

RESEARCH COMMUNICATION

DNA polymerase θ accomplishes translesion synthesis opposite 1, N⁶-ethenodeoxyadenosine with a remarkably high fidelity in human cells

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Here we show that translesion synthesis (TLS) opposite 1, N⁶-ethenodeoxyadenosine (ϵ dA), which disrupts Watson–Crick base pairing, occurs via Pol ι /Pol ζ -, Rev1-, and Pol θ -dependent pathways. The requirement of Pol ι /Pol ζ is consistent with the ability of Pol ι to incorporate nucleotide opposite ϵ dA by Hoogsteen base pairing and of Pol ζ to extend synthesis. Rev1 polymerase and Pol θ conduct TLS opposite ϵ dA via alternative error-prone pathways. Strikingly, in contrast to extremely error-prone TLS opposite ϵ dA by purified Pol θ , it performs predominantly error-free TLS in human cells. Reconfiguration of the active site opposite ϵ dA would provide Pol θ the proficiency for error-free TLS in human cells.

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The 1,N⁶-ethenodeoxyadenosine (ϵ dA) adduct is formed in DNA through interaction with aldehydes derived from lipid peroxidation and by exposure to chemical carcinogens such as vinyl chloride. Lipid peroxidation is a normal chain reaction process that initiates from the oxidation of polyunsaturated fatty acids in cell membranes and results in the formation of a variety of highly reactive aldehydes, including acrolein, malonaldehyde, and *trans*-4-hydroxy-2-nonenal (HNE). Enals such as HNE undergo further oxidation reactions to form epoxyaldehydes and the reaction of epoxyaldehyde with DNA generates the ϵ dA adduct (Chung et al. 1999; Luczaj and Skrzydlewska 2003).

The ϵ dA adduct is highly inhibitory to synthesis by replicative DNA polymerases (Pols) because the exocyclic ring between the N1 and N6 positions of ϵ dA impairs the Watson–Crick (W–C) edge of adenine (Supplemental Fig. S1). The ability of the Pol ι active site to push template purines into a *syn* conformation provides a mechanism by which this polymerase can insert nucleotides opposite DNA lesions which disrupt W–C base pairing. Biochemi-

cal studies have shown that while Pol ι incorporates a T correctly opposite the ϵ dA adduct, it also incorporates a C with an about one fourth efficiency of that of T incorporation (Nair et al. 2006). Crystal structures of Pol ι - ϵ dA-dTTP and Pol ι - ϵ dA-dCTP ternary complexes show that similar to nonadducted A, the ϵ dA adduct adopts a *syn* conformation in the Pol ι active site and its “Hoogsteen edge” participates in hydrogen bonding with the incoming dTTP or dCTP (Nair et al. 2006). Since Pol ι is highly inefficient at extending synthesis from the T or C inserted opposite ϵ dA, the extension reaction would require another TLS Pol and biochemical studies have indicated that Pol ζ can extend synthesis from the ϵ dA-dT or ϵ dA-dC base pair (Nair et al. 2006).

Here we identify the TLS Pols that promote replication through the ϵ dA adduct in human cells and show that replication through this adduct is mediated via three genetic pathways. As expected from biochemical and structural studies, Pol ι and Pol ζ function together in one pathway; however, contrary to biochemical and structural observations indicating a proficiency of Pol ι for misincorporating C opposite ϵ dA, the Pol ι /Pol ζ pathway mediates highly error free TLS opposite this adduct in human cells. Rev1 polymerase activity contributes to a low level of TLS that is mutagenic; in this role, Rev1 functions independent of its scaffolding role with Pol ι in the Pol ι /Pol ζ pathway. Pol θ , a member of A-family Pols, promotes replication through ϵ dA via a third pathway. Although biochemical studies show that purified Pol θ conducts highly error-prone TLS by incorporating an A opposite ϵ dA, in human cells Pol θ mediates predominantly error-free TLS opposite this adduct. We discuss the implications of this marked discrepancy and suggest that opposite DNA lesions such as ϵ dA, Pol θ active site is actively reconfigured in human cells to carry out predominantly error-free TLS.

