Structure
Article

Protein-Template-Directed Synthesis across an Acrolein-Derived DNA Adduct by Yeast Rev1 DNA Polymerase

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SUMMARY

Acrolein is generated as the end product of lipid peroxidation and is also a ubiquitous environmental pollutant. Its reaction with the N^2 of guanine leads to a cyclic γ-HOPdG adduct that presents a block to normal replication. We show here that yeast Rev1 incorporates the correct nucleotide C opposite a permanently ring-closed form of γ-HOPdG (PdG) with nearly the same efficiency as opposite an undamaged G. The structural basis of this action lies in the eviction of the PdG adduct from the Rev1 active site, and the pairing of incoming dCTP with a "surrogate" arginine residue. We also show that yeast Polζ can carry out the subsequent extension reaction. Together, our studies reveal how the exocyclic PdG adduct is accommodated in a DNA polymerase active site, and they show that the combined action of Rev1 and Polζ provides for accurate and efficient synthesis through this potentially carcinogenic DNA lesion.

INTRODUCTION

Cellular DNA is continually exposed to a large variety of external and internal DNA-damaging agents. One common DNA-damaging agent is acrolein, an α,β-unsaturated aldehyde, which is generated in vivo as the end product of lipid peroxidation and during the metabolic oxidation of polyamines, and which is also present as a ubiquitous environmental pollutant formed by the incomplete combustion of organic materials (Chung et al., 1999a, 1999b; Esterbauer et al., 1991; Vaca et al., 1988). Acrolein damages DNA primarily by reacting with the N^2 of guanine, followed by ring closure at N1, leading to the formation of the cyclic adduct γ-hydroxy-1,N^2-propano-2′-deoxyguanosine (γ-HOPdG). Such γ-HOPdG-like adducts have been detected in mammalian tissues at considerable levels, and there is increasing circumstantial evidence that they play a role in the onset of cancer; for example, the level of these adducts is found to be elevated in smokers as compared to non-smokers (Chung et al., 1996, 1999b; Nath and Chung, 1994; Nath et al., 1996). Although γ-HOPdG can also adopt a ring-opened form, from biochemical and NMR studies it has been inferred that, as a templating base, γ-HOPdG would be in the closed cyclic form (de los Santos et al., 2001; Kim et al., 2002; Kozekov et al., 2003), which presents a strong block to DNA synthesis not only by replicative DNA polymerases (Pols), but also by Y-family Pols such as η and κ (Minko et al., 2003; Washington et al., 2004b). The inability of these polymerases to replicate the γ-HOPdG adduct reflects their dependence on Watson-Crick (W-C) base pairing, as the exocyclic ring between N^2 and N1 of cyclic γ-HOPdG impairs the "W-C edge" of guanine.

Rev1 is the most extreme member of the eukaryotic Y-family Pols, as it is highly specific for incorporating a C opposite template G (Haracska et al., 2002; Nelson et al., 1996). Yeast Rev1 misincorporates a G, an A, or a T opposite template G with catalytic efficiencies that are lower by a factor of $\sim 10^3$ than for the incorporation of C (Haracska et al., 2002). Rev1 also incorporates a C (and not the correct nucleotide) opposite the templates A, T, and C with efficiencies that are lower by a factor of $\sim 10^2$–10^3 than for the incorporation of C opposite template G. In this respect, Rev1 differs not only from the replicative and repair polymerases, which incorporate the correct nucleotide opposite all four template bases with nearly equivalent catalytic efficiencies, but it also differs from the other three eukaryotic Y-family Pols, η, ι, and κ. Of these, Polη and Polκ form the four W-C base pairs with similar catalytic efficiencies, whereas Polι incorporates the correct nucleotide opposite template purines with a much higher efficiency than opposite template pyrimidines (Prakash et al., 2005).

The crystal structure of the ternary complex of yeast Rev1 bound to template G and incoming dCTP has revealed the basis of the extreme specificity of this polymerase (Nair et al., 2005b). Strikingly, in the active site of Rev1, template G and dCTP do not pair with each other. Instead, template G is evicted from the DNA helix, and it makes two hydrogen bonds via its Hoogsteen edge (N7 and O^6) with amino acids in a segment of Rev1 called the G loop. Moreover, the incoming dCTP pairs with an arginine in another segment of Rev1 called the N-digit, ensuring the incorporation of dCTP over other incoming nucleotides. Thus, unlike

