Published in final edited form as:

Biochimie. 2016 February ; 121: 161–169. doi:10.1016/j.biochi.2015.12.009.

Kinetic analysis of bypass of 7,8-dihydro-8-oxo-2'-deoxyguanosine by the catalytic core of yeast DNA polymerase η

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Abstract

Reactive oxygen species damage DNA bases to produce 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxoG), which results in G:C to T:A transversions. To better understand mechanisms of dNTP incorporation opposite 8-oxoG, we performed pre-steady-state kinetic analysis of nucleotide incorporation using the catalytic core of yeast DNA polymerase η (Pol η_{core} , residues 1-513) instead of full-length Pol η , eliminating potential effects of the C-terminal C₂H₂ sequence motif on dNTP incorporation. Kinetic analysis showed that Pol η_{core} preferred to incorporate dCTP opposite 8-oxoG. A lack of a pre-steady-state kinetic burst for Pol η_{core} suggested that dCTP incorporation is slower than the dissociation of the polymerase from DNA. The extension products beyond the 8-oxoG were determined by LC-MS/MS and showed that 57% of the products corresponded to the correct incorporation (C) and 43% corresponded to dATP misincorporation. The kinetic analysis of 8-oxoG bypass by yeast DNA Pol η_{core} , provides further understanding of the mechanism of mutation at this oxidation lesion with yeast DNA polymerase η .

Keywords

8-oxoG; yeast DNA polymerase η_{core} ; dNTP incorporation; pre-steady-state enzyme kinetics

1. Introduction

Accurate synthesis of DNA is of great importance for the genomic integrity in all forms of life. DNA replication is generally performed by DNA polymerases with high fidelity. In most cases, the progress of the polymerases is impeded upon encounter of endogenous or

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Conflict of interest

The authors declare no conflict of interest regarding the manuscript.

exogenous DNA damage [1][2]. Reactive oxygen species are a major source of DNA damage. One of the most common free radical-induced oxidative DNA damage is 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxoG). Cellular 8-oxoG DNA damage has been linked to several diseases, including aging, cancer, and neurodegenerative and cardiovascular disorders [3]. Eukaryotic replicative DNA polymerases (Pols) play important roles in the bypass of 8-oxoG lesion by inserting dATP opposite this lesion, resulting in G:C to T:A transversions [4, 5].

Translesion synthesis (TLS) DNA polymerases are found in most organisms and are able to bypass DNA damage. Compared to replicative DNA polymerases, TLS polymerases have relatively larger active sites, allowing them to accommodate mismatched base pairs and bulky DNA lesions at the cost of a lower fidelity [1, 2].

The *Saccharomyces cerevisiae* gene *RAD30* encodes a TLS DNA Pol η . The C-terminal C₂H₂ motif of Pol η contains a ubiquitin-binding zinc finger (UBZ) domain and proliferating cell nuclear antigen (PCNA) interacting peptide (PIP) motif [6][7]. The C₂H₂ sequence motif is highly conserved in the corresponding proteins from many species, e.g. *Saccharomyces cerevisiae*, *Homo sapiens*, *Mus musculus*, and *Drosophila melanogaster* (Fig. 1). The C₂H₂ motif plays a key role in specific protein-protein interactions and post-translational modifications [8]. The N-terminal region of Pol η is the catalytic core of DNA polymerase, which is highly homologous among different species (Fig. 1). It contains palm (I and III, residues 1–30 and 130–286), finger (II, residues 34–127), and thumb (residues 289–378) regions and the polymerase-associated domain (IV and V, PAD; residues 395–509) [9]. Areas I to V correspond the conserved sequences. The thumb skims the DNA minor groove surface; and the PAD makes multiple contacts within the DNA major groove. These PAD-DNA hydrogen bond contacts are conserved in almost all Y-family DNA polymerase ternary structures [10, 11]. The catalytic core of Pol η has a more open active site formed by stubby fingers, the thumb domain, and part of the PAD region. Presumably this open structure allows Pol η to accommodate structural distortion induced by some bulky DNA adducts [1].

Yeast Pol η is able to efficiently and accurately replicate through UV-induced cyclobutane pyrimidine dimers (CPDs). Inactivation of Pol η in *S. cerevisiae* and humans leads to increased UV-induced mutagenesis [12]. For example, a variant form (V) of xeroderma pigmentosum, characterized by an elevated incidence of sunlight-induced skin cancers, was reported due to lack of Pol η in humans [2]. In addition to CPDs, Pol η can also bypass many other DNA lesions with varying degrees of efficiency and fidelity [2, 13–15]. When encountering bulky adducts (e.g., dG-C8-AAF and cisplatin-GG lesions) human and yeast Pol η can incorporate nucleotides opposite the sites at reduced rates [1, 16, 17]. Yeast Pol η bypasses *O*⁶-methylguanine lesions by inserting a C or a T opposite this lesion [18]. The enzyme replicates through 8-oxoG efficiently and accurately by inserting a C opposite lesion and then proficiently extending from the 8-oxoG:C base pair [19, 20]. A role for yeast Pol η in error-free bypass of 8-oxoG lesions has been supported by genetic studies [21, 22], as evidenced by a synergistic increase in the rate of spontaneous mutations in the *ogg1* mutant yeast strain devoid of Pol η (defective in the glycosylase required for 8-oxoG removal [21]).

Silverstein *et al.* have reported the crystal structures of the catalytic core of *S. cerevisiae* Pol η (residues 1-513), Pol η_{core} , in a ternary complex with undamaged DNA and with a *cis-syn* T-T dimer [5]. With minimal changes to the polymerase and the bound DNA, the open active site cleft of Pol η_{core} can also accommodate an 8-oxoG lesion in the *anti* conformation at the insertion and post-insertion steps [5]. Moreover, the active site geometry remains unchanged compared with that of undamaged G [5].

The polymerase nucleotide incorporation reaction is composed of several distinct elementary steps [23], including the nucleotide binding step, conformational change and/or chemistry steps, which are responsible for fidelity during bypass of DNA lesions [23]. Pre-steady-state kinetic studies, which directly measure the incorporation of nucleotides during the first catalytic cycle of the polymerase, make it possible to directly examine the elementary steps of nucleotide binding and incorporation. This approach is not influenced by the slow dissociation of the enzyme from DNA, which limits the subsequent steady-state turnover rate [24].

