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Human DNA Polymerase θ does not harbor intrinsic nuclease activity

Denisse Carvajal-Maldonado[2,3], **Karl Zahn**[4], **Ryan Jensen**[5], **Richard D. Wood**[2,*], **Sylvie Doublié**[1,*]

[1]Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont, USA

[2]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Center, Houston, Texas, USA

[3]Present address: ORIC Pharmaceuticals, 240 E Grand Ave Fl 2, South San Francisco, CA 94080, USA

[4]Repare Therapeutics, 7210 rue Frederick-Banting bureau 100, Saint-Laurent QUEBEC H4S 2A1, Canada

[5]Department of Therapeutic Radiology, Yale School of Medicine, New Haven, CT, 06520, USA

DNA polymerase θ (Pol θ) is the enzyme at the heart of theta-mediated end-joining (TMEJ), a double-strand break repair pathway[1]. The process of aligning microhomologies often results in unpaired 3′ ends that are trimmed by a nuclease before extension by Pol θ. In some polymerases, the same active site can be used to catalyze both polymerase activity and endonucleolytic cleavage. For example, some RNA polymerases reorganize their active site to edit transcripts by cleaving off 1 or 2 nucleotides from the 3′ end[2–4]. Human telomerase can shorten products as well as extend them[5]. Our laboratories previously reported[6] that Pol θ could catalyze DNA polymerase and nuclease activity from the same active site and that the nuclease activity was conducted from a stem-loop intermediate. However, subsequent experiments from our laboratories show that this is incorrect. Instead, we find that Pol θ can use short GC-rich oligonucleotides to perform DNA synthesis. The resulting GC-rich stem loops are stable and happen to migrate faster than the starting ssDNA material on denaturing polyacrylamide gels. Experiments supporting this conclusion are summarized below.

When an active DNA polymerase fragment of Pol θ (aa 1792–2590; Pol θ QM1) is incubated with single-stranded oligonucleotides, the products of some oligonucleotides migrate faster than the starting material (see Figure 1A for three 14-mers). The faster migration occurs on denaturing polyacrylamide gels containing 7 M urea, with samples heated to 95 °C in formamide buffer before loading. We previously concluded that these products arise by nucleolytic trimming of the 3′ ends of oligonucleotides by Pol θ QM1[6]. To further investigate the generation of faster-migrating products, a version of oligonucleotide DCM6 was produced with a 5′ Cy5-label and a 3′ ^{32}P-label (Figure 1B). Following

*Corresponding authors: Sylvie.Doublie@uvm.edu, rwood@mdanderson.org.

incubation of the oligonucleotide with Pol θ QM1 and dNTPs, the faster migrating products retained the 3′ radiolabel (Figure 1C). These data show that the faster migrating products were not the result of end-trimming at the 3′ end but likely arose from DNA synthesis by Pol θ, which can extend short DNA oligonucleotides[7]. This finding is consistent with earlier literature showing that some GC-rich hairpin sequences are resistant to denaturing gel electrophoresis and migrate faster because of their compact form[8,9].

To assess this possibility, we examined the electrophoresis of oligonucleotides simulating the predicted products of stem-loop extension. The 16-mer and 17-mer oligonucleotides (Figure 1D, lanes 10 & 11) migrated faster than the original 14-mer DCM6 and at the same position as DCM6 products processed by Pol θ. This indicates that the faster migrating species produced by Pol θ action on DCM6 are products of extension.

Products were identified definitively by mass spectrometry (MALDI-TOF analysis; Axis-Pharm, San Diego). Mass spectrometry confirmed that the major product formed by Pol θ QM1 had a mass to charge ratio (m/z) consistent with adding two nucleotides (CG) to the original DCM6 oligonucleotide (Figure 1E). With Pol θ ΔCEN protein, which includes both DNA polymerase and DNA helicase domains[10], the major product corresponded with adding three nucleotides (CGC) (Figure 1E).

All results in the earlier paper previously interpreted as end-trimming by Pol θ QM1 should be reinterpreted as templated extension from single-stranded DNA. For example, faster-migrating products are not produced in the presence of incoming dideoxynucleotides, and are not produced by DNA polymerase-defective variants of Pol θ QM1[6]. All the previously reported data are sound and reproducible, but the interpretation of nuclease activity is incorrect. To eliminate confusion and avoid misleading future researchers, we have requested that the paper be retracted. As for the question of which nuclease trims DNA during TMEJ, a recent publication shows that the exonuclease activity of DNA polymerase δ participates in end-trimming[10].

