Supplemental Material

DNA polymerase θ accomplishes translesion synthesis opposite 1,N^6-ethenodeoxyadenosine with a remarkably high fidelity in human cells

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This PDF file includes: Supplemental Methods, Supplemental Tables 1-6, and Supplemental Figures 1-4.

Supplemental Methods

Western blot analysis of siRNA depletion of TLS Pols in HFs and MEFs

48h after siRNA transfection, cells were washed with PBS buffer and lysed with RIPA buffer (1x PBS, 1% IP-40, 0.5% sodium deoxycholate, 0.1% SDS). 30μg of prepared cellular extracts were separated on a 10% SDS-polyacrylamide gel and transferred to a PVDF membrane (Bio-rad). The membranes were probed with antibodies against Polη (rabbit polyclonal antibody against full length of human Polη), Polι (rabbit polyclonal antibody against full length of human Polι), Polκ (Santa Cruz Biothechnology), Rev1 (Santa Cruz Biothechnology), Rev7 (Abcam), or Polθ (Abcam) followed by appropriate secondary antibodies conjugated with horseradish peroxidase. The signals were detected using ECL-Plus (GenDEPOT). For the loading control, anti-β-tubulin antibody (Santa Cruz Biotechnology) was used.

Stable expression of wild type and catalytic mutant of human (1708-2590) Polθ in HFs or MEFs

As described previously (Yoon et al. 2014), wild type Polθ (1708-2590) and its catalytic mutant containing D2540A, E2541A mutations in the active site residues were subcloned into pCMV7-3xFlag-zeo vector (Sigma). The vectors were transfected into HFs (GM637) or MEFs by Lipofectamine 2000 reagent (Invitrogen). After 24h incubation, 0.5 μg of Zeocin (GenDEPOT) were added to the culture media. After 3 days of incubation, cells were washed with PBS buffer and were continuously cultured with the media containing 250 ng of Zeocin for ~ 2 weeks. The protein expression and siRNA knock down efficiency were checked by western blot analysis.

Stable expression of wild type and catalytic mutant of human Rev1 in HFs or MEFs

As described previously (Yoon et al. 2018), siRNA sensitive and siRNA resistant WT and catalytic mutant D570A, E571A Rev1 were expressed in GM637 HFs or MEFs using pCMV7-3x Flag-zeo vector.

Yoon JH, Hodge, RP, Hackfeld, LC, Park, J, Roy Choudhury, J, Prakash, S, and Prakash, L. 2018. Genetic control of predominantly error-free replication through an acrolein-derived minor-groove DNA adduct. *J Biol Chem* **293**: 2949-2958.

Yoon JH, Roy Choudhury, J, Park, J, Prakash, S, and Prakash, L. 2014. A role for DNA polymerase theta in promoting replication through oxidative DNA lesion, thymine glycol, in human cells. *J Biol Chem* **289**: 13177-13185.

Supplemental Table S1

Effects of siRNA knockdown of TLS polymerases on the replicative bypass of the εdA lesion carried on the leading strand template in wild type, Rev1$^{-/-}$, or Polθ$^{-/-}$ MEFs

MEFs	siRNA	Number of *Kan*$^{+}$ colonies	Number of blue colonies among *Kan*$^{+}$	TLS (%)
WT	NC	422	94	22.3
Rev1$^{-/-}$	NC	350	28	8.0
	Polι	308	26	8.4
	Rev3	296	24	8.1
	Polθ	411	4	1.0
Polθ$^{-/-}$	NC	387	44	11.4
	Polι	432	15	3.5
	Rev3	408	14	3.4
	Rev1	367	4	1.1

Supplemental Table S2

Effects of catalytically active (WT) Rev1 or catalytically inactive D570A E571A mutant Rev1 on TLS opposite εdA carried on the leading strand template in human fibroblasts

siRNA	Vector expressing	No. Kan^+ colonies	No. blue colonies among Kan^+	TLS (%)	TLS pathway that remains active
NC	-	404	102	25.2	Polι/Polζ, Rev1 Pol, Polθ
Rev1	-	415	38	9.2	Polθ
Rev1	WT Rev1	317	30	9.5	Polθ
Rev1	siR[a]-WT Rev1	364	85	23.4	Polι/Polζ, Rev1 Pol, Polθ
Rev1	siR catalytic mutant Rev1	294	58	19.7	Polι/Polζ, Polθ

[a]siR, indicates siRNA resistant form of WT or mutant Rev1

Supplemental Table S3

Effects of catalytically active (WT) Rev1 or catalytically inactive D570A E571A mutant Rev1 on TLS opposite εdA carried on the leading strand template in $Rev1^{-/-}$ MEFs

