Nucleic Acids Research, 2003, Vol. 31, No. 2 **517–531**
DOI: 10.1093/nar/gkg137

SURVEY AND SUMMARY

Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: approaches using synthetic oligonucleotides and nucleotides

Hiroyuki Kamiya*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Received August 30, 2002; Revised and Accepted November 6, 2002

ABSTRACT

DNA and DNA precursors (deoxyribonucleotides) suffer damage by reactive oxygen/nitrogen species. They are important mutagens for organisms, due to their endogenous formation. Damaged DNA and nucleotides cause alterations of the genetic information by the mispairing properties of the damaged bases, such as 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) and 2-hydroxyadenine. Here, the author reviews the mutagenic potentials of damaged bases in DNA and of damaged DNA precursors formed by reactive oxygen/nitrogen species, focusing on the results obtained with synthetic oligonucleotides and 2′-deoxyribonucleoside 5′-triphosphates.

INTRODUCTION

DNA is the genetic material, which consists of base, sugar and phosphate moieties. The genetic information is maintained as the alignment of bases. Since DNA bases suffer damage by the chemical attacks, the genetic information is changed by the mispairing properties of the damaged bases thus formed. DNA is modified by many mutagens, such as reactive oxygen species (ROS), ultraviolet, X- and γ-rays and alkylating agents. ROS are recognized as very important sources of mutations and are involved in mutagenesis, carcinogenesis, aging and neurodegeneration (1–3), due to their endogenous formation in addition to their formation by many environmental mutagens and carcinogens.

When ROS react with DNA, various kinds of DNA lesions with modified base and/or sugar moieties are produced (Fig. 1) (4,5). 8-Hydroxyguanine (8-OH-Gua, 7,8-dihydro-8-oxoguanine) (6,7) is believed to be an important lesion because of its mutagenicity in bacterial and mammalian cells, as described below. However, recent studies have suggested that other oxidative DNA lesions may be as important as 8-OH-Gua. Moreover, oxidative lesions formed in the DNA precursors are also considered to be important. The oxidative damage in DNA and in the nucleotide pool provides a basis for understanding the chemical modifications of nucleic acids that disturb the genetic information. The author now reviews the mutagenic potentials of damaged bases in DNA and of damaged DNA precursors formed by ROS. In addition, the author refers to the damage caused by reactive nitrogen species (RNS). Since the relationship between the structures of DNA damage and their mutational properties is clear, the data provided by experiments with synthetic oligonucleotides and 2′-deoxyribonucleoside 5′-triphosphates will mainly be described in this review.

MUTAGENIC POTENTIALS OF OXIDIZED DNA LESIONS

When DNA is attacked by ROS or RNS, DNA lesions with modified base and/or sugar moieties are produced. The DNA lesions thus formed are removed by cellular enzymes, and subsequent enzymatic processes restore the original nucleotide sequences (DNA repair). However, when DNA replication occurs before the repair, the DNA polymerase(s) incorporate a 2′-deoxyribonucleoside 5′-triphosphate opposite the lesions. This translesion synthesis (TLS) is carried out by the replicative DNA polymerase (pol) in some cases, and seems to be conducted by specialized DNA polymerases (see below) in other cases, probably depending on the nature of the lesions. If DNA synthesis is completely blocked by the lesion, then it is lethal. Highly mutagenic lesions appear to block DNA synthesis incompletely, and frequently form base pairs with incorrect nucleotides. *In vitro* DNA synthesis experiments provide valuable information regarding these points, although they are affected by the types of DNA polymerases tested. Site-directed mutagenesis experiments using living cells provide the mutation frequency (MF), which involves the efficiency of DNA repair in addition to these points.

*To whom correspondence should be addressed. Tel: +81 11 706 3733; Fax: +81 11 706 4879; Email: hirokam@pharm.hokudai.ac.jp

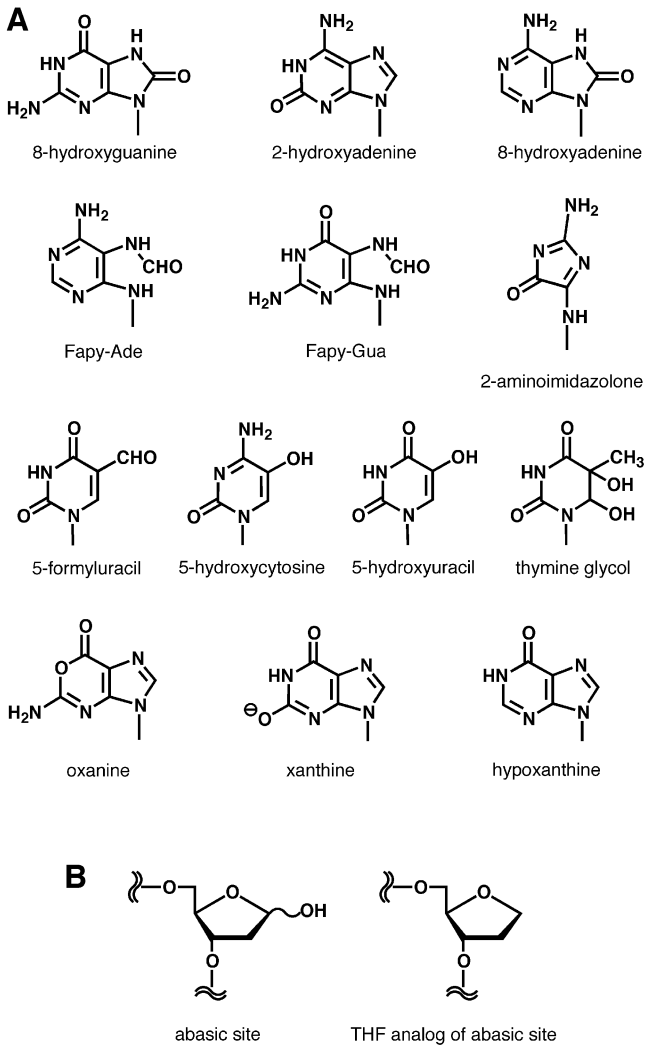

Figure 1. Structures of DNA lesions described in this paper. (**A**) Damaged bases produced by ROS/RNS. Only the base moiety is shown. Oxanine is the base for deoxyoxanosine 5′-triphosphate. (**B**) A true abasic site and its analog.

8-Hydroxyguanine

8-OH-Gua is now recognized as one of the most important DNA lesions, because of its prevalence in DNA and its mutagenicity in bacterial and mammalian cells, as described below. In addition, this compound is a useful marker for DNA oxidation (6,7), since it can be sensitively detected by a high-performance liquid chromatography system equipped with an electrochemical detector (HPLC-ECD). Elevation of 8-OH-Gua content in DNA has been reported in many experiments with cultured cells and animals that were exposed to 'oxidative stress' (6,7).

In vitro DNA synthesis experiments with synthetic templates containing 8-OH-Gua have been carried out. Shibutani *et al.* observed that dCMP and dAMP were incorporated opposite 8-OH-Gua with various misincorporation ratios, depending on the polymerases used [the mammalian DNA polymerases α, β and δ, and *Escherichia coli* pol I and the exonuclease-proficient Klenow fragment (KF^{exo+})] (8). We also examined the miscoding properties of 8-OH-Gua at the sites corresponding to hot spots (5′-GG*C-3′ and 5′-CG*G-3′; G* represents 8-OH-Gua) of the human c-Ha-*ras* protooncogene (9,10). In agreement with the results of Shibutani *et al.*, dCMP and dAMP were incorporated. DNA pol α inserted dAMP exclusively opposite 8-OH-Gua in the 5′-GG*C-3′ sequence, and incorporated dCMP in addition to dAMP opposite 8-OH-Gua in the 5′-CG*G-3′ sequence. The influence of the neighboring sequences around 8-OH-Gua on the efficiency of nucleotide misincorporation was also observed in the case of *Taq* DNA pol (9,10). Efrati *et al.* reported that the incorporation of dAMP opposite 8-OH-Gua was slightly favored over that of dCMP, depending on the 'downstream' sequence context, when human DNA pol β was used (11). Interestingly, they observed a significant increase in dCMP·A and dGMP·A mispairs at the 'upstream' 3′-template site adjacent to the lesion. Errors at these undamaged template sites occurred in four sequence contexts with both gapped and primed single-stranded (ss) DNA templates, but not with pol α instead of pol β (11). Steady-state and pre-steady-state analyses of nucleotide insertion and extension at 8-OH-Gua by several DNA polymerases were reported by Guengerich's group (12–14).

Table 1. Mutational properties of 8-OH-Gua in *E.coli*

Sequence (5′→3′)[a]	ds/ss	MF (%)	Mutations detected[b]	Reference
AG*C	ss	0.7 (G→T)	G→T (21), G→C[c]	15
GG*A	ds[d]	0.8	G→T (22), G→A (1)	16
CG*T	ss	2.2	G→T (10)	17
CG*C	ds	0.1 (G→T)	G→T[e]	18

[a]G* represents 8-OH-Gua.
[b]Number of colonies is shown in parentheses.
[c]It is unclear whether this G→C mutation was induced by 8-OH-Gua, because the same mutation was observed in the case of the unmodified control DNA and the data were not represented in detail.
[d]Approximately 10% of the DNA did not contain 8-OH-Gua, and the complementary strand without 8-OH-Gua contained U residues.
[e]Other mutations were undetectable.

The mutagenic potentials of 8-OH-Gua in *E.coli* have been reported by several groups (15–18) using ss/double-stranded (ds) vectors (Table 1). They observed that 8-OH-Gua elicited G→T transversions with MFs of 0.7–2.2% (ss) and 0.1–0.8% (ds). The MF was increased when a *mutY* or *mutM*/*mutY*, but not a *mutM*, strain was used (17,18).

Several groups have reported the mutagenic potentials of 8-OH-Gua in mammalian cells (Table 2). In 1992 and 1995, we described the mutations induced by 8-OH-Gua in hot spots of the human c-Ha-*ras* protooncogene in mouse NIH3T3 cells (19,20). 8-OH-Gua induced various types of mutations, including the targeted G→T and G→A substitutions and the semi-targeted mutations at the 5′-flanking position in the 5′-GG*C-3′ sequence, and elicited exclusively G→T transversions in the other contexts (Table 2). These results suggest that the neighboring sequences could affect the mutational properties of 8-OH-Gua in living cells. The MFs of 8-OH-Gua were calculated as ~1% (19,20). 8-OH-Gua in ss vectors elicited G→T transversions, with a MF of 4.0–6.8% in COS-7 cells (21–23). In addition to G→T transversions, G→A, G→C and semi-targeted mutations at the 5′-flanking positions were

Table 2. Mutational properties of 8-OH-Gua in mammalian cells

Sequence (5′→3′)[a]	ds/ss	Host	MF (%)	Mutations detected G→T	G→A	G→C	Others	Reference
GG*C	ds	NIH3T3	~1.4[b]	8	3	1	2[c]	19
GG*C	ds	NIH3T3	~1.7[b]	22	8	1	11[d]	20
CG*G	ds	NIH3T3	~0.6[b]	14	1	0	0	20
TG*G	ds	NIH3T3	~1.0[b]	35	0[e]	0[e]	5[f]	20
CG*T	ss	COS-7	4.2	15[g]	0	1	2[h]	21
CG*G	ss	COS-7	4.0	12	0	0	0	22
GG*C	ss	COS-7	4.0	12	0	0	1[i]	22
TG*G	ss	COS-7	5.2	10	1	2	0	23
GG*C	ss	COS-7	6.8	22	3	0	3[j]	23

[a]G* represents 8-OH-Gua.
[b]MF is calculated on the focus formation relative to that of activated c-Ha-*ras* genes.
[c]Two mutants contained a mutation at a flanking site.
[d]Eleven mutants contained a mutation at a flanking site.
[e]Mutations at this position do not activate the c-Ha-*ras* gene. Mutants may fail to be selected due to lack of focus-formation.
[f]Five mutants contained a mutation at a flanking site.
[g]One mutant contained a double mutation (CG*T→ATT).
[h]Two mutants contained a mutation at a flanking site.
[i]The mutant contained a deletion.
[j]The mutants contained a mutation at a flanking site.

detected. The mutagenic potential of 8-OH-Gua in mammalian cells may depend upon the sequence context, the host cells and/or whether ss/ds vectors were used for the experiments.

Synthetic oligonucleotides containing 8-OH-Gua near the 5′-end, mimicking the nucleotide incorporation step, were designed to examine their thermodynamic stabilities (24). We introduced the oxidized guanine into the sites corresponding to hot spots of the human c-Ha-*ras* gene. In duplexes with various bases at the 3′-end, located opposite 8-OH-Gua, remarkably high stability (a small ΔG^0 value) was found in the case of the 8-OH-Gua·T pair in the 5′-GG*C-3′ sequence, while no stabilization was observed with the 8-OH-GuaT pair in the other context. These thermodynamic stabilities may be related to the mutation spectra obtained in our study using NIH3T3 cells (19,20; and see above). On the other hand, the thermodynamic stabilities did not correlate with the mutation spectra when duplexes with a central base pair involving 8-OH-Gua were examined (24). Plum *et al.* measured the thermodynamic stabilities of duplexes containing a base pair involving 8-OH-Gua in the center (25). In agreement with our results, they observed a lack of correlation between the thermodynamic stability of the 'base pairs' and the nucleotides incorporated opposite 8-OH-Gua. Thus, the stability of duplexes containing a modified base at the terminus rather than in the center could be a good parameter for nucleotide insertion.

