DNA damage by reactive species: Mechanisms, mutation and repair

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DNA is continuously attacked by reactive species that can affect its structure and function severely. Structural modifications to DNA mainly arise from modifications in its bases that primarily occur due to their exposure to different reactive species. Apart from this, DNA strand break, inter- and intra-strand crosslinks and DNA–protein crosslinks can also affect the structure of DNA significantly. These structural modifications are involved in mutation, cancer and many other diseases. As it has the least oxidation potential among all the DNA bases, guanine is frequently attacked by reactive species, producing a plethora of lethal lesions. Fortunately, living cells are evolved with intelligent enzymes that continuously protect DNA from such damages. This review provides an overview of different guanine lesions formed due to reactions of guanine with different reactive species. Involvement of these lesions in inter- and intra-strand crosslinks, DNA–protein crosslinks and mutagenesis are discussed. How certain enzymes recognize and repair different guanine lesions in DNA are also presented.

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1. Introduction

DNA damage by reactive species has created profound interest in the medicinal fraternity becuase of the involvement of reactive species in different pathological conditions such as cancer, aging, neurodegenerative diseases, rheumatoid arthritis, etc. (Kirkinezosa and Moraesa 2001; Petersen *et al*. 2005; Waris and Ahsan 2006; Wiseman and Halliwell 1996). Reactive species such as free radicals, one-electron oxidants, different chemicals, etc., can react with different components of DNA to produce a plethora of DNA lesions (Jena and Mishra 2012). These reactive species can modify bases (Jena and Mishra 2005; Jena and Mishra 2006; Jena and Mishra 2007; Jena *et al.* 2008; Shukla *et al.* 2011; Jena and Mishra 2012), induce inter- and intra-strand crosslinks (Bauer and Povirk 1997; Minko *et al.* 2008), promote DNA–protein crosslinks (Johansen *et al.* 2005; Perrier *et al.* 2006; Xu *et al.* 2008) and create strand break (Yermilov *et al.* 1996; Balasubramanian *et al.* 1998).

Several reactive species that contain oxygen such as superoxide radical anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), etc. (scheme 1) are formed inside living cells during normal metabolic activities (Jena and Mishra 2012). For example, leakage of electrons to molecular oxygen (O_2) from mitochondrial electron transport chains consisting of flavoproteins, iron-sulphur proteins, ubiquinone and cytochromes produces $O_2^{\cdot -}$ (scheme 1) (Liu *et al*. 2002a). Dismutation of $O_2^{\cdot -}$ by superoxide dismutase produces H_2O_2 (scheme 1) (Loschen *et al.* 1974). Apart from this, different enzymes such as several oxidases can also produce H_2O_2 in cells. Although catalase and glutathione peroxidase scavenge H_2O_2 by converting it into water, reaction of H_2O_2 with $O_2^{\cdot -}$ can yield OH radical following Haber–Weiss mechanism (scheme 1). Fenton reactions in presence of transition metals (scheme 1) and UV-induced photolysis of H_2O_2 can also generate OH radicals. OH radicals are very reactive and can perturb structures of all components of the DNA. Formation of nitric oxide ($NO^{\cdot}$) catalysed by nitric oxide synthase can produce $ONOO^-$ due to its reaction with $O_2^{\cdot -}$ (scheme 1), which is very reactive. More interestingly, $ONOO^-$ itself is capable of generating other reactive species that are very reactive. For example, the conjugate acid of $ONOO^-$, i.e. ONOOH on homolytic dissociation, can generate reactive NO_2 and OH radicals (Merenyi and Lind 1998). Furthermore, in the presence of carbon dioxide (CO_2), $ONOO^-$ can generate nitrosoperoxycarbonate anion ($ONOOCO_2^-$), which on homolytic dissociation may yield

Keywords. DNA crosslinks; DNA damage; DNA repair; mutation; nucleotide flipping; reactive species

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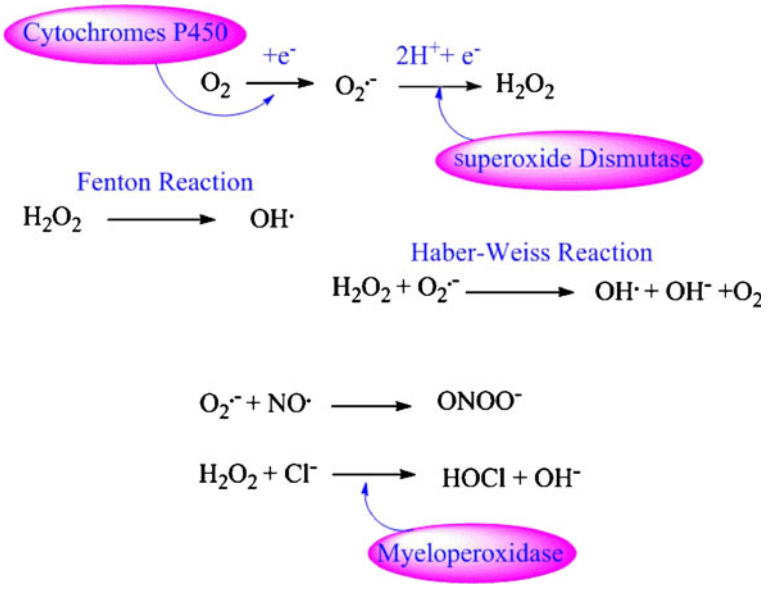


Scheme 1. Formation of different reactive species during cellular metabolic activities.

$CO_3^{\cdot -}$ and $NO_2^{\cdot}$ free radicals (Shafirovich *et al.* 2001). Similarly, in cells, heme myeloperoxidases help in the formation of another powerful oxidising and halogenating species, i.e. HOCl, by catalysing a reaction between H_2O_2 and chlorine anion (Cl^-) (scheme 1) (Gungor *et al.* 2010). HOBr generated by human eosinophils is another potent halogenating agent that readily brominates DNA bases (Weiss *et al.* 1986). In addition to normal metabolic activities, ionizing radiation and surgical resection at any part of the body may help in generation of reactive species (Potenza *et al.* 2011). Other than reactive species, chemicals such as different alkylating and nitrating agents and high-energy radiation are also capable of damaging DNA.