In order to better understand kinetic mechanisms of nucleotide incorporation opposite 8-oxoG by yeast Pol η , we performed pre-steady-state kinetic analysis of nucleotide incorporation and LC-MS/MS sequence analysis of extension products beyond 8-oxoG. Yeast Pol η contains an N-terminal DNA polymerase core and a highly conserved C-terminal C₂H₂ motif [6]. The effects of the C-terminus on the kinetic behavior of dNTP incorporation opposite 8-oxoG and its subsequent extension are unknown. Previous studies have been reported that the C- or N-terminal motifs of DNA polymerases do affect the functions of the catalytic cores. The conformational dynamics of Taq DNA polymerase (relative to the DNA during nucleotide binding and incorporation) were studied using stopped-flow forster resonance energy transfer [25]. The removal of the N-terminal domain of Taq DNA polymerase increased the rate of the global transition. AtPOLK is an Arabidopsis orthologue of mammalian DNA polymerase κ . Removal of the C-terminal 193 amino acids increased the catalytic efficiency and fidelity and also improved the extension of mispaired base pair and the error-free bypass of 8-oxoG [26]. Notably, yeast DNA Pol η_{core} , which lacks the C-terminal C₂H₂ motif, showed decreased DNA binding affinity (2-fold) and reduced base substitution fidelity (3-fold) compared with full-length Pol η [7]. The C-terminus of Pol η may interact with DNA and slightly alter the conformation of the polymerase domain during dNTP incorporation. Therefore, studies using the full-length Pol η may not reveal the intrinsic kinetic behavior of incorporation of dNTP opposite 8-oxoG. Thus, it is necessary to utilize the catalytic core of DNA Pol η to determine its intrinsic kinetic behavior.

We used the catalytic core of yeast Pol η_{core} (residues 1-513) to examine (i) the steady-state dNTP incorporation opposite 8-oxoG, (ii) steady-state next dNTP extension beyond 8-oxoG, (iii) the pre-steady-state dNTP incorporation opposite 8-oxoG, and (iv) LC-MS/MS sequence analysis of full-length extension products. We thus describe the kinetic properties for bypass of 8-oxoG by yeast Pol η_{core} .

2. Materials and methods

2.1 Materials

T4 polynucleotide kinase, restriction endonucleases and unlabeled dNTPs were purchased from New England Biolabs (Beverly, MA). [γ - ^{32}P]ATP (specific activity $3 \times 10^3 \text{ Ci mmol}^{-1}$) was obtained from PerkinElmer Life Sciences (Boston, MA). Bio-spin columns were obtained from Bio-Rad (Hercules, CA). Other reagents were of the highest quality commercially available.

2.2 Alignment of the primary sequences of DNA polymerases η

Amino acid sequences of Pol η of *S. cerevisiae*, *H. sapiens*, *M. musculus*, and *D. melanogaster* were obtained from the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>). GenBank Accession codes of *S. cerevisiae*, *H. sapiens*, *M. musculus*, and *D. melanogaster* are EDN60746.1, BAA81666.1, NP_109640.1 and BAB20905.1, respectively. These amino acid sequences of Pol η were generated by MACAW2.05 [27] with Gibbs Sampler software.

2.2 Expression and purification of Pol η_{core}

The catalytic core of yeast DNA polymerase η (Pol η_{core} , residues 1-513) was expressed and purified as described previously [6, 9, 28]. Yeast Pol η_{core} retains the first N-terminal 513 amino acids of full-length Pol η (1-632). This truncated version of the RAD30 gene was overexpressed in yeast strain BJ5464 as a glutathione transferase (GST)-Rad30 fusion protein under the control of the hybrid GAL PGK promoter in plasmid pHQ241. The GST portion of the fusion protein was removed by treatment with PreScission protease. Purified yeast Pol η_{core} was stored in small aliquots at -30°C in 40 mM Tris-HCl buffer (pH 7.5 at 22°C) containing 100 mM NaCl, 5 mM dithiothreitol, and 50% glycerol (v/v). The concentration of protein was determined by the Bio-Rad Protein Assay bicinchoninic acid (BCA) method with bovine serum albumin as a standard.

2.3 Oligonucleotides

A 13-mer primer, two 14-mer primers, and two 18-mer templates containing G or 8-oxoG (used for kinetic analysis, Table 1) and a 13-mer primer containing uracil (used for LC-MS/MS analysis, dTable 4) were commercially synthesized and purified by HPLC by Midland Certified Reagent Co. (Midland, TX). The ^{32}P -labeled primers and the corresponding primer/template duplexes for steady-state and pre-steady-state kinetic experiments and LC-MS/MS sequence analysis were prepared as described previously [29].

2.4 Reaction conditions for yeast Pol η_{core} assays and product analysis methods

Standard DNA polymerization reactions with yeast Pol η_{core} were performed in a reaction buffer (40 mM Tris-HCl buffer (pH 7.5 at 25°C), 100 mM NaCl, 5 mM dithiothreitol and 3% (v/v) glycerol) at 37°C . All reactions were initiated by mixing a dNTP-MgCl₂ (final MgCl₂ concentration of 5 mM) solution with pre-incubated enzyme-DNA mixtures. The reactions were stopped and the products were analyzed as described previously [30].

2.5 Full-length extension with four dNTPs

Full-length extension reactions were initiated by mixing yeast Pol η_{core} (0, 2, 5, 20, and 50 nM) and 100 nM 13-mer/18-mer DNA mixtures with four dNTPs (each 0.1 mM) and 5 mM MgCl_2 . After reaction for 10 min, each mixture was quenched by the addition of EDTA-formamide solution. The extension products were visualized by phosphorimaging analysis using a Bio-Rad Molecular Imager FX instrument [29].

2.6 Steady-state single dNTP incorporation

Reactions were initiated by mixing 50 nM yeast Pol η_{core} and 120 nM DNA mixtures with 1 mM of each dNTP and 5 mM MgCl_2 . The reactions were quenched every 30 s with EDTA-formamide solution (up to 5 min) (values at zero time points were determined without the addition of dNTP). The products were quantified and analyzed as described above. Products and time were fit to a linear equation to obtain the incorporation rates [32].

2.7 Steady-state one-base incorporation and next-base extension kinetic assays

Steady-state single-base incorporation and next-base extension experiments were performed by adding varied concentrations (twelve in each set) of each individual dNTP and MgCl_2 to the polymerase-DNA complexes in the indicated reaction buffer. The molar ratio of yeast Pol η_{core} to DNA substrate was <10%, and primer conversion to product was kept < 20% by adjusting the polymerase concentration and incorporation time [31]. Plots of incorporation rates versus dNTP concentration were fit to a hyperbolic Michaelis-Menten equation to yield k_{cat} and K_{m} values using nonlinear regression in Prism Version 3.0 (GraphPad, San Diego, CA). The corresponding errors were also obtained to indicate the goodness of fit [29].

