_{\text{correct}} + (k_p/K_d)_{\text{incorrect}}]$. ^bCalculated as defined in equation (8). ^cCalculated using equation (7). ^dAt 23°C (this work).

^eAt 37°C [50]. ^fAt 37°C [66]. ^gAt 37°C, excluding the fidelity contribution from the 3' → 5' exonuclease activity [67]. ^hAt 37°C, excluding the fidelity contribution from the 3' → 5' exonuclease activity [68].

base pair. These large differences may correspond to different rate-limiting steps (e.g., protein conformational change, or phosphodiester bond formation) during nucleotide incorporation [9, 30, 71]. For γ Pol η , kinetic data suggest that correct and incorrect dNTPs are limited by a conformational step preceding chemistry, although, additional studies are needed to confirm these results [28].

4. Conclusions

This work presents the mechanistic basis of the base substitution fidelity of γ Pol η on undamaged DNA, which examined all possible dNTP:dN base pair combinations for the first time. γ Pol η discriminates against incorrect nucleotides at both the ground-state nucleotide binding and incorporation steps. Furthermore, base stacking contributes to tighter binding for a misincorporation. Finally, the 119 residues at the C-terminus have a mild impact on the kinetic mechanism of γ Pol η .

Abbreviations

BSA: Bovine serum albumin
dNTP: 2'-deoxynucleoside 5'-triphosphate
Dpo4: *Sulfolobus solfataricus* P2 DNA polymerase IV
dPTP: Pyrene 5'-triphosphate
EMSA: Electrophoretic mobility shift assay
HPol γ : Human mitochondrial DNA polymerase gamma
PCNA: Proliferating cell nuclear antigen
PIP: PCNA-interacting peptide
PolB1: Exonuclease-deficient DNA polymerase B1 from *sulfolobus solfataricus* P2
Pol ι : DNA polymerase iota
rPol β : Rat DNA polymerase beta
TBE Tris/boric acid/EDTA
UBZ: Ubiquitin-binding zinc finger
YPol η : *Saccharomyces cerevisiae* DNA polymerase eta.

Acknowledgments

This work was supported by the National Institutes of Health Grants CA040463 (to Zucui Suo and John-Stephen Taylor) and GM032431 (to Peter M.J. Burgers). Jessica A. Brown was supported by an American Heart Association Pre-doctoral Fellowship (Grant 0815382D). Shanen M. Sherrer

was supported by a Predoctoral Fellowship from the National Institutes of Health Chemistry-Biology Interface Program at The Ohio State University (Grant 5 T32 GM008512-13). Brown and Zhang contributed equally to this work.

References

- [1] J. D. Fowler and Z. Suo, "Biochemical, structural, and physiological characterization of terminal deoxynucleotidyl transferase," *Chemical Reviews*, vol. 106, no. 6, pp. 2092–2110, 2006.
- [2] M. Garcia-Diaz and K. Bebenek, "Multiple functions of DNA polymerases," *Critical Reviews in Plant Sciences*, vol. 26, no. 2, pp. 105–122, 2007.
- [3] P. M. J. Burgers, E. V. Koonin, E. Bruford et al., "Eukaryotic DNA polymerases: proposal for a revised nomenclature," *Journal of Biological Chemistry*, vol. 276, no. 47, pp. 43487–43490, 2001.
- [4] T. A. Steitz, "DNA- and RNA-dependent DNA polymerases," *Current Opinion in Structural Biology*, vol. 3, no. 1, pp. 31–38, 1993.
- [5] J. D. Fowler, J. A. Brown, M. Kvaratskhelia, and Z. Suo, "Probing conformational changes of human DNA polymerase lambda using mass spectrometry-based protein footprinting," *Journal of Molecular Biology*, vol. 390, no. 3, pp. 368–379, 2009.
- [6] C. Castro, E. D. Smidansky, J. J. Arnold et al., "Nucleic acid polymerases use a general acid for nucleotidyl transfer," *Nature Structural and Molecular Biology*, vol. 16, no. 2, pp. 212–218, 2009.
- [7] A. A. Johnson, K. A. Fiala, and Z. Suo, "DNA polymerases and their interactions with DNA and nucleotides," in *Nucleoside Triphosphates and Their Analogs: Chemistry, Biotechnology, and Biological Applications*, M. M. Vaghefi, Ed., pp. 133–168, Taylor & Francis, Boca Raton, Fla, USA, 2005.
- [8] T. A. Steitz, "DNA polymerases: structural diversity and common mechanisms," *Journal of Biological Chemistry*, vol. 274, no. 25, pp. 17395–17398, 1999.
- [9] C. M. Joyce and S. J. Benkovic, "DNA polymerase fidelity: kinetics, structure, and checkpoints," *Biochemistry*, vol. 43, no. 45, pp. 14317–14324, 2004.
- [10] S. D. McCulloch and T. A. Kunkel, "The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases," *Cell Research*, vol. 18, no. 1, pp. 148–161, 2008.
- [11] H. Ling, F. Boudsocq, R. Woodgate, and W. Yang, "Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication," *Cell*, vol. 107, no. 1, pp. 91–102, 2001.

