Chapter 2
Disturbances of the DNA Replication System

Abstract DNA damage leads to mutation, and bulky DNA damage blocks DNA replication. DNA damage also increases the misincorporation ratio by reducing the correct incorporation efficiency, by increasing the misincorporation efficiency, or by both. DNA damage can also produce frameshifts. DNA–DNA cross-linking can destroy DNA replication.

Keywords Misincorporation ratio · Incorporation efficiency · Blockage of DNA replication · Frameshift · Cross-linking

The molecular mechanisms of mutation caused by DNA damage are still poorly analyzed. Some recent reviews have described various DNA damage, individual DNA polymerase, and information about how these polymerases bypass DNA damage. Herein, based on our own work and recent progress in this area, we analyze these data and proposed four major pathways by which DNA damage leads to mutation: blocking DNA replication, increasing misincorporation ratio, producing frameshifts, and destroying DNA replication by cross-linking.

2.1 DNA Damage Blocks DNA Replication

DNA damage blocks DNA replication. The blockage means that the incoming dNTP cannot form the phosphodiester bond with the 3′-OH at the primer end. At least three reasons can explain these possibilities. Firstly, the incoming dNTP is repulsed outside of the active site and is far away from 3′-OH at the primer end, such as in the case of N^2,N^2-diMeG. Secondly, the incoming dNTP is accommodated at the active site but paired with the template base via nonstandard W-C pairing modes, such as in the case of $N^{2,3}$-εG. Thirdly, the DNA adduct is too bulky to be accommodated and/or disordered in the active site, e.g., PAH-DNA adducts, CPD, DNA-protein cross-link adducts, and AAF. All of these configurations lead to the blockage of DNA replication.

H. Zhang, *DNA Replication - Damage from Environmental Carcinogens*,
SpringerBriefs in Biochemistry and Molecular Biology 17,
DOI 10.1007/978-94-017-7212-9_2

Repulsion of dNTP outside the active site: T7 DNA polymerase, HIV reverse transcriptase, pol κ, pol ι, pol η, and Dpo4 were strongly blocked by N^2,N^2-dialkylG. The efficiency of dCTP incorporation against N^2,N^2-dialkylG was decreased 160,000-fold compared with unmodified G for Dpo4, blocking DNA replication. No obvious fast conformational change was observed, indicating that N^2,N^2-Me_2G strongly perturbs the conformational change. Nearly no burst was observed for the incorporation of dCTP opposite N^2,N^2-Me_2G, indicating less than 5 % active polymerase–DNA–dCTP ternary complex. DNA replication is strongly blocked upon encountering this lesion. The crystal structures showed that the 3′-terminal dideoxycytosine of the primer that should pair with the template N^2,N^2-Me_2G is repulsed outside of the active site and folded back into the minor groove, as a catalytically incompetent complex, explaining the blockage of dNTP incorporation.

Nonstandard W-C paring mode: Lipid peroxidation or oxidation products of vinyl monomers can produce exocyclic adduct N^2,3-εG, which blocks human DNA pol ι and REV1, yielding only 1-base incorporation opposite this lesion. Structurally, pol ι can accommodate an N^2,3-εG:dCTP base pair rather well at the active site without significant conformational changes of protein or nucleic acid, but the phosphate group of the incoming dCTP and primer terminus 3′-OH is misaligned. Two hydrogen bonds were observed in the N^2,3-εG:dCTP base pair, whereas only one appears in the N^2,3-εG:dTTP pair.