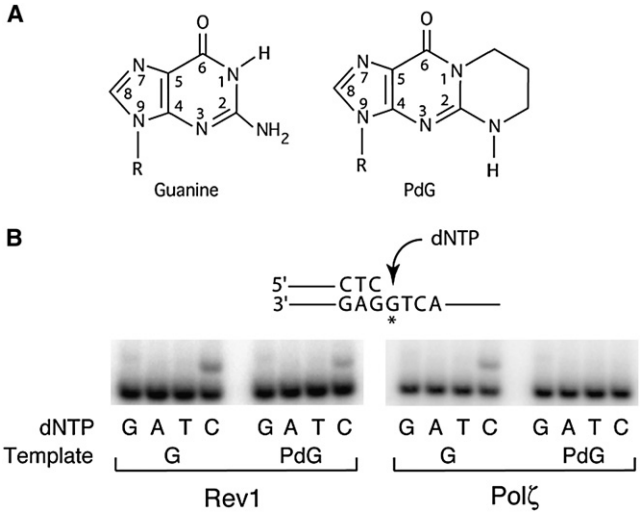


Figure 1. Rev1 and Polζ Activity Opposite the PdG Adduct

(A) Structures of the G and PdG adducts.

(B) DNA synthesis by Rev1 and Polζ on a nondamaged G or PdG adduct containing DNA template. dNTP insertion opposite a G or a PdG adduct by Rev1 or Polζ. Rev1 (0.5 nM) or Polζ (0.4 nM) was incubated with 1 μM or 10 μM dGTP, dATP, dTTP, or dCTP, respectively. A portion of the DNA substrate is shown on top. An asterisk on G in the DNA substrate denotes a G or a PdG adduct.

any other DNA polymerase, in Rev1, specificity for both the templating and the incoming nucleotide is provided by the protein itself rather than the DNA.

The Rev1 structure suggests a specific mechanism for proficient and error-free synthesis through γ-HOPdG and other N^2-adducted guanines that obstruct replication. By excluding N^2-adducted guanines from the DNA helix and pairing incoming dCTP with a "surrogate" arginine, Rev1 can potentially promote synthesis through such lesions. Indeed, yeast Rev1 can incorporate a C opposite γ-HOPdG as efficiently as opposite an undamaged G (Washington et al., 2004a). Because γ-HOPdG is expected to be in a ring-closed form as a templating residue, and because the ring-closed form is highly blocking to synthesis by most DNA polymerases, here we have examined the ability of Rev1 to synthesize opposite the permanently ring-closed form of γ-HOPdG, namely, the 1,N^2-propano-2′-deoxyguanosine (PdG) adduct (Figure 1A). We have carried out biochemical studies to show that Rev1 efficiently inserts a dCTP opposite this adduct, and we have solved the structure of Rev1 in complex with a template-primer containing the PdG adduct and with dCTP as the incoming nucleotide. We also show that yeast Polζ, a member of the B-family of Pols, extends from C incorporated by Rev1 opposite the PdG adduct. Together, our biochemical and structural studies show that the Rev1 active site is uniquely adapted for efficient and accurate synthesis opposite the PdG adduct, and that the sequential action of Rev1 and Polζ can promote efficient and error-free synthesis through the adduct.

RESULTS

Efficient Nucleotide Incorporation Opposite PdG by Rev1

To determine whether the PdG adduct posed an impediment to the DNA polymerase activity of Rev1, we first assayed Rev1 activity in the presence of a single nucleotide on a DNA substrate containing a PdG residue at the templating site. As shown in Figure 1B, Rev1 incorporates dCTP opposite PdG nearly as well as opposite the undamaged G. In contrast, Polζ, a member of the B-family of DNA polymerases, is unable to insert any nucleotide opposite the PdG adduct. We next determined the kinetic efficiency with which Rev1 incorporates nucleotides opposite PdG relative to a nonadducted G. As shown in Table 1, Rev1 incorporates a dCTP opposite the PdG lesion with nearly the same kinetic efficiency as it does opposite the nonadducted G. We also determined the efficiency with which Rev1 incorporates the wrong nucleotides opposite PdG and found the misincorporation efficiency to be nearly the same as that opposite a normal G. Thus, Rev1 incorporates a C opposite the PdG lesion with almost the same efficiency and fidelity as opposite the undamaged G.

Structure Determination

We cocrystallized yeast Rev1 with a template-primer containing a PdG adduct and with dCTP as the incoming nucleotide (Figure 2). The cocrystals belong to space group $C222_1$ and have cell dimensions of a = 181.06 Å, b = 199.58 Å, c = 55.68 Å, and α = β = γ = 90°. The structure was solved by molecular replacement by using the undamaged $Rev1_{G.dCTP}$ complex as a search model. Electron density maps showed clear density for the exocyclic ring of the PdG adduct, allowing it and the rest of the ternary complex to be built and refined at 2.41 Å resolution (R_{free} of

Table 1. Efficiency and Fidelity of Nucleotide Incorporation Opposite Template G and PdG by Rev1

