Fidelity of DNA Polymerase ε Holoenzyme from Budding Yeast

Saccharomyces cerevisiae*

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DNA polymerases δ and ε (pol δ and ε) are the major replicative polymerases and possess 3'-5' proofreading exonuclease activities that correct errors arising during DNA replication in the yeast Saccharomyces cerevisiae. This study measures the fidelity of the wild-type pol ε, the 3'-5' exonuclease-deficient pol2–4, a +1 frameshift mutator for homonucleotide runs, pol2C1089Y, and pol2C1089Y pol2–4 enzymes using a synthetic 30-mer primer/100-mer template. The nucleotide substitution rate for wild-type pol ε was 0.47 × 10^-5 for G:G mismatches, 0.15 × 10^-2 for T:G mismatches, and less than 0.01 × 10^-5 for A:G mismatches. The accuracy for A opposite G was not altered in the exonuclease-deficient pol2–4 pol ε; however, G:G and T:G misincorporation rates increased 40- and 73-fold, respectively. The pol2C1089Y pol ε mutant also exhibited increased G:G and T:G misincorporation rates, 22- and 10-fold, respectively, whereas A:G misincorporation did not differ from that of wild type. Since the fidelity of the double mutant pol2–4 pol2C1089Y was not greatly decreased, these results suggest that the proofreading 3'-5' exonuclease activity of pol2C1089Y pol ε is impaired even though it retains nuclelease activity and the mutation is not in the known exonuclease domain.

The yeast Saccharomyces cerevisiae has three DNA polymerases, (pol α, δ, and ε), which are required for cell growth, chromosomal DNA replication (1), and DNA double-strand break repair (2). Pol α has four subunits (Pol1 (Cdc17), Pol10, Pri1, and Pri2) and is involved primarily in the initiation of DNA replication and priming of Okazaki fragments. Pol δ and pol ε are required during synthesis of the leading and lagging DNA strands at the replication fork; they bind at/or near replication origins and move along DNA with the replication fork (3, 4). The precise roles of pol δ and pol ε during leading and lagging strand DNA synthesis have not yet been defined; however, genetic and biochemical evidence suggest that lagging strand DNA synthesis is carried out by pol α and pol δ (5, 6).

Pol δ of S. cerevisiae has three subunits (Pol3 (Cdc2), Hys2 (Pol31) (7, 8), and Pol32 (8)), which are homologues of Schizosaccharomyces pombe Pol3, Cdc1, and Cdc27, respectively (9). S. pombe pol δ has one additional subunit, Cmt1 (9). Purified yeast pol δ requires accessory factors including PCNA3 and RFC to catalyze processive DNA synthesis (8). Pol δ possesses a 3'-5' exonuclease activity, which performs proofreading/editing during DNA synthesis (10, 11).

The S. cerevisiae pol ε is also a multi-subunit protein complex that includes Pol2, Dpb2, Dpb3, and Dpb4 and like pol δ has a 3'-5' exonuclease activity (1, 12). Pol ε is a highly processive enzyme (12–14). Although pol ε requires PCNA and RFC complex to catalyze processive DNA synthesis on singly primed single-stranded viral DNA (13), these cofactors may not be required for processive DNA synthesis in vivo. Pol2 is the catalytic subunit of pol ε and is encoded by the POL2 gene (15), which is essential for cell growth and required for chromosomal DNA replication (16). The second subunit of pol ε, Dpb2, is encoded by the DPB2 gene and is also essential for yeast cell growth and for chromosomal DNA replication (17). The DPB3 and DPB4 genes encode the third and fourth subunits of pol ε, respectively (18, 19). These genes are not essential for cell growth, although they play an important role in stabilizing the subunit structure of pol ε and in interactions between pol ε and other DNA replication proteins (19). Pol ε interacts with Dpb11 and Sld2, proteins that play an important role in recruiting replication proteins to the replication origin before DNA elongation starts (4, 20, 21).

