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N-terminal domains of human DNA polymerase lambda promote primer realignment during translesion DNA synthesis

David J. Taggart[a], Daniel M. Dayeh[a,b], Saul W. Fredrickson[a,c], Zucai Suo[a,b,*]

[a] Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, USA
[b] The Ohio State Biochemistry Program, The Ohio State University, Columbus, OH, USA
[c] Department of Microbiology, The Ohio State University, Columbus, OH, USA

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ABSTRACT

The X-family DNA polymerases λ (Polλ) and β (Polβ) possess similar 5′-2-deoxyribose-5-phosphate lyase (dRPase) and polymerase domains. Besides these domains, Polλ also possesses a BRCA1 C-terminal (BRCT) domain and a proline-rich domain at its N terminus. However, it is unclear how these non-enzymatic domains contribute to the unique biological functions of Polλ. Here, we used primer extension assays and a newly developed high-throughput short oligonucleotide sequencing assay (HT-SOSA) to compare the efficiency of lesion bypass and fidelity of human Polβ, Polλ and two N-terminal deletion constructs of Polλ during the bypass of either an abasic site or an 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) lesion. We demonstrate that the BRCT domain of Polλ enhances the efficiency of abasic site bypass by approximately 1.6-fold. In contrast, deletion of the N-terminal domains of Polλ did not affect the efficiency of 8-oxodG bypass relative to nucleotide incorporations opposite undamaged dG. HT-SOSA analysis demonstrated that Polλ and Polβ preferentially generated −1 or −2 frameshift mutations when bypassing an abasic site and the single or double base deletion frequency was highly sequence dependent. Interestingly, the BRCT and proline-rich domains of Polλ cooperatively promoted the generation of −2 frameshift mutations when the abasic site was situated within a sequence context that was susceptible to homology-driven primer realignment. Furthermore, both N-terminal domains of Polλ increased the generation of −1 frameshift mutations during 8-oxodG bypass and influenced the frequency of substitution mutations produced by Polλ opposite the 8-oxodG lesion. Overall, our data support a model wherein the BRCT and proline-rich domains of Polλ act cooperatively to promote primer/template realignment between DNA strands of limited sequence homology. This function of the N-terminal domains may facilitate the role of Polλ as a gap-filling polymerase within the non-homologous end joining pathway.

1. Introduction

Oxidative DNA damage is proposed to be a major contributor to carcinogenesis and aging [1,2]. Two of the most common forms of oxidative DNA damage generated within human cells are apurinic/apyrimidinic (AP) sites and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) lesions. It is estimated that approximately 10,000 spontaneous AP sites [3,4] and 1000–10,000 8-oxodG sites [5] are generated in each mammalian cell every day. These lesions are highly mutagenic, as AP sites have lost all coding potential, and 8-oxodG possesses dual coding potential due to its ability to adopt either an *anti* or *syn* conformation within a polymerase active site [6]. While in the *anti* conformation, a templating 8-oxodG correctly base pairs with an incoming dCTP by forming a Watson-Crick base pair. However, while in the *syn* conformation, a templating 8-oxodG lesion utilizes its Hoogsteen edge to preferentially form an incorrect base pair with an incoming dATP [7]. Under normal conditions, AP sites and 8-oxodG lesions are removed through the base excision repair (BER) pathway, and the resulting gap is filled by the action of a repair DNA polymerase such as polymerase λ (Polλ) or β (Polβ). However, under conditions of

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; AP, apurinic/apyrimidinic; BER, base excision repair; BSA, bovine serum albumin; BRCT, BRCA1 C-terminal domain; dNTP, 2′-deoxynucleoside 5′-triphosphate; dPolλ, deletion of human DNA polymerase λ (132-575); dRPase, 5′-2-deoxyribose-5-phosphate lyase domain; fPolλ, full-length human DNA polymerase λ (1-575); HT-SOSA, high-throughput short oligonucleotide sequencing assay; NHEJ, non-homologous end joining; PAGE, polyacrylamide gel electrophoresis; PCNA, proliferating cell nuclear antigen; Polβ, human DNA polymerase β; tPolλ, truncated human DNA polymerase λ (245-575) lacking the N-terminal domains; TLS, translesion DNA synthesis.