Synthetic oligonucleotides containing 8-OH-Gua were used for a structural analysis of the base pairs involving 8-OH-Gua. Figure 2A shows the hydrogen-bonding patterns of 8-OH-Gua·C and 8-OH-Gua·A pairs. It is known that 8-hydroxyguanosine takes the *syn*-conformation (26). An 8-OH-Gua residue in DNA adopted both the *syn*- and *anti*-orientations, depending on the opposite base (27,28). 8-OH-Gua pairs with C in the Watson–Crick geometry, and pairs with A in the Hoogsteen geometry by adopting the *syn*-conformation. The positions of the O^6-, 7*H*- and O^8-atoms of the *syn*-oriented 8-OH-Gua are similar to those of the O^4-, 3*H*- and O^2-atoms of T, which is one of the reasons for the frequent misincorporation of dAMP opposite 8-OH-Gua.

2-Hydroxyadenine

2-Hydroxyadenine (2-OH-Ade, 1,2-dihydro-2-oxoadenine) is another type of DNA lesion produced by ROS. The yields of 2-OH-Ade are similar to those of 8-OH-Gua in the monomeric form, although its formation in DNA is less efficient (29,30). Thus, the incorporation of 2-hydroxy-dAMP (2-OH-dAMP) from the nucleotide pool appears to be the major pathway for the formation of 2-OH-Ade in DNA. It was reported that the treatment of cultured human cells with H_2O_2 induced 2-OH-Ade accumulation in DNA (one-fifth of that of 8-OH-Gua) (31). 2-OH-Ade is more abundant in human cancerous tissues than in non-cancerous tissues (32). Moreover, as shown below, 2-OH-Ade possesses a mutation-inducibility similar to that of 8-OH-Gua in living cells. Thus, 2-OH-Ade seems to be another important form of DNA damage produced by ROS.

To analyze the nucleotide misincorporation induced by 2-OH-Ade in DNA, we used 12 synthetic oligonucleotides containing 2-OH-Ade with different 5′- and 3′-flanking bases as DNA templates for *in vitro* DNA synthesis (Table 3) (33,34). DNA polymerases inserted various nucleotides opposite 2-OH-Ade in the presence of a single dNTP, depending upon the sequence contexts and the DNA polymerases used. The two mammalian DNA polymerases incorporated dCMP most frequently among the 'incorrect' nucleotides, while dGMP was inserted more efficiently than dCMP and dAMP by KF^{exo-}. Interestingly, the three DNA polymerases inserted dAMP opposite 2-OH-Ade in the 5′-TA*A-3′ (A* denotes 2-OH-Ade) sequence. This phenomenon was observed using two different templates containing the 5′-TA*A-3′ sequence. This conclusion was confirmed by a comparison of the full-length products obtained by *in vitro* DNA synthesis in the presence of the four nucleotides (33,34).

The mutations induced by 2-OH-Ade in *E.coli* and simian COS-7 cells were analyzed using a shuttle vector (35,36)

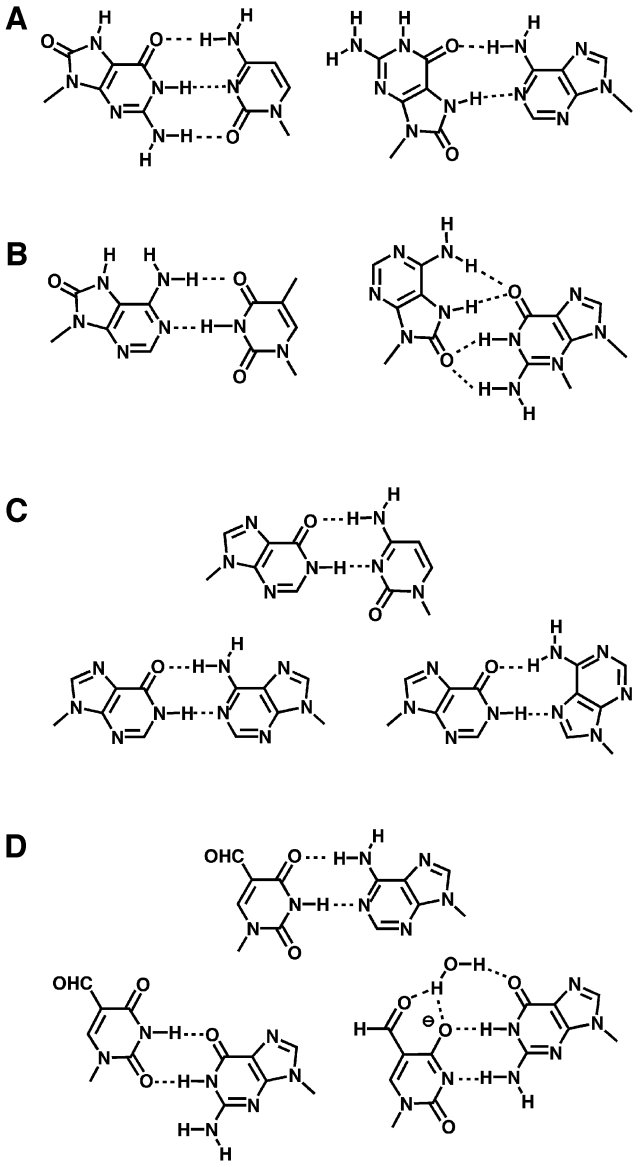

Figure 2. Hydrogen-bonding patterns of (**A**) 8-OH-Gua·C and 8-OH-Gua·A pairs, (**B**) 8-OH-Ade·T and 8-OH-Ade·G pairs, (**C**) Hyp·C and Hyp·A pairs and (**D**) 5-CHO-Ura·A and 5-CHO-Ura·G pairs.

(Table 4). The oxidized adenine was incorporated into 5′-GA*C-3′ and 5′-TA*A-3′ sequences, based on the results of the *in vitro* experiments. In addition, the differences in the mutagenic properties of 2-OH-Ade in the lagging and leading template strands were studied. The frequency and the spectrum of the induced mutations depended on the sequence contexts and the lagging/leading synthesis. The MFs observed in these experiments were 0.1–0.8%, which are comparable with the MFs of 8-OH-Gua in ds DNA in NIH3T3 cells (19,20). Therefore, 2-OH-Ade in DNA is as mutagenic as 8-OH-Gua. The observed –1 deletions at the 5′-GA*C-3′ and 5′-TA*A-3′ sites can be explained by the incorporation of dCMP and dAMP, respectively, opposite the 2-OH-Ade residue, and the subsequent formation of loop-out intermediates (35). These results suggest the formation of the 2-OH-Ade·C and 2-OH-Ade·A pairs in living cells.

Table 3. Sequence dependent incorporation of nucleotides opposite 2-OH-Ade *in vitro*

Sequence of template (5′→3′)[a]	DNA polymerase		
	pol α	pol β	KF^{exo-}
GCGA*ATATTCCGTCAT	T>C>A~G	T>C	T>A>G>C
GCCA*ATATTCCGTCAT	T>G>C>A	T>G>C	T>G>A>C
GCTA*ATATTCCGTCAT	T>A	A>T	T>A>G
GCAA*ATATTCCGTCAT	T>C~A~G	T>C	T>G~A>C
GCGA*CTATTCCGTCAT	T>C>A	T>C	T>G>A~C
GCTA*CTATTCCGTCAT	T>A~C	T>C	T>G>A>C
GCGA*GTATTCCGTCAT	T>C	T>C>A~G	T>G>A>C
GCTA*GTATTCCGTCAT	T>C	T>C>A	T>G>A>C
GCGA*TTATTCCGTCAT	T>C~G~A	T>C>G	T>G>A~C
GCTA*TTATTCCGTCAT	T>C>G	T>C>G	T>G>C>A
CGTGA*CATTTCTGAT	T>C>A	C>T	T>C
CGTTA*AATTTCTGAT	T>A	A>T	T>A>G

[a]A* represents 2-OH-Ade.

Synthetic oligonucleotides containing 2-OH-Ade near the 5′-end, mimicking the nucleotide incorporation step, were designed to examine their thermodynamic stabilities (37). The order of stability was T > G > C >> A in the 5′-GA*C-3′ sequence and T > A > C > G in the 5′-TA*A-3′ sequence. Since T, G and C, and T and A are the nucleotides incorporated opposite 2-OH-Ade in the 5′-GA*C-3′ and 5′-TA*A-3′ sequences, respectively, *in vitro* (33,34; see above), these results agree with the miscoding properties of 2-OH-Ade residues. A stable 2-OH-Ade·A pair was formed for the 5′-TA*A-3′ sequence, but not for the 5′-GA*C-3′ sequence, in molecular modeling (37).

Since no structural information concerning 'incorrect' base pairs involving 2-OH-Ade is available, the reasons why 2-OH-Ade is mutagenic remain to be resolved. The presence of the 2-hydroxy and 1,2-dihydro-2-oxo tautomers, and the possible presence of the *syn* and *anti* conformers, may lead to various types of base pairs involving 2-OH-Ade (38).

8-Hydroxyadenine

8-Hydroxyadenine (8-OH-Ade, 7,8-dihydro-8-oxoadenine) is an isomer of 2-OH-Ade. *In vitro*, 8-OH-Ade is not formed by Fenton-type oxidation, but by γ-ray irradiation (30). It was reported that 8-OH-Ade was formed in DNA by γ-irradiation of mice (39). Moreover, 8-OH-Ade is present in the neoplastic liver of fish (40) and in human cancerous tissues (32).

Shibutani *et al.* carried out *in vitro* DNA synthesis reactions catalyzed by various DNA polymerases, using synthetic templates with 8-OH-Ade, and observed the almost exclusive incorporation of dTMP opposite the base in the presence of the four nucleotides (41). In contrast, misincorporations of dGMP (pol α, pol β and KF^{exo+}) and dAMP (KF^{exo+}) opposite 8-OH-Ade were found in the presence of a single nucleotide (41). Guschlbauer *et al.* sequenced the PCR product obtained with a template containing 8-OH-Ade by the Maxam–Gilbert procedure, and found that the majority of the product contained A at the 8-OH-Ade position (42). We analyzed the nucleotides incorporated opposite 8-OH-Ade by pol α, pol β, KF^{exo+} and *Taq* DNA pol in the presence of the four nucleotides (43). In contrast to the above results, the mammalian DNA polymerases inserted incorrect nucleotides opposite 8-OH-Ade, although the prokaryotic polymerases, KF^{exo+} and *Taq* pol,

Table 4. Mutational properties of 2-OH-Ade in *E.coli* and COS-7 cells

Sequence (5′→3′)[a]	ds/ss	Host	Lagging/Leading	MF (%)	Mutations detected A→G	A→T	A→C	ΔA
GA*C	ss	*E.coli*	NA[b]	0.67	5	0	0	23
TA*A	ss	*E.coli*	NA[b]	0.06	5	0	8	18
GA*C	ds	*E.coli*	Lagging	0.77	6	0	1	20
TA*A	ds	*E.coli*	Lagging	0.05	2	0	2	10
GA*C	ds	*E.coli*	Leading	0.20	14	0	0	5
TA*A	ds	*E.coli*	Leading	0.07	3	9	1	4
GA*C	ds	COS-7	Lagging	0.60	3	0	0	21
TA*A	ds	COS-7	Lagging	0.09	6	1	0	6
GA*C	ds	COS-7	Leading	0.10	4	3	0	7
TA*A	ds	COS-7	Leading	0.11	10	5	0	0

[a]A* represents 2-OH-Ade.
[b]Not applicable.

exclusively inserted dTMP. Mammalian pol α misincorporated dGMP in addition to dTMP, and pol β inserted dTMP, dGMP and dAMP.

Wood *et al*. constructed a ss viral genome containing 8-OH-Ade (44). The mutagenic potential of 8-OH-Ade in *E.coli* was at least one order of magnitude less than that of 8-OH-Gua. To examine the mutagenic properties of 8-OH-Ade in mammalian cells, we introduced 8-OH-Ade into the second position of codon 61 of the human c-Ha-*ras* gene (43) and observed that the modified base induced targeted A→G and A→C substitutions. Since focus-formation was used as the selection pressure, only a portion of the A→C mutants might be detected (43). One clone contained a mutation at the 5′-flanking position of 8-OH-Ade. Tan *et al*. constructed ss phagemid DNAs containing 8-OH-Ade and transfected them into COS-7 cells (23). An 8-OH-Ade in the 5′-TA*G-3′ (A* represents 8-OH-Ade) sequence elicited an A→C transversion with an MF of 1.2%. On the other hand, 8-OH-Ade in the 5′-GA*C-3′ sequence induced an A→G transition with an MF of 0.24% (one mutant). They observed one mutant with a mutation at the 5′-flanking site.

