Bumps in the road: how replicative DNA polymerases see DNA damage

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Significant advances have been made recently in the study of polymerases. First came the realization that there are many more DNA polymerases than originally thought; indeed, no fewer than 14 template-dependent DNA polymerases are found in mammals. Concurrent structural studies of DNA polymerases bound to DNA and incoming nucleotide have revealed how these remarkable copying machines select the correct deoxynucleoside triphosphate among a sea of nucleotides. A whole new level of insight into DNA replication fidelity has been reached as a result of recently determined crystal structures of DNA lesions in the context of the active sites of repair, replicative and specialized DNA polymerases. These structures illustrate why some lesions can be bypassed readily, whereas others are strong blocks to DNA replication.

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Introduction

Based on sequence alignments, DNA polymerases have been divided into seven families: A, B, C, D, X, Y and reverse transcriptase (RT) [1,2]. Despite substantial structural diversity, all DNA polymerases contain a polymerase domain composed of three subdomains: fingers, palm and thumb [3], or N, C and D subdomains in DNA polymerase β (pol β) [4]. The palm comprises conserved acidic residues that ligate two metals important for catalysis [5]; residues of the fingers interact with the incoming nucleotide and the templating base; and the thumb contacts the minor groove of the duplex DNA. The DNA polymerases of the Y family have an additional subdomain termed little finger, wrist or PAD (for a review, see [6]). DNA polymerases copy DNA in a template-directed manner with an error rate that ranges from 10^0 to 10^{-6} ,

depending on the DNA polymerase and sequence context [7]. When a polymerase harbors intrinsic exonuclease activity, the proofreading activity accounts for an additional 10- to 100-fold reduction in the error rate. How do DNA polymerases select the correct nucleotide? Recent structural work has given credence to the concept of geometric selection [8,9]. Upon binding DNA and the correct incipient nucleotide, the fingers domain closes around the nucleotide. The resulting binding site is snug and appears tailored to readily accommodate only regular Watson–Crick base pairs [10,11]. Although numerous structures of polymerases from five different families (A, B, X, Y and RT) have been reported unliganded or in complex with DNA, nucleotide or both, the field has eagerly awaited structures of DNA lesions in the context of a polymerase active site. Our wishes have been granted in the past year, during which several structures of DNA polymerases bound to 8-oxo-7,8-dihydro-2'-deoxyguanosine (8oxoG), thymine dimers or abasic sites (Figure 1) were elucidated [4,12,13–17]. This review will focus primarily on replicative DNA polymerases of the A and B families, although discussion will include repair and specialized DNA polymerases of the X and Y families for comparative purposes.

Non-blocking mutagenic 8-oxoguanine evades proofreading

Reactive oxygen species are a major source of DNA damage. The oxidized purine 8-oxoguanine (8oxoG) is a prevalent lesion that is particularly pernicious because of its ability to pair with either dC or dA. In the latter case, if the mismatch is not repaired, it results in a G•C to T•A transversion. Crystal structures have been solved in the context of the nascent base pair (insertion site) formed by a templating 8oxoG opposite dCTP for pol β (family X [4]), bacteriophage T7 polymerase (A family [13]) and RB69 DNA polymerase (B family [16]). In addition, post-insertion complexes of a templating 8oxoG opposite dC or dA were obtained for *Bacillus stearothermophilus* DNA polymerase I (BF) (a member of the A family [14]) and bacteriophage T7 polymerase (Figures 2 and 3a). Functional studies have shown that the T7 and RB69 DNA polymerases incorporate dC preferentially opposite 8oxoG [4,13], whereas steady-state kinetic assays showed that BF incorporates dA more readily than dC opposite 8oxoG [14]. In all 8oxoG•dC complexes, the oxidized guanine adopts an *anti* conformation opposite dC (Figure 2). In order to prevent a steric clash between the C8 oxygen and the O4' of the deoxyribose, the template strand is distorted and pulls away from the polymerase active site, although the conforma-

Figure 1

Schematic diagrams of DNA lesions: (a) 8oxoG and an abasic site; (b) CPD.

tional changes are less pronounced and more localized in pol β . In the BF and T7 structures with 8oxoG•dA at the post-insertion site, 8oxoG adopts a *syn* conformation and engages in a Hoogsteen base pair with dA [13^{••},14^{••}]. The unusual base pairing is made possible by the oxidation of the guanine at C8, which turns N7 into a hydrogen bond donor. Remarkably, neither the polymerase active site residues nor the DNA itself are disrupted, which explains why 8oxoG•dA so readily evades proofreading (see below).