- [12] S. Mizukami, T. W. Kim, S. A. Helquist, and E. T. Kool, "Varying DNA base-pair size in subangstrom increments: evidence for a loose, not large, active site in low-fidelity Dpo4 polymerase," *Biochemistry*, vol. 45, no. 9, pp. 2772–2778, 2006.
- [13] C. Xu, B. A. Maxwell, J. A. Brown, L. Zhang, and Z. Suo, "Global conformational dynamics of a Y-family DNA polymerase during catalysis," *PLoS Biology*, vol. 7, Article ID e1000225, 2009.
- [14] H. Ohmori, E. C. Friedberg, R. P. P. Fuchs et al., "The Y-family of DNA Polymerases," *Molecular Cell*, vol. 8, no. 1, pp. 7–8, 2001.
- [15] J. P. McDonald, A. S. Levine, and R. Woodgate, "The *Saccharomyces cerevisiae* RAD30 gene, a homologue of *Escherichia coli* dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism," *Genetics*, vol. 147, no. 4, pp. 1557–1568, 1997.
- [16] R. E. Johnson, S. Prakash, and L. Prakash, "Requirement of DNA polymerase activity of yeast Rad30 protein for its biological function," *Journal of Biological Chemistry*, vol. 274, no. 23, pp. 15975–15977, 1999.
- [17] A. A. Roush, M. Suarez, E. C. Friedberg, M. Radman, and W. Siede, "Deletion of the *Saccharomyces cerevisiae* gene RAD30 encoding an *Escherichia coli* DinB homolog confers UV radiation sensitivity and altered mutability," *Molecular and General Genetics*, vol. 257, no. 6, pp. 686–692, 1998.
- [18] R. E. Johnson, S. Prakash, and L. Prakash, "Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol η ," *Science*, vol. 283, no. 5404, pp. 1001–1004, 1999.
- [19] S.-L. Yu, R. E. Johnson, S. Prakash, and L. Prakash, "Requirement of DNA polymerase η for error-free bypass of UV-induced CC and TC photoproducts," *Molecular and Cellular Biology*, vol. 21, no. 1, pp. 185–188, 2001.
- [20] C. Masutani, R. Kusumoto, A. Yamada et al., "The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η ," *Nature*, vol. 399, no. 6737, pp. 700–704, 1999.
- [21] J. Trincão, R. E. Johnson, C. R. Escalante, S. Prakash, L. Prakash, and A. K. Aggarwal, "Structure of the catalytic core of *S. cerevisiae* DNA polymerase η : implications for translesion DNA synthesis," *Molecular Cell*, vol. 8, no. 2, pp. 417–426, 2001.
- [22] A. Alt, K. Lammens, C. Chiochini et al., "Bypass of DNA lesions generated during anticancer treatment with cisplatin by DNA polymerase η ," *Science*, vol. 318, no. 5852, pp. 967–970, 2007.
- [23] K. A. Fiala and Z. Suo, "Mechanism of DNA polymerization catalyzed by *Sulfolobus solfataricus* P2 DNA polymerase IV," *Biochemistry*, vol. 43, no. 7, pp. 2116–2125, 2004.
- [24] V. J. Cannistraro and J.-S. Taylor, "DNA-thumb interactions and processivity of T7 DNA polymerase in comparison to yeast polymerase η ," *Journal of Biological Chemistry*, vol. 279, no. 18, pp. 18288–18295, 2004.
- [25] P. Garg, C. M. Stith, J. Majka, and P. M. J. Burgers, "Proliferating cell nuclear antigen promotes translesion synthesis by DNA polymerase ζ ," *Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23446–23450, 2005.
- [26] K. A. Fiala, J. A. Brown, H. Ling et al., "Mechanism of template-independent nucleotide incorporation catalyzed by a template-dependent DNA polymerase," *Journal of Molecular Biology*, vol. 365, no. 3, pp. 590–602, 2007.
- [27] K. A. Fiala, C. D. Hypes, and Z. Suo, "Mechanism of abasic lesion bypass catalyzed by a Y-family DNA polymerase," *Journal of Biological Chemistry*, vol. 282, no. 11, pp. 8188–8198, 2007.