Vector expressing	Number of *Kan*$^+$ colonies	Number of blue colonies among *Kan*$^+$	TLS (%)	TLS pathway that remains active
—	458	38	8.3	Polθ
WT Rev1	364	70	19.2	Polι/Polζ, Rev1 Pol, Polθ
Catalytic mutant Rev1	306	48	15.7	Polι/Polζ, Polθ

Supplemental Table S4

Effects of catalytically active (WT) or catalytically inactive D2540A E2541A mutant (1708-2590) Polθ on TLS opposite εdA carried on the leading strand template in human fibroblasts

siRNA	Vector expressing	No. Kan^+ colonies	No. blue colonies among Kan^+	TLS (%)
NC	-	404	102	25.2
Polθ	-	336	52	15.5
Polθ	WT Polθ	324	48	14.8
Polθ	siR[a]-WT Polθ	315	74	23.5
Polθ	siR catalytic mutant Polθ	287	42	14.6

[a]siR, indicates siRNA resistant form of WT or mutant Polθ

Supplemental Table S5

Effects of catalytically active (WT) or catalytically inactive D2540A E2541A mutant (1708-2590) Polθ on TLS opposite εdA carried on the leading strand template in Polθ$^{-/-}$ MEFs

Vector expressing	Number of *Kan*$^+$ colonies	Number of blue colonies among *Kan*$^+$	TLS (%)	TLS pathways that remain active
—	381	42	11.0	Polι/Polζ, Rev1 Pol
WT Polθ	332	73	22.0	Polι/Polζ, Rev1 Pol, Polθ
Catalytic mutant Polθ	295	31	10.5	PolιPolζ, Rev1 Pol

Supplemental Table S6

Effect of wild type Polθ, catalytically active (WT) Rev1, or catalytically inactive D570A E571A mutant Rev1 on mutation frequencies and nucleotides inserted opposite εdA carried on the leading strand DNA template in wild type, Rev1$^{-/-}$, or Polθ$^{-/-}$ MEFs

MEFs	Vector expressing, siRNAs	No. of *Kan*+ blue colonies sequenced	Nucleotide inserted				Mutation frequency (%)	Error-prone TLS pathway that remains active
			A	G	C	T		
WT	None	96 (12)[a]	3	0	9	84	12.5	Rev1,Polθ
Polθ$^{-/-}$	Vector control	96 (9)	1	0	8	87	9.4	Rev1
Rev1$^{-/-}$	Vector control	96 (8)	4	0	4	88	8.3	Polθ
Rev1$^{-/-}$ (mPolθ siRNA)[b]	Flag-Mutant - Rev1	90 (0)	0	0	0	90	0	none

[a]Number of colonies where TLS occurred by insertion of a nucleotide other than T are shown in parentheses.

[b]Rev1$^{-/-}$ MEFs were treated with mPolθ siRNA. In these MEFs carrying Flag-mutant Rev1, only the Polι/Polζ dependent error-free pathway remains active.

Supplemental Figure Legends

Supplemental Figure S1

Assay for TLS opposite εdA. *(A)*, Chemical structure of A and εdA. *(B),* The target 16mer sequence containing εdA at $\overset{*}{A}$ is shown on top, and the lacZ' sequence in the leading strand in the pBS vector containing the adduct at $\underset{*}{A}$ is shown below. The εdA-containing DNA strand is in-frame and it carries the *Kan*+ gene. TLS through the adduct generates Kan+ blue colonies.

Supplemental Figure S2

Efficiency of siRNA knockdown of TLS Pols in HFs and MEFs. **(A)** Western blot analyses of siRNA knockdown of TLS Pols in GM637 HFs. NC: negative control siRNA. **(B)** Western blot analyses of siRNA knockdown of TLS Pols in Rev1$^{-/-}$ MEFs. **(C)** Western blot analyses of the efficiency of siRNA knockdown of TLS Pols in Polθ$^{-/-}$ MEFs. In (A), (B), and (C), β-tubulin was used as the loading control. **(D)** RT-PCR analyses of the efficiency of siRNA knockdown of Rev3 in HFs (left) and MEFs (right). In both panels, the first lanes contains molecular weight markers. GAPDH is used as a loading control for RT-PCR. RT-PCR analyses were done as described (Yoon et al. 2009).