Minor groove interactions between conserved polymerase residues and the universal hydrogen bond acceptors O2 and N3 from the base pair situated at the primer 3' end (i.e. the post-insertion site) have been proposed to sense the presence of a mismatch [18,19]. The hydrogen bonding interactions between conserved palm residues (Arg615 and Gln797 in BF; Arg429 and Gln615 in T7) and the minor groove side of the 8oxoG•dA Hoogsteen pair at the post-insertion site are remarkably similar to those described for a normal Watson–Crick base pair. In the *syn* conformation, the O8 is precisely where O2 or N3

would be ordinarily (Figure 2b). 8oxoG•dA thus mimics a cognate base pair and so defeats the proofreading mechanism. Complexes with 8oxoG•dC at the post-insertion site and within the upstream duplex DNA show that there are local distortions of the sugar–phosphate backbone of the template strand, which may explain why mismatched 8oxoG•dA is more readily extended than the correct 8oxoG•dC.

How well do specialized polymerases bypass 8oxoG? DNA polymerase η , a polymerase that specializes in bypassing thymine dimers, preferentially incorporates dC compared to dA opposite 8oxoG [20]. How this specialized enzyme manages to bypass 8oxoG in an essentially error-free manner when high-fidelity polymerases of the A family stumble is a conundrum that should be resolved by the elucidation of the structure of DNA polymerase η bound to the oxidative DNA lesion.

Thymine dimers bring replicative polymerases to a screeching halt

Thymine dimers occur when DNA is exposed to UV radiation. The most common form, *cis-syn* cyclobutane pyrimidine dimer (CPD), is a strong block to replicative DNA polymerases. A series of structures of the high-fidelity T7 DNA polymerase in complex with CPD-containing DNA suggests that the first thymine of the CPD (3'T) is non-instructional, whereas the second base of the CPD (5'T) engages in base pairing with dATP (Figure 4a) [17]. Complexes with nucleotide inserted opposite 3'T or 5'T are referred to as CPD-3' or CPD-5', respectively. In CPD-3' complexes, the entire CPD is situated outside the polymerase active site, in the cleft located at the junction of the fingers and palm subdomains. The fingers subdomain is in an open conformation. In fact, in all three CPD-3' structures, which differ in the amount of ddATP soaked in the crystals, the fingers are open and their closure is hindered by the CPD. Two structures of CPD-5' have been elucidated, in open (CPD-5'a) and closed (CPD-5'b) forms, the latter requiring the addition of 10 mM ddATP to the crystal harvest buffer. In the closed form, the 5'T pairs with ddATP, although the resulting base pair exhibits a high propeller twist (50° versus 17° for a cognate A•T base pair [21]). With no added nucleotide, the DNA slides back and the CPD is pushed towards the major groove. In all T7 CPD structures, the distances from conserved residues Arg429 and Gln615 to the minor groove O2 and N3 are longer than those reported for a regular Watson–Crick base pair. The presence of the CPD lesion disrupts the interactions between the palm residues and the DNA, which probably results in destabilization of the DNA, leading to its switching to the proofreading site. It should be noted that, for obvious reasons, the polymerase used for co-crystallization experiments with the lesion is exonuclease deficient. Disabling the exonuclease activity increases