Results and Discussion

Requirement of Pol ι /Pol ζ and Pol θ for Replication through the ϵ dA adduct in human cells

To identify the TLS Pols required for replicating through the ϵ dA adduct, we analyzed the effects of siRNA depletion of TLS Pols individually and in combinations (Supplemental Fig. S2) on TLS frequencies resulting from replication through the lesion present on the leading or the lagging strand template of the SV40 based duplex plasmid. TLS opposite the ϵ dA adduct carried on the leading strand template in normal human fibroblasts (HFs) and treated with control (NC) siRNA occurs with a frequency of ~25% and TLS frequency was not affected in cells depleted for Pol η or Pol κ , indicating that these Pols play no significant role in TLS opposite this adduct (Table 1). In contrast, TLS frequency was reduced to ~14% in Pol ι -depleted cells. A similar reduction in TLS frequency occurred upon depletion of the Rev3 or the Rev7 subunit of Pol ζ , and depletion of Pol θ reduced TLS frequency to

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~17% (Table 1). The observation that TLS frequency remained the same in cells codepleted for Polι with Rev3 or Rev7 as that in cells depleted for any of these Pols alone (Table 1) indicated that Polι and Polζ function together in one TLS pathway. In contrast, codepletion of Polι with Polθ or of Rev3 or Rev7 with Polθ caused a drastic reduction in TLS frequency to ~4% (Table 1), indicating that Polθ functions independently of Polι and Polζ and that TLS through the εdA adduct is mediated by two separate pathways dependent on either Polι/Polζ or Polθ. We verified this conclusion for TLS opposite εdA present on the lagging strand template (Table 1).

Noncatalytic and catalytic roles of Rev1 in TLS opposite εdA

In human cells, Rev1 functions as an indispensable noncatalytic scaffolding component of Y-family Pols (Yoon et al. 2015). Since ~15% of total TLS persists in HFs codepleted for Polθ with Polι or Polζ (Table 1), we considered the possibility that in addition to its role as a noncatalytic scaffolding component of Polι in the Polι/Polζ pathway, Rev1 contributes to TLS as a DNA polymerase and in this role it functions independently of Polι. To test for this possibility, we first determined whether there was evidence for Polι-dependent and Polι-independent roles of Rev1, and analyzed TLS opposite εdA in wild-type (WT), Rev1^{-/-} (Yoon et al. 2015), and Polθ^{-/-} (Yoon et al. 2019) mouse embryonic fibroblasts (MEFs) depleted for different TLS Pols (Supplemental Fig. S2; Supplemental Table S1). In WT MEFs, TLS opposite εdA occurs with a frequency of ~22% and TLS is reduced to ~8% in Rev1^{-/-} MEFs treated with NC siRNA or depleted for Polι or Polζ. The epistasis of Rev1 over Polι in the Polι/Polζ pathway is consistent with a scaffolding role of Rev1 with Polι. To unravel the Polι-independent role of Rev1, we examined TLS in Polθ^{-/-} MEFs depleted for Polι or Polζ, since then only the Polι/Polζ-independent role of Rev1 would remain functional. Our observation that TLS frequency is reduced to ~3.5% in Polθ^{-/-} MEFs depleted for Polι or Polζ strongly suggested that this residual TLS is mediated by the Polι/Polζ-independent role of Rev1. Furthermore, the almost abolition of TLS in Polθ^{-/-} MEFs depleted for Rev1 or in Rev1^{-/-} MEFs depleted for Polθ provided confirmatory ev-

idence for the Polι-dependent and Polι-independent Rev1 roles.