Supplemental Figure S3

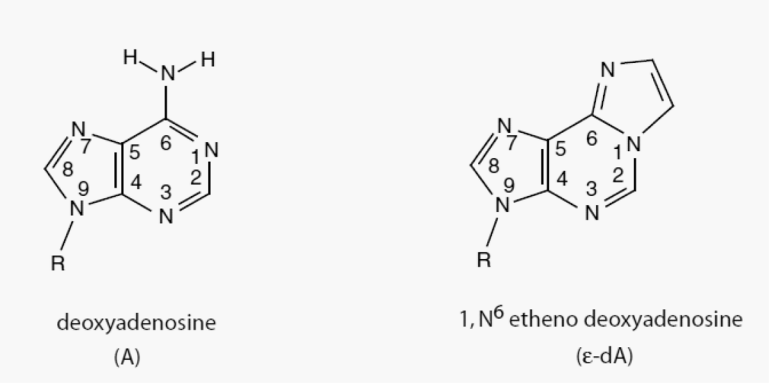
Nucleotide incorporation opposite A or εdA by Rev1. 0.5 nM Rev1 was incubated with 10 nM DNA substrate and 25 uM of either dGTP, dATP, dTTP, dCTP, or all 4 dNTPs for 5 min at 37C. Reactions containing a single dNTP or all 4 dNTPs (N) are indicated. The template in lanes 1-5 contains an undamaged template A at the primer terminus. Lanes 6-20 harbor εdA at the primer terminus followed by either T (lanes 6-10), A (lanes 11-15) or G (lanes 16-20) residue, indicated by D in the template sequence. X in the template sequence indicates the site of undamaged A or εdA.

Supplemental Figure S4

TLS pathways for replicating through εdA in human cells. Replication through εdA occurs *via* 3 different pathways in which the Polι/Polζ pathway conducts error-free TLS and Rev1 functions as a non-catalytic component of Polι in this pathway. Rev1 polymerase activity contributes to error-prone TLS *via* a pathway independent of Polι/Polζ and Polθ functions in another independent error-prone TLS pathway.

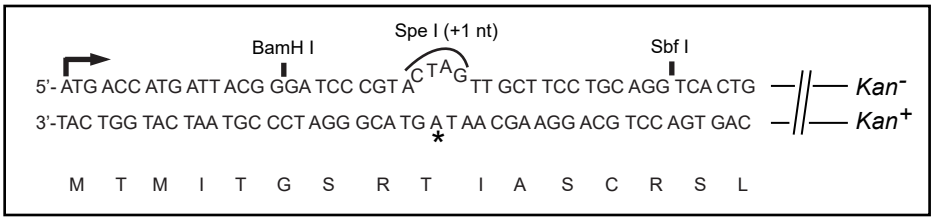
Yoon J-H, Prakash, L, and Prakash, S. 2009. Highly error-free role of DNA polymerase η in the replicative bypass of UV induced pyrimidine dimers in mouse and human cells. *Proc Natl Acad Sci U S A* **106**: 18219-18224.