Synthetic oligonucleotides containing 8-OH-Ade were used for structural analyses of the base pairs involving 8-OH-Ade (42,45). Figure 2B shows the hydrogen-bonding patterns of 8-OH-Ade·T and 8-OH-Ade·G pairs. It is known that 8-hydroxyadenosine takes the *syn*-conformation (26). An 8-OH-Ade residue in DNA adopted both the *syn*- and *anti*-orientations, depending on the opposite base. 8-OH-Ade paired with T in the Watson–Crick geometry, and paired with G in the two reverse three-center hydrogen-bonding system by adopting the *syn*-conformation. Since 8-OH-Ade·G pairs minimally disturb the B-DNA backbone structure (45), the formation of this base pair may cause the misincorporation of dGMP opposite 8-OH-Ade, as observed *in vitro* and in living cells.

Formamidopyrimidines

Formamidopyrimidine (Fapy) lesions, imidazole ring opened purines, are produced by oxidative stress. It was reported that the treatment of cultured human cells with H_2O_2 induced Fapy lesions in DNA (31). They are more abundant in human cancerous tissues than in non-cancerous tissues (32).

Asagoshi *et al.* recently prepared an oligonucleotide template containing a 5-*N*-methylated analog of Fapy-Gua (methyl-Fapy-Gua) and used it in *in vitro* DNA synthesis (46). Methyl-Fapy-Gua constituted a fairly strong, but not absolute, block to DNA synthesis catalyzed by KF^{exo-}, and permitted TLS with limited efficiency. When methyl-Fapy-Gua was bypassed, dCMP was preferentially inserted opposite the lesion. Thus, they concluded that methyl-Fapy-Gua is potentially lethal but not pre-mutagenic.

Tudek *et al.* transfected phage DNA modified with dimethylsulfate and NaOH into SOS-induced *E.coli*, and compared the mutation spectra in the *lacZ*α gene with and without the selective elimination of damaged bases (47). Partial elimination of the methyl-Fapy-Gua from the phage DNA by Fpg digestion resulted in a 2–3-fold decrease in G→T and G→C transversions. Selective depurination of methylated bases (37°C, 9 h, pH 7.0), which resulted in an almost complete loss of 7-methyl-Ade, caused a 9-fold decrease in A→G transitions. They concluded that methyl-Fapy-Ade induced A→G transitions, and that methyl-Fapy-Gua was at least an order of magnitude less mutagenic and elicited G→T and G→C transversions. However, the methyl substituent may affect the hydrogen-bonding, and the response of DNA polymerases to the methylated and unmethylated Fapy lesions may be very different. The recent chemical syntheses of oligonucleotides containing Fapy-Gua and Fapy-Ade (48,49) will provide clearer results.

2-Aminoimidazolone

The formation of 2-aminoimidazolone (Iz) was observed by Mn-TMPyP/$KHSO_5$ and γ-irradiation treatments of dGuo and its derivative, respectively (50,51). In addition, photo-oxidation by riboflavin plus light of an oligodeoxyribonucleotide yielded Iz from G (52).

Kino and Sugiyama carried out an *in vitro* DNA synthesis, using an oligonucleotide template with Iz (53). DNA pol I and KF^{exo-} incorporated dGMP opposite Iz. They mentioned that 98% of the template remained under the conditions used. Thus, this result suggests that Iz causes G·C→C·G transversion mutations, which are often found in ROS-treated DNA. They proposed that the base-pairing pattern of Iz·G resembles the Watson–Crick pairing of C·G (52).

Xanthine

Xanthine (Xan), the deaminated product of guanine, is formed by nitric oxide (NO) treatment of intact human cells and of aerobic solutions of DNA (54).

Eritja *et al.* reported that deoxyribonucleotides were incorporated at the site opposite Xan in the order of dTMP > dCMP >> dAMP and dGMP *in vitro* by *Drosophila* DNA pol α in the presence of a single nucleotide (55). We analyzed the nucleotides incorporated opposite Xan in the presence of the four substrates, and found that mouse pol α inserted dTMP and dCMP with a similar efficiency (9). In contrast, rat pol β and *Taq* pol incorporated dCMP almost exclusively.

A synthetic human c-Ha-*ras* gene with Xan at the second position of codon 12 was constructed, and this ds vector was transfected into NIH3T3 cells (56). The Xan was highly mutagenic: the MF was calculated to be 30–50% in comparison with that of an activated c-Ha-*ras* gene. Analyses of the genes from the transformants revealed a G→A transition almost exclusively.

Hypoxanthine

Hypoxanthine (Hyp), the deaminated product of adenine, is formed by NO treatment of intact human cells and of aerobic solutions of DNA (54).

In *in vitro* DNA synthesis experiments using a synthetic template containing Hyp, we observed the incorporation of dCMP opposite Hyp by *Taq* pol, mouse pol α and rat pol β (9). In the case of pol α, dTMP was also inserted, but much less efficiently. Ohtsuka *et al.* annealed a C-tailed oligonucleotide containing Hyp residues with G-tailed plasmid DNA (57). They then treated the annealed DNA with KF^{exo+} and transfected the DNA into *E.coli* after circularization. All of the Hyp positions were substituted with G, indicating the incorporation of dCMP by KF^{exo+}.

Hill-Perkins *et al.* constructed a ds vector containing Hyp and found that Hyp induced A→G mutations with high frequency (MF was 11%) in *E.coli* (58). We replaced the second base of codon 61 of a synthetic c-Ha-*ras* gene with a Hyp residue in a site-specific manner (59). Transfection of this gene into NIH3T3 cells resulted in very efficient focus formation, and the MF was calculated to be 38–50%. A→G transition mutations were detected almost exclusively. Only one of the 20 clones analyzed contained an A→T mutation. These results indicate that Hyp is a highly mutagenic lesion.

The thermodynamic parameters of the self-complementary oligonucleotides with 2 bp involving Hyp in the middle were reported (60). The stability was in the order of Hyp·C > Hyp·A > Hyp·G. The duplex containing a Hyp·T pair did not show cooperative melting.

The structures of ds oligodeoxyribonucleotides containing Hyp·C and Hyp·A pairs were determined by NMR (61). Hyp can pair with C in the normal Watson–Crick manner (Fig. 2C). The formation of this base pair in the Watson–Crick geometry, together with its high stability, explains the reason for the misincorporation of dCMP opposite Hyp. On the other hand, the Hyp·A base pair structure determined by NMR, is a Watson–Crick type (61), while the Hyp·A base pair is a Hoogsteen type in crystals (Fig. 2C) (62).

5-Formyluracil

5-Formyluracil (5-CHO-Ura) is formed by the oxidation of the 5-methyl group of a thymine base. Its formation was detected in oxidation reactions (30,63–65).

Ono *et al.* conducted an *in vitro* DNA synthesis using KF^{exo+} (66). They observed that this polymerase incorporated dGMP in addition to dAMP. Zhang *et al.* reported that DNA polymerases (KF^{exo+}, KF^{exo-}, *Tth* DNA pol and *Pfu* DNA pol) inserted dAMP and dCMP opposite 5-CHO-Ura *in vitro*, in a sequence-dependent manner (67). On the other hand, Masaoka *et al.* found that dAMP and dGMP were incorporated opposite 5-CHO-Ura in the template DNA by KF^{exo-} (68).

The mutational properties of 5-CHO-Ura in living cells were reported. Miyabe *et al.* conducted site-specific mutagenesis experiments using a ds vector and *E.coli* as a host (69). They observed that the MF of 5-CHO-Ura was lower than 0.05% in repair-proficient bacterial cells. 5-CHO-Ura appeared to induce substitution and deletion mutations.

We recently reported that 5-CHO-Ura residues were weakly mutagenic, and their MFs in ds plasmid vectors were 0.01–0.04% in COS-7 cells (70). These values are lower, by one order of magnitude, than the MFs of 2-OH-Ade in ds vectors in COS-7 cells and of 8-OH-Gua on a chromosome in NIH3T3 cells (19,20,36). The T→G and T→A transversions during lagging and leading strand syntheses, respectively, were the mutations found most frequently. This result suggests the formation of 5-CHO-Ura·C and 5-CHO-Ura·T base pairs.

Recently, the structures of oligonucleotides containing 5-CHO-Ura·A and 5-CHO-Ura·G base pairs were reported (71,72). The latter pairs did not adopt the Watson–Crick geometry (Fig. 2D) and this may be one reason for the infrequent misincorporation of dGMP opposite 5-CHO-Ura. Although the actual mechanisms of the induction of the T→G and T→A transversions are unknown, we hypothesized that these mutations might be explained by the participation of the formyl group in hydrogen-bonding when 5-fU adopts the *syn*-conformation (70).

5-Hydroxypyrimidines

5-Hydroxycytosine (5-OH-Cyt) and 5-hydroxyuracil (5-OH-Ura) are formed by the oxidation of cytosine, and the formation of the latter involves the deamination of cytosine glycol (73). Their formations were observed *in vitro* and in human tissues (30–32,65).

Purmal *et al.* prepared oligonucleotides containing 5-OH-Cyt or 5-OH-Ura in two different sequence contexts, and used them as template DNAs for *in vitro* DNA synthesis conducted by KF^{exo+} (74). In one sequence context, dGMP and dAMP were incorporated opposite 5-OH-Cyt, and dAMP was the principal nucleotide incorporated opposite 5-OH-Ura. In a second sequence context, dCMP was the predominant base incorporated opposite 5-OH-Cyt. In that same sequence context, dCMP was also the predominant nucleotide incorporated opposite 5-OH-Ura. The data suggest that 5-OH-Cyt and 5-OH-Ura have the potential to be pre-mutagenic lesions leading to C→T transitions and C→G transversions.

Kreutzer and Essigmann investigated the mutagenicity of 5-OH-Cyt and 5-OH-Ura in ss DNA in *E.coli* (75). The MF of 5-OH-Cyt was low (0.05%), and 5-OH-Cyt elicited C→T transitions, and to a much lesser extent, C→G transversions. In contrast, as expected, 5-OH-Ura elicited C→T mutations with high frequency (83%).

cis-Thymine glycol

cis-Thymine glycol (Tg) was detected in γ-irradiated DNA and in DNA isolated from γ-irradiated human cells (76,77). Clark

and Beardsley reported that Tg in the template strand strongly blocked synthesis in *in vitro* DNA pol reactions (78). The bypass of Tg by KF^{exo+} was sequence context-dependent, and the 3′–5′ exonuclease activity of the DNA polymerase affected the bypass efficiency. McNulty *et al.* demonstrated that Tg blocked DNA synthesis when human pol β, viral reverse transcriptase, and Sequenase were used (79). TLS was achieved only when KF^{exo-} was added into the reaction mixtures. In this case, the incorporations of dAMP and dGMP opposite Tg were 1700- and 240 000-fold less efficient than the insertion of dAMP opposite T. On the other hand, Purmal *et al.* observed that the insertion of dAMP opposite Tg by KF^{exo-} occurred with 0.2-fold of the efficiency of that opposite T (80). The mutagenicity of Tg in *E.coli* was observed when Tg was present in ss DNA (MF ~0.3%) but not in ds DNA (81). T→C transitions were exclusively generated.

An abasic site and its analogs

DNA residues that lose their bases are called abasic sites. The cleavage of the *N*-glycosyl bond occurs spontaneously, and is enhanced by acid treatment. It was demonstrated that ROS induced significant numbers of AP sites in calf thymus DNA (82). High steady-state levels of AP sites in rat tissues and human liver DNA were also reported (83). An increase in the number of abasic sites was also detected in human cultured cells exposed to H_2O_2 (82). In addition, abasic sites are formed as intermediates during the base-excision repair process conducted by DNA glycosylases, and many oxidized bases are recognized by specific glycosylases. Thus, an abasic site is one type of oxidative DNA damage.

A synthetic abasic site analog, often called a tetrahydrofuran (THF) derivative, was incorporated into synthetic oligonucleotides, which were used as templates in *in vitro* DNA synthesis reactions. Randall *et al.* used *Drosophila* DNA pol α in the reactions, and found that the order of insertion opposite the THF derivative was dAMP >> dGMP > dCMP and dTMP (84). Takeshita *et al.* used viral reverse transcriptase and calf thymus DNA pol α, and found that the former incorporated dAMP opposite the THF derivative with nearly 90% probability, while the latter inserted nucleotides in the order of dAMP (71%) >> dGMP > dTMP > dCMP (85). We carried out *in vitro* DNA synthesis reactions conducted with mouse pol α and *Taq* DNA pol, using a THF analog, and found that dAMP was almost exclusively incorporated (86). Shibutani *et al.* compared nucleotide incorporations opposite a THF derivative and a natural abasic site, in the presence of the four nucleotides (87). They observed that dAMP was incorporated by KF^{exo-} and calf thymus DNA pol α opposite the two types of abasic sites, and that deletions were also induced, depending on the sequence contexts. Interestingly, when human pol β was used in *in vitro* DNA synthesis, the insertion of a nucleotide complementary to the 5′-adjacent base opposite the THF analog was observed (88).