- [28] M. T. Washington, L. Prakash, and S. Prakash, "Yeast DNA polymerase η utilizes an induced-fit mechanism of nucleotide incorporation," *Cell*, vol. 107, no. 7, pp. 917–927, 2001.
- [29] J. A. Brown and Z. Suo, "Elucidating the kinetic mechanism of DNA polymerization catalyzed by *Sulfolobus solfataricus* P2 DNA polymerase B1," *Biochemistry*, vol. 48, no. 31, pp. 7502–7511, 2009.
- [30] K. A. Fiala, S. M. Sherrer, J. A. Brown, and Z. Suo, "Mechanistic consequences of temperature on DNA polymerization catalyzed by a Y-family DNA polymerase," *Nucleic Acids Research*, vol. 36, no. 6, pp. 1990–2001, 2008.
- [31] Y.-C. Tsai and K. A. Johnson, "A new paradigm for DNA polymerase specificity," *Biochemistry*, vol. 45, no. 32, pp. 9675–9687, 2006.
- [32] K. A. Johnson, "Transient-state kinetic analysis of enzyme reaction pathways," *Enzymes*, vol. 20, pp. 1–61, 1992.
- [33] K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J. Sheils, D. C. Tahmassebi, and E. T. Kool, "Factors contributing to aromatic stacking in water: evaluation in the context of DNA," *Journal of the American Chemical Society*, vol. 122, no. 10, pp. 2213–2222, 2000.
- [34] K. A. Fiala, W. W. Duym, J. Zhang, and Z. Suo, "Up-regulation of the fidelity of human DNA polymerase λ by its non-enzymatic proline-rich domain," *Journal of Biological Chemistry*, vol. 281, no. 28, pp. 19038–19044, 2006.
- [35] L. Haracska, C. M. Kondratick, I. Unk, S. Prakash, and L. Prakash, "Interaction with PCNA is essential for yeast DNA polymerase η function," *Molecular Cell*, vol. 8, no. 2, pp. 407–415, 2001.
- [36] N. Acharya, A. Brahma, L. Haracska, L. Prakash, and S. Prakash, "Mutations in the ubiquitin binding UBZ motif of DNA polymerase η do not impair its function in translesion synthesis during replication," *Molecular and Cellular Biology*, vol. 27, no. 20, pp. 7266–7272, 2007.
- [37] Z. Zhuang, R. E. Johnson, L. Haracska, L. Prakash, S. Prakash, and S. J. Benkovic, "Regulation of polymerase exchange between Pol η and Pol δ by monoubiquitination of PCNA and the movement of DNA polymerase holoenzyme," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 14, pp. 5361–5366, 2008.
- [38] M. T. Washington, R. E. Johnson, S. Prakash, and L. Prakash, "Fidelity and processivity of *Saccharomyces cerevisiae* DNA polymerase η ," *Journal of Biological Chemistry*, vol. 274, no. 52, pp. 36835–36838, 1999.
- [39] T. Matsuda, K. Bebenek, C. Masutani, F. Hanaoka, and T. A. Kunkel, "Low fidelity DNA synthesis by human DNA polymerase- η ," *Nature*, vol. 404, no. 6781, pp. 1011–1013, 2000.
- [40] R. E. Johnson, M. T. Washington, S. Prakash, and L. Prakash, "Fidelity of human DNA polymerase η ," *Journal of Biological Chemistry*, vol. 275, no. 11, pp. 7447–7450, 2000.
- [41] Y. Zhang, F. Yuan, H. Xin et al., "Human DNA polymerase κ synthesizes DNA with extraordinarily low fidelity," *Nucleic Acids Research*, vol. 28, no. 21, pp. 4147–4156, 2000.
- [42] E. Ohashi, K. Bebenek, T. Matsuda et al., "Fidelity and processivity of DNA synthesis by DNA polymerase κ , the product of the human DINB1 gene," *Journal of Biological Chemistry*, vol. 275, no. 50, pp. 39678–39684, 2000.
- [43] A. Tissier, J. P. McDonald, E. G. Frank, and R. Woodgate, "pol ι , a remarkably error-prone human DNA polymerase," *Genes and Development*, vol. 14, no. 13, pp. 1642–1650, 2000.
- [44] F. Boudsocq, S. Iwai, F. Hanaoka, and R. Woodgate, "*Sulfolobus solfataricus* P2 DNA polymerase IV (Dp04): an