2.8 Pre-steady-state single dCTP incorporation

Rapid quench experiments were performed in a RQF-3 KinTek Quench Flow Apparatus (KinTek, Austin, TX). Drive syringes on both sides contained 50 mM Tris-HCl buffer (pH 7.4) and the middle (quench) syringe contained 500 mM EDTA. Reactions were initiated by mixing 50 nM Pol η_{core} /120 nM oligonucleotide mixtures with dCTP (varying concentrations) complexed with 5 mM MgCl_2 . After varying times, the reactions were quenched by the addition of 500 mM EDTA from the central syringe line. The products were analyzed and quantitated as described earlier. Product concentration and reaction time were fit to Eq. 1 (where P is product concentration; $[E]_0$ is total DNA polymerase concentration; and t is time) to obtain the incorporation rate k_{SS} :

$$P = [E]_0 k_{\text{SS}} t \quad (\text{Eq. 1})$$

All analysis used GraphPad Prism Version 3.0. The corresponding SE values were included to indicate the goodness of fit [32].

2.9 LC-MS/MS analysis of primer extension products

The primers were extended by incubating yeast Pol η_{core} (4 μM), DNA (10 μM), a mixture of four dNTPs (1 mM each), and MgCl_2 (5 mM) in Tris-HCl buffer (pH 7.5, final volume

100 μ l) at 37 °C for 4 h. The reaction was stopped by removing the unreacted dNTPs with a Bio-Spin 6 chromatography column, and the concentration of Tris-HCl, DTT, and EDTA were adjusted to 50, 5, and 1 mM, respectively. *Escherichia coli* uracil DNA glycosylase (20 units, Sigma-Aldrich, St. Louis, MO) was added to the reaction. After incubation at 37 °C for 6 h to remove the uracil residues from the extended primer, 0.5 M piperidine was added to the solution and it was heated at 95 °C for 1 h, and then dried by vacuum centrifugation [29, 30]. The dried sample was resuspended in 100 μ l of H₂O for MS analysis.

LC-MS/MS analysis was performed using a Waters Acquity UPLC system (Waters, Milford, MA) connected to a Finnigan LTQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA) operating in the ESI negative ion mode, as described previously [29, 30]. An acquity UPLC BEH octadecylsilane (C₁₈) column (1.7 μ m, 1.0 mm $\times$ 100 mm) was used with the following LC conditions (all at 50 °C) with Buffer A (10 mM NH₄CH₃CO₂ plus 2% CH₃CN (v/v)) and Buffer B (10 mM NH₄CH₃CO₂ plus 95% CH₃CN (v/v)). The calculations of the CID fragmentations of oligonucleotide sequences were done using a program linked to the Mass Spectrometry Group (Medicinal Chemistry) of the University of Utah (www.medlib.med.utah.edu/massspec) [29, 30].

3. Results

3.1 Primer extension by Pol η_{core} using all four dNTPs

Oligonucleotide complexes containing nmodified G and 8-oxoG could be extended to full-length with increasing concentrations of Pol η_{core} (Fig. 2) (sequences of DNA oligonucleotides shown in Table 1). The 13-mer primer was readily extended to an 18-mer. Compared with the oligonucleotide containing unmodified G, extension beyond 8-oxoG was partially inhibited, i.e. unextended primer was still observed in the reaction containing 2, 5, or 20 nM Pol η_{core} . The 14-mer product was observed for 8-oxoG, suggesting that the extensions were partially blocked after incorporation of a single dNTP opposite 8-oxoG. Higher concentrations of Pol η_{core} resulted in complete extension beyond 8-oxoG, yielding the full-length 18-mer products. These results showed that 8-oxoG partially inhibited full-length extension by yeast Pol η_{core} compared with unmodified G.

3.2 Steady-state kinetic analysis of dNTP incorporation opposite G or 8-oxoG by yeast Pol η_{core}

Steady-state kinetic parameters (k_{cat} and K_{m}) were measured for the incorporation of each single dNTP opposite G or 8-oxoG (Table 2). The incorporation of dCTP opposite G or 8-oxoG yielded similar k_{cat} and K_{m} values, as well as incorporation efficiencies ($k_{\text{cat}}/K_{\text{m}}$). The efficiencies for the (mis)incorporation of dATP, dGTP, or dTTP opposite G were similar, ~ 3 orders of magnitude relative to dCTP incorporation, yielding misincorporation ratios of $\sim 10^{-4}$. The misincorporation of dGTP or dTTP opposite 8-oxoG was 700- to 900-fold lower than for dCTP opposite 8-oxoG, giving misincorporation ratios of $\sim 10^{-3}$. Notably, the incorporation efficiency of dATP opposite 8-oxoG was reduced only 56-fold compared with dCTP incorporation and was much higher than that of dGTP or dTTP, yielding a misincorporation ratio of 10^{-2} . These data show that misincorporation ratios of dGTP, dTTP,

and dATP opposite 8-oxoG were one or two orders higher than the corresponding misincorporation ratios opposite unmodified G.

3.3 Steady-state kinetic analysis of next-base extension from primer-template terminus containing G or 8-oxoG by yeast Pol η_{core}

Steady-state kinetic parameters (k_{cat} and K_{m}) were measured for the next-base extension beyond G or 8-oxoG (Table 3). The base C or A at the end of primer paired or mispaired with template G or 8-oxoG, respectively (Table 1). dGTP is incorporated opposite the next template base C. The C:G (primer:template) pair was much more readily extended than A:G mismatched pair by yeast Pol η_{core} , yielding a 740-fold higher dGTP incorporation efficiency. Both C:8-oxoG and A:8-oxoG can also be readily extended by Pol η_{core} , giving only 2-fold difference in dGTP incorporation efficiency. These results indicate that yeast Pol η_{core} extends a pair or mispair containing 8-oxoG with almost similar efficiency.