Template Base	Incoming Nucleotide	k_{cat}(min^{-1})	K_M (μM)	k_{cat}/K_M	f_{inc}	Efficiency Relative to G
G	dGTP	2.5 ± 0.06	31 ± 2.8	8.1×10^{-2}	6.3×10^{-3}	-
	dATP	N.D.[a]	≥1250	1.3×10^{-4}[b]	1.0×10^{-5}	-
	dTTP	2.5 ± 0.1	161 ± 18	1.6×10^{-2}	1.3×10^{-3}	-
	dCTP	0.51 ± 0.04	0.04 ± 0.01	12.8	1	-
PdG	dGTP	2.7 ± 0.03	48 ± 2	5.6×10^{-2}	5.2×10^{-3}	0.7
	dATP	N.D.	≥1250	1.2×10^{-4}	1.1×10^{-5}	0.9
	dTTP	2.2 ± 0.03	210 ± 7.2	1.1×10^{-2}	1.0×10^{-3}	0.7
	dCTP	0.43 ± 0.02	0.04 ± 0.01	10.8	1	0.8

[a] N.D., not determined.

[b] Because nucleotide incorporation remained linear throughout the dNTP concentration, the efficiency was determined from the slope of the line.

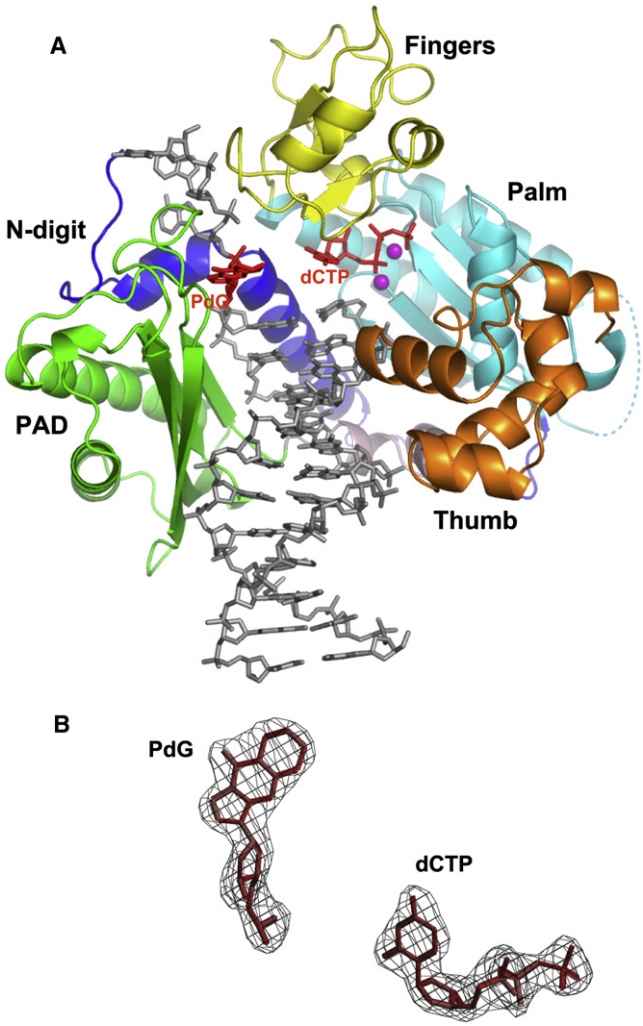


Figure 2. The Rev1:DNA(PdG):dCTP Ternary Complex

(A) Overall view of the complex; the palm, fingers, and thumb domains as well as the PAD are shown in cyan, yellow, orange, and green, respectively. The linker joining the thumb to the PAD is shown in pink. The N-digit in Rev1 is shown in dark blue. DNA is gray, template PdG and incoming dCTP are red, and the putative Mg^{2+} ions are magenta. The cyan, dashed line indicates an unstructured loop in the palm domain.

(B) A simulated annealing $F_o - F_c$ omit map (contoured at 4.0σ; 2.41 Å resolution), showing clear density for the exocyclic ring of the PdG adduct and for the incoming nucleotide dCTP.

24.1%; R_{crys} of 23.1%). The refined $Rev1_{PdG.dCTP}$ structure includes Rev1 residues 305–477 and 484–738 (there is no density for 8 residues at the N and C termini of the construct and for a small loop in the palm domain spanning residues 478–483), nucleotides 1–16 for the template strand, nucleotides 1–12 for the primer strand, incoming dCTP, 2 Mg^{2+} ions, and 158 water molecules. The structure has excellent stereochemistry; >91% of the residues are in the most favored regions of the Ramachandran plot, and only 0.3% are in the disallowed regions.

