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DNA synthesis across an abasic lesion by yeast Rev1 DNA polymerase

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Summary

Abasic (AP) sites are amongst the most abundant DNA lesions in humans and they present a strong block to replication. They are also highly mutagenic because when the replicative DNA polymerases do manage to insert a nucleotide opposite the lesion they prefer to insert an A. Rev1, a member of the Y-family of DNA polymerases, does not obey the A-rule. This enzyme inserts a C opposite an abasic lesion with much greater catalytic efficiency than an A, G, or a T. We present here the structure of yeast Rev1 in ternary complex with DNA containing an abasic lesion and with dCTP as the incoming nucleotide. The structure reveals a mechanism of synthesis across an abasic lesion that differs from that in other polymerases. The lesion is driven to an extra-helical position and the incorporation of a C is mediated by an arginine (Arg324) that is conserved in all the known orthologs of Rev1, including in humans. The hydrophobic cavity that normally accommodates the unmodified G is instead filled with water molecules. Since Gs are especially prone to depurination through spontaneous hydrolysis of the glycosidic bond, the ability of Rev1 to stabilize an abasic lesion in its active site and to employ a surrogate arginine to incorporate a C provides a unique means for the “error-free” bypass of this non-instructional lesion.

INTRODUCTION

Cellular DNA is continually exposed to large variety of external and internal DNA damaging agents. Abasic sites are amongst the most abundant DNA lesions in humans, with up to 10,000 purine bases estimated to be lost per day in each cell due to cleavage of the N-glycosidic bond ^{1, 2}. Hydrolysis of the N-glycosidic bond occurs at a high rate spontaneously, though oxidative stress and exposure to ionizing radiation and alkylating agents can further add to the incidence of base loss. Indeed, it has been estimated that each human liver cell contains ~50,000 apurinic/aprimidinic (AP) sites under normal conditions ³. Although abasic lesions can be removed by the base excision repair and nucleotide excision repair pathways ^{4, 5}, lesions that escape repair can block replication, and are also highly mutagenic because when replicative DNA polymerases (Pols) do manage (inefficiently) to insert a nucleotide opposite the lesion they prefer to insert an A, a

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Coordinates

Coordinates and structure factors have been deposited in the PDB with an accession code 3OSP.

phenomenon known as the A-rule^{6–14}. The mechanistic basis for the A-rule has remained under debate for a long time despite the numerous biochemical and structural studies^{6–10; 12–29}.

The most dramatic example of abasic lesion bypass - and a breakdown of the A-rule - is provided by Rev1^{30; 31}, member of the Y-family DNA polymerases which possess the ability to replicate through DNA lesions³². In addition to Rev1, humans have three other Y-family Pols - Polη, Polκ and Polι - each with a unique DNA damage bypass and fidelity profile³². Rev1 incorporates a C opposite an abasic lesion with much greater catalytic efficiency than A, G, or T³¹. The structures of yeast and human Rev1 bound to undamaged template G and incoming dCTP have revealed a novel protein-template-directed mechanism of DNA synthesis, wherein the template G is evicted from the DNA helix and an arginine makes specific hydrogen bonds with the Watson-Crick (W-C) edge of incoming dCTP^{33; 34}. To see whether this protein-template-directed mechanism of DNA synthesis mechanism holds for the bypass of an abasic lesion, we determined the structure of yeast Rev1 in complex with DNA containing an abasic lesion (a tetrahydrofuran moiety) at the templating position and with dCTP as the incoming nucleotide. The structure shows that Rev1 accommodates an abasic lesion without any major alteration in conformation in the protein, the DNA, or the incoming nucleotide.