- archaeal DinB-like DNA polymerase with lesion-bypass properties akin to eukaryotic pol η ,” *Nucleic Acids Research*, vol. 29, no. 22, pp. 4607–4616, 2001.
- [45] R. J. Kokoska, K. Bebenek, F. Boudsocq, R. Woodgate, and T. A. Kunkel, “Low fidelity DNA synthesis by a Y family DNA polymerase due to misalignment in the active site,” *Journal of Biological Chemistry*, vol. 277, no. 22, pp. 19633–19638, 2002.
- [46] L. Haracska, S. Prakash, and L. Prakash, “Yeast Rev1 protein is a G template-specific DNA polymerase,” *Journal of Biological Chemistry*, vol. 277, no. 18, pp. 15546–15551, 2002.
- [47] A. Vaisman, H. Ling, R. Woodgate, and W. Yang, “Fidelity of Dpo4: effect of metal ions, nucleotide selection and pyrophosphorolysis,” *EMBO Journal*, vol. 24, no. 17, pp. 2957–2967, 2005.
- [48] Y. Zhang, X. Wu, O. Rechkoblit, N. E. Geacintov, J.-S. Taylor, and Z. Wang, “Response of human REV1 to different DNA damage: preferential dCMP insertion opposite the lesion,” *Nucleic Acids Research*, vol. 30, no. 7, pp. 1630–1638, 2002.
- [49] M. T. Washington, R. E. Johnson, L. Prakash, and S. Prakash, “The mechanism of nucleotide incorporation by human DNA polymerase η differs from that of the yeast enzyme,” *Molecular and Cellular Biology*, vol. 23, no. 22, pp. 8316–8322, 2003.
- [50] K. A. Fiala and Z. Suo, “Pre-steady-state kinetic studies of the fidelity of *Sulfolobus solfataricus* P2 DNA polymerase IV,” *Biochemistry*, vol. 43, no. 7, pp. 2106–2115, 2004.
- [51] M. T. Washington, R. E. Johnson, L. Prakash, and S. Prakash, “Human DNA polymerase ι utilizes different nucleotide incorporation mechanisms dependent upon the template base,” *Molecular and Cellular Biology*, vol. 24, no. 2, pp. 936–943, 2004.
- [52] C. A. Howell, S. Prakash, and M. T. Washington, “Pre-steady-state kinetic studies of protein-template-directed nucleotide incorporation by the yeast Rev1 protein,” *Biochemistry*, vol. 46, no. 46, pp. 13451–13459, 2007.
- [53] S. M. Sherrer, J. A. Brown, L. R. Pack et al., “Mechanistic studies of the bypass of a bulky single-base lesion catalyzed by a Y-family DNA polymerase,” *Journal of Biological Chemistry*, vol. 284, no. 10, pp. 6379–6388, 2009.
- [54] O. Potapova, N. D. F. Grindley, and C. M. Joyce, “The mutational specificity of the Dbh lesion bypass polymerase and its implications,” *Journal of Biological Chemistry*, vol. 277, no. 31, pp. 28157–28166, 2002.
- [55] F. Boudsocq, R. J. Kokoska, B. B. Plosky et al., “Investigating the role of the little finger domain of Y-family DNA polymerases in low fidelity synthesis and translesion replication,” *Journal of Biological Chemistry*, vol. 279, no. 31, pp. 32932–32940, 2004.
- [56] R. E. Johnson, C. M. Kondratick, S. Prakash, and L. Prakash, “hRAD30 mutations in the variant form of xeroderma pigmentosum,” *Science*, vol. 285, no. 5425, pp. 263–265, 1999.
- [57] M. T. Washington, L. Prakash, and S. Prakash, “Mechanism of nucleotide incorporation opposite a thymine-thymine dimer by yeast DNA polymerase η ,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 21, pp. 12093–12098, 2003.
- [58] L. Haracska, S.-L. Yu, R. E. Johnson, L. Prakash, and S. Prakash, “Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase η ,” *Nature Genetics*, vol. 25, no. 4, pp. 458–461, 2000.
