**ABSTRACT**

Most eukaryotic DNA replication is performed by A- and B-family DNA polymerases which possess a faithful polymerase activity that preferentially incorporates correct over incorrect nucleotides. Additionally, many replicative polymerases have an efficient 3′→5′ exonuclease activity that excises misincorporated nucleotides. Together, these activities contribute to overall low polymerase error frequency (one error per 10^6–10^8 incorporations) and support faithful eukaryotic genome replication. Eukaryotic DNA polymerase ε (Pole) is one of three main replicative DNA polymerases for nuclear genomic replication and is responsible for leading strand synthesis. Here, we employed pre-steady-state kinetic methods to determine the overall fidelity of human Pole (hPole) by measuring the individual contributions of its polymerase and 3′→5′ exonuclease activities. The polymerase activity of hPole has a high base substitution fidelity (10^{-4}–10^{-7}) resulting from large decreases in both nucleotide incorporation rate constants and ground-state binding affinities for incorrect relative to correct nucleotides. The 3′→5′ exonuclease activity of hPole further enhances polymerization fidelity by an unprecedented 3.5 x 10^2 to 1.2 x 10^4-fold. The resulting overall fidelity of hPole (10^{-6}–10^{-11}) justifies hPole to be a primary enzyme to replicate human nuclear genome (0.1–1.0 error per round). Consistently, somatic mutations in hPole, which decrease its exonuclease activity, are connected with mutator phenotypes and cancer formation.

**INTRODUCTION**

DNA polymerases (Pols) perform a wide variety of biological functions that are critical to the proliferation and maintenance of genomic DNA including DNA replication, DNA repair and translesion DNA synthesis. DNA polymerases are organized into seven families (A, B, C, D, X, Y and RT) and they share a structurally similar polymerase core consisting of finger, palm and thumb domains that together form a right-hand geometry (1–3). Besides the conserved polymerase core, DNA polymerases from different families possess additional domains and structural features that broaden their functional diversity in vitro. For instance, many replicative A- and B-family DNA polymerases possess a 3′→5′ exonuclease domain containing conserved carboxylate residues that are required for coordinating divalent metal ions to catalyze the excision of mismatched bases from the primer 3′ terminus (3–7).

Highly accurate DNA synthesis is critical for eukaryotic genome replication and stability. To ensure that DNA is faithfully copied from generation to generation, cells employ high-fidelity DNA polymerases that make only a single error per 10^6–10^8 nucleotide incorporation events (8–11). Kinetically, the polymerase active site alone in a replicative DNA polymerase has been found to exhibit a nucleotide selectivity of 10^2–10^7 (10–14). It was originally hypothesized that the amplification of free energy differences between correct and incorrect nucleotide incorporation by DNA polymerases was sufficient to account for the fidelity of DNA replication (15). More recently, the measured energetic difference between correct and incorrect nucleotide incorporation by three DNA polymerases account for most of the high fidelity displayed by these enzymes (16). Overall, nucleotide selection by DNA polymerases is guided by a wide variety of factors, such as base stacking (17), nucleotide desolvation (18), induced-fit conformational changes (14) and shape complementarity (17). In addition to the contributions of these factors to DNA polymerase fidelity, the 3′→5′ proofreading activity found in
most A- and B-family DNA polymerases further improves the fidelity of DNA replication by as much as 200-fold (11,19,20).

In eukaryotes, three replicative DNA polymerases from the B-family, Polε, Polδ and Polκ, are responsible for the majority of DNA replication (21). Human Polε (hPolε) is a heterotetramer, consisting of a catalytic subunit, p261, as well as three smaller subunits: p59, p12 and p17 (22). Though the structure of hPolε remains elusive, the crystal structure of the truncated catalytic subunit of yeast Polε (yPolε) was recently solved and shows the canonical right-hand configuration consisting of finger, thumb and palm domains in addition to an N-terminal domain and a 3′→5′ exonuclease domain. Surprisingly, the palm domain of yPolε was found to contain additional structural elements, including a previously unidentified ‘P domain’ which may play a role in aiding processive DNA synthesis catalyzed by Polε (23,24).