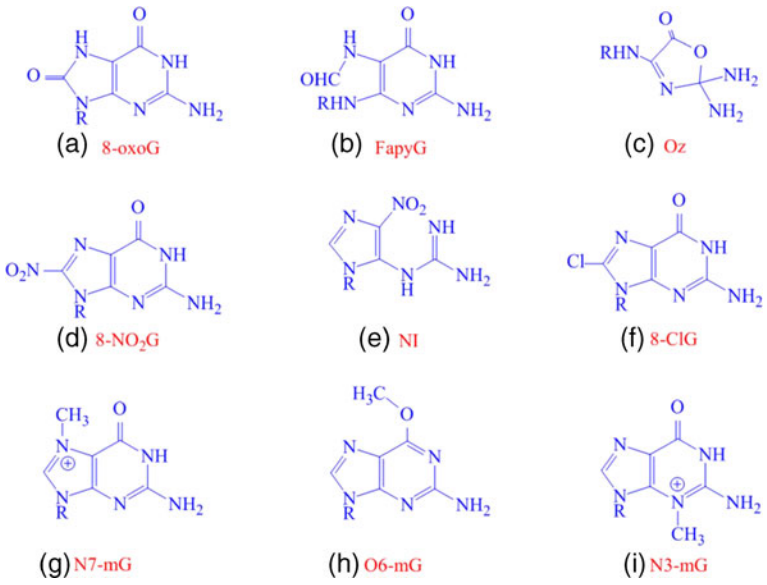
Among all the DNA bases, guanine has the least oxidation potential, because of which it is frequently attacked by different reactive species. Modification of guanine can result a plethora of lethal lesions that may arise due to its oxidation, nitration, halogenation, alkylation, etc. (Jena and Mishra 2012). Structures of some of these lesions have been depicted in scheme 2. Different guanine lesions formed in this way can induce mutagenesis, crosslinks between DNA strands and proteins, thereby affecting DNA replication and transcription (Abdulnur and Flurry 1976; Niles *et al.* 2006).

Given the broad spectrum of DNA damaging species and their involvement in many lethal processes, it is astonishing that under normal circumstances vast majorities of cellular components are error free. This is due to the fact that living cells are evolved with intelligent enzymes that protect DNA from erroneous and hazardous effects by executing about 10^{16}–10^{18} repair events per cell per day (Schärer 2003). These DNA repair machineries have been proposed to function in several innovative ways like base reversal (BR), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MR), double strand break repair (DSBR), etc. (Friedberg *et al.* 1995; Hoeijmakers 2001). However, the way enzymes actually facilitate DNA repair is still a mystery. Two important aspects of DNA repair *in vivo* are to first find out the damaged lesion among millions of undamaged DNA moieties and then to recruit repairing enzyme residues to retrieve normal DNA. Understanding of lesion recognition will not only enrich our understanding of DNA repair but also enlighten causes of many nuclear processes like replication and transcription (Halford and Marko 2004). The main aim of this review is to provide an overview of different guanine lesions formed due to reactions of guanine with different reactive species. Interlinking of these lesions in inter- and intra-strand crosslinks, DNA–protein crosslinks and mutagenesis are discussed. Recognition and repair of these lesions in DNA by different enzymes are also discussed.

2. DNA damage due to guanine modification

2.1 *Oxidation*

Among several oxidation products of DNA involving guanine, 8-oxoguanine (8-oxoG) (scheme 2a) is the ubiquitous product formed in living cells. Other than 8-oxoG (Alhama *et al.* 1998), formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) (Tudek 2003) (scheme 2b) and 2,2-diamino-4-(2-deoxy-b-D-erythropentofuranosyl)amino]-5(2H)-oxazolone (oxazolone, Oz), (Scheme 2c) (Matter *et al.* 2006) in cellular DNA have also been observed.



Scheme 2. Structures of some of the guanine lesions formed due to oxidation (**a–c**), nitration (**d,e**), halogenation (**f**) and alkylation (**g–i**).

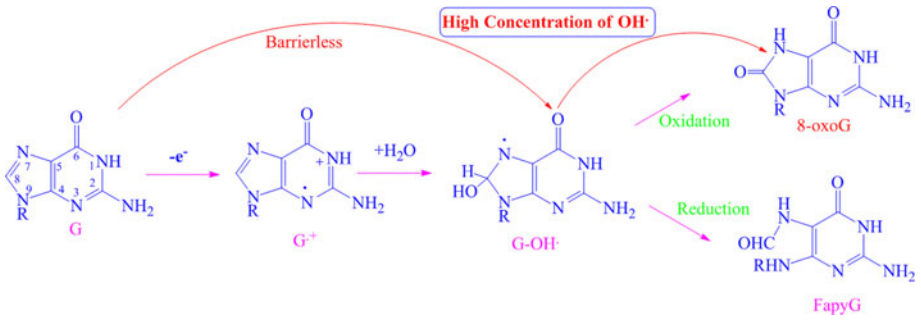
It has been proposed that one electron oxidation of guanine (G) generates guanine radical cation ($G^{\cdot+}$), which upon reaction with a water molecule, yields 8-hydroxy-7,8-dihydroguanyl radical (G-OH$^{\cdot}$) as an relatively stable intermediate (Jena and Mishra 2012). This intermediate upon further oxidation may yield 8-oxoG, while its reduction would generate FapyG (scheme 3) (Matter *et al.* 2006; Jena and Mishra 2012). Apart from this, a previous modelling study (Jena and Mishra 2005) has demonstrated that under high concentration of OH radicals, guanine would directly be converted into 8-oxoG, as the first step related to formation of G-OH$^{\cdot}$ is barrierless (scheme 3). 8-oxoG may also be formed due to oxidation of guanine by other reactive species such as $ONOO^{-}$, HOCl, etc. (Ravanat and Cadet 1995; Whiteman *et al.* 1997; Cheng *et al.* 2003; Yu *et al.* 2005; Niles *et al.* 2006; Jena and Mishra 2007; Jena *et al.* 2008; Yadav and Mishra 2012).