RESULTS

Structure determination

The Rev1_{Abasic.dCTP} cocrystals belong to space group C222₁ with cell dimensions of $a = 180.0 \text{ \AA}$, $b = 200.24 \text{ \AA}$, and $c = 55.73 \text{ \AA}$. The structure was solved by molecular replacement using the undamaged yeast Rev1_{G.dCTP} complex as a search model³⁴. Electron density maps showed clear density for the abasic lesion, allowing it and the rest of the ternary complex to be built and refined at $2.5 \text{ \AA}$ resolution (R_{crys} of 23.7 %; R_{free} of 25.1%). The refined Rev1_{Abasic.dCTP} structure includes Rev1 residues 305–477 and 484–738 (there is no density for eight 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, two Mg^{2+} ions, and 151 water molecules. The structure has excellent stereochemistry, with ~98% of the residues in the favored regions of the Ramachandran plot and only 0.3% 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, and the PAD (polymerase associated domain; residues 621–738) unique to Y-family polymerases (Figure 1A). 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 are primarily made 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, connected by a long linker, which is mostly helical rather than extended as in other Y family polymerases. The right-handed grip of palm, fingers, thumb and the PAD on the template-primer is augmented by loose α -helical substructure at the N-terminus, an N-digit (residues 305–355), which interacts with incoming dCTP. Also, the Rev1 PAD is exceptional in that it has an extra long loop, a G loop (Figure 1A).

Mode of lesion bypass

The structure reveals for the first time how an abasic lesion is accommodated by Rev1 (Figures 1 and 2). The conformation of Rev1 is almost identical to that in the undamaged Rev1_{G.dCTP} ternary complex³⁴, superimposing with an r.m.s. deviation of only 0.22 Å (for 433 Cα pairs) (Figure 3). In the undamaged Rev1_{G.dCTP} ternary complex, the template G is evicted from the active site by Leu325 (from the N-digit), which inserts into the DNA helix. The guanine base slips into a small hydrophobic cavity delineated by aliphatic portions of Met685 and Lys681 that stem from G-loop and the indole ring of Trp417 from the fingers domain (Figure 2c). At the same time, two hydrogen bonds are established between N7 and O6 at the Hoogsteen edge of G and the main-chain amides of Met685 and Gly686 on the G-loop (Figure 2c). In addition, the O6 of G also makes a hydrogen bond to a water molecule linked to Lys681, and N3 of G makes an out-of-plane hydrogen bond with a water molecule that is fixed in position by Asp399, Trp417, and Lys681³⁴ (Figure 2c). As with unmodified G, the abasic lesion in the Rev1_{Abasic.dCTP} complex is extra-helical and Leu325 occupies the space vacated within the DNA helix (Figure 2a). Compared to the Rev1_{G.dCTP} complex, the sugar (or tetrahydrofuran moiety) shifts by ~1 Å towards the hydrophobic cavity (Figure 3) and is thus closer to residues Lys681, Met685, Tyr320, and Trp629 where, for example, the distance between atoms C1' of the sugar and CD1 of Trp629 decreases from 5.6 to 4.7 Å. The sugar also acquires a C3'-endo conformation as compared to C4'-exo conformation in the undamaged complex (Figures 2b and 3). The hydrophobic cavity, left “empty” due to the absence of a base, is occupied by three new ordered water molecules (Figures 2b and 4). Water molecule 1 (WM1) is in the same position as O6 of G in the undamaged complex and interacts with the main chain amide of Gly686 (2.74 Å). Water Molecule 2 (WM2) is at a similar position as N7 of G in the undamaged complex and likewise makes a hydrogen bond with the main chain amide of Met685 (2.76 Å) as well as a hydrogen bond with the carbonyl of Asn628 (2.65 Å). Water molecule 3 (WM3) occupies the same position as the imidazole ring of G and is involved in possible hydrogen bonds with WM1 (3.3 Å) and WM2 (3.4 Å). Additionally, another water molecule (WM4) that is involved in forming water-mediated hydrogen bonds with unmodified G and main chain amide of Lys681 (3.1 Å) is present at an identical position in the Rev1_{Abasic.dCTP} structure (Figures 2b, 2c and 4). Due to the lack of a templating base in the Rev1_{Abasic.dCTP} structure, the direct and water-mediated interactions observed between unmodified G and main chain amides of M685, G686 and K681 in the Rev1_{G.dCTP} structure are absent (Figures 2b and 2c). The water-mediated hydrogen bonds between N3 of unmodified G and Asp399, Trp417, and Lys681 are also absent (Figure 2b and 2c). However, in spite of this, the Met685, Lys681 and Trp417 side chains maintain almost the same conformation as in the undamaged complex even though there is no base in the hydrophobic cavity (Figure 2b and 2c). Indeed, all of the residues that interact with unmodified G (Tyr320, Ser323, Arg324, Leu325, Trp417, Asn628, Lys681, Gly684, Met685 and Gly686) superimpose with an rmsd of only 0.18 Å (for all atoms) with the residues in the abasic structure.