Figure 2

Structures of high-fidelity DNA polymerases in complex with 8oxoG. **(a)** Interactions between T7 DNA polymerase and the minor groove of the terminal primer base pair (at position $n-1$, the post-insertion site). From left to right: C•G base pair upstream of an 8oxoG•ddCTP pair at the insertion site (position n) (PDB code 1TK0); 8oxoG (*anti*)•dC pair (PDB code 1TKD); 8oxoG (*syn*)•dA Hoogsteen base pair (PDB code 1TK8) [13**]. **(b)** Interactions between BF and the minor groove of the terminal primer base pair. From left to right: 8oxoG (*anti*)•dC pair (PDB code 1U47); 8oxoG (*syn*)•dA Hoogsteen base pair (PDB code 1U49) [14**]. For comparison, a G•A mismatch is shown (PDB code 1NKO) [33**]. The red dashed lines represent hydrogen bonds. Below each base pair is a schematic drawing showing the locations of the base pair at the post-insertion site (yellow oval) and the lesion (green). In all figures, the primer strand is orange, the template is tan and the lesion is shown in green. The base or nucleotide opposite the lesion, where applicable, is shown in red. Figures 2–5 were made with PyMol (<http://pymol.sourceforge.net/>).

the efficiency of lesion bypass, by favoring partitioning to the polymerase active site. Increasing the nucleotide concentration is also known to shift the balance in favor of polymerization [7**].

In contrast to replicative DNA polymerases, Y-family polymerases can efficiently bypass thymine dimers. *Escherichia coli* DNA polymerase V incorporates A opposite a CPD 93% of the time *in vivo* [22]; *in vitro*, incorporation of A opposite both Ts is 50-fold more efficient than incorporation of G. This is in contrast to the rather small preference (twofold) for A over G opposite a site of base loss, suggesting that both thymines of the CPD are instructional [23]. Eukaryotic DNA polymerase η can replicate through a CPD as efficiently as through unda-

maged DNA [24] and, correspondingly, mutations in human DNA polymerase η cause a cancer-prone syndrome, the variant form of *Xeroderma pigmentosum* [25,26]. A recent crystal structure of Dpo4, an archaeal homolog of human DNA polymerase η , showed that both bases of the CPD fit in the open active site of the specialized polymerase [12*] (Figure 4b). The 3'T engages in a Watson–Crick base pair with dAMP at the 3' end of the primer. To base pair with the 5'T, ddATP adopts a *syn* conformation. The asymmetry of base pairing across the CPD is consistent with functional studies of DNA polymerase η and Dpo4, which showed that these polymerases incorporate A with lower fidelity opposite 3'T compared to 5'T [27]. Furthermore, a recent structure of DNA polymerase ι revealed that this Y-family polymerase replicates DNA

Figure 3

Minor groove interactions of RB69 DNA polymerase encountering DNA lesions. **(a)** Ternary complex of RB69 DNA polymerase attempting to insert dCTP opposite 8oxoG (PDB code 1Q9Y) [16**]. The geometry of the base pair is almost identical to that of the cognate base pair in the ternary complex with undamaged DNA [32]. **(b)** An open ternary complex of RB69 DNA polymerase attempting to insert dGTP (only dGMP is visible) opposite furan (PDB code 1Q9X) [16**]. The geometry of the DNA is also similar to that of the complex with normal DNA [32]. **(c)** A binary complex of RB69 DNA polymerase after successful incorporation of dAMP (the preferred nucleotide) opposite furan (PDB code 1RV2) [15**]. Distortions in the duplex DNA disrupt the minor groove interactions between protein and DNA, as well as impede translocation. For the sake of clarity, the phosphodiester backbone of the template strand is not shown.

via Hoogsteen base pairing, which might provide a mechanism to bypass modified purines with minor groove adducts [28*]. These structures have clearly revealed that Hoogsteen base pairing is part of the base-pairing repertoire used by polymerases that specialize in lesion bypass.