Similarly, simultaneous one-electron and one-proton loss from guanine can generate deprotonated guanine neutral radical (G-H)$^{\cdot}$, which upon exposure to $O_2^{\cdot-}$ followed by decarboxylation, hydrolysis and rearrangement, may generate another oxidized guanine lesion, imidazolone (Iz) (Jena and Mishra 2012) (scheme 4). Iz on subsequent hydration can yield Oz (Jena and Mishra 2012) (scheme 4). From NMR studies (Gasparutto *et al.* 1998), it has been inferred that Oz may exist in two tautomeric conformations as illustrated in scheme 4. In addition to above lesions, formation of other oxidatively damaged products of guanine such as guanidinohydantoin (Gh), spiroiminodihydantoin (Sp), oxaluric acid (Oa), etc., have also been reported based on *in vitro* studies (Gasparutto *et al.* 1998; Duarte *et al.* 2000; Seguy *et al.* 2001; Chworos *et al.* 2002; Jena and Mishra 2012). However, quantification of these lesions in cellular DNA is still obscure.

2.2 *Nitration and halogenation*

Among various possible nitration products of guanine, 8-nitroguanine (8-NO_2G) is an important and lethal nitrating product. As discussed earlier, nitric oxide synthases release NO$^{\cdot}$ in high amounts, which can be used by macrophages to kill pathogens. However, when NO$^{\cdot}$ is converted to $ONOO^{-}$ or its derivatives, its reactivity increases significantly. For example, it has been found that NO$^{\cdot}$ does not react with G directly. However, when it is converted to $ONOO^{-}$ and $ONOOCO_2^{-}$, it reacts with G to yield 8-nitroguanine (8-NO_2G) (Niles *et al.* 2006; Jena and Mishra 2007) and 5-nitro-guanidinohydantoin (NI) (Niles *et al.* 2006; Jena and Mishra 2012). However, recent studies have revealed that NO$^{\cdot}$ may react with the guanine radical cation ($G^{\cdot+}$) or deprotonated guanine radical (G(−H)$^{\cdot}$) to yield 8-NO_2G and NI (Liu *et al.* 2006; Agnihotri and Mishra 2009; Agnihotri and Mishra 2010; Jena and Mishra 2012). These studies have further revealed that in presence of water molecules, yield of both 8-NO_2G and NI involving $G^{\cdot+}$ would be more than that of G(−H)$^{\cdot}$ (Liu *et al.* 2006; Agnihotri and Mishra 2009; Agnihotri and Mishra 2010). It has been proposed that after one-electron oxidation of G, if the radical centre exists at the C8 position, it would lead to the formation of 8-NO_2G, while the C5 radical centre would ultimately yield NI (scheme 5) (Liu *et al.* 2006).

Halogenation of DNA bases is also carcinogenic and harmful for tissues under inflammatory conditions. HOCl,

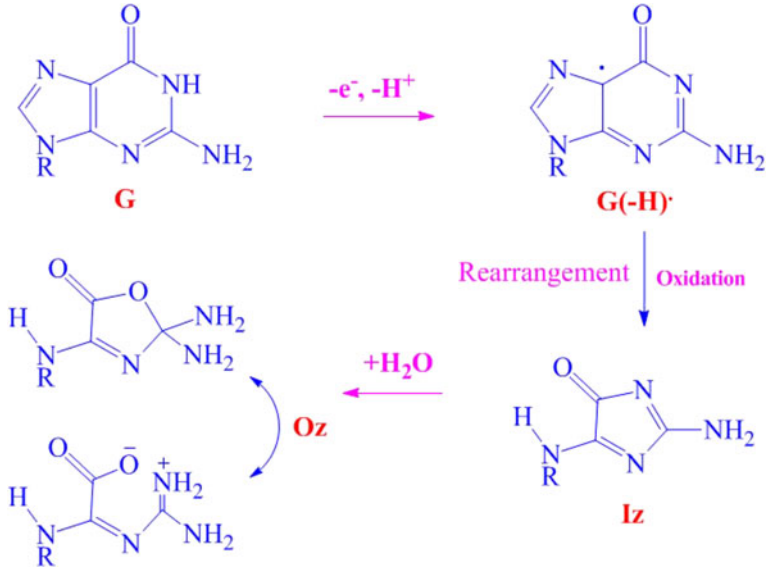


Scheme 3. Mechanisms of formation of 8-oxoG and FapyG from G. For detailed mechanism, refer to Jena and Mishra (2012).

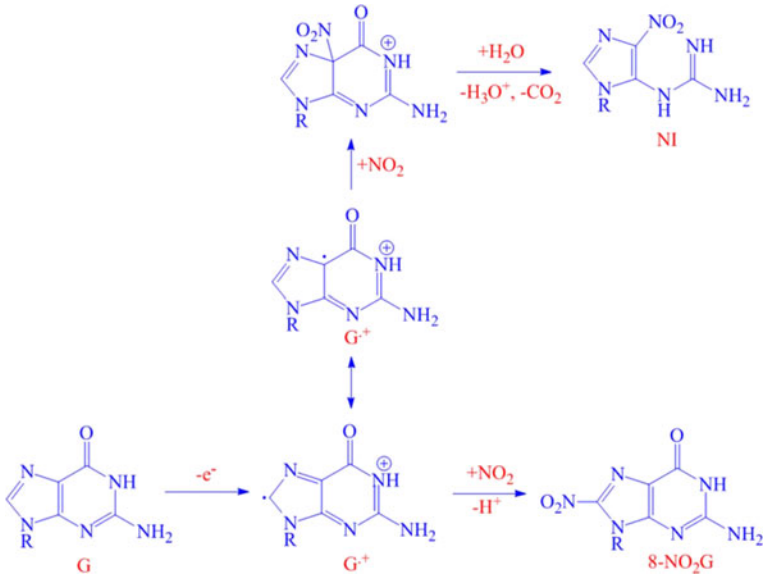
N-chloroamines, HOBr, etc., are the potent halogenated compounds that can affect DNA structure and function severely (Henderson *et al.* 1999; Jiang *et al.* 2003; Sasa *et al.* 2011). Among various DNA halogenating agents, HOCl is the most abundant in cells and reacts with guanine to form 8-chloroguanine (8-ClG) (scheme 2f) (Henderson *et al.* 1999; Masuda *et al.* 2001; Stanley *et al.* 2010). It has been demonstrated by a modelling study that homolytic dissociation of HOCl into HO˙ and Cl˙ is the initial stage of guanine chlorination (Jena *et al.* 2008). The dissociated Cl˙ can react with the N7 or C8 position of guanine giving rise to 7-ClG˙ or 8-ClG˙ respectively as intermediates. Subsequent rearrangement of these radical intermediates would ultimately produce 8-ClG (Jena *et al.* 2008). Although in cells, bromination of uracil and cytosine by HOBr has been detected (Hu *et al.* 2006), significant level of guanine bromination has not been documented. However, *in vitro* studies have demonstrated that formation of 8-BrG is possible in Z-DNA (Moller *et al.* 1984).