Genetic studies have shown that Polε is primarily responsible for synthesizing the leading strand during DNA replication (25–28). To serve this role effectively, Polε must be able to synthesize DNA efficiently and accurately. Recently, our lab utilized pre-steady-state kinetics to elucidate a minimal kinetic mechanism of correct nucleotide incorporation catalyzed by an exonuclease-deficient version of the N-terminal fragment (residues 1–1189) of the catalytic subunit p261 of hPolε (hPolε exo-) (29). Our studies reveal that hPolε inserts the correct nucleotide via an induced-fit mechanism and the rate-determining step is a protein conformational change step that occurs prior to phosphodiester bond formation. The proposed kinetic mechanism has been observed in most kinetically characterized DNA polymerases (8,30–36). For hPolε exo-, forward mutation assays estimated that it has a base substitution fidelity of 10−5, which is similar to the background of the assays and thus the error rate may even be overestimated (37). However, the overall fidelity of hPolε, as a function of its two enzymatic functions, has not yet been determined through pre-steady-state kinetic methods. In this paper, we determined the base substitution fidelity of hPolε exo- using pre-steady-state kinetic methods. Moreover, we investigated the contributions of mismatch extension and exonuclease activity to the overall fidelity of the wild-type, exonuclease-proficient N-terminal fragment of p261 of hPolε (hPolε exo+).

**MATERIALS AND METHODS**

**Materials**

The chemicals used for experiments were purchased from the following sources: [γ-32P]ATP from Perkin-Elmer Life Sciences (Boston, MA, USA); Optokinase from USB (Cleveland, OH, USA) and dNTPs from Bioline (Taunton, MA, USA). Both the wild-type (hPolε exo+) and the exonuclease-deficient triple mutant (D275A/E277A/D368A, hPolε exo-) forms of the truncated hPolε catalytic subunit were overexpressed and purified as described previously (29).

**DNA substrates**

The DNA substrates listed in Table 1 were purchased from Integrated DNA Technologies, Inc. (Coralville, IA, USA) and purified as described previously (38). The 21- and 22-mer primer strands were 5′-radiolabeled by incubation with [γ-32P]ATP and Optokinase for 3 h at 37°C, and then purified from free [γ-32P]ATP by passing through a Bio-Spin 6 column (Bio-Rad). The 5′-radiolabeled primers were then annealed to the 41-mer templates by incubating the primer with a 1.15-fold excess of template at 95°C for 5 min before cooling slowly to room temperature over several hours.

**Polymerase and exonuclease single-turnover assays**

All assays using hPolε exo- or hPolε exo+ were performed at 20°C in reaction buffer E (50 mM Tris-ΟAc, pH 7.4 at 20°C, 8 mM Mg(OAc)2, 1 mM DTT, 10% glycerol, 0.1 mg/ml bovine serum albumin and 0.1 mM ethylenediaminetetraacetic acid (EDTA)). Fast reactions were carried out using a rapid chemical quench-flow apparatus (KinTek). Notably, all reactions were performed at 20°C since the rate constant for correct nucleotide incorporation at 37°C was too fast (kₚ > 500 s⁻¹) to be measured accurately by using the rapid chemical quench-flow apparatus. For polymerization single-turnover assays, a pre-incubated solution of hPolε exo- (260 nM) and a 5′-radiolabeled DNA substrate (20 nM) in buffer E was rapidly mixed with Mg²⁺ (8 mM) and varying concentrations of dNTP. For exonuclease assays, a pre-incubated solution of hPolε exo+ (200 nM) and a 5′-radiolabeled DNA substrate (20 nM) in buffer E was rapidly mixed with Mg²⁺ (8 mM) in the absence of nucleotide to initiate the excision reaction. All reactions were quenched with the addition of 0.37 M EDTA. All reported concentrations are final. Most data, unless otherwise specified, were collected from single trials due to insufficient amount of hPolε to repeat each measurement in triplicate.

**Product analysis**

Reaction products were separated by denaturing polyacrylamide gel electrophoresis (17% acrylamide, 8 M urea and 1x TBE running buffer) and quantified using a Typhoon TRIO (GE Healthcare) and ImageQuant (Molecular Dynamics).

**Data analysis**

All kinetic data were fit by nonlinear regression using KaleidaGraph (Synergy Software). Data from polymerization assays under single-turnover conditions were fit to Equation (1)

\[
[\text{product}] = A[1 - \exp(-k_{obs}t)]
\]

where A is the amplitude of product formation and kₖₒₜₖ is the observed single-turnover rate constant.