3.4 Steady-state and pre-steady-state kinetic analysis of dNTP incorporation opposite G or 8-oxoG by yeast Pol η_{core}

Generally, incorporation of a correct dNTP by most DNA polymerases shows a biphasic character (i.e. burst phase and linear phase), indicating that the dNTP incorporation step is much faster than events occurring after phosphodiester bond formation. The burst phase corresponds to the fast dNTP incorporation step in the first turnover and the linear phase corresponds to the subsequent turnovers, which are limited by steps after nucleotide transfer. In order to determine the elementary steps of dNTP incorporation during the first turnover by yeast Pol η_{core} , pre-steady-state kinetic methods were used to examine the incorporation of dNTP opposite 8-oxoG. dCTP was preferentially incorporated opposite G or 8-oxoG by yeast Pol η_{core} (Fig. 2). Incorporation of dCTP opposite G or 8-oxoG was completed in the first 30 s; while the misincorporation of dTTP, dGTP, or dATP opposite G or 8-oxoG increased linearly over 90 s (Fig. 2A,C). Incorporation of dCTP opposite G or 8-oxoG was detected within the first 30 s. Surprisingly, the product and reaction time showed a linear shape, not a biphasic shape (Fig. 2B,D), suggesting that dCTP incorporation is not faster than the dissociation of yeast Pol η_{core} from DNA or other steps following nucleotide transfer. The incorporation rates were 0.094 s^{-1} and 0.088 s^{-1} for the incorporation of dCTP opposite G or 8-oxoG, similar to the steady-state k_{cat} values of 0.22 s^{-1} and 0.21 s^{-1} , respectively.

3.5 Analysis of primer incorporation/extension products beyond G or 8-oxoG by LC-MS/MS sequence analysis

Misincorporation frequencies can be estimated from the steady-state kinetic analysis were calculated based on the ratio of incorporation efficiencies at each individual experiment containing only one single dNTP. LC-MS/MS sequence analysis provides an efficient way to determine incorporation during DNA polymerization with all four dNTPs in one reaction.

The sequences of the extension products formed by yeast Pol η_{core} were analyzed by LC-MS/MS (Figs. 3 and 4). Insertion of uracil residues in the primer strand (Table 4) and the use of uracil DNA glycosylase (to reduce the product length) simplified MS sequence analysis. Primers were fully extended under the conditions used, as confirmed by denaturing PAGE

(data not shown), and each component in the oligonucleotide product mixture was identified and quantified by LC-MS/MS (Figs. 3 and 4). Several possible extension products were detected, including 17-mer or 18-mer extension products, misincorporations, frameshift, blunt-end additions, and their different combinations. The peaks corresponding to -3 charged ions were used for the quantification. Other peaks corresponding to -2 or -4 charged ions (and some -3 ions plus sodium) were confirmed by MS/MS. For the identically ionized products, the amount of each product was proportional to its corresponding peak area in LC analysis and the percentage of each product can be calculated based on the percentage of each peak area [29, 30].

The major ions of extension products beyond 8-oxoG were observed at m/z 724.2, 732.4, 820.5, 825.5, and 828.8, corresponding to the -3 ions, m/z 605.8 corresponding to the -4 ion, and m/z 1086.6 and 1098.6 corresponding to the -2 ions (Fig. 3B). Because the -3 ions had higher abundance than the other ions, the -3 ions were selected for sequence analysis and quantitation. The total ion trace for the m/z 724.2 ion MS/MS is shown in Fig. 3C, and CID analysis of the m/z 724.2 ion resulting in the fragmentation pattern is shown in Fig. 3D. The major ions in the fragmentation pattern were consistent with the sequence 5'-pTC CGTGA-3' (Table S1), which corresponds to the insertion of C opposite 8-oxoG and accurate full-length extension of the primer. The total ion trace for the m/z 732.4 ion MS/MS is shown in Fig. 3E, and CID analysis of the m/z 732.4 ion (resulting in the fragmentation pattern) is shown in Fig. 3F. The major ions in the fragmentation pattern were consistent with the sequence 5'-pTCAGTGA-3' (Table S2), which corresponds to the insertion of A opposite 8-oxoG and accurate full-length extension of the primer. The other ions were also identified and related to the corresponding sequences (Fig. 4 and Tables S3–S5).

With unmodified G in the template, all extension products corresponded to the correct incorporation of dCTP opposite G followed by accurate extension with some blunt-end additions (Table 4), consistent with the preferential dCTP incorporation in steady-state and pre-steady-state kinetic analysis. No frameshift products were observed. With 8-oxoG, 57% of the products corresponded to incorporation of dCTP opposite 8-oxoG and 43% corresponded to misincorporation of dATP opposite 8-oxoG (Tables 4 and S1–S5). No other misincorporation or frameshift products were observed for 8-oxoG.

4. Discussion

Yeast DNA Pol η contains an N-terminal catalytic core and a C-terminal highly conserved C₂H₂ motif. The C₂H₂ motif plays a key role in specific protein-protein interactions and may affect dNTP incorporation. It has been reported that yeast DNA Pol η_{core} , which lacks the C-terminal C₂H₂ motif, has less DNA binding affinity (2-fold) and lower base substitution fidelity (3-fold) compared with full-length Pol η [7]. In order to study the elementary steps for bypass of 8-oxoG, pre-steady-state kinetic analysis of dNTP incorporation was done using yeast Pol η_{core} . Pol η_{core} contains only the catalytic core of the polymerase (residues 1–513), eliminating the effects of the C₂H₂ motif on dNTP incorporation and subsequent extension. The truncated Pol η (residues 1–452), with further deletion of other residues in the C terminus, is devoid of DNA polymerase activity [6].

The kinetic analysis of dNTP incorporations opposite G or 8-oxoG has also been studied using full-length yeast Pol η [21]. For both Pol η and Pol η_{core} , dCTP was preferentially incorporated opposite G or 8-oxoG, and the misincorporation ratios were 10^{-4} for G and 10^{-2} to 10^{-3} for 8-oxoG. The incorporation efficiencies of dCTP opposite G and 8-oxoG were similar for Pol η and Pol η_{core} .

K_m values for dNTP incorporation (measures of how efficient the enzyme utilizes dNTP) were increased for Pol η_{core} compared with Pol η . The K_m values for incorporation of dCTP opposite G and 8-oxoG were $7 \mu\text{M}$ for Pol η_{core} and $0.2 \mu\text{M}$ for full-length Pol η . This significant difference in K_m may be attributable to the stability of the polymerase-DNA complex, the active site of DNA polymerase, or base pairing with the incoming dNTP, regardless of the reaction conditions. It has been reported that DNA binding affinity was weakened 2-fold for Pol η_{core} compared with full length Pol η [7], indicating that the removal of C-terminal motif from Pol η reduces the stability of the DNA polymerase-DNA complex. The less stable Pol η_{core} -DNA complex may reduce the utilization efficiency of dNTP and increase the K_m values.

