Perspectives in Science (2015) 4, 24–31

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REVIEW

Structure and function of the translesion DNA polymerases and interactions with damaged DNA ☆

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Received 21 October 2013; accepted 4 December 2014
Available online 24 December 2014

KEYWORDS
DNA modification;
DNA polymerase;
Pre-steady-state kinetics;
Base-pairing

Abstract
Modification of DNA is a common event, due to reaction with both exogenous and endogenous factors. The resulting DNA adducts cause blockage of replicative DNA polymerases and also replication errors in cases in which the adducts can be bypassed. Translesion DNA polymerases exist in all forms of life and can replicate past bulky lesions, although with low fidelity. Our research has focused on the interactions of these polymerases with damaged DNA.
Pre-steady-state kinetic analysis has been used to develop minimum kinetic models with rate constants of (the eight) individual reaction steps in the catalytic cycle. The use of single-tryptophan mutants of *Sulfolobus solfataricus* Dpo4 and human (h) pol κ has led to discernment of the steps for the conformation change (associated with dNTP binding and relocation) and nucleotidyl transfer. X-ray crystal structures have been obtained for a number of the DNA adduct/DNA polymerase pairs in both binary and ternary complexes. Two isomeric etheno guanine adducts differ considerably in their interactions with DNA polymerases, explaining the base preferences. Further, even when several DNA polymerases cause the same mispairs with a single DNA adduct, the structural bases for this can differ considerably.

Contents

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http://dx.doi.org/10.1016/j.pisc.2014.12.005

Introduction

The high-fidelity replication of DNA is one of the main features of biochemistry. DNA polymerases do this efficiently in all types of organisms (Friedberg et al., 2006). However, problems exist because DNA is not a perfect collection of A, C, G, and T. The nucleophilic atoms in the bases are subject to attack by electrophiles (and free radicals) (Figure 1). Each cell of the human body has 50,000-100,000 damaged bases, and some examples (and their incidence) are shown in Figure 2. Damage can result from the ingestion of chemicals (e.g., pollutants, natural products) that damage DNA or from the contributions of natural cellular processes (e.g., generation of reactive oxygen species, aberrant methylation by S-adenosylmethionine, misinsertion of uracil followed by action of uracil DNA glycosylase) (Friedberg et al., 2006).

DNA polymerases have difficulties in dealing with many of these lesions. With many, normal Watson-Crick base pairing is not possible and alternate pairing schemes are used (Figure 3) (Saenger, 1984). Some of these are even used in non-damaged, canonical DNA. Several problematic outcomes for the encounter of DNA polymerases with modified DNA are blockage (resulting in cell death), miscoding, and frameshifting due to "skipping" a base (Friedberg et al., 2006).

Our laboratory has been studying the interaction of DNA polymerases with carcinogen-modified DNA (Eoff et al., 2010a, 2010b; Guengerich et al., 2011). Among the approaches used are steady-state and pre-steady-state kinetic analysis of individual catalytic steps that change because of damage, X-ray crystallography, and mass spectrometry. Early work in this laboratory utilised bacteriophage T7 (exonuclease minus) and HIV reverse transcriptase (Choi and Guengerich, 2004; Furge, and Guengerich, 1997; Zang et al., 2005b) but more recent studies have focused on

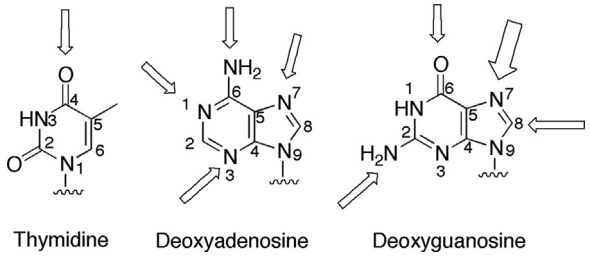

Figure 1 Prominent sites of modification of DNA bases. The N7 atom of deoxyguanine is the most nucleophilic site.