Bulkier adducts disordering active site: The reactive 7,8-diol-9,10-epoxides of benzo[a]pyrene form N^2-B[a]P guanine and N^6-B[a]P adenine adducts, which strongly block dNTP incorporation. dATP is preferentially incorporated by T7 DNA polymerase, but the catalytic efficiency is decreased by at least four orders of magnitude. No fast burst phases were observed for dNTP incorporation opposite all of these lesions, indicating that the rate-limiting step is at or before phosphodiester bond formation. Dpo4 gave the same results as T7 DNA polymerase in bypassing N^6-B[a]P A. The incorporation is blocked and dATP was preferably inserted. Crystal structure of Dpo4 containing this lesion shows that polymerization of incoming dNTP with the 3′-OH at the primer end is inhibited because both of which are separated beyond for a chemical reaction. Mouse pol κ can bypass N^2-B[a]P G efficiently and accurately, but a mutant with the reduced gap size was strongly blocked by this lesion, suggesting that the presence of this gap is essential for the DNA adduct bypass. Structurally, the gap physically accommodates the bulky aromatic adduct and keeps the active site ordered, explaining crucial functions of the gap in pol κ in maintenance of the active site for translesion DNA synthesis.

Human pol κ can insert dATP opposite the 5′-T of a cis-syn T-T dimer but cannot insert nucleotides opposite the 3′-T of the dimer, leading to the blockage of DNA replication. Structurally, the active site of pol κ can only accommodate the incoming dATP with the 5′-T of the T-T dimmer, leaving the 3′-T misaligned at active site and blocking DNA replication. Single-molecule fluorescence resonance energy transfer (smFRET) and protein-induced fluorescence enhancement (smPIFE) experiments show that *Escherichia coli* DNA polymerase I (Klenow fragment) binds to AAF-dG in an intermediate orientation, which is very unstable, and then rapidly transfers DNA from the active site to a more stable exonuclease site, thus blocking DNA replication.

2.2 DNA Damage Increases Misincorporation Ratio

Misincorporation ratio is dependent on the relative efficiency of correct dNTP incorporation relative to misincorporation. Correct incorporation is much faster than incorrect ones for most DNA polymerases. In the presence of DNA adducts, the incorporation efficiency may decrease and/or the incorrect incorporation efficiency may increase, increasing misincorporation ratio.

Decreasing correct incorporation efficiency: Some DNA adducts reduce only correct incorporation efficiency but do not affect the misincorporation, for example, N^2-alkylG and O^6-alkylG adducts.

The bypass of N^2-alkylG adducts has been studied by *Sulfolobus solfataricus* Dpo4. Dpo4 preferentially incorporates dCTP opposite to these adducts, but incorporation efficiencies (k_{cat}/K_m) are decreased 3- to 125-fold compared with the unmodified G. The misincorporation efficiencies remain almost unchanged, thus reducing incorporation fidelities by 100-fold. Some bulkier N^2-alkylG also strongly perturbs the fast conformation change. X-ray crystal structures show that the incoming dCTP is severely buckled and locates outside the active site if N^2-NaphG residue is in the *trans*-form, indicating a nonproductive complex; dCTP can also pair with the *cis*-form of the N^2-NaphG residue via a Hoogsteen mode to continue DNA polymerization. For N^2,N^2-Me_2G, the dCTP incorporation efficiency (k_{cat}/K_m) was drastically decreased by 16,000-fold compared with G, but the misincorporation frequencies are almost unchanged, increasing the misincorporation ratios up to 0.36–2.3. N^2,N^2-Me_2G leads to a random misincorporation and completely destroying the incorporation fidelity.

O^6-MeG and O^6-BzG were also studied by Dpo4. Compared with unmodified G, incorporation of dCTP opposite O^6-MeG is inhibited by three orders of magnitude, but the misincorporation efficiencies are unchanged, thus reducing the incorporation fidelity by three orders of magnitude compared with unmodified G. Finally, about 70 % of dCTP, 20 % of dTTP, and 10 % of dATP were incorporated opposite O^6-MeG. Bypass of O^6-BzG is similar to O^6-MeG except for a greater decrease in incorporation efficiency. The dCTP incorporation efficiency is strongly inhibited by 5000-fold for O^6-BzG compared with G while the misincorporation efficiencies are reduced only 10-fold, finally decreasing the incorporation fidelity about three orders compared with unmodified G. Structurally, O^6-MeG:C or O^6-BzG:C formed a wobble base pair with two hydrogen bonds between O^6-alkylG lesions and dCTP, shifting the base-pairing orientation from the standard W-C mode to the wobble mode, thus decreasing the correct incorporation efficiency.