2.3 *Alkylation*

DNA can be alkylated due to reactions of nitrogen mustards, alkyl halides, alkyl sulphate, alkyl sulfonates, diazo compounds, consumption of nitrosamines, chemotherapeutic drugs, etc. (Singer 1975). These damaging species produce a large spectrum of DNA alkylated products (Shrivastav *et al.* 2010). Alkylation of guanine may occur at multiple sites giving rise to 1-methylguanine (1-mG), 3-methylguanine (3-mG) (scheme 2i), O6-methylguanine (O6-mG) (scheme 2h), 7-methylguanine (7-mG) (scheme 2g), 8-methylguanine (8-mG), 1,2-ethylguanine (1,2-eG) and 2,3-ethylguanine (2,3-eG) (Shrivastav *et al.* 2010). From modelling studies, it has been inferred that among all these sites of guanine, the N7 position is the most reactive for direct alkylation (Ekanayake and Libreton 2007; Shukla and Mishra 2010). It has been further found that the GC-rich regions of different genes are the favoured site for DNA methylation (Mattes *et al.* 1988).



Scheme 4. Mechanism of formation of Oz from G. For detailed mechanism, refer to Jena and Mishra (2012).



Scheme 5. Mechanism of formation of 8-NO_2G and NI from G. For detailed mechanism, refer to Jena and Mishra (2012).

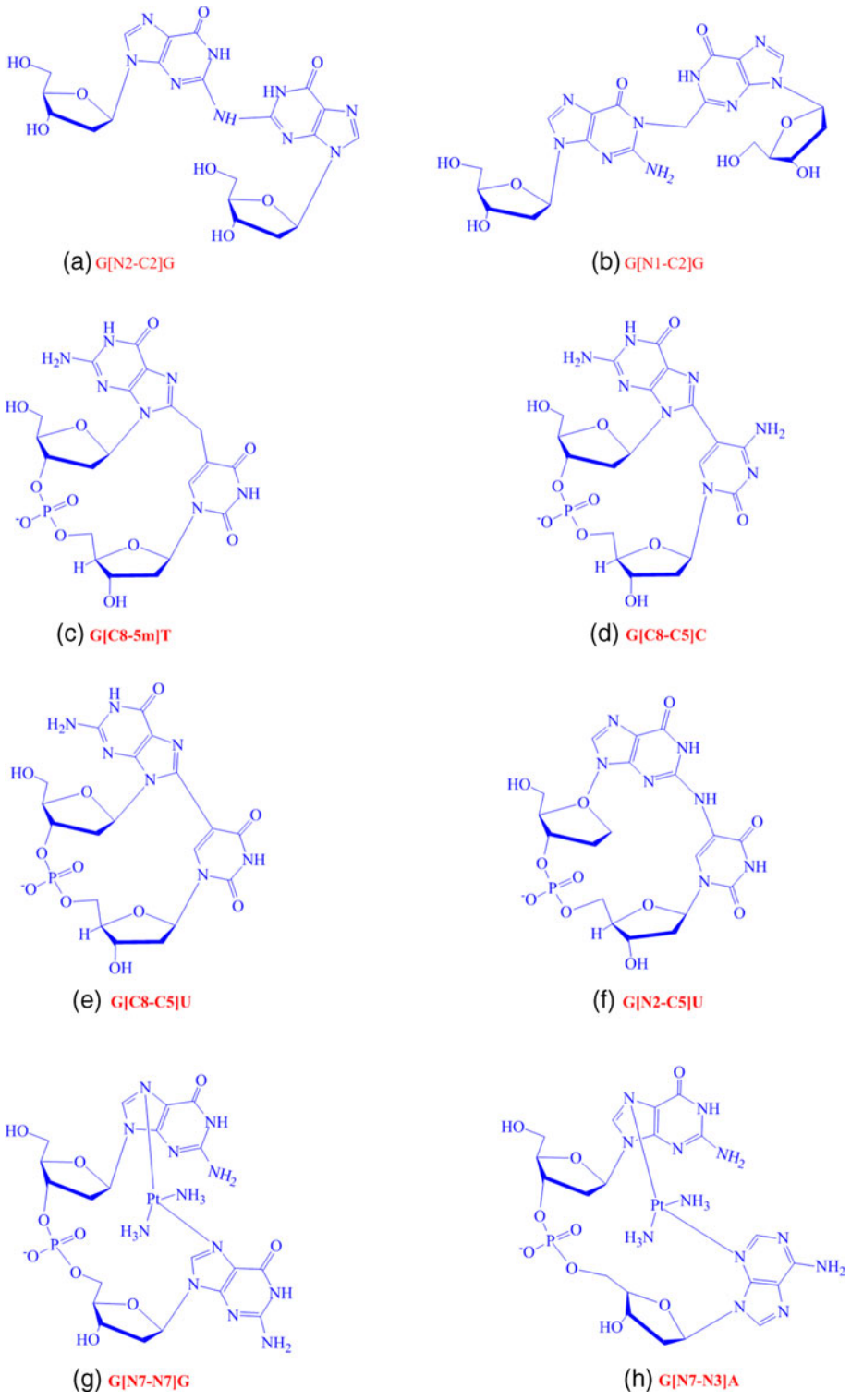
3. DNA damage due to crosslinks

3.1 *Inter- and intra-strand crosslinks*

DNA inter- and intra-strand crosslinks are formed due to covalent bond formation between nucleotides of opposite strands and the same strand respectively. However, determination of the accurate structures of these crosslink products is difficult. It has been established that nucleotide modifications either by reactive species (Wang 2008) or UV irradiation can facilitate formation of different DNA crosslink products. Other than this, interactions of several anticancer and chemical agents with DNA can also generate various DNA crosslink adducts (Coste *et al.* 1999; Hofr and Brabek 2001; Hofr *et al.* 2001). These products are believed to be mutagenic (Hong *et al.* 2007) and can block DNA replication and transcription. Occurrence of these products in DNA can also distort DNA heavily (Hofr *et al.* 2001). Although, formation of several purine–purine (Malinge *et al.* 1999), pyrimidine–pyrimidine (Edfeldt *et al.* 2004) and purine–pyrimidine (Dizdaroglu and Simic 1984) DNA crosslink products have been recently observed, this article is primarily focussed on inter- and intra-strand crosslinks with relevance to guanine.

