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Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites

Magnar Bjørås, Luisa Luna, Barbro Johnsen, Elsebeth Hoff, Terje Haug[1], Torbjørn Rognes and Erling Seeberg[2]

Department of Molecular Biology, Institute of Medical Microbiology, University of Oslo, The National Hospital, N-0027 Oslo and [1]Centre for Occupational and Environmental Medicine, The National Hospital, N-0027 Oslo, Norway

[2]Corresponding author
e-mail: e.c.seeberg@labmed.uio.no

The guanine modification 7,8-dihydro-8-oxoguanine (8-oxoG) is a potent premutagenic lesion formed spontaneously at high frequencies in the genomes of aerobic organisms. We have characterized a human DNA repair glycosylase for 8-oxoG removal, hOGH1 (human yeast OGG1 homologue), by molecular cloning and functional analysis. Expression of the human cDNA in a repair deficient mutator strain of *Escherichia coli* (*fpg mutY*) suppressed the spontaneous mutation frequency to almost normal levels. The hOGH1 enzyme was localized to the nucleus in cells transfected by constructs of hOGH1 fused to green fluorescent protein. Enzyme purification yielded a protein of 38 kDa removing both formamidopyrimidines and 8-oxoG from DNA. The enzymatic activities of hOGH1 was analysed on DNA containing single residues of 8-oxoG or abasic sites opposite each of the four normal bases in DNA. Excision of 8-oxoG opposite C was the most efficient and was followed by strand cleavage via β-elimination. However, significant removal of 8-oxoG from mispairs (8-oxoG: T >G >A) was also demonstrated, but essentially without an associated strand cleavage reaction. Assays with abasic site DNA showed that strand cleavage was indeed dependent on the presence of C in the opposite strand, irrespective of the prior removal of an 8-oxoG residue. It thus appears that strand incisions are made only if repair completion results in correct base insertion, whereas excision from mispairs preserves strand continuity and hence allows for error-free correction by a postreplicational repair mechanism.

Keywords: AP-lyase/base excision/DNA glycosylase/DNA repair/8-oxoguanine

Introduction

Reactive oxygen species are formed in aerobic organisms as by-products of the electron transport machinery and during oxidative stress conditions. Several types of oxidative DNA lesions are being formed, including strand breaks, baseless sugars (AP-sites) and a range of different oxidized base residues (reviewed by Demple and Harrison, 1994). Oxidative DNA damage has been implicated in mutagenesis and carcinogenesis, and may also play an important role in the pathogenesis of ageing (Fraga *et al*., 1990; Lindahl, 1993; Ames *et al.*, 1995). However, DNA repair mechanisms have evolved specifically to counteract the biological effects of oxidation damage. Of particular importance is the base excision repair pathway which is initiated by the action of DNA glycosylases removing different types of modified bases by cleavage of the *N*-glycosylic bonds. The AP-sites thus formed are recognized by AP-endonucleases or AP-lyases producing DNA strand breaks 5′ or 3′ to the baseless sugars, respectively. The termini are subsequently 'cleaned' by nucleases or phosphodiesterases and the repair is completed by resynthesis and ligation (Seeberg *et al*., 1995).

Purines undergo oxidation of the ring atoms leading to various chemical modifications. Most notably, the highly mutagenic guanine residue 7,8-dihydro-8-oxoguanine (8-oxoG) is formed in large quantities (Kasai *et al.*, 1986; Kasai and Nishimura, 1991), and imidazole-ring fragmented lesions formamidopyrimidines (faPy) are also abundant (Imlay *et al.*, 1988; Boiteux *et al*., 1992; Lindahl, 1993). The 8-oxoG lesion has strong miscoding properties and both bacterial and eukaryotic DNA polymerases insert A opposite 8-oxoG with high frequencies (Shibutani *et al*., 1991; Maki and Sekiguchi, 1992; Grollman and Moriya, 1993). Consequently, the presence of 8-oxoG residues in the template during replication induces G:C→T:A transversions (Wood *et al.*, 1990; Moriya *et al*., 1991; Cheng *et al*., 1992). Moreover, A:T→C:G transversions arise from the incorporation of 8-oxoG-containing deoxyribonucleotides formed in the precursor pool. In contrast, faPy residues represent blocks to DNA replication and have mostly cytotoxic effects (Boiteux and Laval, 1983).