Increasing incorrect incorporation efficiency: Some DNA adducts only partially affect the correct incorporation efficiency but drastically increase the misincorporation efficiency, thus reducing incorporation fidelity, for example, 8-oxoG.

The efficiency of incorporation of dCTP opposite 8-oxoG is decreased 80-fold compared with unmodified G for human DNA polymerase κ (hpol κ), but dATP misincorporation efficiency is increased 100-fold compared with unmodified G. Therefore, the incorporation fidelity was decreased 8000-fold for 8-oxoG compared with unmodified G, leading to a conversion from G:C pairing to T:A

pairing. In the pre-steady-state kinetic analysis, the efficiency ($k_{pol}/K_{d,dCTP}$) for the insertion of dCTP opposite 8-oxoG was decreased 65-fold compared with G, but dATP incorporation showed a fast burst, different from the absence of burst for the incorporation of dATP opposite G. The catalytic efficiency ($k_{pol}/K_{d,dCTP}$) for insertion of dATP opposite 8-oxoG is increased up to 0.63 s^{-1} μM^{-1}, 20-fold higher than that for incorporation of dCTP opposite 8-oxoG. From structures, the N-terminal extension of hpol κ stabilizes its little finger domain, which surrounds the Hoogsteen base pair of 8-oxoG and incoming dATP, explaining the increase in efficiency for the incorporation of dATP opposite 8-oxoG.

For Dpo4, the insertion of dCTP opposite G or 8-oxoG has similar catalytic efficiency, whereas the efficiency for incorporation of dATP opposite 8-oxoG was increased 250-fold compared with G, thus decreasing fidelity 200-fold opposite 8-oxoG relative to unmodified G. Crystal structures show that the Arg-332 residue in the little finger domain of Dpo4 can form a hydrogen bond with the oxygen atom at the C8 position of 8-oxoG and stabilizes the Watson–Crick base pair of incoming dCTP and 8-oxoG. Notably, no stabilization function is observed for W-C pairing of 8-oxoG and dCTP at the active site of hpol κ. The Hoogsteen pairing of 8-oxoG:dATP is favorable relative to 8-oxoG:dCTP pair in hpol κ, leading to structurally different bypass of 8-oxoG by Dpo4 and hpol κ.

2.3 DNA Damage Forms Frameshift During DNA Replication

DNA damage leads to frameshifts by adding or subtracting nucleotides in the newly synthesized DNA strand, disturbing DNA sequence, genetic information, RNA transcription, and protein expression. -1 frameshift means one nucleotide is missing after DNA replication. Four different mechanisms have been proposed to explain how -1 deletion frameshifts are produced (Fig. 2.1).

Streisinger template-slippage mechanism: -1 frameshift produced in the replication of repetitive DNA sequences can be explained by Streisinger template-slippage mechanism. The primer misaligns on a repetitive template DNA strand, forming an unpaired template base in the newly synthesized DNA, that is, one base shorter than the original template. Y-family DNA polymerase Dbh generates -1 frameshift in repetitive sequences with error frequencies up to 50 %. In the crystal structure of Dbh, a cleft between the polymerase domain and the C-terminal domain provides ample space to accommodate extrahelical template bases. Tyr-249 and Arg-333 in the C-terminal domain stabilize the extrahelical base at the -3 position of template, and residues in the flexible loop of Dbh interact with the bulged base at the -2 position of template. Therefore, Dbh does not appear to strictly regulate the entry of template bases into the active site, allowing template misalignment to occur more readily.