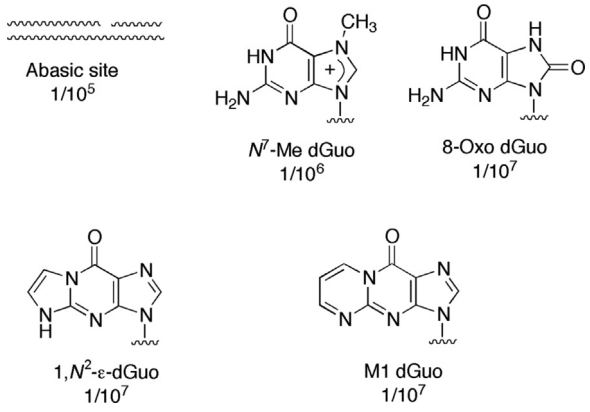

Figure 2 Some common DNA lesions and their abundance in DNA cells of humans and experimental animals (adducts/number normal bases) (Billson et al., 2009; Chastain et al., 2010; Morinello et al., 2001; Rouzer et al., 1997).

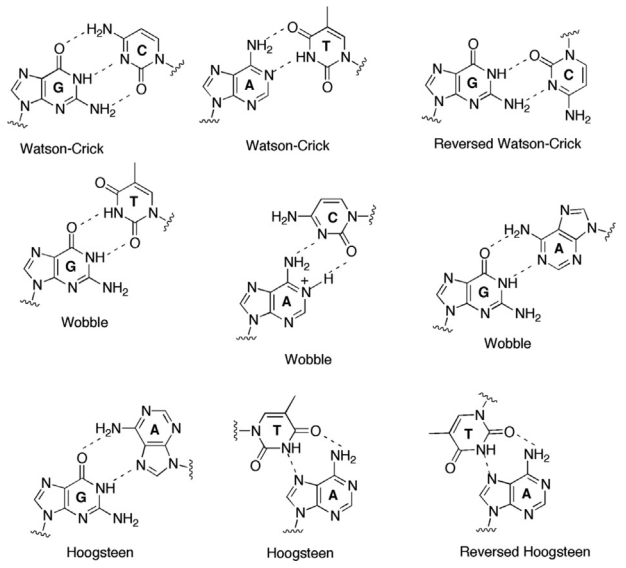

Figure 3 Some modes of base pairing in DNA (Saenger, 1984). Several of these modes (e.g., Hoogsteen) have been reported to occur in canonical double-stranded DNA (Nikolova et al., 2011).

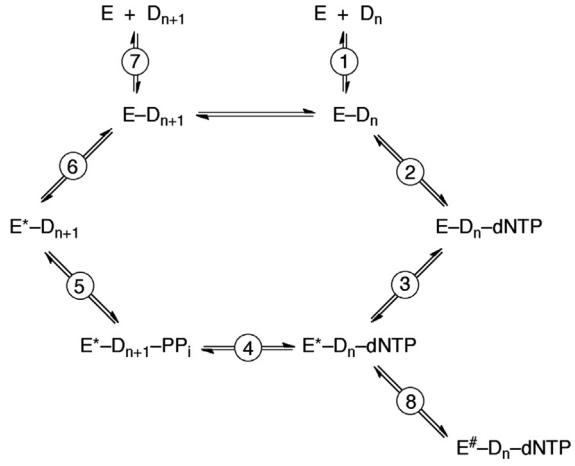

Figure 4 Classic DNA polymerase catalytic cycle (Kuchta et al., 1987; Mizrahi et al., 1985).

the so-called translesion DNA polymerases, which are able to replace the normal "replicative" DNA polymerases in bypassing damage, often remaining on the DNA to replicate for several steps (Eoff et al., 2010a, 2010b; Guengerich et al., 2011). Among these translesion DNA polymerases are *S. solfataricus* Dpo4 and human (h) DNA polymerase (pol) η, ι, and κ and REV1, all of which are in the so-called Y-Family of DNA polymerases.