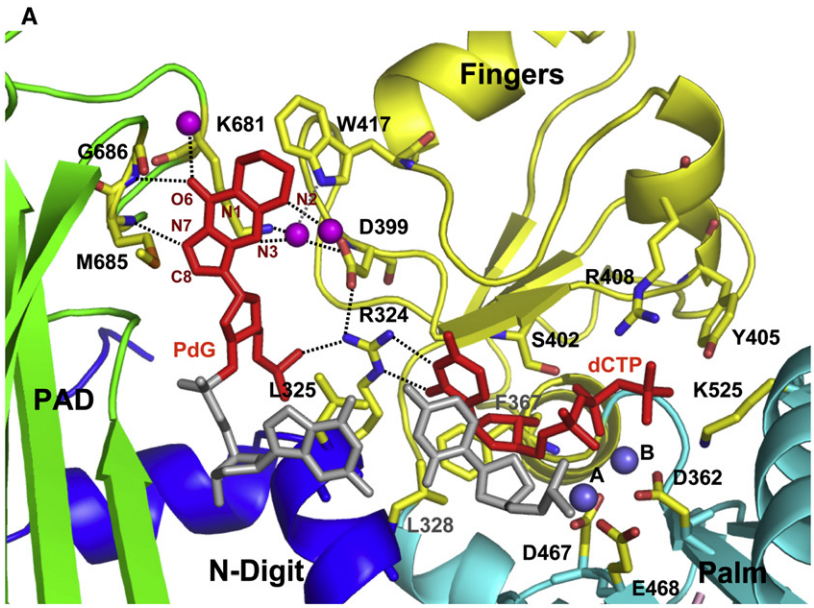
Overall Arrangement

Rev1 embraces the template-primer with its palm (residues 356–365, 438–536), fingers (residues 366–437), and thumb (537–603) domains as well as the PAD (polymerase associated domain; residues 621–738) unique to Y-family polymerases (Figure 2A). The palm carries the active site residues (Asp362, Asp467, and Glu468) that catalyze the nucleotidyl transfer reaction. The fingers domain lies over the replicative end of the template-primer, but, unlike other DNA polymerases, it makes very few contacts with the templating base; instead, interactions primarily occur with incoming dCTP and the unpaired nucleotides at the 5′ end of the template. The thumb and the PAD approach the template-primer from opposite sides and are connected by a long linker, which is mostly helical rather than extended, as in other Y-family polymerases. The right-handed grip of the palm, fingers, and thumb domains and the PAD on the template-primer is further augmented by a loose α loop substructure at the N terminus, an N-digit (residues 305–355), which interacts with incoming dCTP (Figure 2A). Also, the Rev1 PAD is exceptional in that it has an extra long loop, a G loop, which interacts directly with the PdG adduct.

PdG and Incoming dCTP Do Not Pair with Each Other

The structure reveals for the first time, to our knowledge, how a closed-ring PdG adduct is accommodated by a DNA polymerase (Figure 3A). As with unmodified G (Nair et al., 2005b), the adduct is evicted from the Rev1 active site by Leu325 (from the N-digit), which inserts into the DNA helix. PdG slips into a small hydrophobic cavity delineated by aliphatic portions of Met685 and Lys681 that stem from the G loop and by the indole ring of Trp417 that extends from the fingers domain (Figure 3). The PdG exocyclic ring between N^2 and N1 of guanine is localized toward the outer edge of this cavity, well away from the catalytic center of the enzyme, and making van der Waals contacts with the indole ring of Trp417 (Figure 3). The pattern of hydrogen bonding is strikingly similar to that observed with unmodified G, wherein a segment of Rev1—the G loop—and not the incoming nucleotide, mediates the bonding. That is, two hydrogen bonds are established between N7 and O6 at the Hoogsteen edge of PdG and the main chain amides of Met685 and Gly686 on the G loop (Figure 3A). In addition, O6 of PdG hydrogen bonds to a water molecule linked to Lys681, and N3 makes an out-of-plane hydrogen bond with a water molecule that is fixed in position by Asp399, Trp417, and Lys681. In addition to these stabilizing polar interactions, the hydrophobic cavity delineated by the G loop and the fingers domain is shaped optimally to accommodate the PdG adduct (Figure 3B).

Incoming dCTP pairs with an arginine (Arg324) that emanates from the N-digit (Figure 3A). Two hydrogen bonds are established between N3 and O^2 at the W-C edge of dCTP and the Nη2 and Nε donor groups of Arg324, respectively (Figure 3A). Arg324 is buttressed as the surrogate templating residue by a network of hydrogen bonds with Asp399 and the 5′ phosphate of the ejected PdG adduct. By pairing dCTP with Arg324, Rev1 ensures the incorporation of a correct nucleotide opposite the PdG, as substitution by any other incoming nucleotide (dTTP, dATP, or dGTP) would lead to loss of hydrogen bonds, as well as to unfavorable electrostatic and steric intrusion. The catalytic residues, Asp362, Asp467, and Glu468, are arrayed between the dCTP triphosphate moiety and the primer terminus, and two octahedrally coordinated Mg^{2+} ions—analogous to metals "A" and "B" in replicative DNA polymerases (Doublie et al., 1998;