Abasic sites stop the polymerase dead in its tracks

Abasic sites, or sites of base loss, result from depurination or hydroxyl radical attack on deoxyribose. They are also intermediate species generated by the base excision repair pathway [29]. Estimates bring the number to 10 000 abasic sites per mammalian cell per day [30]. Replicative and repair DNA polymerases stall at sites of base loss. In the event of a bypass, which depends on the polymerase and sequence context, A is preferentially incorporated, followed by G and then, at much lower levels, C and T — a principle referred to as the ‘A rule’ [31**]. The order of incorporation preference, not unexpectedly, corresponds to the stacking ability of the four different bases and, possibly, to their solvation state [31**]. Two crystal structures of RB69 DNA polymerase were recently described, with either dGTP [16**] or incorporated dAMP [15**] opposite tetrahydrofuran (furan or F), a stable analog of an abasic site. In both cases, the fingers domains are open. In the ternary complex with an incoming dGTP nucleotide opposite furan, the DNA is undistorted and its minor groove geometry is similar to that seen in the 8oxoG•dC structure (Figure 3a,b) [16**] and, *a fortiori*, in the complex with undamaged DNA [32]. Allowing the polymerase to insert a nucleotide opposite furan by using an extendable pri-

mer resulted in a binary complex of A incorporated opposite a site of base loss (Figure 3c) [15**]. In this complex, the DNA duplex becomes distorted and pulls away from the active site, thus breaking all minor groove contacts. The DNA fails to translocate, in contrast to what is observed in mismatched structures of BF [33**]. The DNA distortions and failure to translocate explain why a site of base loss is a strong block to replicative DNA polymerases.

The crystal of the F•dAMP binary complex serendipitously yielded four unique structural conformations (two with DNA bound to the polymerase active site and two with DNA in the exonuclease active site) that appear to show the coordinated movement of protein domains as the primer strand of the DNA switches between the polymerization and proofreading active sites [15**]. A β hairpin of the editing domain swings downward towards the palm domain by about 30° and contacts the template strand as the primer strand switches between the polymerase and exonuclease active sites (Figure 5a,b). In the complex with DNA in the exonuclease active site, the β hairpin is disordered (Figure 5c), which might be necessary to allow the primer strand to switch between the two active sites. The β -hairpin movement observed in the crystal structure correlates well with a proposed role for this motif in strand separation and active site switching [34].

Unlike replicative DNA polymerases, Dpo4 replicates efficiently past a site of base loss. Recent crystal structures of Dpo4 bound to furan-containing DNA illustrate how the abasic site is bypassed [35*]. The lesion bulges out

Figure 4

Structures of high-fidelity replicative and lesion bypass DNA polymerases encountering a CPD. **(a)** Structures of T7 DNA polymerase encountering a *cis-syn* CPD [17^{**}]. In the CPD-3'a (nucleotide insertion opposite 3'T) binary complex, the CPD is pushed outside the active site and the fingers domain is in an open conformation (PDB code 1SKS). CPD-5'a and CPD-5'b are complexes with nucleotide insertion opposite the 5'T (PDB codes 1SL2 and 1SL1). A high concentration of nucleotide is required to stabilize the closed conformation of CPD-5'b, in which both Ts base pair with the 3'-terminal primer base and the incoming nucleotide. With no added nucleotide (CPD-5'a), the DNA slides back and the CPD is pushed towards the major groove of the DNA. For comparison, a ternary complex of a cognate T•ddATP base pair in the closed conformation is shown (PDB code 1SKR). **(b)** Structures of the Y-family polymerase Dpo4 encountering a CPD [12^{*}]. TT-1 is a ternary complex with an incoming ddATP opposite the 3'T of the CPD (PDB code 1RYR) and TT-2 is a ternary complex with an incoming ddATP opposite the 5'T of the CPD (PDB code 1RYS). Note the breadth and depth of the Dpo4 active site compared to that of T7. The surfaces shown comprise atoms within 10 Å of the DNA. For the sake of clarity, residues from the thumb domain are not shown. In all figures, the primer strand is orange, the template is tan and the lesion is shown in green. The base or nucleotide opposite the lesion, where applicable, is shown in red.

and the nucleotide base pairs in an instructional manner with the template base located 5' to the furan — the authors refer to this as the '5' rule'. The outcome will be a single base deletion if the bulge is conserved; alternatively, if the template realigns, the resulting product will have a base substitution. The ability of a Y polymerase to bypass an abasic site is not universal: Dbh, for example,

bypasses a site of base loss with low efficiency [36]. The little finger subdomain seems to play a part in the ability of a polymerase to bypass a lesion. Indeed, when the little finger subdomains of Dpo4 and Dbh are interchanged, the biochemical characteristics of Dbh become more Dpo4 like, in that it bypasses abasic sites more efficiently [36].