The next-base extension reaction has also been studied using full-length yeast Pol η (Table 3) [21]. With unmodified G, both Pol η and Pol η_{core} preferentially extend the matched base pair rather than the mismatched base pair. Pol η showed a 7-fold preference to extend C:8-oxoG relative to A:8-oxoG; while Pol η_{core} showed only a 2-fold preference. Compared with Pol η , Pol η_{core} enhanced the extension from the mismatched A:8-oxoG base pair.

Most DNA polymerases incorporate dNTP faster than the dissociation of polymerase from DNA, as indicated by a kinetic burst phase. Incorporation of dATP opposite T by full-length Pol η showed a kinetic burst, with a k_{pol} value of 1.3 s^{-1} (25 mM Tris-HCl buffer (pH 7.5), 5 mM MgCl_2 , 5 mM DTT, and 10% glycerol at 30°C) [28]. Incorporation of dCTP opposite G or 8-oxoG by Pol η also showed a burst with a k_{pol} value of 2.1 or 2.2 s^{-1} , respectively, in the same buffer at 22°C [33]. Incorporation of dATP opposite G or 8-oxoG showed no burst phase, indicative of a lower efficiency than incorporation of dCTP. Pol η incorporates dATP more efficiently opposite 8-oxoG than opposite G [33]. In this work, incorporation of dCTP opposite G or 8-oxoG using pol η_{core} did not show a pre-steady-state burst but gave a linear shape, with a rate of 0.094 and 0.088 s^{-1} , respectively, in a similar reaction buffer (40 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 5 mM dithiothreitol, and 3% (v/v) glycerol) at 37°C). The absence of kinetic bursts for pol η_{core} indicates that the incorporation step was not faster than steps following nucleotidyl transfer. The possible reasons may be due to higher temperature (37°C), higher NaCl concentrations (100 mM), and/or the removal of C_2H_2 motif from Pol η .

Steady-state incorporation efficiencies and misincorporation ratios were calculated based on each individual experiment with a single dNTP. In these assays, Pol η_{core} incorporated dCTP opposite 8-oxoG with 57-fold higher efficiency than that of dATP (Table 2). However, DNA polymerization is a continuous and dynamic process when all four dNTP are present in one reaction system. In the LC-MS/MS analysis. assays, about 57% of the products corresponded to the incorporation of dCTP and 43% products to the incorporation of dATP (Table 4). These data show that Pol η_{core} did not discriminate as well between dATP and

dCTP during 8-oxoG bypass if four dNTPs were present together, a condition that mimics the physiological conditions in yeast cell. These conclusions are different from the kinetic data which were calculated from each individual reaction containing only a single dNTP. One possible explanation is that four dNTPs compete with each other during the DNA polymerization. The DNA polymerase may bind incorrect dNTPs and retard the conformation change or the chemical step and then emit the wrong dNTP and select the correct dNTP. The simultaneous presence of nucleotides affects the bypass of DNA damage, in a manner that is not revealed in the analyses of the kinetic constants in steady-state kinetic analysis with individual dNTPs.

Full-length primer extension past 8-oxoG was partially inhibited compared to the G (Fig. 2). Steady-state kinetic analysis showed that the major dNTP incorporated opposite G or 8-oxoG was dCTP (Table 2). These results suggest that dCTP incorporation should be inhibited opposite 8-oxoG compared with regular G. However, Pol η_{core} incorporated dCTP opposite G or 8-oxoG with the same steady-state kinetic efficiency (Table 2). Extension efficiency beyond C:8-oxoG or C:dG base pair was also comparable (Table 3). Therefore, we propose that Pol η_{core} tends to incorporate dATP at a higher frequency than expected in the presence of all four dNTPs. The inefficient incorporation of dATP opposite 8-oxoG and its subsequent extension could inhibit full-length extension beyond 8-oxoG. The LC-MS/MS sequence analysis confirmed that dATP was incorporated more than would be estimated based on the basis of the steady-state kinetic analysis, i. e. incorporation of dATP opposite 8-oxoG was 43%, similar to the 57% for incorporation of dCTP opposite 8-oxoG. Therefore, more dATP was incorporated opposite 8-oxoG with a mixture of dNTPs than predicted using only a single dNTP.

Crystal structures of yeast Pol η_{core} in complex with DNA containing 8-oxoG have been reported [5]. The open active site cleft of Pol η_{core} can accommodate an 8-oxoG lesion in a *syn*-pair with C and *anti*-pair with A at both insertion and postinsertion steps, giving similar active site geometry as the undamaged complex [5]. Therefore, it is reasonable that both dCTP and dATP could be almost equally incorporated opposite 8-oxoG, and both matched and mismatched base pair containing 8-oxoG could be almost equally extended.

Human DNA polymerase κ (hPol κ) prefers to insert dATP rather than dCTP opposite 8-oxoG, leading to a conversion from G:C pairing to T:A pairing [41]. The efficiency ($k_{\text{cat}}/K_{\text{m}}$) for incorporation of dCTP opposite 8-oxoG was decreased 80-fold compared with unmodified G. However, the efficiency for misincorporation of dATP opposite 8-oxoG was increased 100-fold compared with unmodified G. Crystal structures of the complex of hPol κ and DNA containing 8-oxoG showed that the N-terminal extension of hPol κ stabilizes its little finger domain, which surrounds the Hoogsteen base pair of 8-oxoG and incoming dATP. This structure explains the increased efficiency for the incorporation of dATP opposite 8-oxoG [38].

Bypass of the 8-oxoG lesion has also been studied using *S. solfataricus* Dpo4. The insertion of dCTP opposite G or 8-oxoG has similar catalytic efficiency, whereas the efficiency for incorporation of dATP opposite 8-oxoG was increased 250-fold compared with G, thus decreasing fidelity 200-fold opposite 8-oxoG relative to unmodified G [39]. Crystal

structures of the ternary complex of Dpo4, 8-oxoG, and dCTP showed that the Arg-332 residue in the little finger domain of Dpo4 can form a hydrogen bond with the oxygen atom at the C8 position of 8-oxoG and stabilize the Watson-Crick base pair of incoming dCTP and 8-oxoG. No such bonding was observed to stabilize W-C pairing of 8-oxoG and dCTP at the active site of hPol κ . In hPol κ , Hoogsteen pairing of 8-oxoG:dATP appears more favorable relative to a 8-oxoG:dCTP pair, leading to structurally different bypass of 8-oxoG by Dpo4 and hPol κ [40].