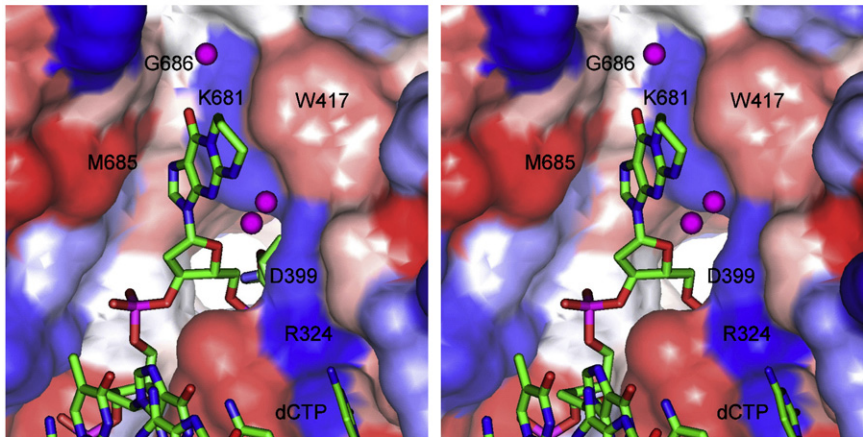


Figure 3. PdG in the Rev1 Active Site

(A) A close-up view of the Rev1 active site region. The N-digit, fingers, and palm domains as well as the PAD are shown in dark blue, yellow, cyan, and green, respectively. The DNA is colored gray, and template PdG and incoming dCTP are shown in red. The putative Mg^{2+} ions are dark blue, and the three displayed water molecules are magenta. Highlighted and labeled are the catalytic residues (D362, D467, and E468); residues (S402, Y405, R408, and K525) that interact with the triphosphate moiety of incoming dCTP; R324, which makes hydrogen bonds with the dCTP base; L325, which pushes template PdG out of the DNA helix; and residues (M685, G686, K681, and W417), which interact with the extra-helical template PdG.

(B) Stereo view of the Rev1 surface near the PdG adduct. The surface is colored according to a spectrum of hydrophobicity: dark red corresponds to maximum hydrophobicity, and dark blue corresponds to maximum hydrophilicity. DNA is shown in stick representation, and the relevant water molecules are shown as magenta spheres.

Li et al., 1998; Steitz, 1999)—complete the Rev1 active site (Figure 3A). In all, Rev1 is well poised for dCTP insertion, with the putative 3′ oxygen (at the primer terminus) located ~4 Å from the dCTP α-phosphate.

Rev1 Is Primed to Accept PdG with Little or No Alteration in Structure

To accommodate the exocyclic ring between N^2 and N1 of PdG, we had considered a model in which the adduct causes a change in Rev1 conformation. This is clearly not the case, as the conformation of Rev1 is almost identical to that with undamaged G (Nair et al., 2005b), superimposing with an rmsd of only 0.17 Å (for Cα atoms) (Figure 4). Furthermore, all atoms of residues that interact with PdG or G (Tyr319, Ser323, Arg324, Leu325, Trp417, Asn628, Lys681, Gly684, Met685, and Gly686) superimpose with an rmsd of only 0.15 Å. The main difference between the two structures is the stacking between the indole ring of Trp417 and the puckered exocyclic ring of PdG. Overall, the Rev1 PAD (encompassing the G loop) and the fingers domain exhibit minimal change upon binding the PdG nucleotide and are remarkably well adapted or juxtaposed to "receive and hold" this potentially carcinogenic lesion for error-free replication through it (Figures 3 and 4).

Primer Extension Opposite PdG by Polζ

We next determined whether Polζ could carry out the extension of a primer terminus opposite PdG. For this purpose, we used a DNA substrate that has a C residue at the primer terminus opposite PdG. As shown in Figure 5, Polζ is able to extend the primer terminus opposite the PdG adduct by inserting the next correct nucleotide, dATP. Next, we determined the kinetic efficiency with which Polζ carries out the extension reaction. As shown in Table 2, compared to extension opposite a nonadducted G, Polζ extends past PdG ~35-fold less efficiently. Interestingly, this drop in Polζ efficiency results primarily from an ~20-fold increase in the K_M for dATP, from 0.04 to 0.84 μM.

DISCUSSION

The reaction of acrolein with N^2 of guanine leads to a cyclic γ-HOPdG adduct that presents a strong block to normal replication. We show here that Rev1 incorporates a C opposite PdG, a permanently ring-closed form of γ-HOPdG, with nearly the same efficiency as opposite an undamaged G. The crystal structure of the $Rev1_{PdG.dCTP}$ complex provides a molecular explanation for this action. The adduct is driven to its extra-helical position by Leu325 (from the N-digit), which takes up much of the space (within the DNA helix) vacated by PdG. The PdG adduct is then accommodated "comfortably" in a large (solvent-filled) void between the PAD and the fingers domain. The adduct is thus moved to a site on the polymerase where its exocyclic