To determine whether Rev1 polymerase function was required for its Polι-independent role, we examined TLS in HFs expressing siRNA-sensitive WT Rev1 or the siRNA-resistant form of either the WT or the catalytically inactive Rev1 D570A, E571A mutant. TLS in cells depleted for Rev1 and carrying the vector control or the siRNA-sensitive WT Rev1 was reduced to ~9% as compared to ~25% in control siRNA-treated cells (Supplemental Table S2). Expression of the siRNA-resistant WT Rev1 restored TLS frequency nearly to normal levels (~23%), while expression of siRNA-resistant D570A, E571A catalytic mutation reduced TLS to ~20% (Supplemental Table S2). This reduction in TLS frequency closely resembles the reduction in TLS frequency that occurs in HFs codepleted for Polι and Polθ (Table 1) or in Polθ^{-/-} MEFs depleted for Polι (Supplemental Table S1). This result suggested that Rev1 polymerase activity accounts for a small proportion of total TLS opposite εdA and that this TLS operates independently of Rev1's scaffolding role with Polι, which will remain intact in Rev1 catalytic mutant. To further ascertain the contribution of Rev1 polymerase activity to TLS, we examined the effect of Rev1 catalytic mutation on TLS opposite εdA in Rev1^{-/-} MEFs. In Rev1^{-/-} MEFs expressing WT Rev1, TLS occurred at a frequency of ~19% and TLS was reduced to ~16% in these MEFs expressing catalytic mutant Rev1 (Supplemental Table S3). This result reinforces the evidence that Rev1 polymerase activity contributes to a relatively small proportion of total TLS opposite εdA. Overall, from analyses of TLS in WT HFs, WT MEFs, Rev1^{-/-} MEFs, and Polθ^{-/-} MEFs, we conclude that TLS opposite εdA is mediated by two major Polι/Polζ- and Polθ-dependent pathways, respectively, and by a relatively minor Rev1 polymerase-dependent pathway (Table 1; Supplemental Tables S1–S3).

Requirement of Polθ polymerase activity for TLS opposite εdA in human cells

Human Polθ is a 290 kDa protein comprised of an N-terminal ATPase/helicase domain, a large central domain, and a C-terminal polymerase domain that shares homology with A-family DNA Pols such as *Escherichia coli*

Table 1. Effects of siRNA knockdown of TLS polymerases on the replicative bypass of the εdA lesion carried on the leading or lagging strand template in HFs (GM637)

siRNA	Leading strand			Lagging strand		
	Number of Kan ⁺ colonies	Number of blue colonies among Kan ⁺	TLS (%)	Number of Kan ⁺ colonies	Number of blue colonies among Kan ⁺	TLS (%)
NC	659	168	25.5	502	116	23.1
Polη	623	146	23.4	460	108	23.5
Polκ	658	161	24.5	403	96	23.8
Polι	589	80	13.6	367	48	13.1
Rev3	615	82	13.3	352	38	10.8
Rev7	308	40	13.0	401	45	11.2
Polθ	612	102	16.7	428	60	14.0
Polι + Rev3	623	78	12.5	394	51	12.9
Polι + Rev7	412	49	11.9	294	34	11.6
Polι + Polθ	674	26	3.9	408	16	3.6
Rev3 + Polθ	236	9	3.8	426	18	4.2
Rev7 + Polθ	405	15	3.7	314	11	3.5

Pol I (Yousefzadeh and Wood 2013). We have shown previously that Pol θ comprised of residues 1708–2590 performs proficient TLS opposite thymine glycol in human cells (Yoon et al. 2014) and provide evidence here that Pol θ (1708–2590) is sufficient for TLS opposite ϵ dA. Whereas TLS in control siRNA-treated HF occurs with a frequency of ~25%, this frequency declines to ~15% in cells depleted for genomic Pol θ and carrying either an empty vector or one expressing siRNA-sensitive WT Pol θ (1708–2590) (Supplemental Table S4). Expression of an siRNA-resistant WT Pol θ (1708–2590), however, restores normal TLS levels in HF cells depleted for genomic Pol θ , confirming that the C-terminal polymerase domain is sufficient for Pol θ -dependent TLS opposite the ϵ dA lesion. TLS was reduced to ~15% in HF cells expressing the siRNA-resistant Pol θ (1708–2590) D2540A, E2541A mutant protein defective in DNA synthesis, indicating that Pol θ polymerase activity is required for its role in TLS opposite ϵ dA (Supplemental Table S4). We confirmed the requirement of Pol θ polymerase activity for TLS opposite ϵ dA in Pol $\theta^{-/-}$ MEFs (Supplemental Table S5).