In an NMR study, G-G inter-strand crosslink product induced due to the interaction of nitrous acid with the DNA preferably at the d(CpG) site has been observed (Malinge *et al.* 1999). This product is formed due to the covalent bond formation between the N2 of guanine in one strand with the C2 of another guanine located in the opposite strand. Although the resulting G[N2-C2]G inter-strand crosslink product (scheme 6a) was observed to be planar with slightly different propeller twist, it, however, pushes cytidine bases paired with each guanine out of the DNA helix through the minor groove (Malinge *et al.* 1999). Similarly, nitrosative deamination of guanine has been proposed to crosslink with cytosine to form G[N1-C2]G inter-strand crosslink product (scheme 6b) (Glaser *et al.* 2005). By employing modelling studies, it has been further found that both G[N2-C2]G and G[N1-C2]G inter-strand crosslinks are of comparable thermodynamic stability (Qian and Glaser 2005) and hence both products can be formed in DNA.

It has been observed that formation of thymine radical can lead to covalent bond formation between the neighbouring C8 atom of guanine and adenine of same strand producing G[C8-C5]T (scheme 6c) (Hong *et al.* 2006) and A[C8-C5]T (Bellon *et al.* 2002;Xerri *et al.* 2006) intra-stand crosslink products respectively. Alternatively, addition of an OH radical to the C6 position of thymine has also been observed to induce intra-strand crosslink between C5 of thymine and C8 of guanine giving rise to T[C5-C8]G adduct (Labet *et al.* 2008). In the LC-MS/MS study performed in both aerobic and anaerobic conditions, it has been further found that due to radical formation at the C5 site of cytosine, it can be covalently bonded to the C8 position of guanine of same strand to form G[C8-C5]C intra-strand crosslink product (scheme 6d) (Box *et al.* 1997, 1998). Crosslink between C5 of cytosine and N2 of guanine (C[C5-N2]G) (Cao and Wang 2009) has also been proposed. Methylation of cytosine at the CpG site can also induce G[C8-C5]C and G[C8-5m]C intra-strand crosslinks (Cao and Wang 2007). Crosslink between C8 and C2 of guanine with C5 of uracil formed due to deamination of cytosine has also been detected (scheme 6e and f) (Crean *et al.* 2008); one-electron oxidation



Scheme 6. Structures of different inter-strand (**a,b**) and intra-strand (**c–h**) crosslink products of DNA involving guanine.

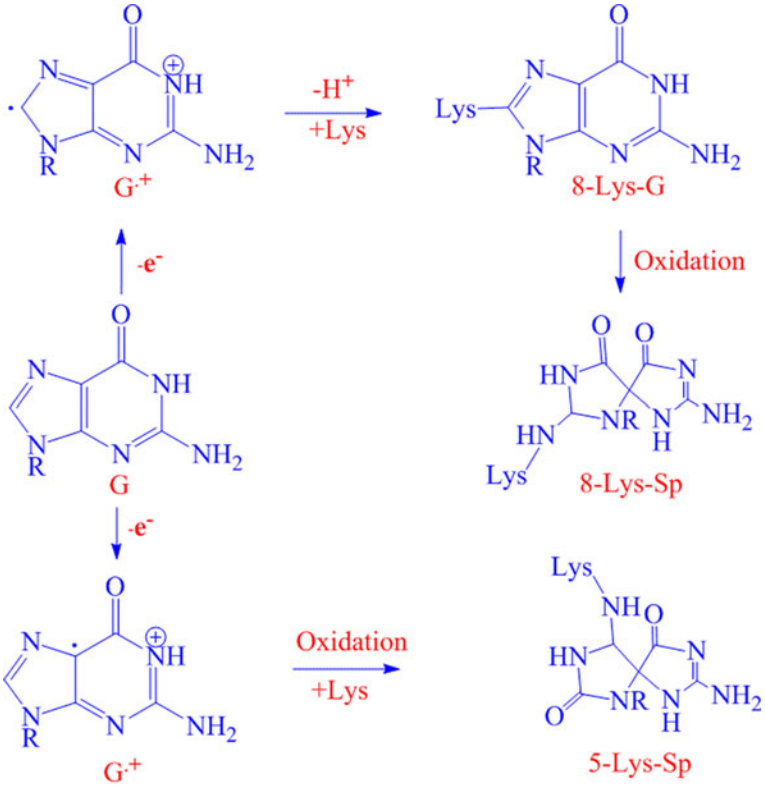
of both guanine and uracil lead to the formation of this crosslink (Churchill *et al.* 2011). Binding of the anticancer drug cisplatin (Pt-$(NH_3)_2$) to guanine has also been detected to promote G[N7-N7]G (scheme 6g) and G[N7-N3]A (scheme 6h) intra-strand crosslinks (Liu *et al*. 2002b; Hegmans *et al.* 2004; Harrington *et al.* 2010).

Apart from the base–base crosslinks, covalent bond formation between a base and a sugar in the same or the opposite strands of DNA results in inter- or intra-strand base–sugar crosslinks (Sonntag 1987; Balasubramanian *et al.* 1998; Burrows and Muller 1998; Cooke *et al.* 2003; Sczepanski *et al.* 2008; Geacintov and Broyde 2010;). Formation of base–sugar crosslinks has been explained to arise mainly due to hydrogen abstraction from the carbon centres of the sugar moiety by reactive species. For example, It is found that the hydrogen abstraction from the C5′ of a deoxyguanosine would induce a covalent bond formation between the C5′ of sugar (S) and C8 positions of G, leading to the intramolecular cyclization to form a N7-centred radical intermediate with a rate constant of $1.6\times10^5\ s^{-1}$ (Geacintov and Broyde 2010). Oxidation of this radical intermediate would generate a lethal intra-strand crosslink product i.e. 8-5′-cyclodeoxyguanosine (S[C5′-C8]G) (Dizdaroglu 1986; Jasti *et al.* 2011).