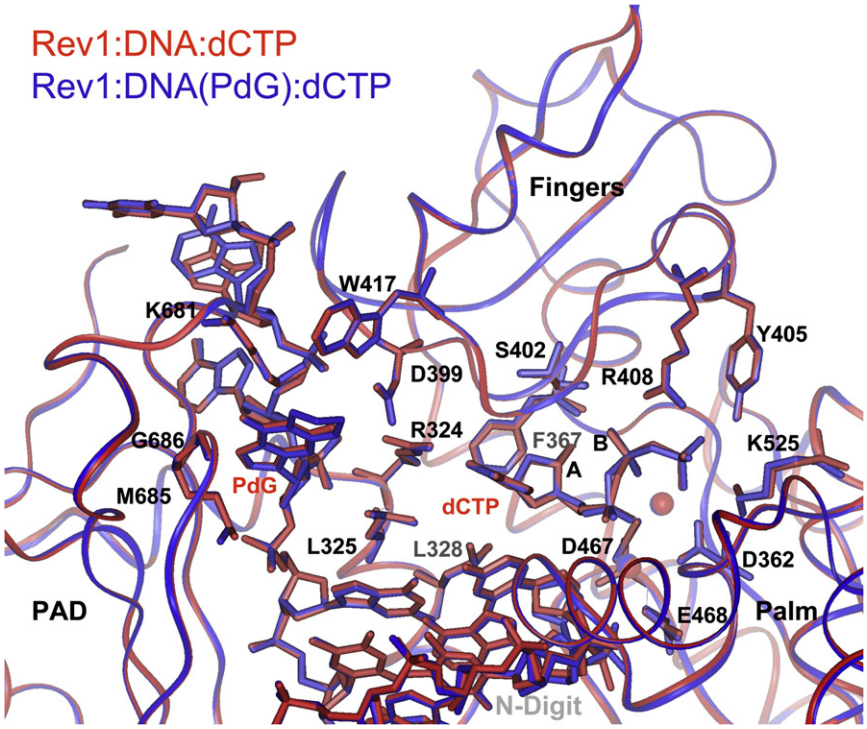


Figure 4. Superposition of Rev1 Ternary Complexes with Damaged and Undamaged DNAs

The undamaged Rev1:DNA:dCTP complex is colored red, and the damaged Rev1:DNA(PdG):dCTP complex is colored blue. The protein is displayed in ribbon representation, and the active site and dCTP-interacting and template-interacting residues are displayed in stick representation. Rev1 accommodates the PdG exocyclic ring with only a minimal change in conformation.

ring cannot interfere with the selection and incorporation of the incoming nucleotide. The incorporation of "correct" dCTP is ensured by a surrogate arginine (Arg324) residue that is conserved in all of the known orthologs of Rev1, including in humans.

Polι is the only other Y-family polymerase that has been found to be capable of inserting a nucleotide opposite the closed-ring PdG (or γ-HOPdG) adduct (Washington et al., 2004b; Wolfle et al., 2005). However, the mechanism by which Polι bypasses this adduct is different from the protein-template-directed strategy employed by Rev1. Polι is a polymerase that is specialized for Hoogsteen base pairing (Nair et al., 2004, 2005a), and, presumably, PdG adopts a syn conformation in its active site. Thus, whereas Rev1 excludes the entire PdG adduct from its active site, Polι presumably evades the "interfering" exocyclic ring of PdG by driving the adduct into the syn conformation. Interestingly, despite these differences, both polymerases make use of the PdG Hoogsteen edge. Rev1 uses it to recognize PdG as a guanine adduct, whereas Polι uses it to specify the identity of the incoming dCTP.

Although Rev1 is able to insert a C opposite the PdG adduct, it is unable to carry out the subsequent extension reaction (data not shown). We examined whether Polζ, a member of the B-family of polymerases, could perform extension of primer opposite PdG, as Polζ is highly adept at extending primer termini opposite a diverse array of lesions (Prakash et al., 2005). We show that although Polζ is strongly inhibited at the nucleotide incorporation reaction opposite PdG, it promotes the subsequent extension reaction opposite this lesion. Similar to extension from the normal G-C base pair, Polζ also extended the primer containing a C paired with PdG. Also, assays done under steady-state conditions to determine the efficiency with which Polζ extended the damaged and undamaged DNA substrates showed that extension by Polζ of C paired with PdG is about 35-fold less efficient than when C is paired opposite the normal G. This drop in efficiency results primarily from an increase in the K_M for dATP from 0.04 μM to 0.84 μM. We do not know at present the conformation of a PdG:C base pair after it is released by Rev1, but, unlike a G:C base pair, it will not be W-C base paired, and this may lend to a distortion at the primer terminus that weakens subsequent dNTP binding. Whether such distortion is indeed transmitted to the Polζ dNTP-binding site may only become clearer with the crystal structure of Polζ. Even so, this decrease in efficiency may not be as refractory to PdG:C extension (by Polζ) in vivo, because the cellular dNTP levels are much