Figure 5

Active site switching in RB69 DNA polymerase. **(a)** Open ternary complex of unincorporated dGMP opposite an abasic site (PDB code 1Q9X) [16**]. **(b)** Open binary complex in which dAMP is incorporated opposite an abasic site (PDB code 1RV2) [15**]. Notably, the DNA did not translocate after phosphoryl transfer. The β hairpin (black) swings down towards the palm domain, as the primer strand heads towards the exonuclease active site. **(c)** Binary complex with the primer in the exonuclease active site [15**]. The single-stranded template containing the abasic site and the tip of the β hairpin are disordered. In all images, only the exonuclease and thumb domains are shown. The curved arrows illustrate the protein movements that accompany the primer switch from the polymerase to exonuclease active sites (ovals).

Conclusions

The recently determined crystal structures of DNA polymerases bound to DNA lesions have revealed why some lesions are readily bypassed whereas others are strong blocks. Generally, lesions that maintain minor groove interactions in high-fidelity polymerases are more likely to evade their proofreading mechanism. Much remains to be done concerning, for example, the asymmetry of the efficiency of incorporation, which differs depending on whether the lesion is the templating base or the nucleotide being incorporated [37]. Comparison of replicative DNA polymerases with specialized polymerases has shown how the latter make use of a more open active site, in combination with the ability to use non-Watson-Crick base pairing, to efficiently bypass lesions that stump their high-fidelity counterparts. The fact that these two types of polymerases handle lesions differently is not surprising, considering that specialized DNA polymerases are recruited to rescue stalled replication forks. The next challenge will be to elucidate the mechanisms that lead to the switch between a replicative and a specialized DNA polymerase when the former hits a speed bump on the replication highway.

Update

Crystal structures of replicative DNA polymerases bound to two classes of carcinogenic DNA adducts — N-(2'-deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF), its deacetylated derivative N-(2'-deoxyguanosin-8-yl)-2-aminofluorene (dG-AF) [38,39] and benzo[a]pyrene [40] — were recently described. Crystal structures of T7 DNA polymerase encountering a templating dG-AAF or dG-AF provide insight into why these very similar lesions

have strikingly different effects on replication [38]. The crystal structure of T7 DNA polymerase with dG-AAF in the templating position shows that the dG-AAF adduct adopts a *syn* conformation, which places the AAF moiety in a hydrophobic pocket of the fingers domain, thus locking the polymerase in an open, inactive conformation [38]. In contrast, the weak electron density for dG-AF suggests that the adduct can switch between the distorting *syn* and relatively unperturbed *anti* conformations, which may explain why dG-AF is more readily bypassed than dG-AAF. In addition, the BF complex with dG-AF revealed that, although dG-AF is in an *anti* conformation at the post-insertion site, where it base pairs with the cognate dC, there are distortions in the active site that result from the protrusion of the AF moiety into the major groove [39]. Interactions between the protein and the minor groove of the DNA are disrupted in a manner consistent with that discussed in this review and suggest why replicative DNA polymerases pause when they encounter a dG-AF adduct. In contrast to dG-AF and dG-AAF adducts, in benzo[a]pyrene adducts to dG ([BP]dG), the polycyclic aromatic moiety lies in the minor groove of the DNA. The crystal structure of BF bound to a templating [BP]dG at the post-insertion site revealed extensive disruption of the polymerase–DNA interactions, which explains why [BP]dG is such a strong block to replicative DNA polymerases [40].

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