Lawrence *et al.* constructed ss DNA containing a natural abasic site (89). Replication of the modified DNA in SOS-uninduced and -induced *E.coli* cells occurred with 0.3–0.6% and 5.0–6.9% efficiencies, respectively. The positions where abasic sites had been incorporated were primarily converted to T (~50 and 80%) followed by C and A in SOS-induced cells, suggesting the predominant insertion of dAMP. The same research group prepared gapped-heteroduplexes containing a natural abasic site and transfected them into *Saccharomyces cerevisiae* (90). They observed that dCMP was preferentially (62–85%) inserted opposite an abasic site in yeast, in contrast to the situation in *E.coli*. At present, this discrepancy is interpreted to be due to the differences in the specialized DNA polymerases that bypass abasic sites: yeast Rev1 and *E.coli* DNA pol V (91–93).

We constructed synthetic c-Ha-*ras* genes with a THF analog in the first or the second position of codon 12, and transfected them into NIH3T3 cells (86). The MFs of the analog were calculated as ~10%. The c-Ha-*ras* genes present in the transformants predominantly contained a point mutation to A in the modified position. In addition, point mutations in the adjacent position were also detected. These results indicate that dTMP was mainly incorporated into the sites opposite the abasic site analog (except for dCMP), and that incorrect deoxynucleotides were incorporated in the position adjacent to the abasic site analog. We also obtained similar results with a true abasic site, but the flanking mutations were detected more frequently (94). Gentil *et al.* constructed ds vectors containing a natural abasic site and transfected them into simian COS-7 cells (95,96). The MF was 0.6–2.6%. They observed that the mutation spectra of the abasic sites were dependent on the 'complementary' base of the abasic site, and that, on average, all of the nucleotides appeared to be incorporated. They also reported that the four nucleotides were inserted with a similar frequency when ss DNA containing a natural abasic site was transfected (97). The detailed mechanism of the 'random' mutagenesis induced by an abasic site is unknown and may be related to the involvement of different specialized DNA polymerases in mammalian cells. Table 5 summarizes the mutational properties of abasic sites and their analogs in mammalian cells.

Ide *et al.* measured the thermodynamic stability of model template–primer DNA duplexes containing a THF derivative at their 5′-termini (98). They observed that the duplexes containing purine bases opposite the abasic site exhibited higher stability than those with pyrimidine bases. Gelfand *et al.* incorporated a THF analog into the center of 13mer DNA duplexes, in which the base opposite the lesion (A, C, G or T) and the base pairs neighboring the lesion (C·G or G·C) were systematically varied (99). They concluded that the thermodynamic impact of the lesion was dominated by the identity of the neighboring base pairs, with the cross-strand partner base exerting only a secondary thermodynamic effect on the duplex properties.

Other forms of damage

Oxidation of 5-methylcytosine leads to the formation of 5-formylcytosine (5-CHO-Cyt) (100). The relative misincorporation frequency opposite 5-CHO-Cyt was similar to that opposite 5-methylcytosine during *in vitro* DNA synthesis conducted by KF^{exo-} (101). In contrast, we found that 5-CHO-Cyt was mutagenic, and its MF in ds vectors was 0.03–0.28% in mammalian cells (102). The mutation spectrum of 5-CHO-Cyt was broad, and included targeted (5-CHO-Cyt→G, 5-CHO-Cyt→A and 5-CHO-Cyt→T) and untargeted mutations.

In vitro, KF^{exo-} incorporated dAMP opposite uracil glycol, oxidized deaminated cytosine, suggesting that this modified base is highly mutagenic (80). In accordance with this result,

Table 5. Mutational properties of abasic sites in mammalian cells

Sequence (5′→3′)[a]	Abasic site	ds/ss	Host	MF (%)	Mutations detected X→A	X→G	X→C	X→T	Others	Reference
CXT	Natural	ds[b]	COS-7	0.80	10	6	NA[c]	11		95
CXT	Natural	ds[d]	COS-7	0.95	15	NA[c]	4	24		96
CXT	Natural	ds[e]	COS-7	0.61	NA[c]	4	3	4		96
CXT	Natural	ds[f]	COS-7	2.6	15	24	6	NA[c]		96
AXT	Natural	ss	COS-7	NA[c]	43	59	50	62		97
CXG	THF	ds[d]	NIH3T3	~7.2[g]	12	NA[c]	2	3	13[h]	86
GXC	THF	ds[d]	NIH3T3	~13[g]	16	NA[c]	0	4	5[i]	86
CXG	Natural	ds[d]	NIH3T3	~7.5[g]	4	NA[c]	0	1	8[j]	94
GXC	Natural	ds[d]	NIH3T3	~5.8[g]	5	NA[c]	0	2	9[k]	94

[a]X represents an abasic site.
[b]Complementary base is G.
[c]Not applicable.
[d]Complementary base is C.
[e]Complementary base is T.
[f]Complementary base is A.
[g]MF is calculated on focus formation relative to that of activated c-Ha-*ras* genes.
[h]Mutations of the 3′-flanking G position.
[i]Mutations of the 5′-flanking G position.
[j]Mutations of the 3′-flanking G position.
[k]Mutations of the 5′-flanking G position.

Kreutzer and Essigmann showed that the mutagenicity of uracil glycol in ss DNA in *E.coli* was high (MF ~80%) and that a C→T transition was elicited (75).

Henderson *et al.* constructed three ss DNAs containing a secondary oxidation product of 8-OH-Gua (oxaluric acid, oxazalone or cyanuric acid) (103). All three lesions were readily bypassed in *E.coli*, causing G→T transversions with frequencies of 86–97%. This result suggests that the G→T transversions observed in ROS-treated DNA may be elicited by these highly mutagenic lesions, rather than by the direct action of 8-OH-Gua.

MUTATGENIC POTENTIALS OF OXIDIZED DNA PRECURSORS

The fact that an *E.coli* mutator gene (*mutT*) encodes a hydrolyzing enzyme for 8-OH-dGTP indicates the incorporation of this oxidized nucleotide by DNA polymerases and its importance as a source of mutations (104). Tajiri *et al.* reported that the incorporation of 8-hydroxy-dGMP (8-OH-dGMP) from the nucleotide pool contributed almost equally to the direct oxidation of G bases in DNA and to the accumulation of 8-OH-Gua in DNA (105). In addition, the fact that the damaged nucleotides incorporated into bacterial cells elicit chromosomal gene mutations (see below) provides direct evidence that damaged DNA precursors act as mutagens. It is noteworthy that damaged DNA precursors appear to exert few biological effects if they are not incorporated into DNA by DNA polymerases. Since a damaged nucleotide 'competes' with the normal nucleotides and much larger amounts of normal nucleotides exist in cells, its incorporation efficiency is very important. Thus, determining the relative incorporation frequency in comparison with the normal pairing event is necessary in *in vitro* DNA synthesis experiments, in addition to the ratio of 'incorrect' to 'correct' insertions. In contrast, the DNA damage within DNA chains, once formed, does not 'compete' with the normal bases. The other important point is that a damaged precursor acts as a mutagen when the template base opposite which the damaged nucleotide is incorporated and the nucleobase incorporated opposite the damaged base in the DNA during the next round of replication are different. dUTP, formed from dCTP, does not appear to be mutagenic because dUMP is exclusively inserted opposite A, and dAMP is exclusively incorporated opposite U.

At present, the actual concentrations of oxidized DNA precursors are unknown. Shen *et al.* reported that the cytosolic 8-OH-Gua pool is increased by the exposure of mammalian cells to HClO and hypoxia (106). Since they used HPLC-ECD after formic acid treatment, they measured 8-OH-Gua in all monomers, including nucleosides, mono-, di- and tri-phosphate derivatives. Recently, Tassotto and Mathews tried to measure 8-OH-dGTP in *E.coli* by HPLC-ECD, and concluded that its intracellular concentration is below 0.34 μM (the limit of detection) (107). However, the ratio of 0.34 μM of 8-OH-dGTP to the estimated concentration of dGTP in the bacterium (100 μM) (108) is 3.4×10^{-3}, and this ratio seems to be too high when the *in vivo* situation is considered. Since the mutagenesis by the damaged deoxyribonucleoside triphosphates certainly depends on their concentration, it is important to establish sensitive methods to determine their intracellular amounts.

8-Hydroxy-dGTP

Maki and Sekiguchi examined the incorporation of 8-OH-dGMP by the α (catalytic) subunit of *E.coli* DNA pol III using synthetic templates (104). 8-OH-dGMP was inserted opposite A and C with nearly equal efficiency. The kinetic parameters that they determined indicated that these incorporations were ~30-fold less efficient than those of the normal pairing. Purmal *et al.* carried out a kinetic analysis of the incorporation of 8-OH-dGMP by KF^{exo-}, and found that they were 1400–2300-fold less efficient than those of the normal pairing (109). In

contrast, Einolf *et al.* reported that the incorporations of 8-OH-dGMP by KF^{exo-} were 150 000–350 000-fold less efficient than those of the normal pairing (110). They also determined the kinetic parameters of 8-OH-dGMP incorporation by other DNA polymerases. The misincorporation opposite A depended upon the DNA polymerase, and the ratio of the incorporation of 8-OH-dGMP opposite A to that opposite C varied (0.05–30).

Cheng *et al.* added 8-OH-dGTP to the deoxyribonucleotides used for the gap-filling reaction by KF^{exo-}, and transfected the synthesized DNA into *E.coli* (16). A→C transversions were almost exclusively detected. Similar results were obtained when other DNA polymerases, including the *E.coli* DNA pol III holoenzyme, were used (111,112). Pavlov *et al.* studied the effects of 8-OH-dGTP on the fidelity of replication conducted by a HeLa cell extract (112). The replicated DNA was transfected into *E.coli* and induced A·T→C·G mutations. The induction of A→C and A·T→C·G mutations by 8-OH-dGTP was dependent on the MutY activity: The frequency of these mutations decreased when *mutY* strains were used as host cells (111,112).

The mutagenicity of 8-OH-dGTP *in vivo* was examined by a new evaluation method using *E.coli* as a host (113). This method consists of the direct incorporation of a damaged nucleotide by heat treatment of *E.coli* cells with $CaCl_2$, and the isolation of *lacI*$^-$ and *lacO*^{c} mutants. We treated wild type (wt) *E.coli* W3110 with 8-OH-dGTP, and the frequencies of *lacI*$^-$ and *lacO*^{c} mutations were measured. The treatment with 8-OH-dGTP increased the frequency of substitution mutations, in contrast to the treatment with either dGTP or dATP. The substitution mutation found most frequently in the 8-OH-dGTP-induced mutants was an A·T→C·G transversion. This type of mutation makes up 90% of the substitution mutations.

2-Hydroxy-dATP

We incubated synthetic oligonucleotide templates with mammalian DNA pol α in the presence of 2-hydroxy-dATP (2-OH-dATP), and found the incorporation of 2-OH-dAMP opposite T and C (29). The misincorporation of 2-OH-dAMP opposite C was observed with three different DNA templates. The kinetic parameters indicated that the incorporation of 2-OH-dAMP opposite T was disfavored by a factor of 290 over that of dAMP, and that the insertion of 2-OH-dAMP opposite C was disfavored by a factor of 1300 over that of dGMP. The incorporation of 2-OH-dAMP opposite T was favored only 4.5-fold over that opposite C. In contrast, the *E.coli* DNA pol III α (catalytic) subunit inserted 2-OH-dAMP opposite T and G, but not opposite C (114). This incorporation mode was observed with two different DNA templates. Based on the kinetic parameters obtained, the insertion of 2-OH-dAMP opposite T was disfavored by a factor of 70 over that of dATP. The insertion of 2-OH-dAMP opposite G was 800 times less frequent as compared with that of dCTP. Thus, the insertion of 2-OH-dAMP opposite T was calculated to be favored only 10-fold over that opposite G. Similar *in vitro* DNA synthesis experiments were carried out with KF^{exo-} (114). The insertion of 2-OH-dAMP opposite T was disfavored by a factor of 90 over that of dAMP. The insertion of 2-OH-dAMP opposite G was disfavored by a factor of 20 000 over that of dCMP. The insertion of 2-OH-dAMP opposite T by KF^{exo-} was favored 200-fold over that opposite G.

We added 2-OH-dATP to the deoxyribonucleotides used for the gap-filling reaction by the *E.coli* DNA pol III holoenzyme, and transfected the synthesized DNA into *E.coli* (111). G→T transversions were almost exclusively detected. The mutagenicity of 2-OH-dATP was higher than that of 8-OH-dGTP.