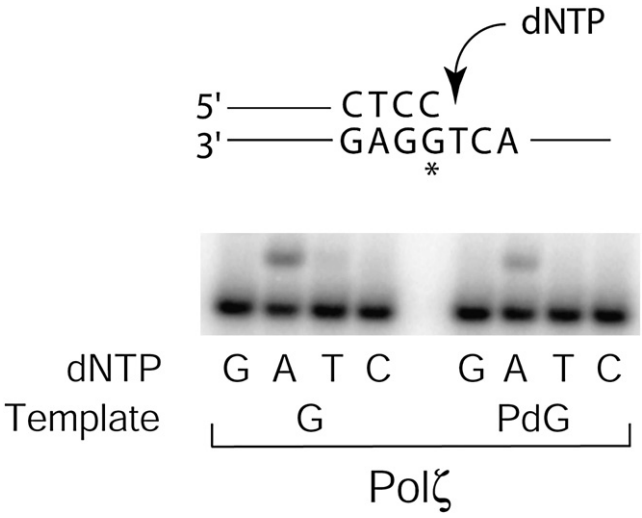


Figure 5. Primer Extension Past a G or PdG by Polζ

Polζ (0.4 nM) was incubated with 10 μM dGTP, dATP, dTTP, or dCTP for 10 min at 30°C. A portion of the DNA substrate is shown on top. An asterisk on G in the DNA substrate denotes a G or a PdG adduct.

Table 2. Efficiency of Primer Extension from C Base Paired Opposite Template G or PdG by Polζ

Template Base	Incoming Nucleotide	k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M	Efficiency Relative to G
G	dATP	0.55 ± 0.03	0.04 ± 0.01	13.8	-
PdG	dATP	0.33 ± 0.02	0.84 ± 0.15	0.39	0.028 (↓ 35×)

higher than the K_Ms measured here. Overall, Polζ is an efficient extender of primers opposite a variety of DNA lesions, and its ability to extend the PdG:C base pair adds to the diversity of DNA lesions that Polζ can handle at the extension step.

The polyunsaturated fatty acid residues of phospholipids in membranes are extremely prone to attack by free radicals. Lipid peroxidation initiates a chain reaction that results in the generation of a great diversity of aldehydes, including acrolein, crotonaldehyde, malondialdehyde, and trans-4-hydroxy-2-noenal (HNE) (Chung et al., 1999a, 1999b; Esterbauer et al., 1991; Vaca et al., 1988). All of these adducts react with the N^2 group of guanine in DNA and form a cyclic ring resulting from ring closure at the N1 of guanine. These various adducts have been identified in DNAs of human and rodent tissues at relatively high levels (Chung et al., 1996, 1999b; Nath and Chung, 1994; Nath et al., 1996). Although these adducts differ in their level of structural complexity, they all share the ring-closure attribute of PdG. The ability of Rev1 to accommodate PdG in its active site and to proficiently insert a dCTP opposite it strongly suggests that Rev1 would promote proficient dCTP incorporation opposite the large array of N^2 adducts resulting from cellular oxidation reactions. Because the N^2-adducted moiety protrudes from a three-sided pocket and faces the solvent (Figure 3B), larger N^2 adducts such as HNE that result from cellular oxidation can be easily accommodated by Rev1. Thus, the combined action of Rev1 and Polζ would promote proficient and error-free replication through such adducts. A similar role for these polymerases in human cells would be important for keeping the incidence of mutagenesis, and hence of carcinogenesis, low.

EXPERIMENTAL PROCEDURES

Protein and DNA Preparation

The *S. cerevisiae* GST-Rev1 (residues 297–746) fusion protein was expressed in yeast strain BJ5464 from plasmid pBJ954. The Rev1 (297–746) protein is as catalytically active as the full-length protein, and it has the same nucleotide incorporation specificity as the wild-type protein. The fusion protein was purified by $(NH_4)_2SO_4$ precipitation, followed by affinity purification with a glutathione Sepharose column. Rev1 (297–746) lacking the GST tag was eluted from the column, following cleavage on the resin with PreScission protease (Amersham Pharmacia). The protein was further purified over a gel-filtration SD200 column, concentrated, and used for cocrystallization. The primer was synthesized as a 12 nucleotide oligonucleotide, with a dideoxy cytosine at its 3′ end (5′-ATCCTCCCCTAC^{dd}-3′). This was annealed with a 16 nucleotide template (5′-TAA(**PdG**)GTAGGGGAGGAT-3′) to yield a 12/16 primer-template. Prior to annealing, the oligonucleotides were purified by reverse phase over a C18 column, desalted, and lyophilized.