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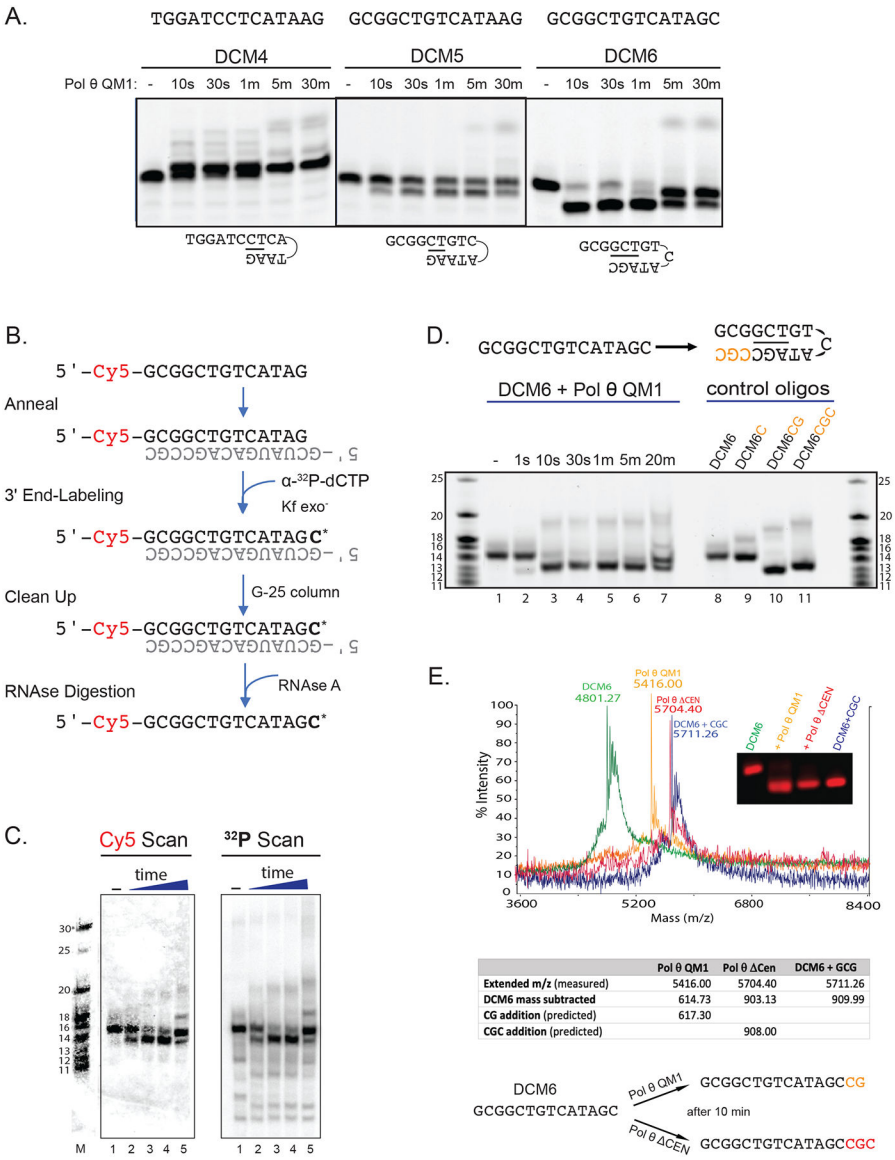

Figure 1.

Pol θ can extend short oligonucleotides to produce stable structures with anomalous migration on denaturing polyacrylamide gels.

A) Pol θ polymerase domain (aa 1792–2590, "Pol θ QM1") was incubated with three different 14-mer oligodeoxynucleotides: DCM4, DCM5 (designated ss14AG in ref. 6) and DCM6 (designated ss14GC in ref. 6). Conditions: 25 nM DNA, 125 nM Pol θ QM1 and 100 μM dNTPs. Products were separated on a 20% acrylamide gel containing 7 M urea. Diagrams beneath the gel image show putative pairing by which Pol θ QM1 could initiate extension.

B) Generation of DCM6 labeled at the 5′ end with Cy5 and at the 3′ end with ^{32}P. *E. coli* DNA polymerase I Klenow fragment (Kf exo$^-$) was used to add a ^{32}P -labeled C residue (C*) to the 3′ end of a 13-mer by using α–^{32}P -dCTP with a complementary RNA template.

Unincorporated nucleotide was removed with a Sephadex G-25 column. The RNA was then digested using DNAse-free RNAse A.

C) The doubly labeled DCM6 was incubated with Pol θ QM1 and products separated on a denaturing polyacrylamide gel. The additional bands in the ^{32}P image arise from impurities present in the original oligonucleotide. Incubation times (sec), lanes 1–5: 0, 10, 30, 300, 1800.

D) A denaturing polyacrylamide gel showing products of migration of Pol θ QM1-extended DCM6 (lanes 1–7) and the predicted products of templated extension (lanes 8–11). Conditions: 25 nM DNA, 125 nM enzyme and 100 μM dNTPs.

E) Mass spectrometry (MALDI-TOF) analysis results, showing 14-mer DCM6, the products of DCM6 extension with Pol θ QM1 and Pol θ ΔCEN, and a 17-mer control oligonucleotide representing the predicted product of extension (DCM6 + CGC). DCM6 oligonucleotide (125 nM) was incubated with 625 nM enzyme and 2.5 mM each dNTP in a 400 μL reaction mixture containing 25 mM potassium phosphate [pH 7.0], 5 mM $MgCl_2$, 5 mM DTT, 100 μg/mL BSA and 10% glycerol for 10 min at 37 °C. Reactions were stopped by heating at 95°C for 5 min, incubated with proteinase K, phenol-chloroform extracted, and ethanol precipitated. DNA was purified for mass spectrometry using ZipTips with 0.6 μL C_{18} resin following the recommended protocol (Axis-Pharm, San Diego) by binding to tips in 0.1 M TEAA (pH 7.0), washing thoroughly in the same buffer and then in nuclease free water, and eluting with 50% acetonitrile. To visualize outcomes, 0.25 μL of each sample was mixed with 10 μL of denaturing stop buffer and separated on a denaturing polyacrylamide gel (inset). The remainder of the sample was used for mass spectrometry with results as shown. At this 10 min point, the product of Pol θ QM1 synthesis is one nucleotide shorter than the product formed by Pol θ ΔCEN.