3.2 *DNA–protein crosslinks*

DNA–protein crosslink generally refers to the formation of a covalent bond between a base or sugar and an amino acid. DNA–protein crosslink can be formed due to (a) exposure of DNA and proteins to reactive species and chemotherapeutic drugs, (b) processing of DNA by replication and recombination proteins and (3) base excision repair of DNA damages. These bulky lesions can inhibit DNA replication and transcription and promote disorders in cells.

DNA–protein crosslink involving thymine and several amino acid residues of histone proteins have been observed both *in vivo* and *in vitro* (Gajewski *et al.* 1988; Gajewski and Dizdaroglu 1989a, b; Gajewski and Dizdaroglu 1990). It was proposed that hydrogen abstraction from these amino acid residues by an $OH^{\cdot}$ followed by oxidation of the thymine–amino acid radical adduct are the main causes of the DNA–histone crosslink. In an *in vitro* study (Perrier *et al.* 2006) involving an oligonucleotide containing thymine–guanine–thymine and tri-lysine peptide, it has been demonstrated that a crosslink between guanine and lysine may occur due to initial oxidation of guanine giving rise to $G^{\cdot+}$ or $G(-H)^{\cdot}$. On subsequent addition of the side chain of lysine at the C8 position of these one-electron oxidized guanine products would generate 8-Lys-G as a crosslink lesion (scheme 7) (Perrier *et al.* 2006). On subsequent oxidation, 8-Lys-G would be converted to another complex crosslink, lesion i.e. 8-Lys-Sp (Sp=spiroiminodihydantoin) (scheme 7) (Perrier *et al.* 2006). The C5 position of guanine has also been suggested to be reactive enough to produce the 5-Lys-Sp



Scheme 7. Mechanism of formation of different guanine–lysine crosslinks.

crosslink lesion (scheme 7) (Xu *et al.* 2008). By using synthetic oligonucleotides in presence of HOCl, $ONOO^-$ and one-electron oxidants, it has been proposed that covalent crosslink between C5 of 8-oxoG and the side chain of lysine is possible, which may induce several Lys-G crosslink lesions (Johansen *et al.* 2005).

Similar to base–amino acid crosslinks, formation of sugar–amino acid crosslinks has also been observed. For example, recently, in nucleosome core particle, crosslinking between apurinic/apyrimidinic (AP) lesion and histone proteins has been observed (Sczepanski *et al.* 2010). It should be mentioned that different AP lesions can be formed due to either spontaneous hydrolysis of damaged and undamaged nucleotides or during processing of damaged nucleotides by the BER proteins.

4. Mutagenesis due to guanine lesions

As mentioned earlier, reactive-species-mediated guanine lesions are involved in mutagenesis. Different possible mutations that may arise due to guanine modifications (Neeley *et al.* 2004; Valko *et al*. 2004; Suzuki 2006; Colis *et al.* 2008; Jena and Mishra 2012) are presented in table 1. From this table it is clear that oxidized, nitrated and halogenated guanine lesions are associated mainly with the G-A and G-T mutations. In addition to these mutations, alkylated guanine lesions are involved with the G-C mutation. While G to G inter-strand and G to T intra-strand crosslinks are associated mainly with the G-T mutation, the base–sugar intra-strand crosslink induces both the G-T and G-A mutations. Both experimental and modelling studies have demonstrated that miscoding properties of different guanine lesions are interlinked with their base pairing abilities with the complementary bases in the opposite strand of DNA (Swann 1990; Feig and Loeb 1993; Jena and Bansal 2011). It has been inferred that the mispaired nucleotides have the following characteristics: (a) deviation from the normal Watson–Crick type of structural alignment and (b) perturbation in the strength of the hydrogen bonding, stacking and hydrophobic interactions (Chabarria *et al*. 2011). These factors together contribute to mutagenesis (scheme 8). It should be noted that deviation from the Watson–Crick type of alignment may arise due to (a) tautomeric arrangement of one of the mispaired nucleotides, (Aquilina 1994; Venkateswarlu and Leszczynski 1998), (b) participation of the protonated or ionized mispairs due to solvent–base interaction (Sowers *et al.* 1986; Leonard *et al.* 1990; Aquilina 1994; Lyngdoh 1994), or (c) *anti* to *syn* conformational change by glycosidic bond rotation (Aquilina 1994; Beard *et al.* 2010) (scheme 8).

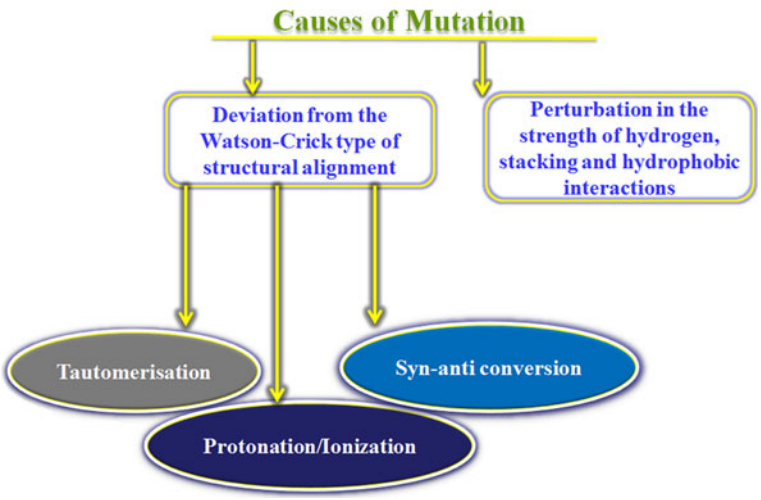
Table 1. Possible mutations associated with different guanine lesions

Lesion	Mutagenesis
8-oxoG	G-A, G-T, G-G
FapyG	G-T
Oz	G-A
8-NO_2G	G-A
NI	G-A, G-T, G-C
8-ClG	G-A
1-mG	G-T, G-A, G-C
O6-mG	G-A
7-mG	G-C, G-T
8-mG	G-C
1,2-eG	G-T, G-C
2,3-eG	G-A
G[N2-C2]G, G[N1-C2]G	G-T
G[C8-5m]T	G-T
S[C5'-C8]G	G-A, G-T