Recent studies in S. cerevisiae and S. pombe suggest that pol ε may not play an essential enzymatic role during DNA replication. The essential function of pol ε may relate to its noncatalytic C-terminal domain (22–24). Deletion of the polymerase catalytic domain of pol ε is not lethal and does not block chromosomal DNA replication (22–24). However, a pol2 deletion mutant is temperature-sensitive, exhibits severe defects in chromosomal DNA replication at the permissive temperature, and undergoes premature senescence (25). This suggests that other DNA polymerases can substitute for the polymerase function of pol ε but that another unspecified function of pol ε is essential in yeast. Yeast deficient in the 3'-5' exonuclease activities of either pol δ or pol ε are spontaneous mutators (26). Furthermore, a mutation near the DNA polymerase domain of pol ε is also a +1 frameshift mutator (27). These results are

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1 The abbreviations used are: PCNA, proliferating cell nuclear antigen; RFC, replication factor C; HE, holoenzyme.
consistent with the notion that both pol δ and pol ε participate in chromosomal DNA replication.

Despite extensive characterization of pol δ and ε (1, 28), little is currently known about their fidelity. Goodman and collaborators (29) developed a gel-based method for measuring polymerase kinetics that is useful for evaluating enzyme fidelity. Kinetic parameters can be measured at specific sites in a primer/template DNA in the presence of proofreading and polymerase accessory proteins (29). This was used to assess the fidelity of calf thymus pol δ in the presence or absence of PCNA using normal (30) and abasic (31) DNA substrates. More recently, Goodman and colleagues (32) compared the base substitution fidelity even in the presence of its 3′-5′ exonuclease activity. B, the band intensities were quantified using a Fuji Image analyzer. The ratio of the amount of unextended primer and extended primer was plotted versus dNTP concentration. The method of quantification was described previously (29, 32).

**EXPERIMENTAL PROCEDURES**

**Materials**—A series of isogenic strains were constructed from CG379 (MATa ade5–1 his7–2 leu2–3, −112 trp1–289 ura3–52) containing the pol2 mutation: CG379 pol2–4 (10), CG379 pol2C1089Y (27), and CG379 pol2–4 pol2C1089Y (this study). Fast protein liquid chromatography-purified nucleotides were purchased from Amersham Biosciences. T4 polynucleotide kinase was purchased from New England Biolabs.

**DNA Substrates**—The template/primer DNA was a synthetic 100-mer template annealed to a complementary 30-mer primer or to a 35-mer primer containing a single noncomplementary base at its 3′-end. The 30-mer primer is complementary to DNA sequence in the middle of the template, forming a template/primer with a 35-nucleotide ssDNA overhang on the 5′- and 3′-sides (29). The template/primer with a mismatched 5′ terminus was formed using a 35-mer primer whose 3′-terminal base was A, G, or T paired with template base G. Synthetic DNA oligomers, which were gel-purified, were purchased from Amersham Biosciences. The DNA sequences were (29):

\begin{verbatim}
5′-AGTCATTATCGAGACTTTGCTAGTGCTGTCGTTTTACGAAACACCGGTTCTAAGGAT
3′-TGCTTTGTGGCCAAGATTCCTA
\end{verbatim}

The primer was 5′,32P-end-labeled with T4 polynucleotide kinase at 30 °C for 60 min. DNA strands were annealed in a ratio of 1 primer to 1.2 template molecules by heating to 90 °C and gradually cooling to room temperature. The concentration of annealed DNA was 100 nM (primer termini).

**Purification of pol ε from Wild-type, pol2–4, pol2C1089Y, and pol2–4 pol2C1089Y Mutant Cells**—pol ε HE, which consists of Pol2, Dpb2, Dpb3, and Dpb4 (12, 18, 31), was purified to near homogeneity from S. cerevisiae CB001 (wild type), CG379 pol2–4 (10), CG379 pol2C1089Y (26), or CG379 pol2–4 pol2C1089Y (this study). Cells were grown in YPD as described previously (12, 14).
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### TABLE I

| pol \( \varepsilon \) | DNA polymerase activity | Exonuclease activity | Ratio (relative) Exo/Pol |
|------------------|-------------------------|----------------------|-------------------------|
| Wild type (POL2) | 100                     | 31.5                 | 0.315 (1)               |
| pol2-4           | 102                     | 2.0                  | 0.019 (0.06)            |
| pol2C1089Y       | 105                     | 35.3                 | 0.336 (1.07)            |
| pol2-4 pol2C1089Y| 103                     | <1.0                 | <0.010 (<0.03)          |

* DNA polymerase activity was measured as described previously (12) using poly(dA)\(-1000\)/dATP (20:1) as a template/primer and about 0.1 unit of pol \( \varepsilon \).