The Pol η /Pol ζ pathway conducts error-free TLS opposite ϵ dA in human cells

TLS opposite ϵ dA incurs a high level of mutagenesis (Table 2). In control (NC) siRNA-treated cells, ~16% of *Kan^r* blue colonies derived from TLS opposite ϵ dA carried on the leading strand template harbored a mutation that resulted from the incorporation of primarily a C, but also of an A or a G opposite ϵ dA. Of the incorrect nucleotides, C was incorporated at a frequency of ~11%, whereas the A and G nucleotides were incorporated at a combined frequency of ~5% (Table 2). As expected from the lack of requirement of Pol η or Pol κ for TLS opposite ϵ dA, the spectrum and frequency of mutagenic TLS opposite this adduct was unchanged upon their depletion. Importantly, depletion of Pol η , Rev3, or Rev7 raised the frequency of mutagenic

TLS to ~27% and Pol θ depletion reduced mutagenic TLS to ~10% (Table 2). These results as well as analyses of mutagenic TLS opposite ϵ dA carried on the lagging strand template indicated that Pol η /Pol ζ -dependent TLS operates in an error-free manner, whereas Pol θ -mediated TLS is error-prone.

To get an estimate of the overall contributions of the Pol η /Pol ζ and Pol θ pathways to error-free and mutagenic TLS, respectively, we pooled the mutation data for the two DNA strands. Overall, mutagenic TLS opposite ϵ dA in WT HF cells occurs with a frequency of ~14%, this frequency rises to ~27% in cells depleted for Pol η or Pol ζ , and declines to ~9% in Pol θ -depleted cells (Table 2). Whereas incorporation of C occurs with a frequency of ~10% in control siRNA-treated cells, it rises to ~20% in cells depleted for Pol η or Pol ζ and declines to ~5% in Pol θ -depleted cells. These data suggested that Pol θ -mediated TLS contributes to C misincorporation opposite ϵ dA and raised the possibility that Rev1 polymerase activity contributes to mutagenic TLS that persists in Pol θ -depleted cells.

The DNA polymerase activities of Pol θ and Rev1 account for error-prone TLS opposite ϵ dA

To test whether the Rev1 polymerase activity contributes to mutagenic TLS and to determine whether Rev1 and Pol θ polymerase activities provide alternative routes of mutagenic TLS, we analyzed the effects of mutations in active site residues in their polymerase domains on mutagenic TLS opposite ϵ dA. As shown in Table 3, in Pol θ -depleted cells expressing siRNA-sensitive WT Pol θ , ~10% of TLS products harbor mutations, and the frequency of mutagenic TLS rises to ~16% in cells expressing siRNA-resistant WT Pol θ , suggesting that ~6% of mutations result from Pol θ -mediated TLS. Expression of the siRNA-resistant catalytic mutant of Pol θ also reduced mutation frequency to ~10%, indicating that Pol θ 's polymerase activity is required for its role in mutagenic TLS. In Rev1-depleted cells

Table 2. Effects of siRNA knockdowns of TLS polymerases on mutation frequencies and nucleotides inserted opposite ϵ dA carried on the leading or lagging strand template in HF cells (GM637)

ϵ dA-containing DNA strand	siRNA	Number of <i>Kan^r</i> blue colonies sequenced	Nucleotide inserted				Mutation frequency (%)
			A	G	C	T	
Leading strand	NC	220 (35) ^a	7	3	25	185	15.9
	Pol η	164 (25)	3	2	20	139	15.2
	Pol κ	166 (26)	3	2	21	140	15.7
	Pol ι	160 (41)	5	6	26	119	25.6
	Rev3	260 (68)	13	8	47	192	26.2
	Rev7	96 (28)	5	2	21	68	29.2
	Pol θ	196 (19)	3	5	11	177	9.7
Lagging strand	NC	170 (20)	2	2	16	150	11.8
	Pol η	96 (11)	2	2	7	85	11.5
	Pol ι	188 (50)	5	1	44	138	26.6
	Rev3	96 (26)	4	0	22	70	27.1
	Rev7	104 (32)	5	0	27	72	30.8
	Pol θ	180 (13)	1	3	9	167	7.2
	Leading or lagging strand	NC	390 (55)	9	5	41	335
Pol ι		348 (91)	10	7	74	257	26.1
Rev3		460 (126)	22	8	96	334	27.4
Rev7		200 (60)	10	2	48	140	30.0
Pol θ		376 (32)	4	8	20	344	8.8

^aNumber of colonies where TLS occurred by insertion of a nucleotide other than T are shown in parentheses.