The mutagenicity of 2-OH-dATP in *E.coli* was examined by a new evaluation method as described above (113). When 2-OH-dATP was incorporated into wt *E.coli*, the MF was increased. 2-OH-dATP seemed to be more mutagenic than 8-OH-dGTP. The substitution mutation found most frequently in the 2-OH-dATP-induced mutants was a G·C→T·A transversion (84% of the total substitution mutations). This type of mutation appears to occur by the incorporation of 2-OH-dAMP opposite G residues in DNA by *E.coli* DNA polymerase(s), in agreement with the *in vitro* data. These results imply that the mutagenic potential of 2-OH-dATP is as important as that of 8-OH-dGTP, when present in the nucleotide pool.

2-Aminoimidazolone-2′-deoxyriboside 5′-triphosphate

Kino and Sugiyama examined primer extension in the presence of dGTP, dATP and dTTP, with or without 2-aminoimidazolone-2′-deoxyriboside 5′-triphosphate (53). They reported that the nucleotide was incorporated opposite G instead of dCTP, although no kinetic data were shown.

Deoxyxanthosine 5′-triphosphate and deoxyoxanosine 5′-triphosphate

Suzuki *et al.* examined the incorporation of deoxyxanthosine 5′-monophosphate (dXMP) and deoxyoxanosine 5′-monophosphate (dOMP) by KF^{exo-} (115). They found that dXMP was incorporated opposite C with much less efficiency (15 000-fold) in comparison with the insertion of dGMP opposite C. The insertion of dOMP opposite C was 5000-fold less efficient than that of dGMP opposite C, and the misinsertion of dOMP opposite T occurred with a 2-fold further decreased efficiency.

5-Formyl-dUTP

Terato *et al.* examined the incorporation of 5-formyl-dUMP (5-CHO-dUMP) by KF^{exo-} and observed that the damaged nucleotide was incorporated opposite A and opposite G 2- and 34 500-fold, respectively, less efficiently than the normal pairing under pH 7.4 (116). They also observed a higher misincorporation efficiency when the pH was increased. We employed 5-formyl-dUTP (5-CHO-dUTP) in an *in vitro* gap-filling reaction conducted by the *E.coli* DNA pol III holoenzyme (at pH 7.5), and transfected the synthesized DNA into *E.coli* (111). However, the MF was not increased significantly as compared with the control experiment, in which the gap-filling was carried out without 5-CHO-dUTP.

When we introduced 5-CHO-dUTP directly into wt *E.coli*, the nucleotide elicited *lacI*$^-$ and *lacO*^{c} mutants as efficiently as 8-OH-dGTP (117). 5-CHO-dUTP induced G·C→A·T, A·T→G·C and G·C→T·A mutations. Since pol III did not seem to misincorporate 5-CHO-dUMP (111), other DNA polymerase(s) may be involved in the mutations induced by 5-CHO-dUTP *in vivo*.

5-Hydroxy-dCTP

Purmal *et al.* compared the incorporations of 5-hydroxy-dCMP (5-OH-dCMP) opposite G and opposite A by KF^{exo-} (109). 5-OH-dCMP was inserted with 0.15-fold of the efficiency of dCMP opposite G. The incorporation of 5-OH-dCMP opposite A was 1000-fold less efficient than that opposite G (7000-fold less efficient than those of the normal pairing).

Feig *et al.* treated dCTP with an ROS-generating system, and separated the modification products by HPLC (118). They then used the human immunodeficiency virus reverse transcriptase to incorporate each product into a DNA that contains a target gene for scoring mutations. One of the mutagenic species isolated was identified as 5-hydroxy-dCTP (5-OH-dCTP), which causes C→T transitions in *E.coli*. We employed 5-OH-dCTP in the *in vitro* gap-filling reaction conducted by the *E.coli* DNA pol III holoenzyme, and transfected the synthesized DNA into *E.coli* (111). However, the MF was not increased significantly as compared with the control experiment, in which the gap-filling was carried out without 5-OH-dCTP, in contrast to the results from Feig *et al.*

When we introduced 5-OH-dCTP directly into wt *E.coli*, the nucleotide elicited *lacI*$^{-}$ and *lacO*^{c} mutants as efficiently as 8-OH-dGTP (117). 5-OH-dCTP induced A·T→C·G, A·T→G·C, and G·C→T·A mutations. However, since pol III did not seem to misincorporate 5-OH-dCMP (111), other DNA polymerase(s) may be involved in the mutations induced by 5-OH-dCTP *in vivo*.

Other damaged nucleotides

8-Hydroxy-dAMP was incorporated only opposite T, with 7700-fold less efficiency than that of the normal pairing by KF^{exo-} (109). Ide *et al.* found that thymidine glycol 5′-monophosphate was not incorporated when KF^{exo+} was used (119). In contrast, they observed that 5,6-dihydrothymidine 5′-monophosphate was incorporated in place of dTMP during a primer elongation catalyzed by KF^{exo-} (119). The rate of incorporation of 5,6-dihydrothymidine 5′-monophosphate was ~10–25-fold lower than that of dTMP. Deoxyuridine glycol 5′-monophosphate was incorporated opposite A by KF^{exo-} with 100-fold less efficiency than dTMP (120).

DNA REPAIR AND NUCLEOTIDE POOL SANITIZATION

As described above, many damaged nucleic acids produced by ROS/RNS, including DNA lesions and damaged DNA precursors, have mutagenic potentials. The damage in DNA, formed either directly or by the incorporation of a damaged DNA precursor, is removed by cellular DNA repair proteins. The repair efficiency of a DNA lesion is one of the determinant factors of the MF in living cells. For example, as shown above, the MF of 8-OH-Gua in *E.coli* is dependent on the presence of the MutM and MutY proteins, which are major repair enzymes preventing mutagenesis by the modified base (17,18). Since many reviews of DNA repair have been published (e.g. refs 4,121–123), the author will simply summarize the data on the removal of damaged nucleotides from the DNA precursor pool. Nucleotide pool sanitization is very important for organisms to remove mutagenic DNA precursors, as described below.

MutT is the gene product of the *E.coli* mutator gene, *mutT*. Disruption of the *mutT* gene led to an increased MF of A·T→C·G (105,124). Maki and Sekiguchi found that MutT hydrolyzes 8-OH-dGTP to form the corresponding monophosphate (104). Their report highlighted the role of 'nucleotide pool sanitization' to prevent the mutagenesis induced by damaged DNA precursors. A similar enzymatic activity was found in mammalian cells, and the cloning of the gene was subsequently reported by the same group (125,126). This enzyme, MTH1 (MutT Homolog), hydrolyzes 8-OH-dGTP to form the cognate monophosphate, and the *MTH1* gene could partially complement a *mutT*-deficiency-induced mutator phenotype (126,127). In addition to 8-OH-dGTP, the human MTH1 hydrolyzes 2-OH-dATP more efficiently than 8-OH-dGTP (128). This preferential hydrolysis of 2-OH-dATP over 8-OH-dGTP was attributed to the higher affinity for 2-OH-dATP than for 8-OH-dGTP. These results imply that 2-OH-dATP is an intrinsic substrate for the human MTH1 protein. Moreover, the MTH1 protein hydrolyzes 8-hydroxy-dATP with an efficiency similar to that for 8-OH-dGTP (128). The fact that a greater number of tumors were formed in the lungs, livers and stomachs of MTH1-deficient mice than wt mice suggests that MTH1 functions as an antimutator protein (129).

Recently, we found that 2-OH-dATP, and, less efficiently, 8-OH-dGTP, were hydrolyzed by the *E.coli* Orf135 protein (130), suggesting that this protein may be involved in the prevention of mutations induced by these oxidized deoxynucleotides. Kobayashi *et al.* found that the overproduction of the *E.coli ribA* gene product (GTP cyclohydrolase II) reduced the increased MF level of the *mutT* strain to almost the normal level (131). They also observed that the purified GTP cyclohydrolase II protein efficiently hydrolyzed 8-OH-dGTP to 8-OH-dGMP. This enzyme may also protect genetic material from the untoward effects of ROS.

An inosine triphosphatase, which hydrolyzes ITP, dITP and xanthosine 5′-triphosphate, was found (132 and references therein). Although the activity has not been demonstrated, it may act on dXTP as well as dITP. Thus, this enzyme may contribute to the elimination of damaged deoxyribonucleotides formed by NO. A similar enzyme, dUTP pyrophosphatase, which hydrolyzes the deamination product of dCTP, is ubiquitously present in organisms (133 and references therein). These proteins remove damaged nucleotides in the nucleotide pool, and are called sanitizing enzymes.

ROLE OF SPECIALIZED DNA POLYMERASES

Recently, a family of DNA polymerases involved in TLS has been identified in prokaryotes and eukaryotes, including human cells (134–136). It is the accepted idea that these specialized DNA polymerases act to minimize the cell death resulting from replication blockage. For example, *E.coli* has three specialized DNA polymerases (DNA polymerases II, IV and V). It was shown that the bypass of *N*-2-acetylaminofluorene guanine adducts located within a mutation hot spot requires pol II for –2 frameshifts, but pol V for error-free TLS (137). On the other hand, error-free and –1 frameshift TLS at a benzo[*a*]pyrene adduct requires both pol IV and pol V (137). In the case of an abasic site, the involvement of *E.coli* DNA

pol V in TLS has been suggested by the *in vitro* results (92,93), supporting the observation that TLS is not observed in an *umuDC* (pol V deficient) *E.coli* strain (138). However, the actual TLS polymerase(s) for abasic sites in mammalian cells is unclear. To date, for most oxidized DNA lesions, there has not been a distinctive correspondence of a specialized DNA pol with a DNA lesion, and the role of these polymerases in the mutagenesis remains to be resolved. In the near future, *in vitro* DNA synthesis reactions may be re-investigated by using the responsible DNA pol that actually bypasses the lesion *in vivo*.

CONCLUSION AND PERSPECTIVE

Approaches using synthetic oligodeoxyribonucleotides containing a defined DNA lesion have been successfully employed in DNA synthesis experiments *in vitro* and in living cells, to elucidate the mutagenic potential of the lesion of interest. To date, only the lesions that have moderate chemical stability have been examined, due to stringent conditions during the oligonucleotide synthesis. Further efforts, such as the development of new protecting groups that can be removed under very mild conditions, are necessary. Some oligonucleotides with a DNA lesion have been prepared by the post-synthetic (chemical or enzymatic) conversion of the precursor oligonucleotides (e.g. refs 70,78,89), by the enzymatic incorporation of nucleotides (ref. 74), and their combination (e.g. ref. 46). The development of these procedures is also important to synthesize oligonucleotides with specific lesions. In addition, the mutagenic potential of chemically unstable lesions must be addressed. Although they may be difficult to detect in the DNA, due to their instability, they may be highly mutagenic. In particular, the mutagenicity of an unstable lesion in living cells is important, and should be studied in detail. The hurdles toward the achievement of this issue may be overcome by the use of an oligonucleotide with a good analog, or the establishment of a novel and complete conversion method from the precursor in vector DNA.

Another important issue is the use of ds and ss vectors. In general, the repair of a DNA lesion appears to be more efficient in ds DNA than in ss DNA, in living cells. Thus, the use of a ss vector may be better to study the misincorporation frequency in cells. However, a ds vector may be more appropriate for an accurate elucidation of the mutagenicity in cells, considering both the repair and misincorporation efficiencies. The possibility that ss DNA replication may be carried out by different machinery than that for ds DNA is noteworthy. Interestingly, some DNA lesions showed different mutational properties when located in the leading and lagging template strands of ds vectors (e.g. refs 35,36,70). This is another important point to consider when evaluating the actual mutagenicity of DNA lesions in cells, in addition to the interest in discerning the mechanism of this phenomenon.

As described in this paper, the mutagenic potentials of some DNA lesions depended on the sequence context (e.g. refs 10,20,34). It is unclear whether all lesions show sequence-dependency, because very few sequence contexts have been used. Recently, Delaney and Essigmann have developed a novel system, the restriction endonuclease and post-labeling assay, and have used this method in experiments using O^6-methylguanine in 16 sequence contexts (139). This method enabled them to quantify the MF very rapidly, without an analysis of each colony or plaque. This procedure seems to be very useful to examine the effects of the sequence around a DNA lesion on the mutagenicity and the mutation spectrum. Hatahet *et al.* synthesized oligonucleotides containing either 8-OH-Gua, a natural abasic site, or the THF derivative surrounded by four randomized bases on both the 5′ and 3′ sides, and used them as templates for synthesis by T4 DNA polymerase holoenzyme (140,141). Successful bypass products were purified after (8-OH-Gua) or without (abasic sites) DNA repair enzyme treatment, and were subcloned to determine the sequence. They observed that biases for and against certain nucleotides were readily noticeable across the entire randomized region. This type of approach appears to be useful to determine the effect of the sequence contexts and may be extended to the *in vivo* experiments.