5. Conclusions

In this work, we studied pre-steady-state kinetics of nucleotide incorporation opposite 8-oxoG using Pol η_{core} to eliminate the potential effects of the C-terminal C₂H₂ sequence motif on dNTP incorporation. Kinetic analysis indicate that Pol η_{core} showed preference for the incorporation of dCTP opposite 8-oxoG and its subsequent extension but yielded no kinetic burst for any incorporation, suggesting that the incorporation step is not faster than subsequent steps, including the dissociation of the polymerase from DNA. LC-MS/MS sequence analysis shows that 57% of extension products corresponded to the correct incorporation while 43% corresponded to incorrect dATP incorporation. More dATP was incorporated opposite 8-oxoG with a mixture dNTPs than that predicted by steady-state kinetic assays using only single dNTPs. This study revealed some of kinetic aspects of bypass of 8-oxoG by yeast DNA Pol η_{core} , providing more understanding of the mutagenic mechanism of the oxidation lesion with yeast DNA Pol η .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge financial support by the Natural Science Foundation of China (NSFC Nos. 31370793 and 814220410 and the United States National Institutes of Health grant R01 ES010546..

References

1. Vooradi V, Romano LJ. Effect of N-2-Acetylaminofluorene and 2-Aminofluorene Adducts on DNA Binding and Synthesis by Yeast DNA Polymerase η . *Biochemistry*. 2009; 48:4209–4216. [PubMed: 19354292]
2. Swanson AL, Wang JS, Wang YS. In Vitro Replication Studies of Carboxymethylated DNA Lesions with *Saccharomyces cerevisiae* Polymerase η . *Biochemistry*. 2011; 50:7666–7673. [PubMed: 21809836]
3. Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA. Gene regulation and DNA damage in the ageing human brain. *Nature*. 2004; 429:883–891. [PubMed: 15190254]
4. Einolf HJ, Guengerich FP. Fidelity of nucleotide insertion opposite 8-Oxo-7,8-dihydroguanine by mammalian DNA polymerase δ . Steady-state and pre-steady-state kinetic analysis. *J Biol Chem*. 2001; 276:3764–3771. [PubMed: 11110788]
5. Silverstein TD, Jain R, Johnson RE, Prakash L, Prakash S, Aggarwal AK. Structural Basis for Error-free Replication of Oxidatively Damaged DNA by Yeast DNA Polymerase η . *Structure*. 2010; 18:1463–1470. [PubMed: 21070945]

6. Kondratieck CM, Washington MT, Prakash S, Prakash L. Acidic residues critical for the activity and biological function of yeast DNA polymerase η . *Mol Cell Biol*. 2001; 21:2018–2025. [PubMed: 11238937]
7. Brown JA, Zhang L, Sherrer SM, Taylor J-S, Burgers PMJ, Suo Z. Pre-Steady-State Kinetic Analysis of Truncated and Full-Length *Saccharomyces cerevisiae* DNA Polymerase ϵ . *Journal of nucleic acids*. 2010; 2010
8. Johnson RE, Washington MT, Prakash S, Prakash L. Bridging the gap: a family of novel DNA polymerases that replicate faulty DNA. *Proc Natl Acad Sci USA*. 1999; 96:12224–12226. [PubMed: 10535901]
9. Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S, Aggarwal AK. Structural basis for the suppression of skin cancers by DNA polymerase ϵ . *Nature*. 2010; 465:1039–U1096. [PubMed: 20577207]
10. Nair DT, Johnson RE, Prakash L, Prakash S, Aggarwal AK. Rev1 employs a novel mechanism of DNA synthesis using a protein template. *Science*. 2005; 309:2219–2222. [PubMed: 16195463]
11. Lone S, Townson SA, Uljon SN, Johnson RE, Brahma A, Nair DT, Prakash S, Prakash L, Aggarwal AK. Human DNA polymerase κ encircles DNA: implications for mismatch extension and lesion bypass. *Mol Cell*. 2007; 25:601–614. [PubMed: 17317631]
12. Washington MT, Johnson RE, Prakash L, Prakash S. The mechanism of nucleotide incorporation by human DNA polymerase δ differs from that of the yeast enzyme. *Mol Cell Biol*. 2003; 23:8316–8322. [PubMed: 14585988]
13. Patra A, Nagy LD, Zhang Q, Su Y, Mueller L, Guengerich FP, Egli M. Kinetics Structure, and Mechanism of 8-Oxo-7,8-dihydro-2'-deoxyguanosine Bypass by Human DNA Polymerase ϵ . *J Biol Chem*. 2014; 289:16867–16882. [PubMed: 24759104]
14. Patra A, Nagy LD, Zhang Q, Su Y, Mueller L, Guengerich FP, Egli M. How DNA Polymerase ϵ Deals with DNA Damage. *J Biol Chem*. 2014; 289:16883.
15. Zhao B, Xie ZW, Shen HY, Wang ZG. Role of DNA polymerase ϵ in the bypass of abasic sites in yeast cells. *Nucleic Acids Res*. 2004; 32:3984–3994. [PubMed: 15284331]
16. Vaisman A, Masutani C, Hanaoka F, Chaney SG. Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase ϵ . *Biochemistry*. 2000; 39:4575–4580. [PubMed: 10769112]
17. Chaney SG, Campbell SL, Bassett E, Wu YB. Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. *Crit Rev Oncol Hematol*. 2005; 53:3–11. [PubMed: 15607931]
18. Haracska L, Washington MT, Prakash S, Prakash L. Inefficient bypass of an abasic site by DNA polymerase ϵ . *J Biol Chem*. 2001; 276:6861–6866. [PubMed: 11106652]
19. Yung CW, Loakes D, Arimoto S, Negishi K, Negishi T. Kinetics of dCTP incorporation opposite to 7,8-dihydro-8-oxoguanine with different 5' nearest neighbors by yeast polymerase ϵ . *Nucleic acids symposium series*. 2008; 52:531–532.
20. McCulloch SD, Kokoska RJ, Garg P, Burgers PM, Kunkel TA. The efficiency and fidelity of 8-oxoguanine bypass by DNA polymerases δ and ϵ . *Nucleic Acids Res*. 2009; 37:2830–2840. [PubMed: 19282446]
21. Haracska L, Yu SL, Johnson RE, Prakash L, Prakash S. Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase ϵ . *Nature Genetics*. 2000; 25:458–461. [PubMed: 10932195]
22. Yuan F, Zhang Y, Rajpal DK, Wu X, Guo D, Wang M, Taylor JS, Wang Z. Specificity of DNA lesion bypass by the yeast DNA polymerase ϵ . *J Biol Chem*. 2000; 275:8233–8239. [PubMed: 10713149]
23. Zhang HD, Guengerich FP. Effect of N-2-Guanyl Modifications on Early Steps in Catalysis of Polymerization by *Sulfolobus solfataricus* P2 DNA Polymerase Dpo4 T239W. *J Mol Biol*. 2010; 395:1007–1018. [PubMed: 19969000]
24. Washington MT, Prakash L, Prakash S. Mechanism of nucleotide incorporation opposite a thymine-thymine dimer by yeast DNA polymerase ϵ . *Proc Natl Acad Sci U S A*. 2003; 100:12093–12098. [PubMed: 14527996]
25. Xu C, Maxwell BA, Suo Z. Conformational Dynamics of *Thermus aquaticus* DNA Polymerase I during Catalysis. *J Mol Biol*. 2014; 426:2901–2917. [PubMed: 24931550]