* Corresponding author at: 880 Biological Sciences, 484 West 12th Ave., Columbus, OH 43210, USA. Tel.: +1 614 688 3706; fax: +1 614 292 6773.
E-mail address: suo.3@osu.edu (Z. Suo).

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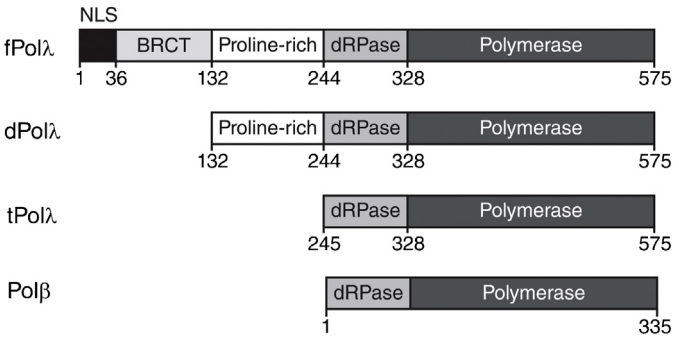

Fig. 1. Schematic domain structures of human fPolλ, dPolλ, tPolλ and Polβ. The nuclear localization signal (NLS), BRCA1 C-terminal (BRCT) domain, proline-rich domain, 5′-2-deoxyribose-5-phosphate lyase (dRPase) domain, and polymerase core domain of each full-length or truncated enzyme are labeled, and the amino acid residue numbers are indicated below each diagram.

significant oxidative stress, both strands of genomic DNA may become damaged, and therefore Polλ or Polβ may be required to bypass damaged sites, a process termed translesion DNA synthesis (TLS).

Polλ and Polβ are members of the X-family, one of six families of DNA polymerases (A, B, C, D, X, and Y). Although both Polλ and Polβ are template-directed DNA polymerases, possess 5′-2-deoxyribose-5-phosphate lyase (dRPase) activity, lack 3′→5′ exonuclease activity, and exhibit low processivity when replicating non-gapped DNA substrates, these enzymes have distinct biological roles *in vivo* (reviewed in reference [8]). For instance, Polβ plays a critical role in short-patch base excision repair (BER) [9,10] and may catalyze strand-displacement synthesis during PCNA-independent long-patch BER [11–13]. In contrast, Polλ has been proposed to act as a gap-filling polymerase during repair of double-stranded DNA breaks within the nonhomologous end joining (NHEJ) pathway [14–16], although Polλ also appears to possess a secondary role during BER of oxidative lesions [17,18].

Human Polλ and Polβ both possess conserved dRPase and polymerase domains (Fig. 1), and share 32% amino acid sequence identity (54% sequence homology) within these domains [19,20]. However, unlike Polβ, Polλ additionally contains a purported nuclear localization signal (NLS), a breast cancer susceptibility gene 1 C-terminal (BRCT) domain and a proline-rich domain at the N terminus (Fig. 1). BRCT domains are known to mediate protein-DNA and protein-protein interactions, and this domain is required for Polλ to function within reconstituted NHEJ systems [14,21]. The proline-rich domain has been shown to limit Polλ strand-displacement synthesis [15], and influence the rate and fidelity of Polλ nucleotide incorporation into undamaged DNA substrates [22,23]. The proline-rich domain is also phosphorylated by the cyclin-dependent kinase Cdk2/cyclin A complex [24], an event which may regulate the *in vivo* functions of Polλ. Although the N-terminal domains of Polλ likely contribute to the unique biological activities of this enzyme, the exact roles of these domains are unclear.

To investigate the potential functions of the N-terminal domains of Polλ, we sought to determine how the N-terminal domains influence the efficiency of lesion bypass and the fidelity of Polλ nucleotide incorporation during TLS of AP and 8-oxodG sites. To quantify the types and frequencies of errors induced by oxidative lesion bypass, we utilized a high-throughput short oligonucleotide sequencing assay (HT-SOSA) that was recently developed in our laboratory [25]. Our data support a model wherein the N-terminal domains of Polλ increase the frameshift error rate of Polλ opposite damaged sites by promoting primer/template realignment. This function of the N-terminal domains of Polλ may enable Polλ to catalyze the extension of primer/template junctions containing damaged bases or limited sequence homology, such as those encountered during the gap filling step of NHEJ.