Accumulating evidence indicates the importance of damaged nucleotides in the mutation process. Interestingly, Nunoshiba *et al.* reported that the mutations found in an *E.coli* strain lacking superoxide dismutases and a repressor for iron-uptake systems were G·C→T·A and A·T→C·G transversions, and they reported that these mutations might be induced by 2-OH-dATP and 8-OH-dGTP, respectively (142,143). From this viewpoint, the nucleotide pool sanitization enzymes should be studied in detail, because they may be as important as DNA repair enzymes.

In conclusion, the mutagenic potentials of oxidized nucleic acids are determined by various cellular factors: the efficiencies of the DNA repair/nucleotide pool sanitization, the misincorporation by replicative DNA polymerase(s), and the misincorporation by specialized DNA polymerase(s). Since repair and sanitization efficiencies may differ between organisms and strains, due to variations in the type and the expression of these enzymes, the mutagenicity of a DNA lesion probably depends on the cell type. Likewise, the involvement of specialized DNA polymerases will differ in each cell. Thus, DNA repair proteins, nucleotide pool sanitization enzymes, and specialized DNA polymerases that deal with a certain DNA lesion have to be examined in detail and to be corresponded to a lesion. The experimentally observed MFs will be understood by the functions of these parameters, as revealed by *in vitro* experiments. Chemically synthesized oligonucleotides and nucleotides will be useful to clarify the events that occur in living cells.

ACKNOWLEDGEMENTS

The author wishes to thank the collaborators who participated in the experiments described in this paper. The author also thanks Naoko Murata-Kamiya for reading the manuscript. Work in our laboratory was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Ames,B.N. (1983) Dietary carcinogens and anticarcinogens. *Science*, **221**, 1256–1264.
2. Harman,D. (1981) The aging process. *Proc. Natl Acad. Sci. USA*, **78**, 7124–7128.

3. Ames,B.N., Shigenaga,M.K. and Hagen,T.M. (1993) Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl Acad. Sci. USA*, **90**, 7915–7922.
4. Wallace,S.S. (1998) Enzymatic processing of radiation-induced free radical damage in DNA. *Radiat. Res.*, **150**, S60–S79.
5. Halliwell,B. and Aruoma,O.I. (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.*, **281**, 9–19.
6. Kasai,H. and Nishimura,S. (1991) Formation of 8-hydroxydeoxyguanosine in DNA by oxygen radicals and its biological significance. In Sies,H. (ed.), *Oxidative Stress: Oxidants and Antioxidants*. Academic Press, Inc., New York, NY, pp. 99–116.
7. Kasai,H. (1997) Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat. Res.*, **387**, 147–163.
8. Shibutani,S., Takeshita,M. and Grollman,A.P. (1991) DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.
9. Kamiya,H., Sakaguchi,T., Murata,N., Fujimuro,M., Miura,H., Ishikawa,H., Shimizu,M., Inoue,H., Nishimura,S., Matsukage,A., Masutani,C., Hanaoka,F. and Ohtsuka,E. (1992) *In vitro* replication study of modified bases in ras sequences. *Chem. Pharm. Bull.*, **40**, 2792–2795.
10. Kamiya,H., Murata-Kamiya,N., Fujimuro,M., Kido,K., Inoue,H., Nishimura,S., Masutani,C., Hanaoka,F. and Ohtsuka,E. (1995) Comparison of incorporation and extension of nucleotides *in vitro* opposite 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-ras gene. *Jpn. J. Cancer Res.*, **86**, 270–276.
11. Efrati,E., Tocco,G., Eritja,R., Wilson,S.H. and Goodman,M.F. (1999) 'Action-at-a-distance' mutagenesis. 8-oxo-7,8-dihydro-2′-deoxyguanosine causes base substitution errors at neighboring template sites when copied by DNA polymerase β. *J. Biol. Chem.*, **274**, 15920–15926.
12. Lowe,L.G. and Guengerich,F.P. (1996) Steady-state and pre-steady-state kinetic analysis of dNTP insertion opposite 8-oxo-7,8-dihydroguanine by *Escherichia coli* polymerases I exo- and II exo-. *Biochemistry*, **35**, 9840–9849.
13. Furge,L.L. and Guengerich,F.P. (1997) Analysis of nucleotide insertion and extension at 8-oxo-7,8-dihydroguanine by replicative T7 polymerase exo- and human immunodeficiency virus-1 reverse transcriptase using steady-state and pre-steady-state kinetics. *Biochemistry*, **36**, 6475–6487.
14. Furge,L.L. and Guengerich,F.P. (1998) Pre-steady-state kinetics of nucleotide insertion following 8-oxo-7,8-dihydroguanine base pair mismatches by bacteriophage T7 DNA polymerase exo-. *Biochemistry*, **37**, 3567–3574.
15. Wood,M.L., Dizdaroglu,M., Gajewski,E. and Essigmann,J.M. (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry*, **29**, 7024–7032.
16. Cheng,K.C., Cahill,D.S., Kasai,H., Nishimura,S. and Loeb,L.A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *J. Biol. Chem.*, **267**, 166–172.
17. Moriya,M. and Grollman,A.P. (1993) Mutations in the mutY gene of *Escherichia coli* enhance the frequency of targeted G:C→T:A transversions induced by a single 8-oxoguanine residue in single-stranded DNA. *Mol Gen. Genet.*, **239**, 72–76.
18. Wagner,J., Kamiya,H. and Fuchs,R.P.P. (1997) Leading versus lagging strand mutagenesis induced by 7,8-dihydro-8-oxo-2′-deoxyguanosine in *E. coli*. *J. Mol. Biol.*, **265**, 302–309.
19. Kamiya,H., Miura,K., Ishikawa,H., Inoue,H., Nishimura,S. and Ohtsuka,E. (1992) c-Ha-ras containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. *Cancer Res.*, **52**, 3483–3485.
20. Kamiya,H., Murata-Kamiya,N., Koizume,S., Inoue,H., Nishimura,S. and Ohtsuka,E. (1995) 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-ras gene. Effects of sequence contexts on mutation spectra. *Carcinogenesis*, **16**, 883–889.
21. Moriya,M. (1993) Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted GC→TA transversions in simian kidney cells. *Proc. Natl Acad. Sci. USA*, **90**, 1122–1126.
22. LePage,F., Margot,A., Grollman,A.P., Sarasin,A. and Gentil,A. (1995) Mutagenicity of a unique 8-oxoguanine in a human Ha-ras sequence in mammalian cells. *Carcinogenesis*, **16**, 2779–2784.
23. Tan,X., Grollman,A.P. and Shibutani,S. (1999) Comparison of the mutagenic properties of 8-oxo-7,8-dihydro-2′-deoxyadenosine and 8-oxo-7,8-dihydro-2′-deoxyguanosine DNA lesions in mammalian cells. *Carcinogenesis*, **20**, 2287–2292.
24. Koizume,S., Kamiya,H., Inoue,H. and Ohtsuka,E. (1994) Synthesis and thermodynamic stabilities of damaged DNA involving 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in a ras gene fragment. *Nucleosides Nucleotides*, **13**, 1517–1534.
25. Plum,G.E., Grollman,A.P., Johnson,F. and Breslauer,K.J. (1995) Influence of the oxidatively damaged adduct 8-oxodeoxyguanosine on the conformation, energetics and thermodynamic stability of a DNA duplex. *Biochemistry*, **34**, 16148–16160.
26. Uesugi,S. and Ikehara,M. (1977) Carbon-13 magnetic resonance spectra of 8-substituted purine nucleotides. Characteristic shift for the syn conformation. *J. Am. Chem. Soc.*, **99**, 3250–3253.
27. Oda,Y., Uesugi,S., Ikehara,M., Nishimura,S., Kawase,Y., Ishikawa,H., Inoue,H. and Ohtsuka,E. (1991) NMR studies of a DNA containing 8-hydroxydeoxyguanosine. *Nucleic Acids Res.*, **19**, 1407–1412.
28. Kouchakdjian,M., Bodepudi,V., Shibutani,S., Eisenberg,M., Johnson,F., Grollman,A.P. and Patel,D.J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7*H*-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7*H*-dG(*syn*)dA(*anti*) alignment at lesion site. *Biochemistry*, **30**, 1403–1412.
29. Kamiya,H. and Kasai,H. (1995) Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damaged nucleotide and its incorporation by DNA polymerases. *J. Biol. Chem.*, **270**, 19446–19450.
30. Murata-Kamiya,N., Kamiya,H., Muraoka,M. and Kasai,H. (1997) Comparison of oxidation products from DNA components by γ-irradiation and Fenton-type reactions. *J. Radiat. Res.*, **38**, 121–131.
31. Jaruga,P. and Dizdaroglu,M. (1996) Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Res.*, **24**, 1389–1394.
32. Olinski,R., Zastawny,T., Budzbon,J., Skokowski,J., Zegarski,W. and Dizdaroglu,M. (1992) DNA base modifications in chromatin of human cancerous tissues. *FEBS Lett.*, **309**, 193–198.
33. Kamiya,H., Ueda,T., Ohgi,T., Matsukage,A. and Kasai,H. (1995) Misincorporation of dAMP opposite 2-hydroxyadenine, an oxidative form of adenine. *Nucleic Acids Res.*, **23**, 761–766.
34. Kamiya,H. and Kasai,H. (1996) Effects of sequence contexts on misincorporation of nucleotides opposite 2-hydroxyadenine. *FEBS Lett.*, **391**, 113–116.
35. Kamiya,H. and Kasai,H. (1997) Substitution and deletion mutations induced by 2-hydroxyadenine in *Escherichia coli*: effects of sequence contexts in leading and lagging strands. *Nucleic Acids Res.*, **25**, 304–310.
36. Kamiya,H. and Kasai,H. (1997) Mutations induced by 2-hydroxyadenine on a shuttle vector during leading and lagging strand syntheses in mammalian cells. *Biochemistry*, **36**, 11125–11130.
37. Kawakami,J., Kamiya,H., Yasuda,K., Fujiki,H., Kasai,H. and Sugimoto,N. (2001) Thermodynamic stability of base pairs between 2-hydroxyadenine and incoming nucleotides as a determinant of nucleotide incorporation specificity during replication. *Nucleic Acids Res.*, **29**, 3289–3296.
38. Kamiya,H. and Kasai,H. (2000) 2-Hydroxyadenine formation by reactive oxygen species and mutagenic effects. *Recent Res. Dev. Biochem.*, **2**, 41–50.
39. Mori,T., Hori,Y. and Dizdaroglu,M. (1993) DNA base damage generated *in vivo* in hepatic chromatin of mice upon whole body γ-irradiation. *Int. J. Radiat. Biol.*, **64**, 645–650.
40. Malins,D.C. and Haimanot,R. (1990) 4,6-Diamino-5-formamidopyrimidine, 8-hydroxyguanine and 8-hydroxyadenine in DNA from neoplastic liver of English sole exposed to carcinogens. *Biochem. Biophys. Res. Commun.*, **173**, 614–619.
41. Shibutani,S., Bodepudi,V., Johnson,F. and Grollman,A.P. (1993) Translesional synthesis on DNA template containing 8-oxo-7,8-dihydrodeoxyadenosine. *Biochemistry*, **32**, 4615–4621.
42. Guschlbauer,W., Duplaa,A.M., Guy,A., Teoule,R. and Fazakerley,G.V. (1991) Structure and *in vitro* replication of DNA templates containing 7,8-dihydro-8-oxoadenine. *Nucleic Acids Res.*, **19**, 1753–1758.
43. Kamiya,H., Miura,H., Murata-Kamiya,N., Ishikawa,H., Sakaguchi,T., Inoue,H., Sasaki,T., Masutani,C., Hanaoka,F., Nishimura,S. and