Incoming dCTP pairs with Arg324 that emanates from the N-digit (Figure 2). Two hydrogen bonds are established between N3 and O² at the Watson-Crick edge of dCTP and the Nη2 (3.1 Å) and Nε (2.8 Å) donor groups of Arg324, respectively (Figures 1b and 2a). Arg324 is buttressed as the “surrogate” templating residue by a network of hydrogen bonds with Asp399 and the 5' phosphate of the abasic site lesion. By pairing dCTP with Arg324, Rev1 ensures the incorporation of a C opposite an abasic lesion. Substitution by any other incoming nucleotide would lead to loss of hydrogen bonds with Arg324, as well as to unfavorable electrostatic and steric intrusion. Incoming dCTP is further stabilized by interactions with side chains of three residues that are conserved in homologous proteins - Tyr405, Arg408 and Lys525 (Figure 2a). In addition the deoxyribose sugar of dCTP stacks against the aromatic ring of Phe367. 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^{35–37} – complete the Rev1 active site (Figure 2). In all, Rev1 is well poised for dCTP insertion opposite an abasic lesion, with the putative 3' oxygen (at the primer terminus) located $\sim 4\text{\AA}$ from the dCTP α -phosphate.

DISCUSSION

Rev1 exhibits minimal changes on binding an abasic lesion. Its active site is remarkably well adapted for the bypass of this common lesion. The non-instructional nature of abasic lesions makes them potentially cytotoxic and highly mutagenic. Almost all high fidelity DNA polymerases prefer to incorporate an A opposite the lesion, but Rev1 is one of the few DNA polymerases that does not obey the A-rule. Rev1 incorporates a C opposite an abasic lesion with much greater (>80 -fold) catalytic efficiency than A, G, or T³¹. We show here that the lesion is driven to an extra-helical position and the incorporation of a C opposite it is mediated by an arginine (Arg324) that is conserved in all the known orthologs of Rev1, including in humans. The specificity for a C derives from a pattern of hydrogen bonds between Arg324 and incoming dCTP that would not be satisfied by other incoming nucleotides. In all, the structure we present here provides a first view of how Rev1 bends the traditional A-rule and incorporates a C much more proficiently than other nucleotides opposite an abasic lesion.

Although, Rev1 has a strong preference for incorporating a C opposite an abasic lesion, the overall catalytic efficiency is ~ 25 -fold lower than that opposite undamaged G³¹. From steady state kinetics, this decrease in efficiency is primarily due to a reduction in the apparent K_m for the nucleotide, whereas the k_{cat} stays approximately the same³¹. The similarity in k_{cat} values is reflected in an active site geometry that is almost identical in the undamaged Rev1_{G,dCTP} and the damaged Rev1_{Abasic,dCTP} ternary complexes. In both complexes Rev1 is well poised for dCTP insertion with the putative 3' oxygen (at the primer terminus) located $\sim 4\text{\AA}$ from the dCTP α -phosphate. The difference in K_m s likely reflects the loss of interactions between the unmodified G and the polymerase in the Rev1_{G,dCTP} complex³⁴. Although, in the abasic structure the space occupied by G is filled by three water molecules, two of which are at similar positions as atoms O6 and N7 of G and partake in hydrogen bonds with the polymerase, they do not substitute for the range of polar and non-polar interactions observed in the undamaged Rev1_{G,dCTP} complex (Figures 2b and 2c). The entropic cost of retaining the water molecules in the hydrophobic cavity may further reduce the affinity of Rev1 for DNA containing an abasic site relative to the undamaged duplex.

Apart from Rev1, Polt is the other Y-family polymerase that has been found capable of inserting a nucleotide opposite an abasic lesion. Polt can insert all four nucleotides opposite an abasic lesion, but with a small preference for a G³⁸. From the structures, the ability of Polt to insert all four nucleotides opposite an abasic lesion derives from the same structural features that make it conducive to Hoogsteen base pairing, namely a constricted active site that reduces the C1'-C1' distance across the sugars in the active site cleft^{23; 39; 40}. The small preference for a G appears to derive from better stacking interactions with the base pair at the primer terminus as well as a direct hydrogen bond between N2 of G and Gln59 of Polt²³. Thus, although Rev1 and Polt have evolved different strategies (and nucleotide preferences) to cope with a non-instructional template, both polymerases employ an amino acid to incorporate the preferred nucleotide. An analogous amino acid templating mechanism may also operate in polymerases from the A-family. In a recent structure of *Thermus aquaticus* DNA polymerase, a member of the A-family, with an abasic lesion, a conserved tyrosine (Tyr671) fills the space of the absent base and supports a hydrogen bond