Data from the plot of kₖₒₜₖ versus dNTP concentration were fit to Equation (2)

\[
k_{obs} = k_p[dNTP]/(K_d + [dNTP])
\]

where kₚ is the maximum rate constant of nucleotide incorporation and K_d is the equilibrium dissociation constant for
dNTP binding. When $K_d$ is very large, the plot of $k_{obs}$ versus dNTP concentration was fit to Equation (3)

$$k_{obs} = \left( k_p / K_d \right) [\text{dNTP}]$$

(3)

to yield the substrate specificity constant, $k_p/K_d$.

Data from exonuclease assays under single-turnover conditions were fit to Equation (4)

$$[\text{product}] = A[\exp(-k_{exo}t)] + C$$

(4)

where $A$ is the reaction amplitude and $k_{exo}$ is the overall DNA excision rate constant.

All reported errors were generated by fitting the data to the above equations through Kaleidagraph.

RESULTS

Substrate specificity of hPolexo-

In our recent publication we revealed through pre-steady-state kinetics that hPol, like all other kinetically characterized polymerases, catalyzes correct nucleotide incorporation via an induced-fit mechanism (29). At 20°C, hPol exo- binds and incorporates correct dTTP opposite dA with a maximum rate constant, $k_p$, of 248 s⁻¹ and an equilibrium dissociation constant, $K_d$, of 31 μM (29). However, $k_p$ and $K_d$ for an incorrect incoming nucleotide have not yet been determined. We expected that hPol, like other replicative DNA polymerases, exhibits high selectivity for correct incoming nucleotides versus incorrect nucleotides through the combination of both a faster incorporation rate constant and a higher ground-state binding affinity (1/Kd).

To confirm this hypothesis, we measured the substrate specificities ($k_p/K_d$) for each of the 15 remaining possible incoming nucleotide and templating base combinations through four perfectly matched DNA substrates (D-1, D-6, D-7 and D-8) listed in Table 1. As examples, the plots of $k_{obs}$ versus dNTP concentration for the extension of the 21-mer primer in D-6 are shown for correct dCTP and incorrect dATP in Figure 1A and B, respectively. The plot in Figure 1A was fit to Equation (2) (see Materials and Methods) to obtain a $k_p$ of 268 ± 14 s⁻¹ and a $K_d$ of 19 ± 4 μM as well as a calculated $k_p/K_d$ of 14 μM⁻¹s⁻¹ for correct dCTP incorporation. Likewise, the plot in Figure 1B was fit to Equation (2) to yield a $k_p$ of (8.8 ± 0.4) × 10⁻² s⁻¹, a $K_d$ of (9 ± 1) × 10² μM and a $k_p/K_d$ of 9.8 × 10⁻⁶ μM⁻¹s⁻¹ for incorrect dATP incorporation. Similarly, the kinetic parameters for both correct and incorrect nucleotide incorporation are listed in Table 1 at 20°C were determined and are listed in Table 2.

Mismatch extension fidelity of hPolexo-

After a misincorporation event, hPol will excise the nascent mismatched base pair, dissociate from the DNA substrate or further extend the mismatched base pair. Following selective inhibition of its 3’→5’ exonuclease activity by mutating three highly conserved carboxylate residues (D275/E277/D368) at the exonuclease active site to alanine (29), we were able to determine the $k_p/K_d$ values for the incorporation of both a correct nucleotide and an incorrect nucleotide on DNA substrates containing a single mismatched base at the primer 3’ terminus (M-1, M-7 and M-8 in Table 1). As an example, the plot of $k_{obs}$ versus dCTP concentration for the extension of M-7 (Figure 2) was fit to Equation (2) (see Materials and Methods) to yield a $k_p$ of (4.3 ± 0.4) × 10⁻² s⁻¹ and a $K_d$ of (1.6 ± 0.2) × 10³ μM. Notably, M-7 contains a C:T mismatch at the primer-template junction, but is otherwise identical to the four correctly matched DNA substrates (D-1, D-6, D-7 and D-8 in Table 1). Interestingly, both correct dCTP and incorrect dGTP with M-7 had very low substrate specificities which were comparable to the values measured for incorrect nucleotide incorporation into a correctly matched DNA substrate (Table 3). Similarly, the kinetic parameters for correct dCTP and incorrect dGTP incorporation into the other two mismatched DNA substrates, M-1 and M-8, in Table 1 at 20°C were determined and are listed in Table 3.