Table 3. Effect of catalytically active (WT) Polθ, catalytically inactive D2540A E2541A mutant 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 HFs (GM637)

siRNA	Vector expressing	Number of Kan ⁺ blue colonies sequenced	Nucleotide inserted				Mutation frequency (%)	Error-prone TLS pathway that remains active
			A	G	C	T		
Polθ	WT-Polθ	96 (10) ^a	2	1	7	86	10.4	Rev1 Pol
Polθ	siR ^b -WT-Polθ	96 (15)	3	1	11	81	15.6	Rev1 Pol, Polθ
Polθ	siR-mutant Polθ	90 (9)	2	0	7	81	10.0	Rev1 Pol
Rev1	WT-Rev1	96 (7)	2	1	4	89	7.3	Polθ
Rev1	siR-WT-Rev1	96 (16)	3	2	11	80	16.7	Rev1 Pol, Polθ
Rev1	siR-mutant Rev1	88 (7)	3	0	4	81	8.0	Polθ
Rev1 + Polθ	siR-mutant Rev1	80 (6)	2	0	4	74	7.5	Polθ
Rev1 + Polθ	siR-mutant Rev1	96 (0)	0	0	0	96	0.0	Neither

^aNumber of colonies where TLS occurred by insertion of a nucleotide other than T are shown in parentheses.

^b(siR) siRNA-resistant.

expressing siRNA-sensitive WT Rev1, mutagenic TLS occurs at a frequency of ~7%, and expression of the siRNA-resistant WT Rev1 raises the frequency of mutagenic TLS to ~17% (Table 3). This result indicated the requirement of Rev1 for mutagenic TLS. To establish that Rev1 polymerase activity was required for mutagenic TLS and that Rev1 functions in this role independent of Polθ, we expressed siRNA-resistant Rev1 catalytic mutant in Rev1-depleted cells or in cells codepleted for Rev1 and Polθ (Table 3, items 6 and 7). In both cases, mutagenic TLS was reduced to 8%, confirming the Polθ-independent role of Rev1 polymerase in mutagenic TLS. The observation that codepletion of Rev1 and Polθ in cells expressing siRNA-resistant Rev1 catalytic mutant causes a complete inhibition of mutagenic TLS (Table 3, last item) supports the conclusion that Rev1 polymerase and Polθ polymerase activities provide alternative routes for mutagenic TLS opposite εdA (Table 3). Since no mutations occur in cells lacking Polθ and Rev1 polymerase activity but retaining the Rev1 scaffolding function, where only the Polθ/Polζ pathway would remain functional, Polθ/Polζ-dependent TLS must operate in an error-free manner (Table 3, last item).

To further ascertain the contributions of Rev1 polymerase-dependent and Polθ-dependent pathways to mutagenic TLS and to verify the requirement of Polθ for error-free TLS in the Polθ/Polζ pathway, we analyzed the mutation spectrum of TLS products in WT and Polθ^{-/-} MEFs, and in Rev1^{-/-} MEFs lacking or expressing the catalytic mutant Rev1 protein (Supplemental Table S6). The effects of Polθ and Rev1 knockouts on the frequency and mutation spectrum of TLS products are remarkably similar to that in HFs depleted for these Pols (Table 3) and the results that no mutations are recovered in Rev1^{-/-} MEFs expressing the catalytic mutant Rev1 protein and depleted for Polθ (Supplemental Table S6) provide confirmatory evidence that Rev1 polymerase and Polθ provide alternate pathways of mutagenic TLS, and that the Polθ/Polζ pathway, which would remain functional in these MEFs, operates in an error-free manner.

Biochemical analysis of Polθ polymerase activity for TLS opposite εdA

For these studies, we examined DNA synthesis by Polθ across from either an undamaged A or εdA. Whereas oppo-

site an undamaged A template Polθ inserts a T but also a C or a G (Fig. 1, lanes 1–5), opposite εdA Polθ primarily inserts an A (Fig. 1, lanes 9,4,19). Although Polθ can insert each of the four dNTPs opposite εdA, importantly, in the presence of all four dNTPs, dATP is preferentially inserted (Fig. 1, lane 10). Since dATP was preferentially incorporated opposite εdA in a template in which the downstream 5' template nucleotide was a T residue, we determined whether Polθ used a frameshift mechanism to incorporate dATP opposite the downstream template T rather than opposite εdA. To examine this, we used templates containing εdA that harbor either an A or a G as the next templating