Misincorporation–misalignment mechanism: dNTP is firstly misincorporated and then the template misaligns so that dNTP can pair with the next template base

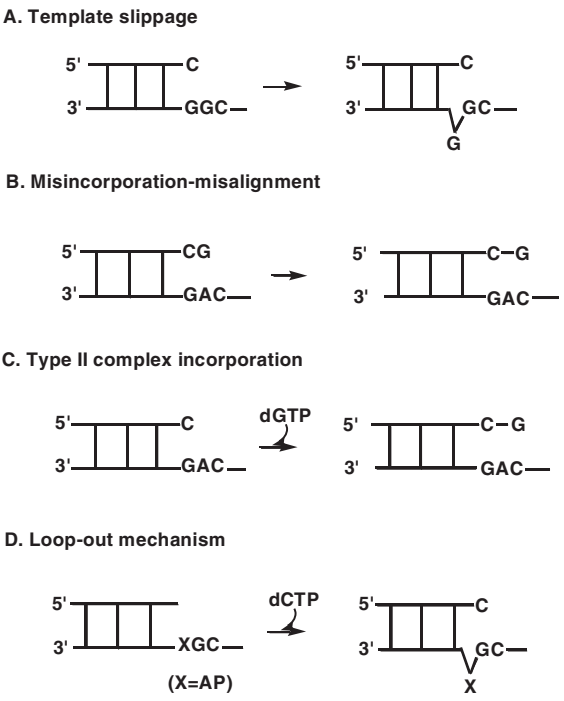

Fig. 2.1 Four mechanisms are proposed for the formation of -1 deletion frameshifts

correctly. DNA polymerase I (Klenow fragment) of bacteriophage M13mp2 catalyzes DNA replication on M13 dsDNA containing a 361-nucleotide ssDNA gap using an imbalanced dNTP pool. -1 frameshifts are preferentially formed when the template sequence has a 5′-neighbor nucleotide that is complementary to the dNTP provided in excess. When a dNTP complementary to the 5′-next nucleotide in the template is first misincorporated opposite a template nucleotide, this misincorporated nucleotide then misaligns and correctly pairs with the next complementary template nucleotide, forming a correct terminal base pair and -1 frameshift. If the mispaired nucleotide at the end of primer is complementary to the 5′-next template nucleotide, the in vivo frequency of -1 frameshift deletion was 58-fold higher than if the nucleotide at the primer end was non-complementary to the 5′-template nucleotide, reflecting the misincorporation–misalignment mechanism.

dNTP-stabilized misalignment mechanism: This mechanism is also called as Type II complex mechanism. Dpo4 produces -1 frameshifts. 0–2 % frameshifts are produced with thymine or cytosine at the incorporation position, 9–12 % with adenine or guanine, and 25–50 % with larger planar cyclic DNA adducts, e.g., 1,N^2-ε-G, which has the strongest stacking interaction with the incoming dNTP. In crystal structures, purines and larger planar DNA adduct at the incorporation position can stack with the incoming dNTP that has paired with the next template base, forming a Type II complex. The conformational change of Dpo4 upon forming the Type II complex is very fast, followed by a slow step for the formation of phosphodiester bond.

Loop-out mechanism: A loop-out mechanism is also proposed to explain the formation of -1 frameshift. In the bypass of an abasic site by *S. solfataricus* Dpo4, the abasic site in the template cannot base stack with an incoming dNTP and then loops out from the duplex. The incoming dNTP subsequently pairs with the 5′ next template base. In crystal structures, the abasic lesion that loops out can be accommodated in the cavity between the fingers and little finger domains of Dpo4.

2.4 DNA–DNA Cross-Linking Destroys DNA Replication

1,3-butadiene (BD), which belongs to bis-electrophilic agents, can produce DNA–DNA or DNA–protein cross-links. BD can be oxidized to 1,2,3,4-diepoxybutane (DEB) (Fig. 2.2). Alkylation of adenine or guanine base by DEB produces 2-hydroxy-3,4-epoxybut-1-yl (HEB) DNA lesion, which contains an inherently reactive oxirane group that can further alkylate neighboring nucleotide bases within the DNA duplex to form DNA–DNA cross-links. The 3,4-epoxy ring can also produce DNA–protein cross-links by nucleophilic attack by amino acid groups in side chains of neighboring proteins. DEB reacting with a protein such as O^6-alkylG DNA alkyltransferase or the tripeptide glutathione is also documented and probably more likely. Acrolein and other α,β-unsaturated aldehydes from cigarette smoke and automobile exhaust can also cross-link (inter-strand) DNA in a similar pathway.