5. Damage recognition and repair

As prolonged persistence of DNA lesions in cells is lethal, these lesions should be excised or repaired before their involvement in different cellular processes. Structures and functions of various DNA repair enzymes have been extensively reviewed (Friedberg *et al.* 1995; Wood 1997; de Laat *et al.* 1999; David *et al.* 2007; Hitomi *et al.* 2007; Helleday *et al.* 2008; Jackson and Bartek 2009) and hence will not be discussed here. However, as the exact mechanisms of lesion recognition and repair are not comprehensively known, these aspects will be briefly discussed, with emphasis DNA repair by nucleotide flipping. It has been proposed that proteins find their target by diffusing along DNA (protein translocation) via several mechanisms. These hypothetical mechanisms include (a) hopping, where a protein moves along the DNA through various microscopic dissociations and rebinding, (b) sliding, where proteins move along DNA through random walks and by continuously contacting DNA backbones without dissociating from it, and (c) inter-segment transfer, in which proteins move from one segment of DNA to another via loops (Gorman and Greene 2008; Blain *et al.* 2009). It may also possible that during target recognition, rotation of either the protein or DNA along the helix axis will facilitate the search process (Blainey *et al.* 2006; Blain *et al*. 2009; Hedglin and O'Brien 2010).

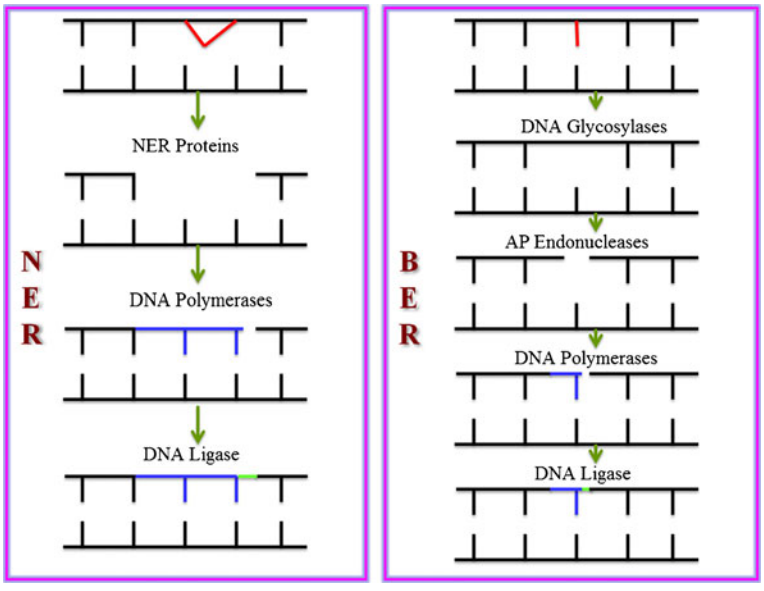
Once the damaged site on DNA is identified, proteins initiate the repair process. Repair of DNA can be executed in several ways depending on the structure of the lesion and its impact on the DNA. For example, bulky lesions (e.g. DNA crosslinks) (Peng *et al.* 2010) in DNA are repaired by the NER proteins by removing a long patch of the single-stranded DNA containing the damaged nucleotide. This creates a vacancy in the single strand, which is subsequently



Scheme 8. Mechanism of mutation caused due to DNA damage by reactive species.

filled by DNA polymerases by substituting a newly synthesized strand. DNA polymerases synthesize a new strand by considering the opposite undamaged strand as a template. Ultimately the broken and synthesized strands get sealed by a DNA ligase (scheme 9). On the other hand, relatively simpler DNA lesions (e.g. 8-oxoG, FapyG, etc.) are repaired by the BER proteins. The BER proteins, in the first step, recruit DNA glycosylases that help in the glycosidic bond scission to remove the damaged nucleotide from the sugar–phosphate backbone creating an AP lesion. In the second step, AP endonucleases help to cleave the phosphodiester bonds between the sugar and phosphate at both the 3′ and 5′ sites of the AP lesion by employing β- and δ-elimination reactions respectively (Liu *et al.* 2007). In the third step, DNA polymerases replace the gap by synthesizing a new nucleotide. In the fourth step, the remaining nick in the DNA single strand is sealed by the DNA ligase (scheme 9). Unlike the NER and BER proteins, different BR proteins repair alkylated lesions by complete reversal of the damaged bases (Volkert 1988).

It should be noted that nucleotide flipping is the primary pathway of DNA repair enzymes such as BER, NER and BR



Scheme 9. Mechanisms of DNA repair by nucleotide excision repair (NER) and base excision repair (BER) proteins. The bases shown in red refer to the damaged ones. The crossed bases refer to the crosslink lesion in DNA. The new strands or nucleotide synthesized by the DNA polymerases and ligase have been shown in violet and green respectively.

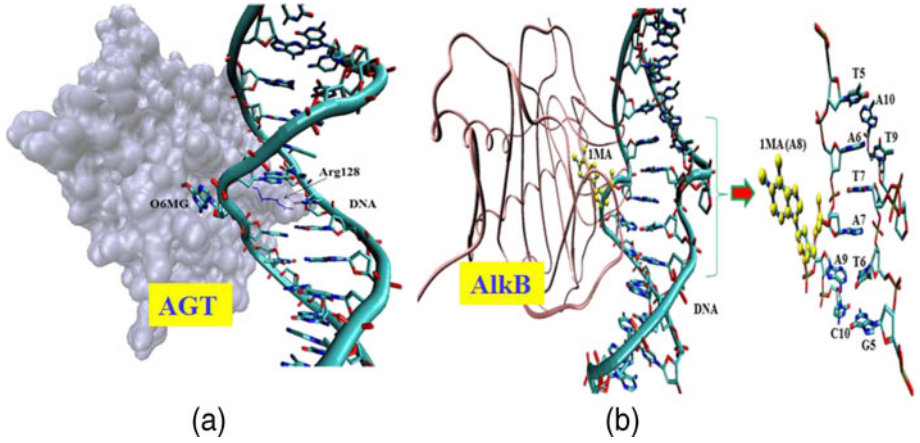
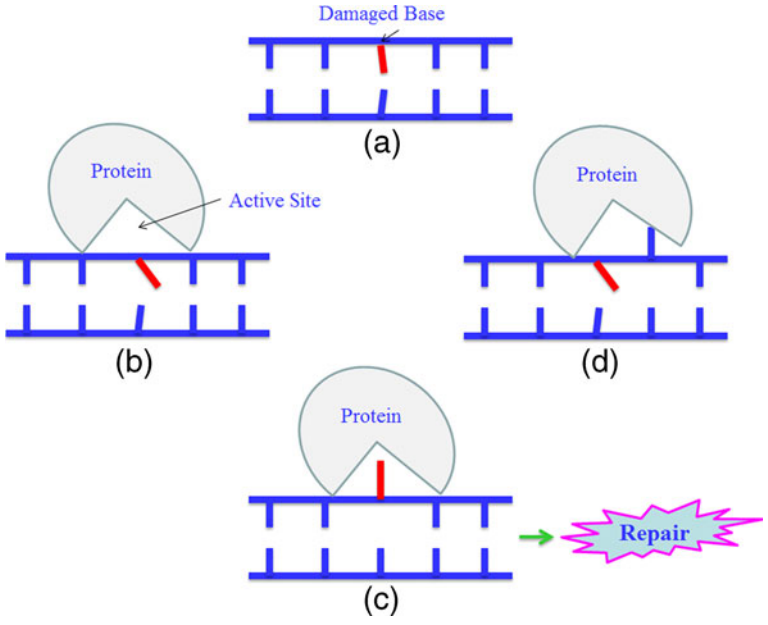