network with incoming dATP²⁵. In contrast, in B-family polymerases, such as those from phage RB69 or T4, base stacking interactions with the neighboring base pair are believed to be the primary determinant of the preference for an A^{27; 28}. Y-family archaeal polymerase Dpo4 and mammalian Pol β display as yet other mechanisms^{21; 29}, where the abasic lesion is either looped out between the fingers domain and the PAD (Dpo4) or shifted upstream (Pol β). It is noteworthy that Pol β also has an arginine (Arg283) close to the abasic site but unlike Rev1 it points away from the incoming nucleotide²⁹. In all, there appear to be multiple mechanisms and pathways for the bypass of abasic sites in cells, and replicative and repair polymerases may both contribute to the bypass of the lesion. However, it is notable that Gs are especially prone to depurination through spontaneous hydrolysis of the glycosidic bond and this vulnerability is heightened through chemical modifications of the base^{9; 41}. Additionally, it has been shown recently that there exist sequences (~50,000 in the human genome) that can self-catalyze the depurination of a guanine base at a specific internal site⁴². These observations imply that depurination of guanine might be responsible for a significant majority of abasic sites in a cell. The ability of Rev1 to stabilize an abasic lesion in its active site and to employ a surrogate arginine to incorporate a C provides a unique means for the “error-free” bypass of this non-instructional and pervasive DNA lesion.

MATERIALS and METHODS

Protein and DNA preparation

The *S.cerevisiae* GST-Rev1 (residues 297–746) fusion protein was expressed in yeast strain BJ5464 using 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 (NH₄)₂SO₄ precipitation followed by affinity purification using 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 co-crystallization. The primer was synthesized as a 12-nt oligonucleotide, with a dideoxy cytosine at its 3' end (5'-ATCCTCCCCTAC^{dd}-3'). This was annealed with a 16-nt template (5'-TAA(**Abasic**)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 (0.3 mM) with the 12/16 primer-template in a molar ratio of 1:1.2 with 10 mM dCTP and 10 mM MgCl₂, and then crystallized from solutions containing 10–16 % PEG 5000 MME and 0.2 M (NH₄)₂SO₄ buffered at a pH of 6.5 (0.1M sodium cacodylate). The cocrystals belong to space group C222₁ with cell dimensions of a = 180.02 Å, b = 200.24 Å, c = 55.72 Å and $\alpha=\beta=\gamma=90^\circ$. For data collection, the cocrystals were cryoprotected by soaks for 5 minutes in mother liquor solutions containing 5%, 10%, 15% and 20% glycerol (v/v), respectively, and then flash frozen in liquid nitrogen.

Structure determination

X-ray diffraction data on the cocrystals (2.5Å) were measured at the Advanced Photon Source (APS; beamline 17-ID) (Table 1). All data were indexed and integrated using DENZO and reduced using SCALEPACK⁴³. The structure was solved by molecular replacement with the program AMoRe⁴⁴ - using the structure of Rev1_{G,dCTP} complex as a search model³⁴. To prepare the search model, the atoms corresponding to the unpaired region in the duplex DNA, incoming nucleotide, water molecules and ions were removed.

The electron density map calculated after a round of refinement showed clear density for the abasic lesion. The abasic site coordinates were extracted from the Pol_I_{Abasic.dGTP} structure²³, and iterative rounds of refinement with CNS⁴⁵, model building with O⁴⁶ and water picking, lowered the R_{free} to 25.1%. The final model includes Rev1 residues 305–477 and 484–738 (there is no density for eight 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, two Mg²⁺ ions, and 151 water molecules. The model has excellent stereochemistry, as shown by RAMPAGE⁴⁷, with over 98% of the residues in the favored regions of the Ramachandran plot and only 0.3% in the disallowed regions. The Rev1_{Abasic.dCTP} structure was compared with that of Rev1_{G.dCTP} using O and the CONTACT program of the CCP4 suite⁴⁸. In order to find out the conformation of the furanose sugar of the templating nucleotide, pseudorotation phase angles were calculated with the help of the PROSIT server (<http://cactus.nci.nih.gov/prosit/>) for the Rev1_{G.dCTP} complex and manually using the equation provided by Altona and Sundaralingam⁴⁹ for the Rev1_{Abasic.dCTP} complex