Excision of matched and mismatched DNA substrates by hPolexo+

hPol, like most A- and B-family replicative DNA polymerases, possesses a 3’→5’ exonuclease proofreading activity that is proficient in removing mismatched bases from the primer 3’ terminus. It is expected that the exonuclease
Table 2. Kinetic parameters for correct and incorrect nucleotide incorporation catalyzed by hPol exo- at 20°C

| dNTP   | $k_p$ (s$^{-1}$) | $K_d$ (μM) | $k_p/K_d$ (μM$^{-1}$s$^{-1}$) | $F_{pol}^b$ |
|--------|----------------|------------|-----------------------------|------------|
| dATP  | 0.61 ± 0.04    | (6 ± 1) x 10$^2$ | 1.0 x 10$^{-3}$            | 1.2 x 10$^{-4}$ |
| dCTP  | 5.2 ± 0.9      | (2.0 ± 0.6) x 10$^3$ | 2.6 x 10$^{-3}$            | 3.2 x 10$^{-4}$ |
| dGTP  | 1.13 ± 0.04 x 10$^{-2}$ | (3.2 ± 0.3) x 10$^2$ | 3.5 x 10$^{-5}$            | 4.4 x 10$^{-6}$ |

Table 3. Kinetic parameters for mismatch extension and excision catalyzed by hPol exo- and hPol exo+ at 20°C

| dNTP   | $k_p$ (s$^{-1}$) | $K_d$ (μM) | $k_p/K_d$ (μM$^{-1}$s$^{-1}$) | $F_{ext}^a$ | $k_{obs}$ (s$^{-1}$)$^b$ | $k_{exo}$ (s$^{-1}$) | $F_{exo}^c$ |
|--------|----------------|------------|-----------------------------|------------|-------------------------|---------------------|------------|
| C:A mismatch (M-1) |
| dCTP  | (4.0 ± 0.4) x 10$^{-2}$ | (5.4 ± 1.3) x 10$^2$ | 7.4 x 10$^{-5}$            | -          | 6.2 x 10$^{-3}$         | -                   | 350        |
| dGTP  | (3.6 ± 0.3) x 10$^{-4}$ | (5.3 ± 1.3) x 10$^2$ | 6.8 x 10$^{-7}$            | 9.1 x 10$^{-3}$ | 5.7 x 10$^{-5}$         | -                   | -          |
| C:T mismatch (M-7) |
| dCTP  | (4.3 ± 0.4) x 10$^{-2}$ | (1.6 ± 0.2) x 10$^1$ | 2.7 x 10$^{-5}$            | -          | 2.5 x 10$^{-3}$         | -                   | -          |
| dGTP  | (6.3 ± 0.5) x 10$^{-4}$ | (6.4 ± 1.0) x 10$^2$ | 9.8 x 10$^{-7}$            | 3.5 x 10$^{-2}$ | 8.5 x 10$^{-5}$         | -                   | -          |
| C:C mismatch (M-8) |
| dCTP  | -              | -          | -                          | -          | 2.9 ± 0.3               | -                   | 1200       |
| dGTP  | (6.1 ± 0.3) x 10$^{-4}$ | (1.5 ± 0.1) x 10$^3$ | 4.1 x 10$^{-7}$            | 0.14       | 3.8 x 10$^{-5}$         | -                   | -          |

activity of hPol will be kinetically favored over its polymerase activity in the presence of a mismatched primer terminus due to a significantly higher rate of excision versus extension. On the other hand, excision of a matched base pair should be much slower than correct nucleotide incorporation to prevent futile competition with 5′→3′ primer extension during processive DNA synthesis. To verify this hypothesis, we measured the overall excision rate constants ($k_{exo}$) of matched versus mismatched base pairs by hPol exo+. The D-8 and M-8 substrates (Table 1) were used to measure the $k_{exo}$ values for a matched and mismatched primer–template pair, respectively. The concentration of remaining substrate was plotted versus time and the data were fit to Equation (4) (see Materials and Methods) to yield $k_{exo}$ (Figure 3). The $k_{exo}$ values were determined to be 0.17 ± 0.02 s$^{-1}$ and 3.0 ± 0.7 s$^{-1}$ for matched (D-8) and mismatched (M-8) primer–template pairs at 20°C, respectively. These measurements were repeated at a lower enzyme concentration and $k_{exo}$ was found to be unaffected by the ratio of hPol exo+ to DNA (data not shown). Notably, the measured $k_{exo}$ is not the true excision rate constant at the exonuclease active site ($k_\text{exo}^b$) since it is a function of $k_\text{exo}$, the forward and backward transfer rates of the primer 3′-terminal nucleotides between the polymerase and exonuclease active sites, and DNA dissociation and rebinding rates from the exonuclease active site. Similarly, we measured $k_{exo}$ for the mismatched DNA substrates M-1 and M-8 (Table 1) and the $k_{exo}$ values are listed in Table 3. Interestingly, Table 3 shows that the overall rate constant of excision was not significantly affected by the identity of the 3′ mismatched base pair in a DNA substrate.
Figure 1. Nucleotide concentration dependence on the pre-steady-state kinetic parameters of correct dCTP and incorrect dATP incorporation opposite dG catalyzed by hPol/exo- at 20°C. (A) A pre-incubated solution of hPol/exo- (260 nM) and 5' -radiolabeled D-6 (20 nM) was mixed with increasing concentrations of correct dCTP and Mg2+ for various times. The plot of product concentration versus time was fit to Equation (1) to yield \( k_{\text{obs}} \) (data not shown). The resulting \( k_{\text{obs}} \) values were plotted against dCTP concentration and fit to Equation (2) to yield a \( k_p \) of 268 ± 14 s\(^{-1}\) and a \( K_d \) of 19 ± 4 µM (B) hPol/exo-and 5'-radiolabeled D-6 were mixed with increasing concentrations of incorrect dATP and Mg2+ as described above. The data were similarly processed to yield a \( k_p \) of (4.3 ± 0.4) × 10\(^{-2}\) s\(^{-1}\) and a \( K_d \) of (1.6 ± 0.2) × 10^3 µM.