A

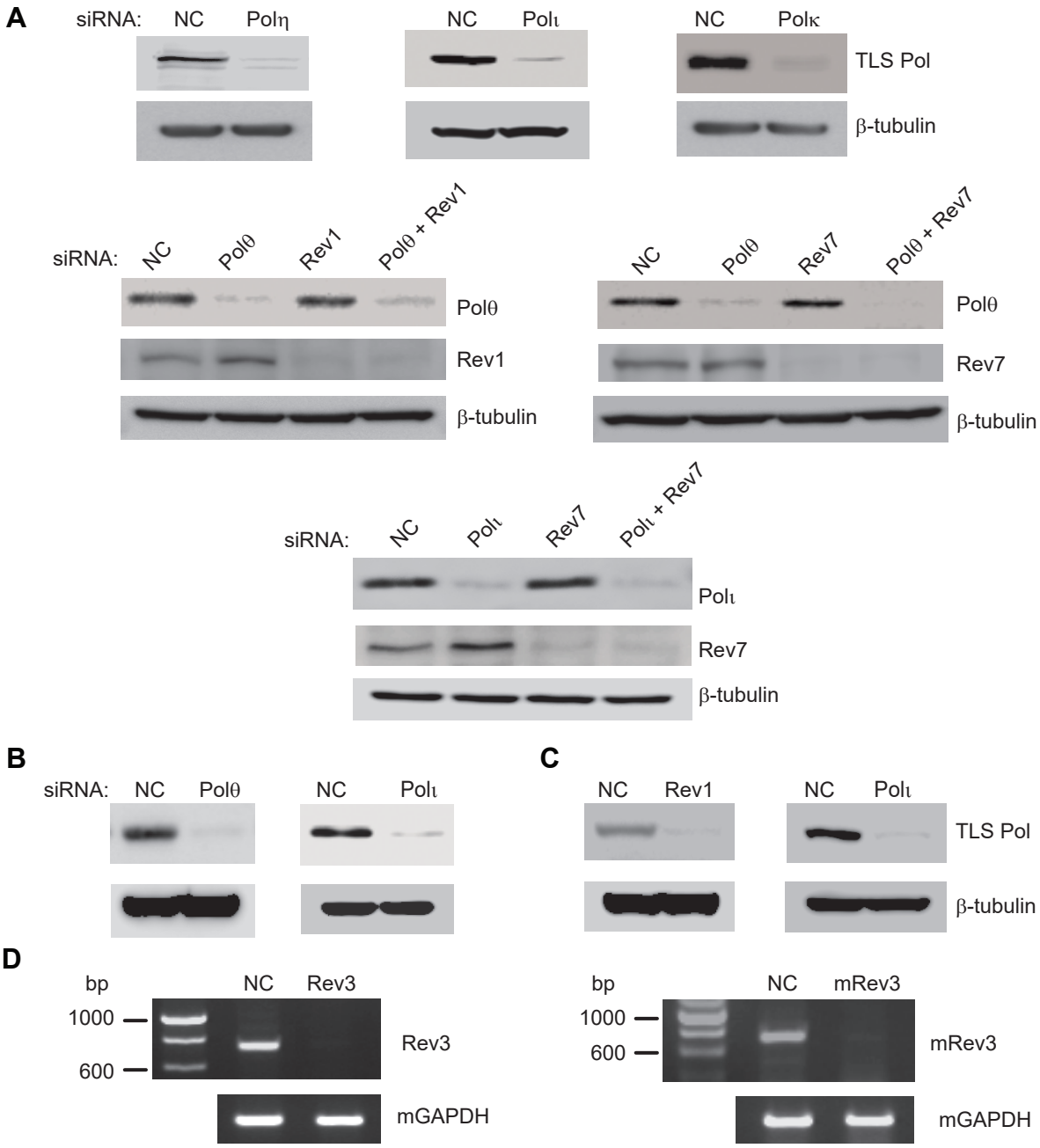


B

5'-GGA AGC AAT Å GTACGG-3'



Leading strand (pBS vector)


A
siRNA:
NC
Polη
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Polι
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Polκ
TLS Pol
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siRNA:
NC
Polθ
Rev1
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Polθ
Rev1
β-tubulin
NC
Polθ
Rev7
Polθ + Rev7
Polθ
Rev7
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Rev7
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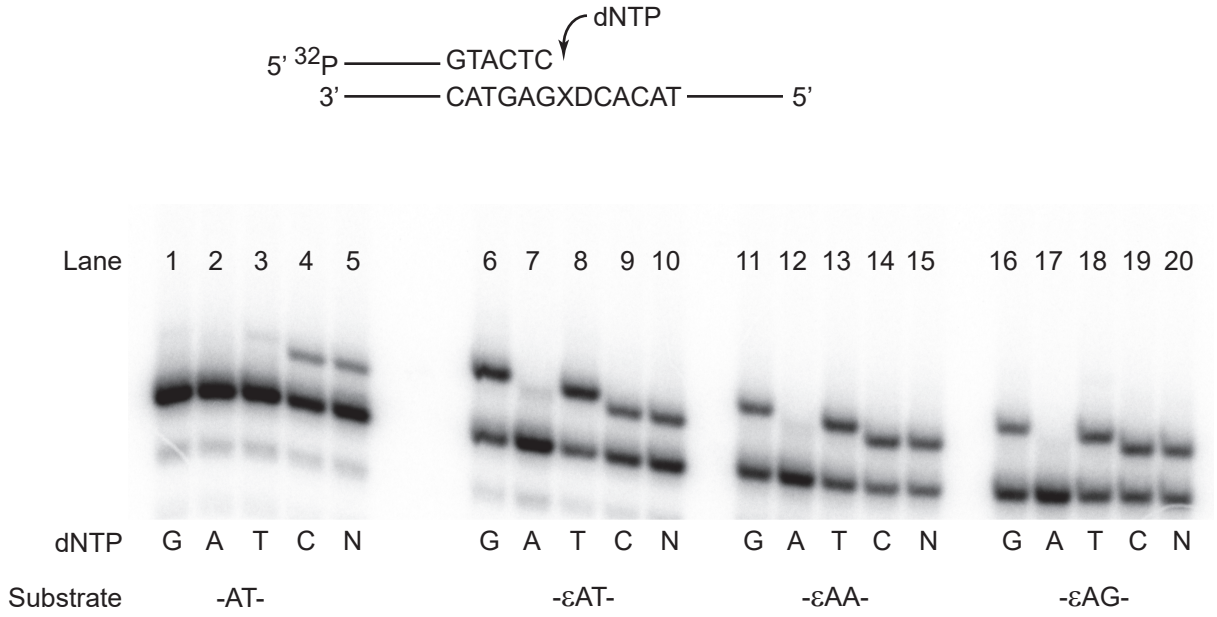
Supplemental_Fig_S3.pdf



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