Cocrystallization

The native complex was prepared by incubating Rev1 with the 12/16 primer-template in a molar ratio of 1:1.2 with 10 mM dCTP and 10 mM $MgCl_2$ and was then crystallized from solutions containing 10%–16% PEG 5000 MME and 0.2 $(NH_4)_2SO_4$ buffered at a pH of 6.5. The cocrystals belong to space group $C222_1$ and have cell dimensions of a = 181.1 Å, b = 199.6 Å, c = 55.7 Å and $\alpha = \beta = \gamma = 90^\circ$. For data collection, the cocrystals were cryoprotected by soaks for 5 min in mother liquor solutions containing 5%, 10%, 15%, and 20% glycerol and were then flash frozen in liquid nitrogen.

Structure Determination

X-ray diffraction data on the cocrystals (2.41 Å) were measured at the Advanced Photon Source (APS; beamline 17-ID) (Table 3). All data were indexed and integrated by using DENZO and were reduced by using SCALEPACK (Otwinowski and Minor, 1997). The structure was solved by molecular replacement with the program AMoRe (Navaza, 1994), by using the structure of the $Rev1_{G.dCTP}$ complex as a search model (Nair et al., 2005b). The electron density map calculated after a round of refinement showed clear density for the exocyclic ring of the PdG adduct. The PdG adduct coordinates were extracted from a DNA hairpin loop structure (PDB ID: 1LAE) (Weisenseel et al., 2002), and iterative rounds of refinement with CNS (Brunger et al., 1998), model building with O (Jones et al., 1991), and water picking lowered the R_{free} value to 24.1%. The final model includes Rev1 residues 305–477 and 484–738 (there is no density for 8 residues at the N and C termini of the construct and for a small loop in the palm domain spanning residues 478–483), nucleotides 1–16 for the template strand, nucleotides 1–12 for the primer strand, incoming dCTP, 2 Mg^{2+} ions, and 158 water molecules. The model has excellent stereochemistry, as shown by PROCHECK (Laskowski et al., 1993); over 91% of the residues are in the most favored regions of the Ramachandran plot, and only 0.3% are in the disallowed regions.

Table 3. Data Collection and Refinement Statistics

	$Rev1_{PdG.dCTP}$
Data Collection	
Space group	C222(1)
Cell dimensions	
a, b, c (Å)	181.06, 199.58, 55.68
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	2.41 (2.5–2.41)[a]
R_{sym} or R_{merge}	4.9 (16.4)
I/σI	32.1 (8.8)
Completeness (%)	99.7 (98.4)
Redundancy	5.4 (4.9)
Refinement	
Resolution (Å)	50–2.41
Number of reflections	38,556
R_{work}/R_{free}	23.1/24.1
Number of atoms	
Protein	3,370
DNA	572
dCTP	28
Mg^{2+} ion	2
Water	158
B factors(Å^2)	
Protein	37.5
DNA	42.0
dCTP	27.5
Mg^{2+} ion B	33.3
Mg^{2+} ion A	47.7
Water	38.5
Rms deviations	
Bond lengths (Å)	0.012
Bond angles (°)	2.05

[a] One crystal was used to collect data for each structure. The highest resolution shell is shown in parentheses.

PdG Bypass and Kinetics

Full-length yeast Rev1 was expressed in yeast as a GST fusion from plasmid pBJ1096. Rev1 protein was purified as described, and the GST tag was removed by cleavage with prescission protease. Polζ (comprised of Rev3 and Rev7) was purified as described (Johnson et al., 2000). To assay the insertion of nucleotides opposite a G or a PdG, the DNA substrate was

made by annealing the 52-mer oligonucleotide 5′-TTCGTATAATGCCTA CACTXGAGTACCGGAGCATCGTCGTGACTGGGAAAAC-3′, where the X denotes either a G or a PdG adduct to a 32-mer 5′-GTTTTCCCAGTCACGAC GATGCTCCGGTACTC-3′. To assay for extension from a C opposite a G or the PdG adduct, a 33-mer consisting of the identical sequence of the 32-mer but containing an additional C at the 3′ end was annealed to the G- or PdG-containing 52-mer. PdG-containing oligonucleotides were prepared by using a PdG phosphoramidite that was generated in the UTMB Organic Chemistry core facility. The insertion and extension assays shown in Figure 1B contained either 0.5 nM Rev1 or 0.4 nM Polζ and 10 nM DNA template. Assays contained 1 μM (for Rev1) or 10 μM (for Polζ) dGTP, dATP, dTTP, or dCTP and were carried out for 10 min at 30°C. To assay for the fidelity of Rev1 opposite G versus PdG, 0.25 or 0.5 nM enzyme was used, and dNTP concentrations were varied from 0.005 to 2500 μM. To assay for the kinetics of extension from a C paired opposite a G or a PdG, 0.4 nM Polζ was used; dATP concentrations ranged from 0.005 to 100 μM. All kinetic reactions were carried out for 5 min at 30°C.

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Accession Numbers

The coordinates and structure factors have been deposited in the Protein Data Bank with accession code 3BJY.