DISCUSSION

To determine if hPol synthesizes DNA with high fidelity as observed with other replicative DNA polymerases, we used pre-steady-state kinetics to measure the kinetic parameters of nucleotide incorporation and excision on both matched and single-base mismatched DNA substrates. First, we calculated the base substitution fidelity of hPol/exo- by measuring the \( k_p \) and \( K_d \) values at 20°C for all 16 possible combinations of incoming nucleotides and templating bases. Correct nucleotides were incorporated with an average \( k_p \)
and $K_d$ of 252 s$^{-1}$ and 23 μM, respectively. The $k_p$ values for incorrect nucleotide incorporation varied widely from $(8.8 \pm 0.4) \times 10^{-1}$ s$^{-1}$ to $5.2 \pm 0.9$ s$^{-1}$ while the $K_d$ values ranged between $(2.4 \pm 0.3) \times 10^3$ to $(2.0 \pm 0.6) \times 10^3$ μM. Strikingly, the $k_p$ difference between correct and incorrect nucleotide incorporation $[(k_p)_{correct}/(k_p)_{incorrect}]$ contrasts broadly, varying by one to four orders of magnitude. A similar result was previously obtained from pre-steady-state kinetic analysis of hPolyexo- (13). Overall, hPolyexo incorporated a correct nucleotide with a 48- to 3.0 × 10$^9$-fold faster rate constant than an incorrect nucleotide, and bound a correct nucleotide with a 10- to 100-fold higher affinity. Thus, the differences in both $k_p$ and $K_d$ were major determinants of the base substitution fidelity of hPolyexo-, which was calculated to be 10$^{-4}$–10$^{-1}$ (Table 2). Similar kinetic patterns of incorrect nucleotide discrimination were determined for other highly accurate replicative DNA polymerases, including hPol, T7 DNA polymerase and RB69 DNA polymerase (13,14,39). Interestingly, all DNA polymerases including hPolyexo-(Table 2) possess sequence-dependent base substitution fidelity. The fidelity of DNA synthesis catalyzed by replicative DNA polymerases is further enhanced by an associated proofreading activity (c) especially on the mismatched M-1 substrate by hPolyexo- (41–43). Strikingly, somatic mutations of the proofreading domain of hPolyexo+ are highly efficient at removing mismatched base pairs, the possibility that hPolyexo+ might partition toward removal of a correctly matched base pair must be considered. For example, the extension rate constant ($k_p$) on the D-8 substrate in the presence of the next correct nucleotide, dGTP, was measured to be 219 ± 13 s$^{-1}$ (Table 2), while the overall extension rate constant $k_p$ was 0.17 ± 0.02 s$^{-1}$ (Figure 3). Since typical cellular nucleotide concentrations (100 μM) are significantly higher than the $K_a$ value (9 μM, Table 2) for dGTP with D-8, the dGTP incorporation rate constant should approach $k_p$. Thus, the probability of matched base pair excision, given by $k_{obs}/k_{exo}$, was calculated to be only 0.08% while the probability of further extension $k_p/(k_p + k_{exo})$ approached 100%. In contrast, for a single base mismatched terminus in a DNA substrate, the kinetic partitioning between excision $k_{obs}/(k_{exo} + k_{obs})$ and extension $k_{obs}/(k_{obs} + k_{exo})$ was calculated to be 99.719–99.991% and 0.009–0.281%, respectively (Table 4). Thus, the 3′→5′ proofreading activity of hPolyexo+ is very efficient at removing mismatched nucleotides without interfering with continuous faithful DNA synthesis. From the combined contributions of both high polymerase selectivity (10$^{-4}$–10$^{-7}$, Table 2) and efficient 3′→5′ proofreading activity (3.5 × 10$^{-2}$ to 1.2 × 10$^4$, Table 3), hPolyexo exhibits overall polymerization fidelity of 10$^{-6}$–10$^{-11}$ in vitro. Such high fidelity of DNA synthesis qualifies hPolyexo+ as a main enzyme to catalyze accurate replication of large human nuclear genome (3 × 10$^9$ base pairs). As the key polymerase responsible for leading strand synthesis during nuclear genomic replication, hPolyexo+ must synthesize long stretches of DNA without making an error. Consistently, the fidelity of DNA replication in normal human cells was estimated to be 10$^{-3}$–10$^{-10}$ (41–43). Strikingly, somatic mutations in the 3′→5′ exonuclease domain of hPolyexo+ impair the proofreading activity, causing a high frequency of errors (>10$^{-4}$ mutations per base) in the leading strand, elevate recurrent nonsense mutation rates in key tumor suppressors, such as TP53, ATM, and PIK3R1, and ultimately lead to the formation of various cancers (27). This error frequency is greater than the high end of the fidelity range of hPolyexo- (10$^{-3}$–10$^{-7}$) measured here. Such a discrepancy suggests other cellular factors also contribute to the high leading strand mutation rate in tumors carrying inactivating mutations of the proofreading domain of hPolyexo+. 
Table 4. Comparison of the contribution of 3′→5′ exonuclease activity to the overall fidelity of replicative DNA polymerases when encountering a single base mismatch in the staggering end of a DNA substrate