Ohtsuka,E. (1995) 8-Hydroxyadenine (7,8-dihydro-8-oxoadenine) induces misincorporation in *in vitro* DNA synthesis and mutations in NIH3T3 cells. *Nucleic Acids Res.*, **23**, 2893–2899.
44. Wood,M.L., Esteve,A., Morningstar,M.L., Kuziemko,G.M. and Essigmann,J.M. (1992) Genetic effects of oxidative DNA damage: comparative mutagenesis of 7,8-dihydro-8-oxoguanine and 7,8-dihydro-8-oxoadenine in *Escherichia coli*. *Nucleic Acids Res.*, **20**, 6023–6032.
45. Leonard,G.A., Guy,A., Brown,T., Teoule,R. and Hunter,W.N. (1992) Conformation of guanine-8-oxoadenine base pairs in the crystal structure of d(CGCGAATT(O8A)GCG). *Biochemistry*, **31**, 8415–8420.
46. Asagoshi,K., Terato,H., Ohyama,Y. and Ide,H. (2002) Effects of a guanine-derived formamidopyrimidine lesion on DNA replication: translesion DNA synthesis, nucleotide insertion and extension kinetics. *J. Biol. Chem.*, **277**, 14589–14597.
47. Tudek,B., Graziewicz,M., Kazanova,O., Zastawny,T.H., Obtulowicz,T. and Laval,J. (1999) Mutagenic specificity of imidazole ring-opened 7-methylpurines in M13mp18 phage DNA. *Acta Biochim. Pol.*, **46**, 785–799.
48. Haraguchi,K. and Greenberg,M.M. (2001) Synthesis of oligonucleotides containing FapydG (N^6-(2-deoxy-α,β-D-erythro-pentofuranosyl)-2,6-diamino-4-hydroxy-5-formamidopyrimidine). *J. Am. Chem. Soc.*, **123**, 8636–8637.
49. Haraguchi,K., Delaney,M.O., Wiederholt,C.J., Sambandam,A., Hantosi,Z. and Greenberg,M.M. (2002) Synthesis and characterization of oligodeoxynucleotides containing formamidopyrimidine lesions and nonhydrolyzable analogues. *J. Am. Chem. Soc.*, **124**, 3263–3269.
50. Vialas,C., Pratviel,G., Claparos,C. and Meunier,B. (1998) Efficient oxidation of 2′-deoxyguanosine by Mn-TMPyP/$KHSO_5$ to imidazolone dIz without formation of 8-oxo-dG. *J. Am. Chem. Soc.*, **120**, 11548–11553.
51. Cadet,J., Berger,M., Buchko,G.W., Joshi,S.R., Raoul,S. and Ravanat,J.L. (1994) 2,2-Diamino-4-[(3,5-di-*O*-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)amino]-5-(2*H*)-oxazolone: a novel and predominant radical oxidation product of 3′,5′-di-*O*-acetyl-2′deoxyguanosine. *J. Am. Chem. Soc.*, **116**, 7403–7404.
52. Kino,K., Saito,I. and Sugiyama,H. (1998) Product analysis of GG-specific photooxidation of DNA via electron transfer: 2-aminoimidazolone as a major guanine oxidation product. *J. Am. Chem. Soc.*, **120**, 7373–7374.
53. Kino,K. and Sugiyama,H. (2001) Possible cause of GC→CG transversion mutation by guanine oxidation product, imidazolone. *Chem. Biol.*, **8**, 369–378.
54. Nguyen,T., Brunson,D., Crespi,C.L., Penman,B.W., Wishnok,J.S. and Tannenbaum,S.R. (1992) DNA damage and mutation in human cells exposed to nitric oxide *in vitro*. *Proc. Natl Acad. Sci. USA*, **89**, 3030–3034.
55. Eritja,R., Horowitz,D.M., Walker,P.A., Ziehler-Martin,J.P., Boosalis,M.S., Goodman,M.F., Itakura,K. and Kaplan,B.E. (1986) Synthesis and properties of oligonucleotides containing 2′-deoxynebularine and 2′-deoxyxanthosine. *Nucleic Acids Res.*, **14**, 8135–8153.
56. Kamiya,H., Shimizu,M., Suzuki,M., Inoue,H. and Ohtsuka,E. (1992) Mutation induced by deoxyxanthosine in codon 12 of a synthetic c-Ha-ras gene. *Nucleosides Nucleotides*, **11**, 247–260.
57. Ohtsuka,E., Matsuki,S., Ikehara,M., Takahashi,Y. and Matsubara,K. (1985) An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. *J. Biol. Chem.*, **260**, 2605–2608.
58. Hill-Perkins,M., Jones,M.D. and Karran,P. (1986) Site-specific mutagenesis *in vivo* by single methylated or deaminated purine bases. *Mutat. Res.*, **162**, 153–163.
59. Kamiya,H., Miura,H., Kato,H., Nishimura,S. and Ohtsuka,E. (1992) Induction of mutation of a synthetic c-Ha-ras gene containing hypoxanthine. *Cancer Res.*, **52**, 1836–1839.
60. Kawase,Y., Iwai,S., Inoue,H., Miura,K. and Ohtsuka,E. (1986) Studies on nucleic acid interactions. I. Stabilities of mini-duplexes ($dG_2A_4XA_4G_2$-$dC_2T_4YT_4C_2$) and self-complementary d(GGGAAXYTTCCC) containing deoxyinosine and other mismatched bases. *Nucleic Acids Res.*, **14**, 7727–7736.
61. Uesugi,S., Oda,Y., Ikehara,M., Kawase,Y. and Ohtsuka,E. (1987) Identification of I:A mismatch base-pairing structure in DNA. *J. Biol. Chem.*, **262**, 6965–6968.
62. Corfield,P.W., Hunter,W.N., Brown,T., Robinson,P. and Kennard,O. (1987) Inosineadenine base pairs in a B-DNA duplex. *Nucleic Acids Res.*, **15**, 7935–7949.
63. Kasai,H., Iida,A., Yamaizumi,Z., Nishimura,S. and Tanooka,H. (1990) 5-Formyldeoxyuridine: a new type of DNA damage induced by ionizing radiation and its mutagenicity to Salmonella strain TA102. *Mutat. Res.*, **243**, 249–253.
64. Wakizaka,A., Aiba,N., Okuhara,E. and Kawazoe,Y. (1987) Production of 5-formyluracil from thymine in an *in vitro* active oxygen-generating system. *Biochem. Int.*, **14**, 289–295.
65. Douki,T., Delatour,T., Paganon,F. and Cadet,J. (1996) Measurement of oxidative damage at pyrimidine bases in gamma-irradiated DNA. *Chem. Res. Toxicol.*, **9**, 1145–1151.
66. Ono,A., Okamoto,T., Inada,M., Nara,H. and Matsuda,A. (1994) Nucleosides and nucleotides. 131. Synthesis and properties of oligonucleotides containing 5-formyl-2′deoxyuridine. *Chem. Pharm. Bull. (Tokyo)*, **42**, 2231–2237.
67. Zhang,Q.M., Sugiyama,H., Miyabe,I., Matsuda,S., Saito,I. and Yonei,S. (1997) Replication of DNA templates containing 5-formyluracil, a major oxidative lesion of thymine in DNA. *Nucleic Acids Res.*, **25**, 3969–3973.
68. Masaoka,A., Terato,H., Kobayashi,M., Ohyama,Y. and Ide,H. (2001) Oxidation of thymine to 5-formyluracil in DNA promotes misincorporation of dGMP and subsequent elongation of a mismatched primer terminus by DNA polymerase. *J. Biol. Chem.*, **276**, 16501–16510.
69. Miyabe,I., Zhang,Q.M., Sugiyama,H., Kino,K. and Yonei,S. (2001) Mutagenic effects of 5-formyluracil on a plasmid vector during replication in *Escherichia coli*. *Int. J. Radiat. Biol.*, **77**, 53–58.
70. Kamiya,H., Murata-Kamiya,N., Karino,N., Ueno,Y., Matsuda,A. and Kasai,H. (2002) Induction of T→G and T→A transversions by 5-formyluracil in mammalian cells. *Mutat. Res.*, **513**, 213–222.
71. Tsunoda,M., Karino,N., Ueno,Y., Matsuda,A. and Takenaka,A. (2001) Crystalization and preliminary X-ray analysis of a DNA dodecamer containing 2′-deoxy-5-formyluridine; what is the role of magnesium cation in crystalization of Dickerson-type DNA dodecamers? *Acta Cryst.*, **D57**, 245–248.
72. Tsunoda,M., Sakaue,T., Naito,S., Sunami,T., Karino,N., Ueno,Y., Matsuda,A. and Takenaka,A. (2001) X-ray analysis of DNA dodecamers containing 2′-deoxy-5-formyluridine. *Nucleic Acids Res. Symp. Ser.*, Suppl. 1, 279–280.
73. Dizdaroglu,M., Holwitt,E., Hagan,M.P. and Blakely,W.F. (1986) Formation of cytosine glycol and 5,6-dihydroxycytosine in deoxyribonucleic acid on treatment with osmium tetroxide. *Biochem J.*, **235**, 531–536.
74. Purmal,A.A., Kow,Y.W. and Wallace,S.S. (1994) Major oxidative products of cytosine, 5-hydroxycytosine and 5-hydroxyuracil, exhibit sequence context-dependent mispairing *in vitro*. *Nucleic Acids Res.*, **22**, 72–78.
75. Kreutzer,D.A. and Essigmann,J.M. (1998) Oxidized, deaminated cytosines are a source of C→T transitions *in vivo*. *Proc. Natl Acad. Sci. USA*, **95**, 3578–3582.
76. Hegi,M.E., Sagelsdorff,P. and Lutz,W.K. (1989) Detection by ^{32}P-postlabeling of thymidine glycol in γ-irradiated DNA. *Carcinogenesis*, **10**, 43–47.
77. Weinfeld,M., Xing,J.Z., Lee,J., Leadon,S.A., Cooper,P.K. and Le,X.C. (2001) Factors influencing the removal of thymine glycol from DNA in γ-irradiated human cells. *Prog. Nucleic Acid Res. Mol. Biol.*, **68**, 139–149.
78. Clark,J.M. and Beardsley,G.P. (1989) Template length, sequence context and 3′-5′ exonuclease activity modulate replicative bypass of thymine glycol lesions *in vitro*. *Biochemistry*, **28**, 775–779.
79. McNulty,J.M., Jerkovic,B., Bolton,P.H. and Basu,A.K. (1998) Replication inhibition and miscoding properties of DNA templates containing a site-specific *cis*-thymine glycol or urea residue. *Chem. Res. Toxicol.*, **11**, 666–673.
80. Purmal,A.A., Lampman,G.W., Bond,J.P., Hatahet,Z. and Wallace,S.S. (1998) Enzymatic processing of uracil glycol, a major oxidative product of DNA cytosine. *J. Biol. Chem.*, **273**, 10026–10035.
81. Basu,A.K., Loechler,E.L., Leadon,S.A. and Essigmann,J.M. (1989) Genetic effects of thymine glycol: site-specific mutagenesis and molecular modeling studies. *Proc. Natl Acad. Sci. USA*, **86**, 7677–7681.
82. Nakamura,J., La,D.K. and Swenberg,J.A. (2000) 5′-Nicked apurinic/apyrimidinic sites are resistant to β-elimination by β-polymerase and