Pre-steady-state kinetics

A general paradigm for DNA polymerase activity is shown in Figure 4, where E denotes the polymerase, D the DNA (actually an oligonucleotide), and N a nucleoside triphosphate (dNTP). Experimental settings usually begin with an E-D complex, which is mixed rapidly with N (dNTP). DNA polymerases must recognise all four of the natural dNTPs and then quickly position the proper one opposite its complementary base. The thermodynamic differences between correct *vs.* incorrect base pairing are not sufficient to account for the high fidelity of DNA replication, and there is extensive evidence that DNA polymerases adjust through conformational changes to increase the fidelity through an induced-fit mechanism (Johnson, 1993, 2008). The conformational change (to E*) is followed by nucleotidyl transfer (phosphodiester bond formation). Pyrophosphate must be released from the enzyme and, because a conformational change occurred, it must be reversed to complete the catalytic cycle. Finally, either the oligonucleotide is released (and a new one binds), or the DNA polymerase relocates to the next position to begin a new cycle.

We and others have provided evidence that unproductive E-D-N complexes can be formed (Eoff et al., 2007a; Furge and Guengerich, 1999; Suo and Johnson, 1998) and exist in equilibrium with productive complexes. Except in cases of strong blockage, burst kinetics are observed in DNA polymerase reactions, i.e., rate-limiting steps follow product formation, and therefore pre-steady-state kinetic analysis is necessary. Some of the evidence for unproductive complexes is the existence of partial bursts with modified oligonucleotides (Furge and Guengerich, 1999) or even non-canonical structures of unmodified DNA (Suo and Johnson, 1998).

One of the difficulties in the kinetic analysis is discerning step 3 from 4 (Figure 4). With the *S. solfataricus* translesion DNA polymerase Dpo4, which is devoid of Trp, we added Trp residues at several locations and found two mutants that were useful in reporting what we interpret to be conformational changes (Figure 5) (Beckman et al., 2008). The rapid first change was dNTP concentration-dependent and was not seen when nucleotidyl transfer was blocked (e.g., with the use of a 3'-dideoxy-terminated primer strand). The slower second phase, in the opposite direction, was slower and independent of the dNTP concentration. We have interpreted these fluorescence changes to be associated with steps 3 and 6 of Figure 4 (Beckman et al., 2008).

Subsequent studies with a series of N^2-alkyl guanine substituted oligonucleotides showed that bulk affected step 4 up to the size of naphthyl(methylene) and that with larger residues step 3 was attenuated (Zhang and Guengerich, 2010). Another use of the system was in demonstrating that frameshift

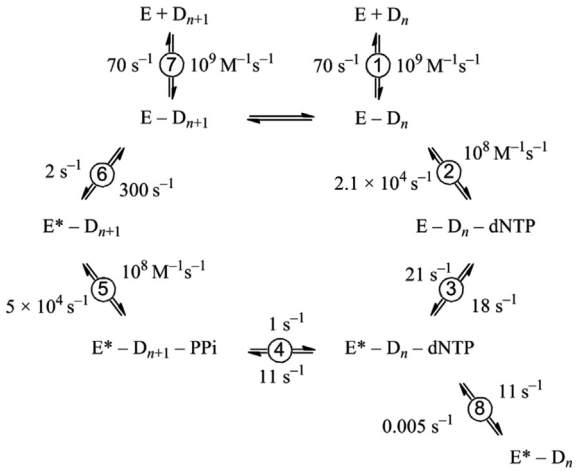

Figure 5 Catalytic cycle for Dpo4 (normal incorporation of dCTP opposite G) minimal mechanism has rate constants compatible with several sets of kinetic data (Beckman et al., 2008). This research was originally published in (Beckman et al. (2008). ©The American Society for Biochemistry and Molecular Biology.

insertion by Dpo4 occurs by initial base insertion followed by slippage/readjustment of the oligonucleotide, rather than vice-versa (Zhang et al., 2009).