* Exonuclease activity was also measured in the reaction mixture containing \(^{32}\)P-labeled \( E. \) coli DNA (which was preheated at 95°C for 5 min and quickly ice-chilled) and 0.1 unit of pol \( \varepsilon \) at 30°C for 30 min (12).

Gel Kinetic Fidelity Analysis—A gel-based fidelity assay was used to determine the kinetics of incorporation of dNTPs opposite the target site (29, 32). Primer extension reactions for pol \( \varepsilon \) were performed as follows: The template/primer DNA and the dNTP to be incorporated opposite the target were mixed together in the reaction buffer. S. cerevisiae pol \( \varepsilon \) and the running-start nucleotide (dATP) were added to initiate the reaction. Reactions proceeded for 3 min at 30°C and incorporation of dCMP or misincorporation of dNMP was measured opposite G. Incorporation of the correct nucleotide was measured under single-hint conditions (i.e., most template/primer DNA molecules encounter one polynucleotide only). The final reaction conditions included 50 \( \mu \)M dATP, 10 \( \mu \)M template/primer DNA, 10 \( \mu \)g/ml (0.2 unit/ml) S. cerevisiae pol \( \varepsilon \), and dNTP as indicated in the figures. Control reactions were for 3 min using only dATP to verify that dATP was not misincorporated opposite the target G. Reactions were terminated, and reaction products were analyzed by gel electrophoresis. Band intensities were integrated and quantified on a Fuji Image analyzer as described previously (29, 32).

Assay for the 3'–3' Exonuclease Activity of pol \( \varepsilon \)—Assay for the 3'–3' exonuclease activity associated with pol \( \varepsilon \) was performed by two different ways. One way was the previously published method (12) using \(^{32}\)P-labeled \( E. \) coli single-stranded DNA as a substrate. The other was performed using a single-stranded 5'–3' end-labeled 30 mer as a substrate as follows. A single-stranded 5'–3' end-labeled 30 mer was incubated at 30°C with 10 \( \mu \)M (0.2 unit/ml) pol \( \varepsilon \) in reaction buffer (20 \( \mu \)l). Aliquots (2 \( \mu \)l) were removed from the reaction and quenched with 5 \( \mu \)l of 20 mM EDTA, 95% formamide at the appropriate time points. Reaction products were separated on a 12% denaturing polyacrylamide gel in the presence of 7 M urea for 3 h at 1600 V.

Proteinase K Assays—The processivity of pol \( \varepsilon \) was measured using poly(dA)\(-1000\)/dATP (20:1) as a template/primer DNA substrate and pol \( \varepsilon \) at 30°C for 3 min in reaction buffer (10 \( \mu \)l) as described previously (12). Reactions were quenched with 25 \( \mu \)l of 20 mM EDTA, 95% formamide at the appropriate time points. Reaction products were separated on a 12% denaturing polyacrylamide gel.

Other Materials and Methods—Other materials and methods used in this study have been described previously (12, 19, 26, 27).

### RESULTS

pol \( \varepsilon \) is one of the principal replication polymerases in budding yeast (1, 33), pol HE, which includes the four subunits Pol2p, Dpb2p, Dpb3p, and Dpb4p (1, 19, 33), catalyzes highly processive DNA synthesis on \( \alpha \)-174 single-stranded DNA primed with a short oligonucleotide in the presence of replica-
tion factor A (RPA) and the absence of PCNA and RFC (14). This study examines the fidelity of wild-type and mutant S. cerevisiae pol ε HE (see “Experimental Procedures”) in the same sequence context used in previous studies of the fidelity of E. coli pol III HE (29) and S. pombe pol δ HE (32).