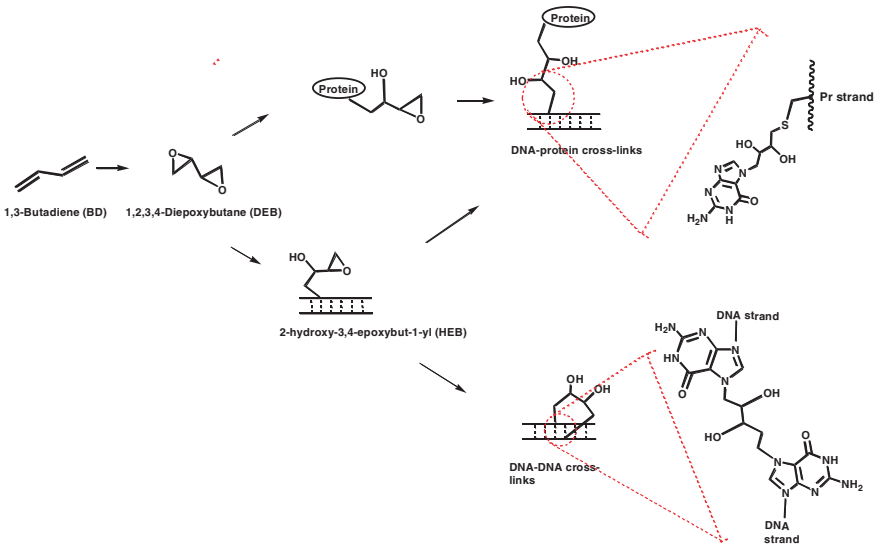

Fig. 2.2 Formation of DNA–DNA or DNA–protein cross-links involving an oxidation product of 1,3-butadiene (BD) (*Pr* protein)

The cross-linking adducts block A-family DNA polymerases. Translesion DNA polymerases, such as *E. coli* pol IV and pol V, are also incapable of bypassing the cross-links. Crystal structures of these complexes have not been reported yet, but the general opinion is that these adducts are too bulky to be accommodated by the active sites of DNA polymerases. However, hpol κ can efficiently bypass DNA-peptide cross-links if the peptide is N-substituted and linked to the N2 position of G, which can be considered an N^2-alkylG adduct. The crystal structure of the complex containing N^2-alkylG shows that pol κ completely encircles the DNA duplex using its unique N-terminal extension. This kind of structure may be flexible enough in the hinge domain to allow pol κ to pass through these kinds of cross-linked bulky lesions.

Further Reading

Irimia A, Eoff RL, Guengerich FP, Egli M (2009) Structural and functional elucidation of the mechanism promoting error-prone synthesis by human DNA polymerase kappa opposite the 7,8-Dihydro-8-oxo-2 '-deoxyguanosine adduct. J Biol Chem 284:22467–22480.

Zang H, Irimia A, Choi JY, Angel KC, Loukachevitch LV, Egli M, Guengerich FP (2006) Efficient and high fidelity incorporation of dCTP opposite 7,8-dihydro-8-oxodeoxyguanosine by Sulfolobus solfataricus DNA polymerase Dpo4. J Biol Chem 281:2358–2372.

Eoff RL, Irimia A, Angel KC, Egli M, Guengerich P (2007) Hydrogen bonding of 7,8-dihydro-8-oxodeoxyguanosine with a charged residue in the little finger domain determines miscoding events in Sulfolobus solfataricus DNA polymerase Dpo4. J Biol Chem 282:19831–19843.

Zhao L, Pence MG, Christov PP, Wawrzak Z, Choi JY, Rizzo CJ, Egli M, Guengerich FP (2012) Basis of Miscoding of the DNA Adduct N^2,3-Ethenoguanine by Human Y-family DNA Polymerases. J Biol Chem 287:35516–35526.