Although step 6 (pyrophosphate (PPi) release) has often been shown after step 7 (conformational relaxation) in many schemes, including our own (e.g., Kuchta et al., 1987; Furge and Guengerich, 1999; Tsai and Johnson, 2006) but not all (Mizrahi et al., 1985), evidence now clearly indicates that PPi release precedes the conformational step with Dpo4 (Beckman et al., 2008). A phosphate binding protein (modified with a coumarin) was used to detect PPi (cleaved with an excess of pyrophosphatase (Hanes and Johnson, 2008)). When this was done, PPi release was as fast as product formation, within error (following mixing of E·D and N in Figure 4). Fitting of the data to a scheme yielded the rate constants shown in Figure 5 (Beckman et al., 2008).

hpol κ is a complex translesion polymerase, but the catalytic core is fully active. The two Trp residues in the catalytic core could be removed without major loss of catalytic activity or selectivity, and we used a previously determined crystal structure of hpol κ (Irimia et al., 2009) to guide selection of placement of Trp residues in the Trp-free sequence (Zhao et al., 2014). Two of these substitutions, Y50W and T408W, provided useful fluorescent changes (Figure 6).

The fluorescence changes were in the opposite direction of those observed with Dpo4 (Beckman et al., 2008) and only ~1/2 as intense but could be utilised in a similar way. As with Dpo4, the first fluorescence change is step 3 (Figure 4) and the slower reverse step is step 6. As with Dpo4, the kinetics measured with the phosphate binding protein showed that PPi release is fast, and step 5 is placed before step 6. Kinetic modelling (KinTek Explorer®) was used to obtain a minimal kinetic model with the rate constants shown in Figure 7. Further studies can be done to analyse the effects of individual DNA adducts on the rates of steps 3 and 4.

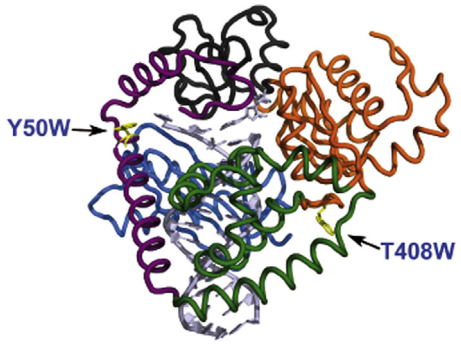

Figure 6 Ribbon diagram of hpol κ (Irimia et al., 2009). The positions of two site-directed mutants used for fluorescence kinetic analysis is shown (Zhao et al., 2014).

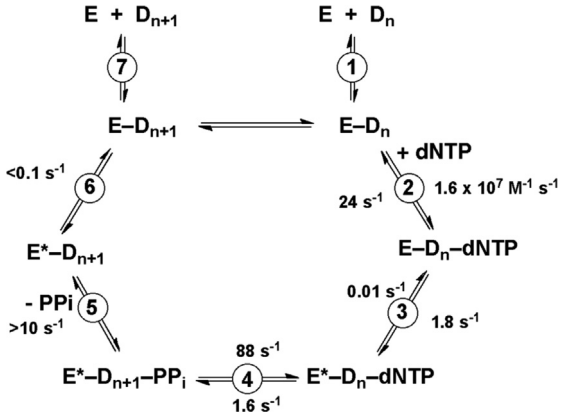

Figure 7 Catalytic cycle for hpol κ (normal incorporation of dCTP opposite G). A minimal mechanism is shown with rate constants consistent with several sets of kinetic data.