Studies with *Escherichia coli* have shown that three different repair activities, MutM/Fpg (Boiteux *et al.*, 1987; Tchou *et al.*, 1991), MutY (Michaels *et al.*, 1990) and MutT (Maki and Sekiguchi, 1992), cooperate to prevent mutations from being formed at 8-oxoG lesions (Michaels and Miller, 1992; Tchou and Grollman, 1993; Tajiri *et al*., 1995). Formamidopyrimidine DNA glycosylase (Fpg) removes 8-oxoG as well as formamidopyrimidines from DNA. MutY is also a DNA glycosylase; it removes A from A:G and A:8-oxoG mispairs. Such mispairs are formed if 8-oxoG remains in the template during replication. The MutT protein hydrolyses 8-oxo-dGTP to 8-oxo-dGMP, thus preventing 8-oxo-dGTP from being incorporated during replication.

Searches for similar gene functions in mammalian cells have identified genes encoding enzymes with sequence homologies to MutY (Slupska *et al*., 1996) and MutT (Sakumi *et al*., 1993). However, mammalian cDNA sequences with homology to Fpg/MutM have not been identified, even though DNA glycosylase activities for 8-oxoG removal have been detected in extracts from

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OGG1     1 ------------------MSYKFGKLAINKSELCLANVLQAGQSFRWI------WDEKLN  36
hOGH1    1 MPARALLPRRMGHRTLASTPALWASIPCPRSELRLDLVLPSGQSFRWREQSPAHWSGVLA  60

OGG1    37 QYSTTMKIGQQEKYSVVILRQDEENEILEFVAVGDCGNQDALKTHLMKYFRLDVSLKHLF  96
hOGH1   61 DQVWTLTQTEEQLHCTVYRGDKSQASRPTP---------DELEA-VRKYFQLDVTLAQLY 110

OGG1    97 DNVWIPSDKAFAKLSP--QGIRILAQEPWETLISFICSSNNNISRITRMCNSLCSNFGNL 154
hOGH1  111 HH-WGSVDSHFQEVAQKFQGVRLLRQDPIECLFSFICSSNNNIARITGMVERLCQAFGPR 169

OGG1   155 ITTIDGVAYHSFPTSEELTSRATEAKLRELGFGYRAKYIIETARKLVNDKAEANITSDTT 214
hOGH1  170 LIQLDDVTYHGFPSLQALAGPEVEAHLRKLGLGYRARYVSASARAILEEQGGLA------ 223

OGG1   215 YLQSICKDAQYEDVREHLMSYNGVGPKVADCVCLMGLHMDGIVPVDVHVSRIAKRDYQIS 274
hOGH1  224 WLQQL-RESSYEEAHKALCILPGVGTKVADCICLMALDKPQAVPVDVHMWHIAQRDYSWH 282

OGG1   275 ANKNHLKELRTKYNALPISRKKINLELDHIRLMLFKKWGSYAGWAQGVLFSKEIGGTSGS 334
hOGH1  283 PTTSQAKG----------PSPQTNKELGNFFRSL---WGPYAGWAQAVLFSADLRQSRHA 329

OGG1   335 TTTGTIKKRKWDMIKETEAIVTKQMKLKVELSDLHIKEAKID                    376
hOGH1  330 QEPPAKRRKGSKGPEG-------------------------                    345
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Fig. 1. Alignment of the amino acid sequences of the *S.cerevisiae* OGG1 and the human hOGH1 proteins. Highlighted amino acids represent identity between sequences. DDBJ/EMBL/GenBank accession Nos are U44855 and Y11838, respectively.