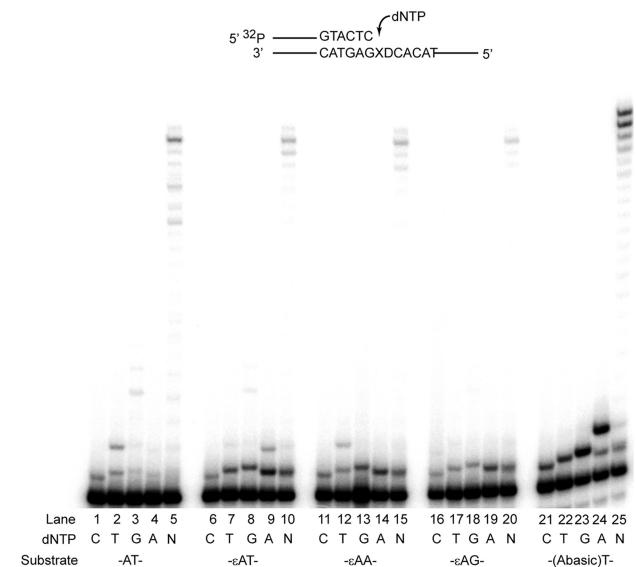


Figure 1. Nucleotide incorporation opposite A or εdA by Polθ. 0.5 or 5 nM Polθ was incubated with 10 nM DNA substrate and 25 μM of either dGTP, dATP, dTTP, dCTP, or all four dNTPs for 5 min at 37°C. Reactions containing a single or all four dNTPs (N) are indicated. The DNA substrate used in lanes 1–5 harbor undamaged template A at the primer terminus, and reactions contained 0.5 nM Polθ. The substrate in lanes 6–20 harbored 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. Synthesis opposite an abasic site is shown in lanes 21–25. Reactions in lanes 6–25 were all carried out with 5 nM Polθ. X in the template sequence indicates the position of undamaged A, εdA, or an abasic site.

residue downstream from the lesion (Fig. 1, lanes 11–20). In each case, an A was the best incorporated nucleotide opposite ϵ dA, suggesting that Pol θ is not frameshifting the template and using the downstream nucleotide, for if it were, then the pattern of insertion would be different for each substrate and a preference for the W–C partner of the downstream nucleotide would be observed.

Pol θ exhibits a high proficiency for replicating through an abasic site by inserting an A opposite it and by extending synthesis (Fig. 1, lanes 24,25; Seki et al. 2004). Although Pol θ is more efficient in performing TLS opposite an abasic site than opposite ϵ dA, the similar pattern of nucleotide incorporation opposite the two DNA lesions (Fig. 1) suggests that the ϵ dA adduct becomes extrahelical and Pol θ incorporates an A opposite an abasic site-like mode.

Biochemical analysis of Rev1 polymerase activity for TLS opposite ϵ dA

Rev1 specifically incorporates dCTP opposite template G (Nelson et al. 1996; Haracska et al. 2002). In the Rev1 active site, template G, and the incoming dCTP do not pair with each other; instead, the template G residue is evicted from the DNA helix and makes hydrogen bonds via its Hoogsteen edge with the amino acids in the G loop of Rev1, while an Arg residue in Rev1 forms hydrogen bonds with the incoming dCTP (Nair et al. 2005; Swan et al. 2009). Rev1 favors nucleotide incorporation opposite template G over other nucleotides because the G loop makes specific hydrogen bonds with the Hoogsteen edge of templating G. Incorporation of dCTP opposite template A is much less favorable, likely due to the presence of the N⁶ H-bond donor on adenine which would sterically hinder binding by the G loop. Since ϵ dA lacks the N⁶ amino group (Supplemental Fig. S1A), it would not sterically clash with the G loop. Accordingly, we found that under identical conditions, nucleotide incorporation opposite ϵ dA is better than opposite template A (Supplemental Fig. S3). In fact, yeast Rev1 incorporates dCTP opposite ϵ dA with a catalytic efficiency only approximately fivefold less than template G, whereas opposite template A, yeast Rev1 is \sim 200-fold less efficient (data not shown). This proposed better binding of ϵ dA by the Rev1 G loop over template A would also account for the incorporation of dGTP and dTTP mispairs opposite ϵ dA that is not observed opposite template A (Supplemental Fig. S3, lanes 1,3 vs. 6,8,11,13,16,18). Since in the presence of all four dNTPs (Supplemental Fig. S3, lanes 10,15,20), Rev1 preferentially incorporates dCTP, dCTP is incorporated opposite ϵ dA with the highest catalytic efficiency.