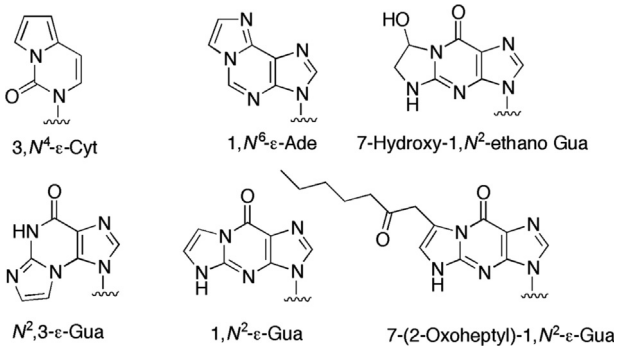

Figure 8 Some etheno (ε) adducts found in DNA (Guengerich et al., 1993; Leonard and Barrio, 1984).

Structural studies with modified DNA

Kinetic studies can implicate conformational changes but cannot reveal actual structures. Our first attempts to obtain diffractable crystals of ternary complexes (E-D-N) with HIV-1 reverse transcriptase were unsuccessful with the DNA adduct 7,8-dihydro-8-oxoG (8-oxoG) and we termed our attention to Dpo4.

The so-called "etheno" (ε) adducts (Figure 8) are of interest because they arise from reaction with oxidation products of the human carcinogen vinyl chloride (Barbin et al., 1975; Guengerich et al., 1993). Gel electrophoresis assays had indicated complex behaviour of dNTP insertion opposite 1,N^2-etheno-G (1,N^2-ε-G) by Dpo4 (Zang et al., 2005a). We developed an HPLC-mass spectrometry (LC-MS) approach for the sequence analysis of extended oligonucleotide primer products, which revealed the presence of four products, two resulting from A incorporation and frameshifting, which could not be detected in the gel assays (Zang et al., 2005a). The crystal structure was "Type II" (Ling et al., 2004), with the polymerase bypassing the lesion with little distortion of either the DNA or the enzyme (Figure 9).

We were also interested in the isomeric DNA adduct N^2,3-ε-G, which is formed at higher concentrations than 1,N^2-ε-G in DNA (Müller et al., 1997). However, only very limited biochemical studies had been done with this base because of its sensitivity to non-enzymatic deglycosylation. We were able to address this problem by using a 2'-fluoro isostere, taking advantage of the electronegativity of the fluorine to destabilise the transition state leading to the oxocarbenium ion intermediate involved in the cleavage. The synthetic oligonucleotide had a $t_{1/2}$ of three weeks at 37 °C (Zhao et al., 2012a). Misincorporation opposite 1,N^2-ε-G and (2'-F) N^2,3-ε-G by several DNA polymerases are shown in Table 1 (Boosalis et al., 1987; Choi et al., 2006; Langouët et al., 1998). N^2,3-ε-G has a tendency to insert C or T in all cases, whereas 1,N^2-ε-G generally leads to insertion of C or A or a-1 frameshift (Zhao et al., 2012a).

Although Dpo4 and hpol ι show similar coding patterns with N^2,3-ε-G (Table 1), the structural basis appears to be very different. Dpo4 positions dCTP (correct base opposite N^2,3-ε-G using pseudo-Watson-Crick pairing) and positions dTTP opposite N^2,3-ε-G using a "sheared" pairing (Figure 10) (Zhao et al., 2012a). hpol ι positions both dCTP and dTTP opposite N^2,3-ε-G using Hoogsteen pairing, but with the correct base (dCTP) forming two hydrogen bonds instead of only one (dTTP) (Figure 11) (Zhao et al., 2012b).

Structural considerations regarding multiplicity of mechanisms with other DNA lesions

We have experience with structures of a number of DNA lesions with translesion DNA polymerases, but the work with 8-oxoG (Figure 2) and O^6-methylG (O^6-MeG) is most relevant to the present consideration of multiple pairing schemes (Table 2).