human cells (Bessho *et al.*, 1993a). It has proven difficult to purify and analyse these enzymes in any detail, due to the low abundance of such enzymes in mammalian cells. However, a functional analogue of the bacterial Fpg enzyme was recently cloned and characterized from the yeast *Saccharomyces cerevisiae* (van der Kemp *et al.*, 1996). The sequence of this enzyme, OGG1, was found to be substantially different from the bacterial enzymes, thus explaining the difficulty in finding mammalian counterparts of the bacterial Fpg (Nash *et al.*, 1996; van der Kemp *et al.*, 1996).

In this communication, we have identified and characterized a human homologue of the yeast OGG1, termed hOGH1 (human OGG1 homologue 1). The purified enzyme of 38 kDa has DNA glycosylase activity towards 8-oxoG and faPy residues in DNA, like yeast OGG1. However, hOGH1 has different reaction patterns depending on the base residing opposite 8-oxoG in the complementary strand and cleaves DNA at AP-sites efficiently only across C in the opposite strand.

Results

Cloning and sequence analysis of the hOGH1 cDNA

Three different oligonucleotide primers were made from sequences derived from human expressed sequence tags (ESTs; EMBL accession numbers N55394 and W04935) translating into protein fragments with homology to yeast OGG1 (Nash *et al.*, 1996; van der Kemp *et al.*, 1996). Total DNA was extracted from five different cDNA libraries and screened by PCR with these primers. PCR fragments of the expected size were amplified from a library isolated from blood cells. The fragments were cloned and sequenced to confirm the identity of the ESTs. With these fragments as probes, seven positive clones with inserts ranging in size from 0.55 kb to 1.7 kb were isolated after plaque hybridization screening of 1.5×10^6 clones from the same blood library. One of these (*HOGH1*-83) contained an insert of 1616 bp with an open reading frame (ORF) translating into a polypeptide of 345 amino acids starting from the first ATG within the ORF. The ORF was preceded by 307 bp of an untranslated leader and succeeded by a 274 bp 3′-tail including a polyadenylation site (EMBL accession number Y11838). The hOGH1 protein is 30 amino acids shorter than yeast OGG1, with 38% overall identity and 58% overall similarity (Figure 1). The hOGH1 sequence contains the helix–hairpin–helix motif characteristic of the DNA glycosylase family including the conserved K^{249} and D^{268} residues (Seeberg *et al.*, 1995; Thayer *et al.*, 1995; Nash *et al.*, 1996).

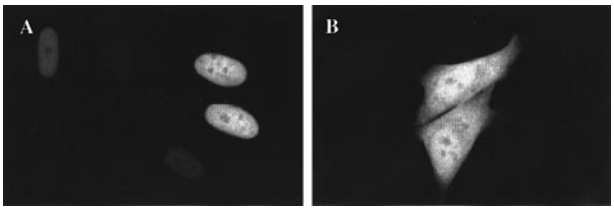

Fig. 2. Confocal laser scanning microscopy of HeLa cells expressing the hOGH1–GFP fusion protein. Cells were transfected with constructs expressing pHOGH1-EGFP-N12 (**A**) and p-EGFP-N1 (**B**).

Nuclear localization and chromosome assignment

A putative nuclear localization signal in the C-terminal region of hOGH1 (positions 333–339, PAKRRKG) was predicted by the PSORT algorithm (Nakei and Kaneisha, 1992). Transient transfection of HeLa cells with an expression vector of hOGH1 fused to green fluorescent protein (pEGFP-N1) produced a fluorescent signal only in the nucleus, thus confirming that hOGH1 is sorted to the nucleus (Figure 2).