Pathways for replicating through ϵ dA in human cells

Based upon our observations in HFs, and in WT, Pol θ ^{-/-}, Rev1^{-/-} MEFs, we conclude that replication through the ϵ dA adduct is mediated via two Rev1-dependent TLS pathways in which Rev1 functions as a noncatalytic component of Pol ι in the Pol ι /Pol ζ pathway and as a DNA polymerase that functions independently of the Pol ι /Pol ζ pathway and via a third pathway dependent on Pol θ (Supplemental Fig. S4). Pol ι /Pol ζ -dependent TLS operates in an error-free manner, Rev1 polymerase activity makes a relatively minor contribution to TLS and it acts in an error-prone manner, and Pol θ polymerase activity provides the alternative route of mutagenic TLS.

Role of Pol ι /Pol ζ -dependent TLS in error-free replication through ϵ dA in human cells

Although structural studies have shown that Pol ι can accommodate dTTP or dCTP opposite ϵ dA via Hoogsteen base pairing, and biochemical studies have shown that Pol ι incorporates a T opposite ϵ dA with only an approximately fourfold higher catalytic efficiency than a C (Nair et al. 2006), our genetic studies reveal that Pol ι -dependent TLS opposite this lesion operates in an error-free manner in human cells. In the Pol ι active site, ϵ dA is pushed into a *syn* conformation and it forms a Hoogsteen base pair with the incoming T or C residue (Nair et al. 2006). While the ability of Pol ι to push the adduct into the *syn* conformation explains its proficiency for TLS opposite ϵ dA, it does not explain how Pol ι avoids C incorporation in human cells. To explain the high fidelity of Pol ι opposite ϵ dA in human cells, we suggest that TLS Pols function as a component of a multiprotein ensemble and that their fidelity is actively regulated in such an ensemble by protein–protein interactions and posttranslational modifications. A similar mechanism would account for predominantly error-free replication by TLS Pols through other DNA lesions (Yoon et al. 2009, 2010, 2017, 2018; Conde et al. 2015).

Reconfiguration of Pol θ active site opposite ϵ dA in human cells

Similar to other A family Pols, Pol θ incorporates nucleotides opposite undamaged A, G, C, or T templates via W–C base pairing. Since ϵ dA disrupts W–C base pairing, purified Pol θ could replicate through this adduct by pushing it out of the DNA helix into an abasic-like mode, then inserting an A opposite the adduct site and extending synthesis. The feasibility of this scenario is suggested from the proficient ability of Pol θ to replicate through an abasic site where it inserts an A opposite the abasic site and then extends synthesis. Structural studies with Pol θ have verified its ability to insert an A opposite an abasic site (Zahn et al. 2015).

Even though Pol θ contributes to error-prone TLS opposite ϵ dA in human/mouse cells, in both HFs and MEFs, it incorporates a T at a frequency of \sim 92%, a C at \sim 5%, and an A at \sim 3% (Table 3; Supplemental Table S6). Thus in spite of the propensity of purified Pol θ for incorporating an A opposite ϵ dA, Pol θ -mediated TLS opposite this adduct is predominantly error-free in human cells. Pol θ could accomplish this by pushing ϵ dA into a *syn* conformation and by forming a Hoogsteen base pair with the incoming T. The low frequency of C insertion could also occur by similar means. Although in its potential to form a Hoogsteen base pair between ϵ dA in *syn* conformation and an incoming T or C in *anti* conformation Pol θ would resemble Pol ι , the two Pols would differ in a number of ways. Pol ι has a preformed active site which enables it to similarly position an undamaged A or an ϵ dA in the *syn* configuration; hence, Pol ι would adopt a similar mechanism for performing TLS in human cells as it portrays in biochemical assays. In striking contrast, Pol θ would need to adopt entirely different means for conducting TLS opposite ϵ dA in human cells than those adopted by purified Pol θ .