With 8-oxoG, DNA polymerases insert C or A. The selectivity appears to be due largely to an *anti* (C) *vs. syn* (A) arrangement of the 8-oxoG base with the deoxyribose sugar moiety (Kouchakdjian et al., 1991; Leonard et al., 1992). With Dpo4, both structures (for pairing C and A) have been established (Zang et al., 2006) and at least part of the basis appears to a H-bond of the 8-oxoG O atom with Arg-332, as subsequently indicated by site-directed mutagenesis studies (Eoff et al., 2007b). hpol κ has a Leu residue in the corresponding position near 8-oxoG and accordingly does not stabilise the *anti*-configuration (Irimia et al., 2009). Site-directed mutagenesis of the Leu (-508) to a Lys group improved the fidelity, in line with our hypothesis. However,

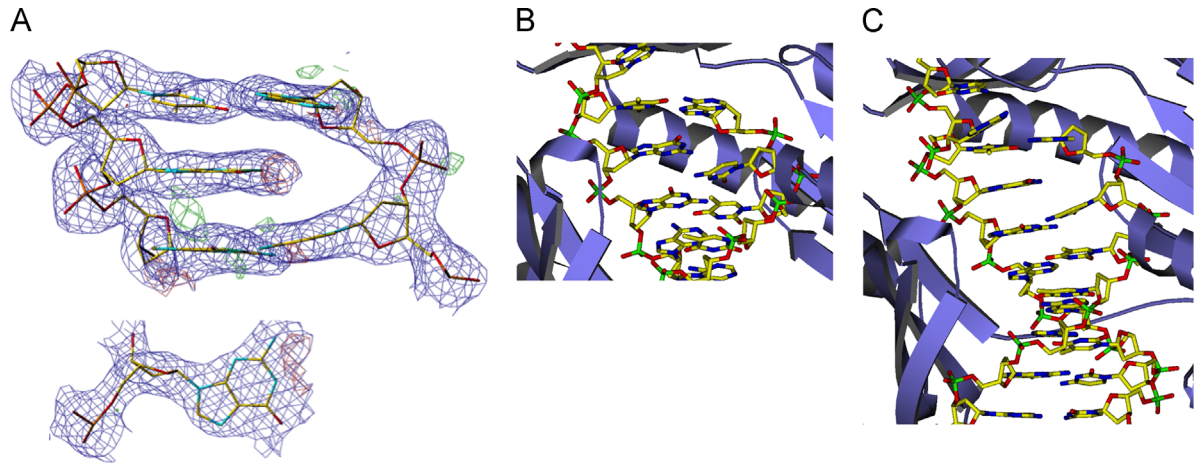

Figure 9 Base pairing region of Dpo4 copying past 1,N^2-ε-guanine (in the crystal structure). The "skip" past the adduct (a "Type II" structure (Ling et al., 2004)) is associated with a frameshift deletion (Zang et al., 2005a). PDB codes 2bq3, 2bqr, 2bqu, 2br0.

Table 1 Misincorporation frequencies (*f*) of DNA polymerases with two ε adducts.[a]

DNA polymerase	Template[b]	Template base: dNTP	k_{cat}/K_m[c]	*f*[c]	Template[a]	dNTP/template base	k_{cat}/K_m[b]	*f*[d]
E. coli pol I (Klenow fragment exō)	3'-GTA	N^2,3-εG:C	0.23	1.0	3'-GTG	1,N^2-εG:C	0.0081	1.0
		N^2,3-εG:T	0.24	1.0		1,N^2-εG:G	0.0087	1.1
						1,N^2-εG:A	0.0016	1.2
Dpo4	3'-GTA	N^2,3-εG:C	0.025	1.0	3'-GTA	1,N^2-εG:C	0.00006	1.0
		N^2,3-εG:T	0.0054	0.22		1,N^2-εG:A (also prominent -1,-2 frameshifts)	0.0008	14
hpol κ	3'-GTA	N^2,3-εG:C	0.022	1.0	3'-GTA	1,N^2-εG:C	0.0012	1.0
		N^2,3-εG:T	0.0081	0.37		1,N^2-εG:A	0.0012	1.0
hpol ι	3'-GTA	N^2,3-εG:C	0.0017	1.0	3'-GTA	1,N^2-εG:C	0.017	1.0
		N^2,3-εG:T	0.0012	0.71		1,N^2-εG:A	0.016	0.96
hpol η	3'-GTA	N^2,3-εG:C	0.08	1.0	3'-GTA	1,N^2-εG:C	0.072	1.0
		N^2,3-εG:T	0.05	0.63		1,N^2-εG:A	0.23	3.3
						1,N^2-εG:G	0.36	5.0