26. Garcia-Ortiz MV, Roldan-Arjona T, Ariza RR. The noncatalytic C-terminus of AtPOLK Y-family DNA polymerase affects synthesis fidelity, mismatch extension and translesion replication. *Febs Journal*. 2007; 274:3340–3350. [PubMed: 17550419]
27. Schuler GD, Altschul SF, Lipman DJ. A workbench for multiple alignment construction and analysis. *Proteins*. 1991; 9:180–190. [PubMed: 2006136]
28. Washington MT, Prakash L, Prakash S. Yeast DNA polymerase eta utilizes an induced-fit mechanism of nucleotide incorporation. *Cell*. 2001; 107:917–927. [PubMed: 11779467]
29. Zhang HD, Eoff RL, Kozekov ID, Rizzo CJ, Egli M, Guengerich FP. Versatility of Y-family *Sulfolobus solfataricus* DNA Polymerase Dpo4 in Translesion Synthesis Past Bulky N-2-Alkylguanine Adducts. *J Biol Chem*. 2009; 284:3563–3576. [PubMed: 19059910]
30. Zhang H, Eoff RL, Kozekov ID, Rizzo CJ, Egli M, Guengerich FP. Structure-Function Relationships in Miscoding by *Sulfolobus solfataricus* DNA Polymerase Dpo4 : guanine N2,N2-dimethyl substitution produces inactive and miscoding polymerase complexes. *J Biol Chem*. 2009; 284:17687–17699. [PubMed: 19542237]
31. Goodman MF, Creighton S, Bloom LB, Petruska J. Biochemical basis of DNA replication fidelity. *Crit Rev Biochem Mol Biol*. 1993; 28:83–126. [PubMed: 8485987]
32. Zhang HD, Beckman JW, Guengerich FP. Frameshift Deletion by *Sulfolobus solfataricus* P2 DNA Polymerase Dpo4 T239W Is Selective for Purines and Involves Normal Conformational Change Followed by Slow Phosphodiester Bond Formation. *J Biol Chem*. 2009; 284:35144–35153. [PubMed: 19837980]
33. Carlson KD, Washington AT. Mechanism of efficient and accurate nucleotide incorporation opposite 7,8-dihydro-8-oxoguanine by *Saccharomyces cerevisiae* DNA polymerase eta. *Mol Cell Biol*. 2005; 25:2169–2176. [PubMed: 15743815]
34. Eoff RL, Irimia A, Egli M, Guengerich FP. *Sulfolobus solfataricus* DNA polymerase Dpo4 is partially inhibited by “wobble” pairing between O-6-methylguanine, cytosine, but accurate bypass is preferred. *J Biol Chem*. 2007; 282:1456–1467. [PubMed: 17105728]
35. Eoff RL, Angel KC, Egli M, Guengerich FP. Molecular basis of selectivity of nucleoside triphosphate incorporation opposite O-6-benzylguanine by *Sulfolobus solfataricus* DNA polymerase Dpo4 - Steady-state and pre-steady-state kinetics and X-ray crystallography of correct and incorrect pairing. *J Biol Chem*. 2007; 282:13573–13584. [PubMed: 17337730]
36. Zhang H, Eoff RL, Kozekov ID, Rizzo CJ, Egli M, Guengerich FP. Versatility of Y-family *Sulfolobus solfataricus* DNA Polymerase Dpo4 in Translesion Synthesis Past Bulky N(2)-Alkylguanine Adducts. *J Biol Chem*. 2009; 284:3563–3376. [PubMed: 19059910]
37. Zhao L, Pence MG, Christov PP, Wawrzak Z, Choi JY, Rizzo CJ, Egli M, Guengerich FP. Basis of Miscoding of the DNA Adduct N-2,3-Ethenoguanine by Human Y-family DNA Polymerases. *J Biol Chem*. 2012; 287:35516–35526. [PubMed: 22910910]
38. Irimia A, Eoff RL, Guengerich FP, Egli M. Structural and Functional Elucidation of the Mechanism Promoting Error-prone Synthesis by Human DNA Polymerase kappa Opposite the 7,8-Dihydro-8-oxo-2'-deoxyguanosine Adduct. *J Biol Chem*. 2009; 284:22467–22480. [PubMed: 19542228]
39. Zang H, Irimia A, Choi JY, Angel KC, Loukachevitch LV, Egli M, et al. Efficient and high fidelity incorporation of dCTP opposite 7,8-dihydro-8-oxodeoxyguanosine by *Sulfolobus solfataricus* DNA polymerase Dpo4. *J Biol Chem*. 2006; 281:2358–2372. [PubMed: 16306039]
40. Eoff RL, Irimia A, Angel K, Egli M, Guengerich FP. Hydrogen bonding of 7,8-dihydro-8-oxodeoxyguanosine with a charged residue in the little finger domain determines miscoding events in *Sulfolobus solfataricus* DNA polymerase Dpo4. *J Biol Chem*. 2007; 282:19831–19843. [PubMed: 17468100]

Highlights

Use of yeast pol η_{core} eliminates the effects of C-terminal C₂H₂ motif on dNTP incorporation.

Pol η_{core} preferentially incorporates dCTP opposite 8-oxoG and extends from it.

Pol η_{core} showed no kinetic burst for incorporation opposite G or 8-oxoG.

Pol η_{core} inserted 57% C and 43% A opposite 8-oxoG followed, by correct extension.

Fig. 1. Alignment of primary sequences of DNA polymerases η
Shaded boxes indicate the positions of the N-terminal catalytic core and the C-terminal conserved C₂H₂ motif. The catalytic core region of DNA polymerases contains the conserved palm (I and III), finger (II) and polymerase associated (IV and V) domains. The C-terminal C₂H₂ motif contains the ubiquitin-binding zinc finger (UBZ) domain and proliferating cell nuclear antigen (PCNA) interacting peptide (PIP) motif. The numbers in parentheses on the right indicate numbers of amino acids in the DNA polymerase η in each species.