Table 1
DNA substrates used for primer extension experiments[a]

18/41-mer-ctl	5′-CGCAGCCGTCCAACCAAC 3′-GCGTCGGCAGGTTGGTTGAGT**A**GCAGCTAGGTTACGGCAGG-5′
18/41-mer-AP-5′G	5′-CGCAGCCGTCCAACCAAC 3′-GCGTCGGCAGGTTGGTTGAGT**X**GCAGCTAGGTTACGGCAGG-5′
18-16/41-mer-ctl	5′-CGCAGCCGTCCAACCAAC $_p$CGATCCAATGCCGTCC-3′ 3′-GCGTCGGCAGGTTGGTTGAGT**A**GCAGCTAGGTTACGGCAGG-5′
18-16/41-mer-AP-5′G	5′-CGCAGCCGTCCAACCAAC $_p$CGATCCAATGCCGTCC-3′ 3′-GCGTCGGCAGGTTGGTTGAGT**X**GCAGCTAGGTTACGGCAGG-5′
18-15/40-mer-ctl	5′-CGCAGCCGTCCAACCAAC $_p$GATCCAATGCCGTCC-3′ 3′-GCGTCGGCAGGTTGGTTGAGT**G**CAGCTAGGTTACGGCAGG-5′
18-15/40-mer-8oxodG	5′-CGCAGCCGTCCAACCAAC $_p$GATCCAATGCCGTCC-3′ 3′-GCGTCGGCAGGTTGGTTGAGT**Y**CAGCTAGGTTACGGCAGG-5′

[a] **X** represents the AP lesion site while **Y** represents the 8-oxodG lesion site. The subscript 'p' indicates that the 5′-end of the downstream primer is phosphorylated.

2. Materials and methods

2.1. Materials

Reagents were purchased from the following companies: T4 DNA ligase from New England Biolabs, OptiKinase from USB Corporation, [γ-^{32}P]ATP from Perkin Elmer, ATP from Thermo Scientific, and dNTPs from GE Healthcare. Full-length human fPolλ (1-575), dPolλ (132-575), tPolλ (245-575) and full-length human Polβ (1-335) were produced and purified as previously described [22,26,27].

2.2. DNA substrates

The synthetic 40-mer DNA template containing 8-oxodG (40-mer-8oxodG) was purchased from Midland Certified Reagent Company (Table 1). All other synthetic DNA oligomers were purchased from Integrated DNA Technologies (Table 1 and Supplementary Table S1). The stable AP site analog tetrahydrofuran was used in place of a natural AP site to improve the stability of damaged DNA templates. All DNA oligomers were gel purified by using denaturing polyacrylamide gel electrophoresis (PAGE, 17% polyacrylamide, 8 M urea). Upstream primers were 5′-[^{32}P]-radiolabeled by incubating the primer of interest with OptiKinase and [γ-^{32}P]ATP for 3 h at 37 °C. Gapped DNA substrates were generated by annealing the indicated 5′-[^{32}P]-radiolabeled upstream primer and a 5′-phosphorylated downstream primer with the corresponding template in a 1.00:1.25:1.15 molar ratio, respectively. The primer-template DNA substrates were similarly prepared by annealing a 5′-[^{32}P]-radiolabeled upstream primer with the appropriate template at a 1.00:1.15 molar ratio, respectively. DNA substrate annealing solutions were heat denatured by incubation at 75 °C for 5 min, followed by slow cooling to 25 °C over several hours.

2.3. Reaction buffer

All kinetic and HT-SOSA reactions were performed in optimized reaction buffer L (50 mM Tris, pH 8.4 at 37 °C, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, 10% glycerol and 0.1 mg/mL BSA) for fPolλ, dPolλ and tPolλ or reaction buffer B (50 mM Tris, pH 7.8 at 37 °C, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, 10% glycerol and 0.1 mg/mL BSA) for Polβ. All reported