are persistent in human cultured cells after oxidative stress. *J. Biol. Chem.*, **275**, 5323–5328.
83. Nakamura,J. and Swenberg,J.A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res.*, **59**, 2522–2526.
84. Randall,S.K., Eritja,R., Kaplan,B.E., Petruska,J. and Goodman,M.F. (1987) Nucleotide insertion kinetics opposite abasic lesions in DNA. *J. Biol. Chem.*, **262**, 6864–6870.
85. Takeshita,M., Chang,C.N., Johnson,F., Will,S. and Grollman,A.P. (1987) Oligodeoxynucleotides containing synthetic abasic sites. Model substrates for DNA polymerases and apurinic/apyrimidinic endonucleases. *J. Biol. Chem.*, **262**, 10171–10179.
86. Kamiya,H., Suzuki,M., Komatsu,Y., Miura,H., Kikuchi,K., Sakaguchi,T., Murata,N., Masutani,C., Hanaoka,F. and Ohtsuka,E. (1992) An abasic site analogue activates a c-Ha-ras gene by a point mutation at modified and adjacent positions. *Nucleic Acids Res.*, **20**, 4409–4415.
87. Shibutani,S., Takeshita,M. and Grollman,A.P. (1997) Translesional synthesis on DNA templates containing a single abasic site. A mechanistic study of the 'A rule'. *J. Biol. Chem.*, **272**, 13916–13922.
88. Efrati,E., Tocco,G., Eritja,R., Wilson,S.H. and Goodman,M.F. (1997) Abasic translesion synthesis by DNA polymerase beta violates the 'A-rule'. Novel types of nucleotide incorporation by human DNA polymerase beta at an abasic lesion in different sequence contexts. *J. Biol. Chem.*, **272**, 2559–2569.
89. Lawrence,C.W., Borden,A., Banerjee,S.K. and Le Clerc,J.E. (1990) Mutation frequency and spectrum resulting from a single abasic site in a single-stranded vector. *Nucleic Acids Res.*, **18**, 2153–2157.
90. Gibbs,P.E. and Lawrence,C.W. (1995) Novel mutagenic properties of abasic sites in *Saccharomyces cerevisiae*. *J. Mol. Biol.*, **251**, 229–236.
91. Nelson,J.R., Lawrence,C.W. and Hinkle,D.C. (1996) Deoxycytidyl transferase activity of yeast REV1 protein. *Nature*, **382**, 729–731.
92. Tang,M., Shen,X., Frank,E.G., O'Donnell,M., Woodgate,R. and Goodman,M.F. (1999) UmuD′(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc. Natl Acad. Sci. USA.*, **96**, 8919–8924.
93. Reuven,N.B., Arad,G., Maor-Shoshani,A. and Livneh,Z. (1999) The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA and SSB and is specialized for translesion replication. *J. Biol. Chem.*, **274**, 31763–31766.
94. Kamiya,H., Suzuki,M. and Ohtsuka,E. (1993) Mutation-spectrum of a true abasic site in codon 12 of a c-Ha-ras gene in mammalian cells. *FEBS Lett.*, **328**, 125–129.
95. Gentil,A., Renault,G., Madzak,C., Margot,A., Cabral-Neto,J.B., Vasseur,J.J., Rayner,B., Imbach,J.L. and Sarasin,A. (1990) Mutagenic properties of a unique abasic site in mammalian cells. *Biochem. Biophys. Res. Commun.*, **173**, 704–710.
96. Gentil,A., Cabral-Neto,J.B., Mariage-Samson,R., Margot,A., Imbach,J.L., Rayner,B. and Sarasin,A. (1992) Mutagenicity of a unique apurinic/apyrimidinic site in mammalian cells. *J. Mol. Biol.*, **227**, 981–984.
97. CabralNeto,J.B., Cabral,R.E., Margot,A., Le Page,F., Sarasin,A. and Gentil,A. (1994) Coding properties of a unique apurinic/apyrimidinic site replicated in mammalian cells. *J. Mol. Biol.*, **240**, 416–420.
98. Ide,H., Murayama,H., Sakamoto,S., Makino,K., Honda,K., Nakamuta,H., Sasaki,M. and Sugimoto,N. (1995) On the mechanism of preferential incorporation of dAMP at abasic sites in translesional DNA synthesis. Role of proofreading activity of DNA polymerase and thermodynamic characterization of model template-primers containing an abasic site. *Nucleic Acids Res.*, **23**, 123–129.
99. Gelfand,C.A., Plum,G.E., Grollman,A.P., Johnson,F. and Breslauer,K.J. (1998) Thermodynamic consequences of an abasic lesion in duplex DNA are strongly dependent on base sequence. *Biochemistry*, **37**, 7321–7327.
100. Murata-Kamiya,N., Kamiya,H., Karino,N., Ueno,Y., Matsuda,A. and Kasai,H. (1999) Formation of 5-formyl-2′-deoxycytidine from 5-methyl-2′-deoxycytidine in duplex DNA by Fenton-type reactions and γ-irradiation. *Nucleic Acids Res.*, **27**, 4385–4390.
101. Karino,N., Ueno,Y. and Matsuda,A. (2001) Synthesis and properties of oligonucleotides containing 5-formyl-2′-deoxycytidine: *in vitro* DNA polymerase reactions on DNA templates containing 5-formyl-2′-deoxycytidine. *Nucleic Acids Res.* **29**, 2456–2463.
102. Kamiya,H., Tsuchiya,H., Karino,N., Ueno,Y., Matsuda,A. and Harashima,H. (2002) Mutagenicity of 5-formylcytosine, an oxidation product of 5-methylcytosine, in DNA in mammalian cells. *J. Biochem.*, **132**, 551–555.
103. Henderson,P.T., Delaney,J.C., Gu,F., Tannenbaum,S.R. and Essigmann,J.M. (2002) Oxidation of 7,8-dihydro-8-oxoguanine affords lesions that are potent sources of replication errors *in vivo*. *Biochemistry*, **41**, 914–921.
104. Maki,H. and Sekiguchi,M. (1992) MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, **355**, 273–275.
105. Tajiri,T., Maki,H. and Sekiguchi,M. (1995) Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*. *Mutat. Res.*, **336**, 257–267.
106. Shen,Z., Wu,W. and Hazen,S.L. (2000) Activated leukocytes oxidatively damage DNA, RNA and the nucleotide pool through halide-dependent formation of hydroxyl radical. *Biochemistry*, **39**, 5474–5482.
107. Tassotto,M.L. and Mathews,C.K. (2002) Assessing the metabolic function of the MutT 8-oxodeoxyguanosine triphosphatase in *Escherichia coli* by nucleotide pool analysis. *J. Biol. Chem.*, **277**, 15807–15812.
108. Mathews,C.K. (1972) Biochemistry of deoxyribonucleic acid-defective amber mutants of bacteriophage T4. 3. Nucleotide pools. *J. Biol. Chem.*, **247**, 7430–7438.
109. Purmal,A.A., Kow,Y.W. and Wallace,S.S. (1994) 5-Hydroxypymidine deoxynucleoside triphosphates are more efficiently incorporated into DNA by exonuclease-free Klenow fragment than 8-oxopurine deoxynucleoside triphosphates. *Nucleic Acids Res.*, **22**, 3930–3935.
110. Einolf,H.J., Schnetz-Boutaud,N. and Guengerich,F.P. (1998) Steady-state and pre-steady-state kinetic analysis of 8-oxo-7,8-dihydroguanosine triphosphate incorporation and extension by replicative and repair DNA polymerases. *Biochemistry*, **37**, 13300–13312.
111. Kamiya,H. and Kasai,H. (2000) 2-Hydroxy-dATP is incorporated opposite G by *Escherichia coli* DNA polymerase III resulting in high mutagenicity. *Nucleic Acids Res.*, **28**, 1640–1646.
112. Pavlov,Y.I., Minnick,D.T., Izuta,S. and Kunkel,T.A. (1994) DNA replication fidelity with 8-oxodeoxyguanosine triphosphate. *Biochemistry*, **33**, 4695–4701.
113. Inoue,M., Kamiya,H., Fujikawa,K., Ootsuyama,Y., Murata-Kamiya,N., Osaki,T., Yasumoto,K. and Kasai,H. (1998) Induction of chromosomal gene mutations in *Escherichia coli* by direct incorporation of oxidatively damaged nucleotides. *J. Biol. Chem.*, **273**, 11069–11074.
114. Kamiya,H., Maki,H. and Kasai,H. (2000) Two DNA polymerases of *Escherichia coli* display distinct misinsertion specificities for 2-hydroxy-dATP during DNA synthesis. *Biochemistry*, **39**, 9508–9513.
115. Suzuki,T., Yoshida,M., Yamada,M., Ide,H., Kobayashi,M., Kanaori,K., Tajima,K. and Makino,K. (1998) Misincorporation of 2′-deoxyoxanosine 5′-triphosphate by DNA polymerases and its implication for mutagenesis. *Biochemistry*, **37**, 11592–11598.
116. Terato,H., Masaoka,A., Kobayashi,M., Fukushima,S., Ohyama,Y., Yoshida,M. and Ide,H. (1999) Enzymatic repair of 5-formyluracil. II. Mismatch formation between 5-formyluracil and guanine during DNA replication and its recognition by two proteins involved in base excision repair (AlkA) and mismatch repair (MutS). *J. Biol. Chem.*, **274**, 25144–25150.
117. Fujikawa,K., Kamiya,H. and Kasai,H. (1998) The mutations induced by oxidatively damaged nucleotides, 5-formyl-dUTP and 5-hydroxy-dCTP, in *Escherichia coli*. *Nucleic Acids Res.*, **26**, 4582–4587.
118. Feig,D.I., Sowers,L.C. and Loeb,L.A. (1994) Reverse chemical mutagenesis: identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl Acad. Sci. USA*, **91**, 6609–6613.
119. Ide,H., Melamede,R.J. and Wallace,S.S. (1987) Synthesis of dihydrothymidine and thymidine glycol 5′-triphosphates and their ability to serve as substrates for *Escherichia coli* DNA polymerase I. *Biochemistry*, **26**, 964–969.
120. Purmal,A.A., Bond,J.P., Lyons,B.A., Kow,Y.W. and Wallace,S.S. (1998) Uracil glycol deoxynucleoside triphosphate is a better substrate for DNA polymerase I Klenow fragment than thymine glycol deoxynucleoside triphosphate. *Biochemistry*, **37**, 330–338.
121. Demple,B. and Harrison,L. (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annu. Rev. Biochem.*, **63**, 915–948.
122. Sancar,A. (1996) DNA excision repair. *Annu. Rev. Biochem.*, **65**, 43–81.

123. Scharer,O.D. and Jiricny,J. (2001) Recent progress in the biology, chemistry and structural biology of DNA glycosylases. *Bioessays*, **23**, 270–281.
124. Yanofsky,C., Cox,E.C. and Horn,V. (1966) The unusual mutagenic specificity of an *E. coli* mutator gene. *Proc. Natl Acad. Sci. USA*, **55**, 274–281.
125. Mo,J.Y., Maki,H. and Sekiguchi,M., (1992) Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc. Natl Acad. Sci. USA*, **89**, 11021–11025.
126. Sakumi,K., Furuichi,M., Tsuzuki,T., Kakuma,T., Kawabata,S., Maki,H. and Sekiguchi,M. (1993) Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *J. Biol. Chem.*, **268**, 23524–23530.
127. Furuichi,M., Yoshida,M.C., Oda,H., Tajiri,T., Nakabeppu,Y., Tsuzuki,T. and Sekiguchi,M. (1994) Genomic structure and chromosome location of the human mutT homologue gene MTH1 encoding 8-oxo-dGTPase for prevention of A:T to C:G transversion. *Genomics*, **24**, 485–490.
128. Fujikawa,K., Kamiya,H., Yakushiji,H., Fujii,Y., Nakabeppu,Y. and Kasai,H. (1999) The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. *J. Biol. Chem.*, **274**, 18201–18205.
129. Tsuzuki,T., Egashira,A., Igarashi,H., Iwakuma,T., Nakatsuru,Y., Tominaga,Y., Kawate,H., Nakao,K., Nakamura,K., Ide,F., Kura,S., Nakabeppu,Y., Katsuki,M.T., Ishikawa,M. and Sekiguchi,M. (2001) Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase. *Proc. Natl Acad. Sci. USA*, **98**, 11456–11461.
130. Kamiya,H., Murata-Kamiya,N., Iida,E. and Harashima,H. (2001) Hydrolysis of oxidized Nucleotides by the *Escherichia coli* Orf135 protein. *Biochem. Biophys. Res. Commun.*, **288**, 499–502.
131. Kobayashi,M., Ohara-Nemoto,Y., Kaneko,M., Hayakawa,H., Sekiguchi,M. and Yamamoto,K. (1998) Potential of *Escherichia coli* GTP cyclohydrolase II for hydrolyzing 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *J. Biol. Chem.*, **273**, 26394–26399.
132. Lin,S., McLennan,A.G., Ying,K., Wang,Z., Gu,S., Jin,H., Wu,C., Liu,W., Yuan,Y., Tang,R., Xie,Y. and Mao,Y. (2001) Cloning, expression and characterization of a human inosine triphosphate pyrophosphatase encoded by the *itpa* gene. *J. Biol. Chem.*, **276**, 18695–18701.
133. McIntosh,E.M., Ager,D.D., Gadsden,M.H. and Haynes,R.H. (1992) Human dUTP pyrophophatase: cDNA sequence and potential biological importance of the enzyme. *Proc. Natl Acad. Sci. USA*, **89**, 8020–8024.
134. Wang,Z. (2001) Translesion synthesis by the UmuC family of DNA polymerases. *Mutat. Res.*, **486**, 59–70.
135. Livneh,Z. (2001) DNA damage control by novel DNA polymerases: translesion replication and mutagenesis. *J. Biol. Chem.*, **276**, 25639–25642.
136. Friedberg,E.C., Wagner,R. and Radman,M. (2002) Specialized DNA polymerases, cellular survival and the genesis of mutations. *Science*, **296**, 1627–1630.
137. Napolitano,R., Janel-Bintz,R., Wagner,J. and Fuchs,R.P. (2000) All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *EMBO J.*, **19**, 6259–6265.
138. Lawrence,C.W., Borden,A. and Woodgate,R. (1996) Analysis of the mutagenic properties of the UmuDC, MucAB and RumAB proteins, using a site-specific abasic lesion. *Mol. Gen. Genet.*, **251**, 493–498.
139. Delaney,J.C. and Essigmann,J.M. (2001) Effect of sequence context on O^6-methylguanine repair and replication *in vivo*. *Biochemistry*, **40**, 14968–14975.
140. Hatahet,Z., Zhou,M., Reha-Krantz,L.J., Morrical,S.W. and Wallace,S.S. (1998) In search of a mutational hotspot. *Proc. Natl Acad. Sci. USA*, **95**, 8556–8561.
141. Hatahet,Z., Zhou,M., Reha-Krantz,L.J., Ide,H., Morrical,S.W. and Wallace,S.S. (1998) *In vitro* selection of sequence contexts which enhance bypass of abasic sites and tetrahydrofuran by T4 DNA polymerase holoenzyme. *J. Mol. Biol.*, **286**, 1045–1057.
142. Nunoshiba,T., Obata,F., Boss,A.C., Oikawa,S., Mori,T., Kawanishi,S. and Yamamoto,K. (1999) Role of iron and superoxide for generation of hydroxyl radical, oxidative DNA lesions and mutagenesis in *Escherichia coli*. *J. Biol. Chem.*, **274**, 34832–34837.
143. Nunoshiba,T., Watanabe,T., Nakabeppu,Y. and Yamamoto,K. (2002) Mutagenic target for hydroxyl radicals generated in *Escherichia coli* mutant deficient in Mn- and Fe-superoxide dismutases and Fur, a repressor for iron-uptake systems. *DNA Repair*, **1**, 411–418.