[a] N^2,3-εG results from Zhao et al. (2012a, 2012b) (using 2'-F isostere). 1,N^2-εG results from Choi et al. (2006) and Langouët et al. (1998).

[b] Only the region of template involved in pairing is shown. The underlined base is the site of dNTP pairing/incorporation (substituted with εG).

[c] Expressed as $min^{-1}\ \mu M^{-1}$ (based on dNTP).

[d] $f = (k_{cat}/K_m)_{dNTP}/(k_{cat}/K_m)_{dCTP}$, where *N* is the incorrect base (Boosalis et al., 1987).

we have recently obtained structures of 8-oxoG-hpol η complexes and, contrary to our earlier prediction (Irimia et al., 2009), determined that the C>A insertion preference and *anti*-configuration (of the base and sugar) are imposed not by an H-bond with an amino acid but by steric restriction due to Arg-61 (Patra et al., 2014).

With O^6-MeG, different DNA polymerases also achieve the same effect *via* different pairing mechanisms (Table 2). Warren et al. (2006) studied *Bacillus stearothermophilus* BF (the major replicative DNA polymerase of that organism) and found a strong preference for misincorporation of T>C. The T pairing was explained by Watson-Crick pairing of a rare tautomer, and the C pairing was attributed to an unusual -OCH_3 H-bond (Warren et al., 2006). In our work with Dpo4 (Eoff et al., 2007c), pairing of C>T was preferred, with C pairing due to a Wobble pair (and T to pseudo-Watson-Crick pairing). With hpol ι, Hoogsteen pairing is observed with both C and T but the former is favoured due to a bifurcated H-bond (Pence et al., 2010).

In conclusion, it is difficult to predict non-canonical base pairing either (i) *a priori*, just drawing reasonable pairing structures, or (ii) analysing NMR structures of paired oligonucleotides. Different polymerases can use different mechanisms to achieve the same net result. Again, we see the power of enzymes to drive similar reactions in their own specific ways.

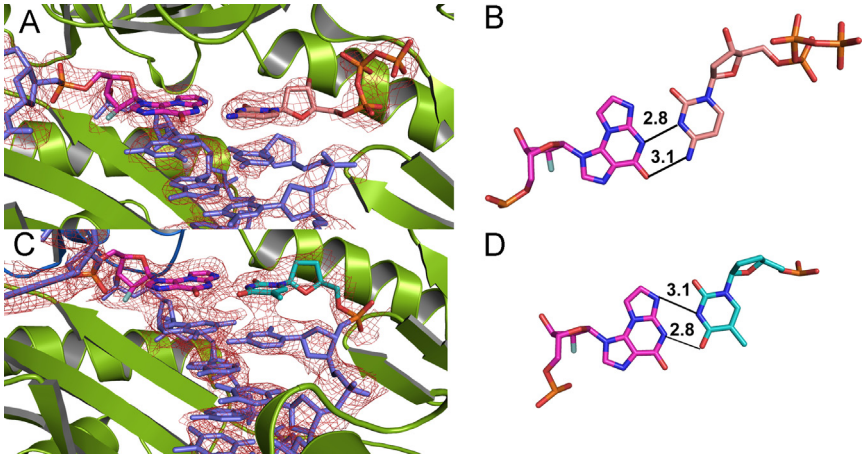

Figure 10 Base pairing region of Dpo4 copying past (2'-F) N^2,3-ε-guanine (in the crystal structure). Both the (A) dCTP and (B) dTTP structures are shown (Zhao et al., 2012a). PDB codes 3V6H, 3V6J, 3V6K.