Fidelity of pol ε HE—Base substitution fidelity measurements were performed with wild-type pol ε HE are described in Fig. 1 (note that all experiments described below were carried out with polymerase HE). When the reaction was initiated, wild-type pol ε extended the 32P-labeled primer by incorporating four running-start As. The fidelity of pol ε was then measured at the fifth template nucleotide, which was a target G. The template DNA sequence is shown along the right side of the assay gel in Fig. 1A. In the presence of dCTP, pol ε incorporated four running-start As and then a C opposite the target G. In addition, a very faint band was detected at the next template C; incorporation opposite downstream template nucleotides T, G, and T increased with increasing dCTP (Fig. 1A, dCTP lane). The amount of shortened primer degraded to the first upstream template G also increased with increasing dCTP (indicated by asterisks in Fig. 1A). These results indicate that the pol ε 3′-5′ exonuclease acts as a proofreading exonuclease during DNA synthesis by pol ε.

Reactions were also carried out in the presence of dGTP, dTTP, and dATP (Fig. 1A). The amount of extended primer was quantified, and the misincorporation efficiency was estimated as described previously (29, 32). The nucleotide misincorporation rate for pol ε was 0.47 × 10^{-5} for G:G mismatches, 0.15 × 10^{-5} for T:G mismatches, and less than 0.01 × 10^{-5} for A:G mismatches (Fig. 1B and Table I). Highly represented degradation products (asterisks in Fig. 1A) correspond to sites of degradation and incorporation in the presence of dGTP or dTTP. Thus, the fidelity of wild-type pol ε is considerably higher than the fidelity of S. pombe pol δ (32) and is comparable with the fidelity of E. coli pol III (29).

Fidelity of 3′-5′ Exonuclease-deficient pol ε—pol ε fidelity was also measured using pol ε mutant pol2–4 deficient in 3′-5′ exonuclease activity (26). This mutant pol ε has no detectable nuclease activity (Table II) and does not degrade the 32P-labeled primer under the conditions in which its fidelity was measured (Fig. 2). Fig. 3 shows the extension products from polymerase reactions with the mutant enzyme. In the presence of the control level of dATP, the 32P-labeled primer was extended by four As up to the target template G, and limited misincorporation was observed at the target template G (Fig. 3A, Control lane). Increasing the dATP led to a misincorporation rate comparable with the wild-type enzyme (0.029 × 10^{-5}). However, the G:G and T:G misincorporation rates were 19.2 × 10^{-5} and 11.0 × 10^{-5}, respectively (40- and 73-fold higher than wild-type pol ε) (Fig. 3B and Table I), and misincorporation of C opposite the next C was greatly increased. These results indicate that pol ε 3′-5′ exonuclease activity efficiently removes a mismatched nucleotide during DNA synthesis and that pol ε has a low A:G misincorporation rate.

Fidelity of pol2C1089Y or pol2–4 pol2C1089Y Mutant pol ε—The pol ε mutant pol2C1089Y was identified as a +1 frameshift mutator for homonucleotide runs (27). The biochemical features of this and the double mutant pol2–4 pol2C1089Y
were examined. We noticed that the processivity of pol2C1089Y or pol2–4 pol2C1089Y pol ε was almost the same as wild-type pol ε using poly(dA)_{1000}/poly(dT)_{10} (20:1) as a template/primer under the condition previously described (12) (data not shown). The misincorporation rates of pol2C1089Y or pol2–4 pol2C1089Y pol ε were determined as described above (Figs. 4 and 5, and Table I). The G:G and T:G misincorporation rate of pol ε pol2C1089Y was 10.3 × 10^{-5} (22-fold higher than wild type) and 1.52 × 10^{-5} (10-fold higher than wild type), respectively. In contrast, the A:G misincorporation rate was 0.016 × 10^{-5} (similar to wild type). The lack of proofreading in the double mutant led to only modest increases: however, there is no increase beyond that due to the proofreading mutation alone (Table I). These results may indicate that the 3′-5′ exonuclease activity of the pol2C1089Y mutant is insufficient to remove a mismatched nucleotide during DNA synthesis. One possible explanation for this result is that the pol2C1089Y mutant undergoes a conformational change that affects the enzyme’s ability to switch from DNA synthesis mode to editing mode when a mismatched nucleotide is incorporated. Therefore, the intrinsic misincorporation might be compromised, but instead the establishment of the mutation could be altered. This idea was tested by challenging the enzyme with a primer/template DNA substrate with a single 3′-terminal mismatched base. The results are shown in Fig. 6. The pol2C1089Y and pol2C1089Ypol2–4 mutant pol ε extend the mismatched primer efficiently, but pol2–4 mutant pol ε extends the mismatched primer very inefficiently. In contrast, these enzymes extend the matched primer with the same efficiency (Fig. 6). Note that regardless of the matched or mismatched primer used for the extension reactions, we observed a short time lag before pol ε starts DNA synthesis (Fig. 6). These results suggest that the pol2C1089Y mutation may affect the proofreading exonuclease activity during DNA synthesis. Nevertheless, the mutant pol2C1089Y has near wild-type exonuclease activity on a single-stranded DNA substrate (Fig. 2 and Table II).