Zhang H, Eoff RL, Kozekov ID, Rizzo CJ, Egli M, Guengerich FP (2009) Structure-Function Relationships in Miscoding by Sulfolobus solfataricus DNA Polymerase Dpo4 guanine N2,N2-dimethyl substiton produces inactive and miscoding polymerase complexes. J Biol Chem 284:17687–17699.

Zang H, Chowdhury G, Angel KC, Harris TM, Guengerich FP (2006) Translesion synthesis across polycyclic aromatic hydrocarbon diol epoxide adducts of deoxyadenosine by *Sulfolobus solfataricus* DNA polymerase Dpo4. Chem Res Toxicol 19:859–867.

Vasquez-Del Carpio R, Silverstein TD, Lone S, Johnson RE, Prakash L, Prakash S, Aggarwal AK (2011) Role of Human DNA Polymerase kappa in Extension Opposite from a cis-syn Thymine Dimer. J Mol Biol 408:252–261.

Minko IG, Yamanaka K, Kozekov ID, Kozekova A, Indiani C, O'Donnell ME, Jiang Q, Goodman MF, Rizzo CJ, Lloyd RS (2008) Replication bypass of the acrolein-mediated deoxyguanine DNA-peptide cross-links by DNA polymerases of the DinB family. Chem Res Toxicol 21:1983–1990.

Vrtis KB, Markiewicz RP, Romano LJ, Rueda D (2013) Carcinogenic adducts induce distinct DNA polymerase binding orientations. Nucl Acids Res 41:7843–7853.

Choi JY, Guengerich FP (2006) Kinetic evidence for efficient and error-prone bypass across bulky N^2-guanine DNA adducts by human DNA polymerase *i*. J Biol Chem 281:12315–12324.

Choi JY, Angel KC, Guengerich FP (2006) Translesion synthesis across bulky N^2-alkylguanine DNA adducts by human DNA. J Biol Chem 2006:21062–21072.

Choi JY, Guengerich FP (2005) Adduct size limits efficient and error-free bypass across bulky N^2-guanine DNA lesions by human DNA polymerase η. J Mol Biol 352:72–90.

Choi YJ, Guengerich FP (2004) Analysis of the effect of bulk at N^2-alkylguanine DNA adducts on catalytic efficiency and fidelity of the processive DNA polymerase T7 exonuclease$^-$ and HIV-1 reverse transcriptase. J Biol Chem 279:19217–19229.

Zhang H, Guengerich FP (2010) Effect of N-2-Guanyl Modifications on Early Steps in Catalysis of Polymerization by Sulfolobus solfataricus P2 DNA Polymerase Dpo4 T239 W. J Mol Biol 395:1007–1018.

Zang H, Harris TM, Guengerich FP (2005) Kinetics of nucleotide incorporation opposite polycyclic aromatic hydrocarbon - DNA adducts by processive bacteriophage T7 DNA polymerase. Chem Res Toxicol 18:389–400.

Ling H, Sayer JM, Plosky BS, Yagi H, Boudsocq F, Woodgate R, Jerina DM, Yang W (2004) Crystal structure of a benzo[*a*]pyrene diol epoxide adduct in a ternary complex with a DNA polymerase. Proc Natl Acad Sci U S A 101:2265–2269.

Kirouac KN, Basu AK, Ling H (2013) Structural Mechanism of Replication Stalling on a Bulky Amino-Polycyclic Aromatic Hydrocarbon DNA Adduct by a Y Family DNA Polymerase. J Mol Biol 425:4167–4176.

Liu Y, Yang Y, Tang TS, Zhang H, Wang Z, Friedberg E, Yang W, Guo C (2014) Variants of mouse DNA polymerase reveal a mechanism of efficient and accurate translesion synthesis past a benzo[a]pyrene dG adduct. Proc Natl Acad Sci U S A 111:1789–1794.

Ling H, Boudsocq F, Woodgate R, Yang W (2001) Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. Cell 107:91–102.