Fig. 2. Extension of ^{32}P -labeled primers beyond G or 8-oxoG by yeast Pol η_{core} with all four dNTPs present

Reactions were performed for 10 min with increasing concentrations of yeast Pol η_{core} (0, 2, 5, 20, or 50 nM) and 100 nM DNA substrate (primer/template) in the presence of all four dNTPs (100 μM each). Reaction products were resolved by denaturing gel electrophoresis with subsequent phosphorimaging analysis. The symbol * in the scheme indicates G or 8-oxoG at the incorporation position.

Fig. 3. Steady-state and pre-steady-state incorporation of a single dNTP opposite G or 8-oxoG by Pol η_{core}

Yeast Pol η_{core} (50 nM) was incubated with 13-mer/18-mer primer:template complex (120 nM each), each individual dNTP (1 mM), and 5 mM Mg^{2+} in a RQF-3 KinTek quench flow apparatus to initiate single-base incorporation reactions. *A*, Incorporations opposite G (scale of minutes); *B*, incorporation of dCTP opposite G (scale of seconds) with a linear rate (slope) of 0.094 s^{-1} ; *C*, incorporations opposite 8-oxoG (scale of minutes); *D*, incorporation of dCTP opposite 8-oxoG (scale of seconds) with a linear rate (slope) of 0.088 s^{-1} .

Fig. 4. LC-MS/MS sequence analysis of full-length extension products beyond 8-oxoG by yeast Pol η_{core}

A, Total ion current trace of the extension products. **B**, ESI mass spectrum of peak eluted at 3.3 min in **A**. **C**, Reconstructed single reaction monitoring profile of m/z 724.2. **D**, CID spectrum of m/z 724.2. See Table S1 for lists of assignments of CID ions. **E**, Reconstructed single reaction monitoring profile of m/z 732.4. **F**, CID spectrum of m/z 732.4. See Table S2 for lists of assignments of CID ions.

Fig. 5. LC-MS/MS sequence analysis of the full-length extension products beyond 8-oxoG by yeast Pol η_{core}

A, Reconstructed single reaction monitoring profile of m/z 820.5. **B**, CID spectrum of m/z 820.5. See Table S3 for lists of assignments of CID ions. **C**, Reconstructed single reaction monitoring profile of m/z 828.8. **D**, CID spectrum of m/z 828.8. See Tables S4 and S5 for lists of assignments of CID ions.

Table 1

Oligodeoxynucleotides used in this study

Name	Sequence	Experiment
13-mer	5'-GGGGGAAGGATTC- 3'	Single dNTP incorporation
18-mer	3'-CCCCCTTCCTAAGG*CACT- 5'	
14A-mer	5'-GGGGGAAGGATTCA- 3'	Next-base extension
14C-mer	5'-GGGGGAAGGATTCC- 3'	
18-mer	3'-CCCCCTTCCTAAGG*CACT- 5'	

G*: G or 8-oxoG

Steady-state kinetic parameters for one-base incorporation opposite G or 8-oxodG by yeast Pol η_{core} ^a**Table 2**

Template	dNTP	$K_{\text{m,dNTP}} \mu\text{M}$	$k_{\text{cat}} \times 10^3 \text{ s}^{-1}$	$k_{\text{cat}}/K_{\text{m}} \times 10^5 \mu\text{M}^{-1} \text{ s}^{-1}$	Efficiency relative to dCTP:G	dNTP selectivity ^b
5'-C	C	6.9 ± 0.9	220 ± 5	3190	1	
	A	83 ± 11	1.9 ± 0.1	2.3	1400-fold less	7.1 × 10 ⁻⁴
	G	180 ± 22	2.3 ± 0.1	1.3	2500-fold less	4.1 × 10 ⁻⁴
	T	950 ± 80	16 ± 0.6	1.7	1900-fold less	5.3 × 10 ⁻⁴
3'-GG*G	C	7.0 ± 0.7	208 ± 4	3000	1.1-fold less	
	A	126 ± 19	65 ± 4	52	62-fold less	1.7 × 10 ⁻²
	G	210 ± 14	10.0 ± 0.2	4.7	680-fold less	1.6 × 10 ⁻³
	T	480 ± 50	18 ± 0.6	3.7	860-fold less	1.2 × 10 ⁻³

^aThe extent of single dNTP incorporation was < 20% in all assays, adjusted by changing the polymerase concentration and reaction time.^bdNTP selectivity ratio = $(k_{\text{cat}}/K_{\text{m}})_{\text{dNTP}}/(k_{\text{cat}}/K_{\text{m}})_{\text{dCTP}}$.

Steady-state kinetic parameters for next-base extension from G (or 8-oxoG) : C (or A) template:primer termini by yeast Pol η_{core} ^a

Table 3

Template G*	Primer X	$K_m, \text{ nM}$	$k_{\text{cat}}, \times 10^3 \text{ s}^{-1}$	$k_{\text{cat}}/K_m, \times 10^5 \mu\text{M}^{-1} \text{ s}^{-1}$	Efficiency relative to G:C
5'-CX	C	5.2 ± 0.3	94 ± 0.8	1800	1
	A	280 ± 20	7.0 ± 0.2	2.5	740-fold less
3'-GG*C	C	5.5 ± 0.2	89 ± 1	1600	1.1-fold less
	A	10 ± 1	83 ± 1	810	2.2-fold less

^aThe extent of extension was < 20% in all assays, adjusted by changing the polymerase concentration and reaction time.

Table 4LC-MS/MS sequence analysis of full-length extension products using Pol η_{core} .^a

5'-GGGGGAAGGAU-TC

3'-CC CCCTT CCTA- AG G* CACT

G*	Extension products	Product, %	
G	TC C GTGA	37	100% C
	TC C GTGA A	26	
	TC C GTGA C	20	
	TC C GTGA G	17	
8-oxodG	TC C GTGA	26	57% C
	TC C GTGA A	10	
	TC C GTGA C	21	
	TC A GTGA	20	44% A
	TC A GTGA C	22	

^aReaction conditions: 4 μM Pol η_{core} , 10 μM 13-mer/18-mer oligonucleotide complex, 1 mM each dNTP, 5 mM Mg^{2+} , 8 h. LC-MS: t_{R} 3.3 min; charge: -3; area: m/z value ± 0.5 .

The position of incorporation is indicated in italics.