Mutation Specificity of pol2C1089Y and pol2–4 pol2C1089Y—The high misincorporation rate of the pol2C1089Y mutant pol ε suggests that it is a base substitution mutator as well as a +1 frameshift mutator for homonucleotide runs (27). Although it was found to have only modest effects on forward mutation in the CAN1 locus (27), we also examined it in a URA3 forward mutation assay (26); mutations were selected as resistant to 5-FOA. As expected, the mutation rate of pol2–4 mutant was ~10-fold higher than wild type (5 × 10^{-7}) (Table III). The spontaneous mutation rate of the pol2C1089Y mutant is 1000-fold higher than

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**Fig. 4. Fidelity of mutant pol2C1089Y pol ε.** Primer extension reactions were carried out with dATP (50 µM), primer/template DNA (10 nM), mutant pol2C1089Y pol ε (10 µg/ml, 0.2 unit/ml), and various concentrations of dNTP as described in the legend for Fig. 1.
wild type (5 × 10⁻⁵ versus 0.005 × 10⁻⁵). The presence of \( \text{pol}2^{-}4 \) mutation increased the rate a further 5-fold (25 × 10⁻⁵).

The mutations were characterized by sequencing 43 randomly picked \( \text{ura}3 \) mutants from the forward mutation assay with wild type, 100 from the \( \text{pol}21089Y \), and 106 from \( \text{pol}2^{-}4 \text{pol}21089Y \) mutant cells (see previously published method (24)). As shown in Fig. 7, 22 (21%) of 107 mutants from \( \text{pol}21089Y \) were \( -1 \) frameshift mutations, and one (0.9%) was a \( +1 \) frameshift mutation. The remaining 84 (78%) were single-nucleotide substitution mutations. On the other hand, 17 (17%) of 100 mutants from the \( \text{pol}2^{-}4 \text{pol}21089Y \) double mutant were \( -1 \) frameshift, 83 (83%) were single-nucleotide substitution mutations, and no \( +1 \) frameshift mutation was found. The high frequency of \( -1 \) frameshift mutations is specific for \( \text{pol}21089Y \) and is not observed with wild-type \( \text{pol} \) \( \) and the \( \text{pol}2^{-}4 \text{pol} \) \( \) mutant (see ref. 26). More importantly, the mutation specificity of \( \text{pol}21089Y \) and \( \text{pol}2^{-}4 \text{pol}21089Y \) was not changed significantly.

**DISCUSSION**

Processive DNA polymerases have enhanced template/primer binding and a decreased probability of dissociating from the template/primer. These enzymes are inherently prone to misincorporating an incorrect DNA base or a nucleotide ana-
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Nucleotide misincorporation rates were increased in the pol ε mutant pol2C1089Y relative to wild type. The observation that the level in the double mutant pol2–4 pol2C1089Y was not greatly increased suggests that the mutant pol2C1089Y is specifically deficient in 3′-5′ exonuclease proofreading activity or that the errors appearing in the mutant are not subject to proofreading. However, the mutant pol2C1089Y pol ε exhibits almost the same specific activity of exonuclease as that of wild-type pol ε (Fig. 2 and Table II) and excises misincorporated nucleotides during DNA synthesis (Fig. 4). One possible explanation is that the pol2C1089Y mutant is defective in coordinating processive DNA synthesis with the excision of misincorporated nucleotides.