| Polymerase | Mismatch | $k_{eox}$ (s$^{-1}$) | $k_{obs}$ (s$^{-1}$)$^a$ | $F_{exox}$ $^b$ | Excision% $^c$ |
|------------|----------|----------------------|------------------------|--------------|--------------|
| hPole$^d$  | C:A      | 2.2                  | 6.2 x 10$^{-3}$        | 350          | 99.719       |
|            | C:T      | 2.9                  | 2.5 x 10$^{-3}$        | 1200         | 99.914       |
|            | C:C      | 3.0                  | 2.6 x 10$^{-4}$        | 12,000       | 99.991       |
| S. solfataricus PolBI$^e$ | A:A      | 1.86                 | 0.012                  | 160          | 99.359       |
| hPol$^f$   | T:T      | 0.4                  | 0.1                    | 4            | 80.000       |
| T7 DNA     | A:A      | 2.3                  | 0.012                  | 190          | 99.481       |
| hPol$^f$   | T:T      | 0.4                  | 0.1                    | 4            | 80.000       |
| T7 DNA     | A:A      | 2.3                  | 0.012                  | 190          | 99.481       |

$^a$ Calculated as $k_{eox} / (k_{eox} + [dNTP])$ during extension from a mismatched primer terminus at an intracellular nucleotide concentration of 100 μM.

$^b$ Calculated as $k_{eox} / k_{obs}$.

$^c$ Calculated as $k_{eox} / (k_{eox} + k_{obs})$ for a single base mismatch.

$^d$ This work (performed at 20°C).

$^e$ Reference (11) (performed at 37°C).

$^f$ Reference (20) (performed at 37°C).

$^g$ Reference (19) (performed at 20°C).

Notably, the lower limit (10$^{-6}$) of the fidelity range of hPole (10$^{-6}$–10$^{-11}$) is significantly higher than the error frequency of normal human genome replication (10$^{-9}$–10$^{-10}$) (41–43). It is likely that this difference is accounted for by post-replication mismatch repair in vivo, which enhances replication fidelity by one to three orders of magnitude in E. coli and Saccharomyces cerevisiae (43–47). Additionally, it is possible that interactions between the p261 catalytic subunit and the smaller subunits or other proteins in the replisome may further enhance the fidelity of DNA replication in vivo. To investigate this hypothesis, we are currently studying the effect of the smaller subunits on the catalytic properties of p261 of hPole.

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