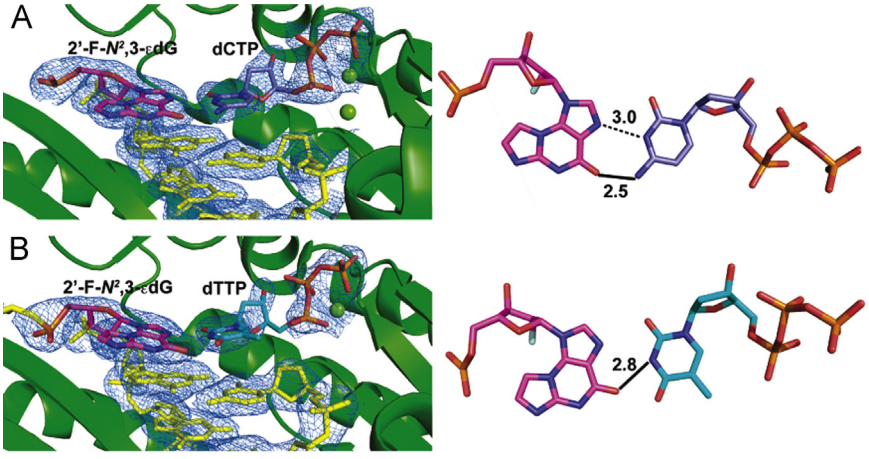

Figure 11 Base pairing region of hpol ι copying past (2'-F) N^2,3-ε-guanine (in the crystal structure). Both the (A) dCTP and (B) dTTP structures are shown (Zhao et al., 2012b). PDB codes 4FS1, 4FS2. This research was originally published in Zhao et al. (2012b). ©The American Society for Biochemistry and Molecular Biology.

Table 2 Comparison of coding opposite two DNA G adducts by different DNA polymerases.

Adduct	Insertion preference	DNA polymerase	Basis
8-oxoG	C > A	Dpo4	*Anti* configuration of base and sugar give current pairing; Arg-332H-bonded to O8 atom of 8-oxoG to maintain *anti* (*vs. syn*) configuration (Eoff et al., 2007b; Zang et al., 2006)
	A > C	hpol κ	Leu-508 at site of Arg-332, fails to H-bond to maintain *anti* configuration; L508K shifts in favour of C (Irimia et al., 2009)
	C ≈ A	hpol η	Arg-61 forces *anti* configuration *via* steric restriction (Patra et al., 2014)
O^6-MeG	T > C	*B. stearothermophilus* pol I (BF)	T-rare tautomer stabilisation *vs.* -OCH_3 H-bond (Warren et al., 2006)
	C > T	Dpo4	Wobble *vs.* pseudo Watson-Crick pairing (Eoff et al., 2007c)
	C ≈ T	hpol η	(in progress)

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This research was supported in part by United States Public Health Services Grants R01 ES010375, R01 ES010546, and P30 ES000267. Thanks are extended to Kathleen Trisler for her assistance in preparation of the manuscript, to Prof. Carmelo J. Rizzo (Vanderbilt) for collaborative efforts in this research, and to a series of graduate students and post-doctoral fellows who have worked in this area, including Mi-Sook Kim, Michael Müller, Sophie Langouët, Laura L. Furge, Heidi J. Einolf, Adrienne M. Woodside, Hong Zang, Jeong-Yun Choi, Robert L. Eoff, Huidong Zhang, Jeffrey W. Beckman, Livia Müller, Qianqian Zhang, and Yan Su.

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