- [59] K. D. Carlson and M. T. Washington, “Mechanism of efficient and accurate nucleotide incorporation opposite 7,8-dihydro-8-oxoguanine by *Saccharomyces cerevisiae* DNA polymerase η ,” *Molecular and Cellular Biology*, vol. 25, no. 6, pp. 2169–2176, 2005.
- [60] A. Vaisman, C. Masutani, F. Hanaoka, and S. G. Chaney, “Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase η ,” *Biochemistry*, vol. 39, no. 16, pp. 4575–4580, 2000.
- [61] C. Masutani, R. Kusumoto, S. Iwai, and F. Hanaoka, “Mechanisms of accurate translesion synthesis by human DNA polymerase η ,” *EMBO Journal*, vol. 19, no. 12, pp. 3100–3109, 2000.
- [62] E. Bassett, A. Vaisman, K. A. Tropea et al., “Frameshifts and deletions during in vitro translesion synthesis past Pt-DNA adducts by DNA polymerases β and η ,” *DNA Repair*, vol. 1, no. 12, pp. 1003–1016, 2002.
- [63] E. Bassett, A. Vaisman, J. M. Havener, C. Masutani, F. Hanaoka, and S. G. Chaney, “Efficiency of extension of mismatched primer termini across from cisplatin and oxaliplatin adducts by human DNA polymerases β and η in vitro,” *Biochemistry*, vol. 42, no. 48, pp. 14197–14206, 2003.
- [64] J.-Y. Choi and F. P. Guengerich, “Adduct size limits efficient and error-free bypass across bulky N 2-guanine DNA lesions by human DNA polymerase η ,” *Journal of Molecular Biology*, vol. 352, no. 1, pp. 72–90, 2005.
- [65] J.-Y. Choi, H. Zang, K. C. Angel et al., “Translesion synthesis across 1,N2-ethenoguanine by human DNA polymerases,” *Chemical Research in Toxicology*, vol. 19, no. 6, pp. 879–886, 2006.
- [66] J. Ahn, V. S. Kraynov, X. Zhong, B. G. Werneburg, and M.-D. Tsai, “DNA polymerase β : effects of gapped DNA substrates on dNTP specificity, fidelity, processivity and conformational changes,” *Biochemical Journal*, vol. 331, part 1, pp. 79–87, 1998.
- [67] L. Zhang, J. A. Brown, S. A. Newmister, and Z. Suo, “Polymerization fidelity of a replicative DNA polymerase from the hyperthermophilic archaeon *Sulfolobus solfataricus* P2,” *Biochemistry*, vol. 48, no. 31, pp. 7492–7501, 2009.
- [68] H. R. Lee and K. A. Johnson, “Fidelity of the human mitochondrial DNA polymerase,” *Journal of Biological Chemistry*, vol. 281, no. 47, pp. 36236–36240, 2006.
- [69] D. T. Nair, R. E. Johnson, S. Prakash, L. Prakash, and A. K. Aggarwal, “Replication by human DNA polymerase- ι occurs by Hoogsteen base-pairing,” *Nature*, vol. 430, no. 6997, pp. 377–380, 2004.
- [70] M. T. Washington, S. A. Helquist, E. T. Kool, L. Prakash, and S. Prakash, “Requirement of Watson-Crick hydrogen bonding for DNA synthesis by yeast DNA polymerase η ,” *Molecular and Cellular Biology*, vol. 23, no. 14, pp. 5107–5112, 2003.
- [71] J. H. Wong, K. A. Fiala, Z. Suo, and H. Ling, “Snapshots of a Y-family DNA polymerase in replication: substrate-induced conformational transitions and implications for fidelity of Dpo4,” *Journal of Molecular Biology*, vol. 379, no. 2, pp. 317–330, 2008.
- [72] J. Petruska, M. F. Goodman, M. S. Boosalis, L. C. Sowers, C. Cheong, and I. Tinoco Jr., “Comparison between DNA melting thermodynamics and DNA polymerase fidelity,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 17, pp. 6252–6256, 1988.