Zhang H, Beckman JW, Guengerich FP (2009) Frameshift Deletion by Sulfolobus solfataricus P2 DNA Polymerase Dpo4 T239 W Is Selective for Purines and Involves Normal Conformational Change Followed by Slow Phosphodiester Bond Formation. J Biol Chem 284:35144–35153.

Zang H, Goodenough AK, Choi JY, Irimia A, Loukachevitch LV, Kozekov ID, Angel KC, Rizzo CJ, Egli M, Guengerich FP (2005) DNA adduct bypass polymerization by Sulfolobus solfataricus DNA polymerase Dpo4 - Analysis and crystal structures of multiple base pair substitution and frameshift products with the adduct 1,N-2-ethenoguanine. J Biol Chem 280:29750–29764.

Streisinger G, Okada Y, Emrich J, Newton J, Tsugita A, Terzaghi E, Inouye M (1966) Cold Spring Harbor Symposia on Quantitative Biology. Cold Spring Harbor Laboratory Press 31:77–84.

Wilson RC, Pata JD (2008) Structural insights into the generation of single-base deletions by the Y family DNA polymerase dbh. Mol Cell 29:767–779.

Kunkel TA, Alexander PS (1986) The base substitution fidelity of eucaryotic DNA polymerases: mispairing frequencies, site preferences, insertion preferences, and base substitution by dislocation. J Bio Chem 261:160–166.

Bebenek K, Kunkel TA (1990) Frameshift errors initiated by nucleotide misincorporation. Proc Natl Acad Sci U S A 87:4946–4950.

Kunkel TA, Soni A (1988) Mutagenesis by transient misalignmen. J Biol Chem 263:14784–14789.

Fiala KA, Hypes CD, Suo Z (2007) Mechanism of abasic lesion bypass catalyzed by a Y-family DNA polymerase. J Biol Chem 282:8188–8198.

Ling H, Boudsocq F, Woodgate R, Yang W (2004) Snapshots of replication through an abasic lesion; structural basis for base substitutions and frameshifts. Mol Cell 13:751–762.

Lone S, Townson SA, Uljon SN, Johnson RE, Brahma A, Nair DT, Prakash S, Prakash L, Aggarwal AK (2007) Human DNA polymerase kapa encircles DNA: implications for mismatch extension and lesion bypass. Mol Cell 25:601–614.

Brenlla A, Markiewicz RP, Rueda D, Romano LJ (2013) Nucleotide selection by the Y-family DNA polymerase Dpo4 involves template translocation and misalignment. Nucleic Acids Res 42:2555–2563.

Zhang H, Eoff RL, Kozekov ID, Rizzo CJ, Egli M, Guengerich FP (2009) Versatility of Y-family Sulfolobus solfataricus DNA Polymerase Dpo4 in Translesion Synthesis Past Bulky N-2-Alkylguanine Adducts. J Biol Chem 284:3563–3576.

Zhang H, Bren U, Kozekov ID, Rizzo CJ, Stec DF, Guengerich FP (2009) Steric and Electrostatic Effects at the C2 Atom Substituent Influence Replication and Miscoding of the DNA Deamination Product Deoxyxanthosine and Analogs by DNA Polymerases. J Mol Biol 392:251–269.

Eoff RL, Irimia A, Egli M, Guengerich FP (2007) *Sulfolobus solfataricus* DNA polymerase Dpo4 is partially inhibited by "Wobble" pairing between O^6-methylguanine and cytosine but accurate bypass is preferred. J Biol Chem 282:1456–1467.

Eoff RL, Angel KC, Egli M, Guengerich FP (2007) Molecular basis of selectivity of nucleoside triphosphate incorporation opposite O^6-benzylguanine by *Sulfolobus solfataricus* DNA polymerase: steady-state and pre-steady-state kinetics and x-ray crystallography of correct and incorrect pairing. J Biol Chem 282:13573–13584.

http://www.springer.com/978-94-017-7211-2

DNA Replication - Damage from Environmental Carcinogens

Zhang, H.

2015, XI, 43 p. 4 illus., 1 illus. in color., Softcover
ISBN: 978-94-017-7211-2