The pol ε catalytic subunit contains a 3′-5′ proofreading exonuclease domain at the N terminus (10, 26). In the known crystal structures of family B members, this domain is folded around a central β-sheet that contains the active site and, together with the exonuclease domain, creates a ring-shaped structure with a central hole, where the template/primer duplex DNA is positioned. The catalytic mechanism leading to the removal of the last incorporated nucleotide by the exonuclease activity is a phosphoryl transfer catalyzed by two metal ions, analogous to the one responsible for polymerization (38). A model between the polymerase and exonuclease activities of family B polymerases can be constructed by comparing the structure of RB69 in its polymerizing (39) and editing (40) modes. A mismatched base pair prevents the fingers from rotating toward the palm to bind the incoming dNTP. This leaves the 3′-mismatched end available for binding to the exonuclease active site, which removes the wrong nucleotide. During the switch between the polymerizing and editing modes, the DNA moves toward the exonuclease active site with a rotation in the double-helix axis. This movement is aided by the tip of the thumb subdomain, which holds contact with the DNA during proofreading by polymerase chain reaction, and sequenced as described previously (26). The URA3 nucleotide sequence is numbered from the first nucleotide of the first codon. Lowercase black letters represent substitutions found in wild-type cells, and lowercase purple and red letters are the substitutions found in pol2C1089Y and pol2–4 pol2C1089Y mutant cells, respectively. The downward triangle and upward triangle show the single-base deletion and addition, respectively. Capital blue letters show the sites where the base substitutions were found in the pol2–4 mutant (26).

Fig. 7. URA3 forward mutation spectra from wild type, pol2C1089Y, and pol2–4 pol2C1089Y double mutant. The URA3 mutants were isolated from 5-fluoroorotic acid-resistant colonies, and loge and to immortalizing the misincorporation by continued DNA synthesis. The polymerase-associated 3′-5′ exonuclease is ideally suited to minimize replication errors (i.e. pol δ- or pol ε-associated proofreading exonuclease). DNA polymerases α, β, and recently discovered lesion bypass polymerases such as pol η, κ, and ζ are not highly processive, lack intrinsic 3′-5′ exonuclease activity, and have low fidelity (34–37).

pol ε is believed to be the primary replicative polymerase for processive DNA synthesis in eukaryotic cells (1, 28, 33). Despite the importance of this enzyme, little is known regarding its fidelity in vitro and in vivo. This study has examined the fidelity of wild-type or mutant pol ε using a previously characterized gel-based assay system (29, 32). Wild-type pol ε extended the primer 35 nucleotides to the end of the template in 3 min (Fig. 1) in the absence of any additional cofactors. For the wild-type enzyme, $f_{\text{inc}}$ for nucleotide misincorporation was 0.15 × 10^{-5} (T:G), 0.47 × 10^{-5} (G:G), and 0.015 × 10^{-5} (A:G); for the pol2–4 mutant, $f_{\text{inc}}$ was 11.0 × 10^{-5} (T:G), 19.2 × 10^{-5} (G:G), and 0.03 × 10^{-5} (A:G) (Fig. 1, Table I), indicating that, the G:G and T:G misincorporation frequencies increase significantly in 3′-5′-exonuclease-deficient pol ε and that the 3′-5′ exonuclease activity is required for high fidelity DNA synthesis by pol ε.