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Figure 1. Rev1:DNA(Abasic):dCTP ternary complex. **(A)** Overall view of the complex with the palm, fingers, thumb domains and the PAD 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 in gray, template abasic site and incoming dCTP are in red, and the putative Mg^{2+} ions are displayed as blue spheres. Cyan dashed line indicates an unstructured loop in the palm domain. **(B)** A simulated annealing Fo-Fc omit map (contoured at 5.0σ ; $2.5\text{\AA}$ resolution), showing clear density for the abasic site lesion and for the incoming nucleotide dCTP.

Figure 2.

Abasic lesion and unmodified G in the Rev1 active site. (A) Close-up view of the Rev1 active site region with the abasic site and dCTP is shown. The N-digit, fingers and palm domains, and the PAD are shown in dark blue, yellow, cyan and green, respectively. The DNA is colored gray, template abasic lesion and incoming dCTP are shown in red, and the putative Mg^{2+} ions are dark blue. Highlighted and labeled are the catalytic residues (D362, D467 and E468), residues (Y405, R408 and K525) that interact with the triphosphate moiety of incoming dCTP, R324 that makes hydrogen bonds with the dCTP base, L325 that pushes the abasic lesion (a tetrahydrofuran moiety) out of the DNA helix, and residues that surround the template base from the N-digit (S423), fingers domain (W417), G-loop (K681, G684, M685 and G686) and PAD (N628 and W629). (B) Close-up view of some residues surrounding the abasic lesion in the $Rev1_{Abasic.dCTP}$ structure. The lesion is colored red, the interacting residues are colored according to element and the relevant water molecules are displayed as red spheres. Hydrogen bonds are shown as dotted lines. (C) Close-up view of some residues surrounding the unmodified G in the undamaged $Rev1_{dG.dCTP}$ structure (PDB

ID 2AQ4). The figure is drawn in the same orientation as figure 2B, with the templating G colored red, the interacting residues colored according to element, and the relevant water molecules displayed as red spheres. Hydrogen bonds are shown as dotted lines.

Figure 3. Comparison of Rev1 ternary complexes with damaged and undamaged DNAs. Superposition of the undamaged Rev1:DNA(dG):dCTP complex (red) and the damaged Rev1:DNA(abasic):dCTP complex (blue). The C α trace of the proteins is displayed in ribbon representation. A close-up view of the superposition of the unmodified G and abasic site to highlight the location and conformation of the sugar ring is shown in the inset box.

Figure 4.

Stereo surface representations of the hydrophobic cavity containing the abasic lesion (top) or the unmodified G (bottom) at the templating position. The surface is colored grey and DNA is shown in stick representation and the relevant water molecules are shown as red spheres. The figure shows that the space occupied by the guanine base in undamaged complex is now occupied by three ordered water molecules in the damaged abasic complex.

Table 1

Data collection and refinement statistics

	Rev1 _{Abasic,dCTP}
Data collection	
Space group	C222(1)
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	180.02, 200.24, 55.73
α , β , γ (°)	90.00, 90.00, 90.00
Resolution (Å)	2.50 (2.59–2.50) *
<i>R</i> _{sym} or <i>R</i> _{merge}	8.2 (28.0)
<i>I</i> / σ <i>I</i>	18.9 (3.6)
Completeness (%)	94.2 (61.8)
Redundancy	5.1 (3.3)
Refinement	
Resolution (Å)	50–2.5
No. reflections	33331
<i>R</i> _{work} / <i>R</i> _{free}	23.7/25.1
No. atoms	
Protein	3419
DNA	559
dCTP	28
Mg ²⁺ ion	2
Water	151
<i>B</i> -factors(Å ²)	
Protein	41.1
DNA	47.4
dCTP	29.6
Mg ²⁺ ion B	21.5
Mg ²⁺ ion A	53.8
Water	43.4
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.50

* One crystal was used to collect data.

* Highest resolution shell is shown in parentheses.