To explain the adoption of different TLS mechanisms by Pol θ in human cells vs in biochemical assays with purified enzyme, we suggest that Pol θ functions in TLS in human cells as a component of a multiprotein ensemble and that protein–protein interactions and posttranslational

modifications in that ensemble actively modulate Polθ active site such that the εdA adduct is pushed and stabilized in a *syn* conformation, allowing for Hoogsteen base pairing primarily with the T. Thus, whereas protein–protein interactions in the Polθ-containing multiprotein ensemble would actively reconfigure Polθ active site for Hoogsteen base pairing opposite εdA enabling it to conduct relatively high fidelity TLS in human cells, in Polι with its preformed active site, the overall mechanism of Hoogsteen base pairing would stay the same in the multiprotein ensemble in human cells as with purified Pol but its fidelity opposite εdA will be greatly enhanced in human cells.

Materials and methods

Construction of εdA plasmid vectors and translesion synthesis assays

The 16-mer oligonucleotides containing εdA, purchased from the Midland Certified Reagent Company, Inc., and the in-frame target sequence of *lacZ'* genes in the resulting vectors are shown in Supplemental Figure S1B. The WT kanamycin gene (*Kan^r*) was placed on the same DNA strand as εdA and *lacZ'* in this DNA strand is in-frame and functional for β-gal. The opposite DNA strand harbors an *SpeI* restriction site containing a +1 frameshift, which makes it nonfunctional for β-gal. This DNA strand carries the *Kan^r* gene. The detailed methods for construction of lesion containing SV40 duplex plasmid, for assays for translesion synthesis, and for mutation analyses of TLS products have been published previously (Yoon et al. 2009, 2010).

Protein expression and purification

Rev1 core (amino acid residues 330–883) and Polθ core (amino acid residues 1708–2590) proteins were each expressed and purified from yeast as fusion proteins harboring an N-terminal Glutathione-S transferase (GST) tag as described (Johnson et al. 2006). Rev1 and Polθ proteins were expressed from plasmids pBJ1228 and pPOL507, respectively. The GST tags were removed by prescission protease during purification.

DNA polymerase assays

Proteins were assayed using the standard DNA polymerase reaction conditions (Johnson et al. 2006). The DNA substrate consisted of the 52mer template 5'-TTTCGTATAATGCCTACACDXGAGTACCGGAGCATCGTCGT GACTGGGAAAAC-3', where D indicates either G, A, or T and X indicates either an undamaged A or εdA annealed to the 32mer ³²P radiolabeled primer 5'-GTTTTCCCAGTCACGACGATGCTCCGGTACTC-3'. Reactions (5 μL), carried out for 5 min at 37°C, contained 10 nM DNA substrate, 25 μM dNTP and protein concentrations as indicated in the figure legends. Reaction products were separated by 15% TBE PAGE containing 8 M urea as described, and visualized by phosphorimaging on a Typhoon FLA 7000 (GE Healthcare).

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Author contributions: J.H.Y. performed the genetic experiments and analyzed the data; R.E.J. performed biochemical experiments and analyzed the data; L.P. and S.P. designed and coordinated the study; J.H.Y., R.E.J., L.P., and S.P. wrote the paper.

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CORRIGENDUM

Genes & Development 33: 282–287 (2019)

Corrigendum: DNA polymerase θ accomplishes translesion synthesis opposite 1,N⁶-ethenodeoxyadenosine with a remarkably high fidelity in human cells

Jung-Hoon Yoon, Robert E. Johnson, Louise Prakash, and Satya Prakash

In [Supplemental Figure S2](#) in the above article, we have been unable to confirm that the Western blots in panels A–C were assembled correctly. Because of our concerns about the accuracy of the data in the Western blots, we are presenting a new set of verified experiments in the new [Supplemental Figure S2](#), which can be found in the Revised Supplemental Material online. This change in no way affects any of the results or conclusions of this study. The authors apologize for this error.

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DNA polymerase θ accomplishes translesion synthesis opposite 1,N⁶-ethenodeoxyadenosine with a remarkably high fidelity in human cells

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<https://genesdev.cshlp.org/content/suppl/2019/12/26/gad.320531.118.DC2>

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