Figure 1. Damaged nucleotide flipping by (**a**) AGT due to intercalation of an amino acid (Arg128) (pdb 1t38) and (**b**) AlkB due to DNA squeezing (pdb 3bie).

proteins (Daniels *et al.* 2004; Malta *et al.* 2006). These proteins selectively flip the damaged nucleotides out of the DNA double helix into their binding pocket where they can excise (BER and NER) or repair (BR) the damaged lesion. Three different mechanisms of damage recognition by nucleotide flipping have been proposed. According to these mechanisms, (a) proteins may find their target by flipping all nucleotides out of the DNA helix into their active site and inspect each of them to identify the damaged one (Yang *et al.* 2009); (b) they may search for the unusual distorted base pairs on DNA first to selectively flip the damaged nucleotide; or (c) proteins may capture an extrahelical lesion already present in the DNA, which might have arisen due to spontaneous breathing dynamics of DNA base pairs (Cao *et al.* 2004; Parker *et al.* 2007; Yang *et al.* 2009) or weak base pairing and instabilities of damaged sites (Krosky *et al.* 2004)

Based on various structural studies, enzyme-induced nucleotide flipping occurs due to intercalation of one or more enzyme residues into the DNA double helix near the damaged site, which push the target out of the DNA double helix (Kunkel and Wilson 1996; Scharer and Campbell 2009; Jena



Scheme 10. Mechanism of DNA repair by nucleotide flipping. Different steps involved in the damaged base recognition and repair are shown schematically from (**a**) to (**d**). (**a**) Local distortion due to base modification. (**b**) Protein binding and promotion of further distortion. (**c**) Nucleotide flipping out into the active site of protein for repair. (**d**) No access for unwanted nucleotide to enter into the active site of protein.

and Bansal 2011) (figure 1). For example, it has been observed that intercalation of Arg128 into DNA can flip O6-methylguanine (O6-mG) DNA damage out of the DNA double helix into the active site of O6-alkylguanine-DNA alkyl transferase (AGT) for repair (figure 1a). Similarly, it has been observed that intercalation of a wedge of four residues (Pro, Tyr, Ile and Pro) into DNA can also flip hypoxanthine from DNA double helix into the active site of Endonuclease V (EndoV) for repair (Dalhus *et al.* 2009). In contrast to the nucleotide flipping by DNA intercalation, damaged nucleotide extrusion without the involvement of protein intercalation has also been observed in many studies (Yu *et al.* 2006; Parker *et al.* 2007). For example, it has been recently observed that without intercalating into DNA, AlkB protein can flip 1-methyladenine (1-mA) DNA damage out of the DNA double helix by squeezing the DNA at the damaged site (figure 1b) (Yu *et al.* 2006). Similarly, the uracil-DNA glycosylase (UDG)-mediated flipping out of uracil has also been suggested to occur without the direct involvement of protein, rather due to DNA dynamics (Parker *et al.* 2007).

Thus, from these studies it is clear that nucleotide flipping occurs in two steps (Jena and Bansal 2011). In the first step, proteins find their target by sensing DNA distortion near the damaged site that arises due to unusual base pairing involving the damaged nucleotide. DNA–protein binding can facilitate further DNA distortion at the damaged site (Scheme 10b) (Jena and Bansal 2011). In the second step, it may employ a push-pull mechanism involving either DNA intercalation or squeezing at the lesion site, reinforcing the damaged nucleotide to flip into its active site (scheme 10c). Interestingly, it has been inferred from structural studies on model systems that proteins will only allow damaged nucleotides to flip into its active site for further processing by rejecting access of any undamaged nucleotides to enter into its active site (scheme 10d) (Benerjii *et al*. 2005). These studies highlight the fact that in addition to above two-step mechanism, proteins may use a gate-keeping strategy to ensure that only damaged nucleotides are repaired without affecting the normal ones (Scheme 10) (Benerjii *et al*. 2005). These mechanisms of nucleotide flipping have been demonstrated to be kinetically and thermodynamically favoured (Jena and Bansal 2011) (Benerjii *et al*. 2005; Hu *et al.* 2008), indicating that this mechanism of damage repair might be operational in cellular DNA.

6. Conclusion

As elaborated, reactive species can damage DNA in a variety of ways. Among several DNA lesions, guanine lesion is the most abundant. This is due to the fact that guanine has the least oxidation potential and hence can be easily modified by reactive species. Guanine lesions arising due to its oxidation, nitration, halogenation and alkylation are mutagenic. Other than inducing mutation, guanine modifications can also promote DNA strand breaks and DNA–protein crosslinks, which are not only mutagenic but can also inhibit replication and transcription. Although guanine lesions are lethal, certain enzymes can repair them by adopting different mechanisms depending on the structure of the lesion and its effect on the DNA. Nucleotide flipping is the initial stage of DNA repair in which the damaged nucleotide is flipped away from the DNA double helix into the active site of the protein for further processing.

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