The pol ε catalytic subunit contains a 3′-5′ proofreading exonuclease domain at the N terminus (10, 26). In the known crystal structures of family B members, this domain is folded around a central β-sheet that contains the active site and, together with the exonuclease domain, creates a ring-shaped structure with a central hole, where the template/primer duplex DNA is positioned. The catalytic mechanism leading to the removal of the last incorporated nucleotide by the exonuclease activity is a phosphoryl transfer catalyzed by two metal ions, analogous to the one responsible for polymerization (38). A model between the polymerase and exonuclease activities of family B polymerases can be constructed by comparing the structure of RB69 in its polymerizing (39) and editing (40) modes. A mismatched base pair prevents the fingers from rotating toward the palm to bind the incoming dNTP. This leaves the 3′-mismatched end available for binding to the exonuclease active site, which removes the wrong nucleotide. During the switch between the polymerizing and editing modes, the DNA moves toward the exonuclease active site with a rotation in the double-helix axis. This movement is aided by the tip of the thumb subdomain, which holds contact with the DNA during proofreading by polymerase chain reaction, and sequenced as described previously (26). The URA3 nucleotide sequence is numbered from the first nucleotide of the first codon. Lowercase black letters represent substitutions found in wild-type cells, and lowercase purple and red letters are the substitutions found in pol2C1089Y and pol2–4 pol2C1089Y mutant cells, respectively. The downward triangle and upward triangle show the single-base deletion and addition, respectively. Capital blue letters show the sites where the base substitutions were found in the pol2–4 mutant (26).
the movement, guiding it on a path between the two sites. Based on the results presented in this study, it is possible that the pol2C1098Y mutation disrupts coordination between processive DNA synthesis and the proofreading exonuclease of pol ε, which may be a role of the thumb subdomain.

*S. cerevisiae* has another major DNA replication polymerase, pol δ. The fidelity of pol δ from *S. cerevisiae* has not yet been characterized. However, the fidelity of *S. cerevisiae* pol ε can be compared with the fidelity of *S. pombe* pol δ, which was studied previously in the presence of PCNA, RFC, and *E. coli* single stranded DNA binding protein (SSB) using the same primer/template DNA substrate (32). The fidelity of *S. cerevisiae* pol ε is considerably higher than *S. pombe* pol δ. The fidelity of *S. cerevisiae* pol ε was 31-fold (T-G mismatch), 11-fold (G-G mismatch), or 30-fold (A-G mismatch) higher than for *S. pombe* pol δ. It has been reported that *S. pombe* pol δ has only a weak 3'-5' exonuclease activity and is unable to correct polymerase insertion errors efficiently in the presence or absence of accessory proteins (32). Therefore, it is possible that the difference in fidelity of these two enzymes may reflect the weak 3'-5' exonuclease of *S. pombe* pol δ. Interestingly, the fidelity of 3'-5' exonuclease-deficient pol2Δ4 mutant pol ε (Fig. 3) is comparable with that of *S. pombe* pol δ in the presence of PCNA, RFC, and SSB. The fidelity of pol2Δ4 mutant is 4.1- and 0.3-fold higher for T-G and G-G mismatches, respectively, and 15-fold higher for A-G mismatches. However, our preliminary results show that *S. cerevisiae* pol δ has a very active 3'-5' exonuclease activity, which has a ratio between the polymerase activity and the exonuclease activity similar to that of *S. cerevisiae* pol ε, but its fidelity under the processive conditions (with PCNA or PCNA and RFC) is lower than the fidelity of *S. cerevisiae* pol ε.2 The error rates of *S. cerevisiae* pol δ HE, which consists of three subunits (12, 8), were 7.60 × 10^{-5} for G-G mismatches, 6.10 × 10^{-5} for T-G mismatches, and 0.54 × 10^{-5} for A-G mismatches.2 Thus, *S. cerevisiae* pol ε may be ~10-fold more accurate than pol δ under processive conditions, and pol δ may be an intrinsically low fidelity enzyme.

Previous studies showed that 3'-5' exonuclease-deficient forms of pol ε or pol δ (pol2Δ4 or pol3Δ01) are spontaneous mutators. However, the spontaneous mutation frequency of the pol δ mutant is ~10 times higher than the pol ε mutant (26). This difference in *in vivo* spontaneous mutation frequency may directly reflect the difference in the intrinsic fidelity of pol ε and pol δ but not the extent to which a DNA polymerase synthesizes inside the cell.

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2 K. Hashimoto, K. Shimizu, and A. Sugino, unpublished results.