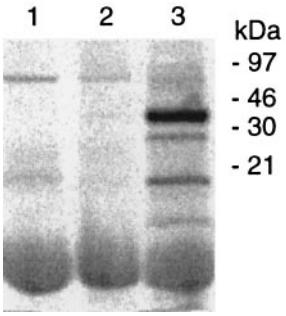

Fig. 3. Expression of *HOGH1* cDNA *in vitro*. Denaturating SDS–PAGE of [^{35}S]methionine-labelled proteins from reticulocyte lysate coupled transcription–translations. Lane 1, no DNA; lane 2, T3-dependent transcription/translation of pBK-CMV-*HOGH1*-83; lane 3, T7-dependent transcription/translation of pBK-CMV-*HOGH1*-83.

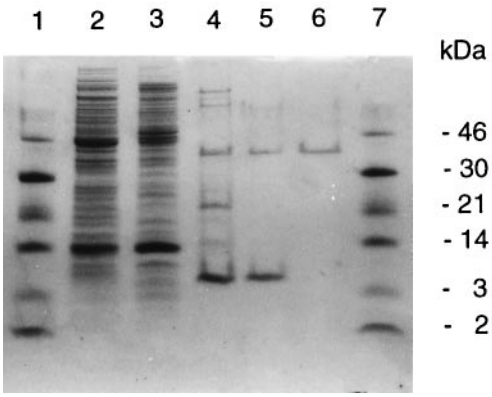

Fig. 4. SDS–PAGE of protein fractions obtained during purification of hOGH1. Lanes 1 and 7, molecular weight size markers as indicated; lane 2, Fraction I, cell extract; lane 3, Fraction II, Affigel Blue; lane 4, Fraction III, DNA–cellulose; lane 5, Fraction IV, MonoQ anion exchange chromatography; lane 6, Fraction V, MonoS cation exchange chromatography.

Table I. Suppression of spontaneous mutations to rifampicin resistance by expression of hOGH1 in *E.coli* BK3002 *(mutY fpg)*

Strain	No. of Rif^r mutants per 10^8 c.f.u.
BK3002/pUC18	533 ± 36
BK3002/pUC18-*HOGH1*-30	14 ± 12
AB1157 (wild type)	3 ± 1

Numbers represent the average of three independent experiments with standard deviations.

Database searches revealed identity between the last 56 bases of *HOGH1* and the last 56 bases (of the complementary strand) of the gene encoding human calcium–calmodulin dependent protein kinase I (CaMKI, EMBL accession no. L41816). Southern blot analysis of total genomic DNA and a human genomic *HOGH1* clone was compatible with the presence of a single genomic locus carrying this sequence (data not shown), indicating that the transcription units of these two genes are in opposite orientations with overlapping exons at the 3′-end. *HOGH1* thus co-localizes with *CAMK1* and maps to chromosome 3 (Haribabu *et al*., 1995).

In vitro expression of the HOGH1 cDNA

In vitro transcription/translation of the *HOGH1*-83 clone revealed a major ^{35}S-labelled product of size corresponding to the predicted M_r of 38 800 (Figure 3). The lysate containing the translation of the *HOGH1*-83 clone could cleave 8-oxoG-DNA, while such activity was not present in extracts from control translations without vector DNA or with transcription in the opposite orientation (data not shown). It thus appears that the human homologue of the yeast OGG1 is also involved in removing 8-oxoG from DNA.

Suppression of spontaneous mutations by expression of hOGH1 in a mutY fpg double mutant strain of E.coli

The Fpg (MutM) and MutY DNA glycosylases are both parts of the 8-oxoG repair system in *E.coli* that protects from the induction of mutations caused by spontaneous 8-oxoG formation. Double mutants carrying *mutY fpg* show a high rate of spontaneous mutations (Table I), mostly G:C to T:A transversions resulting from the misincorporation of A opposite 8-oxoG in the template (Michaels *et al.*, 1992). Expression of hOGH1 in this mutant reduces the spontaneous mutation frequency ~50-fold (Table I). However, the mutation frequency is still higher than in the wild type, implying that hOGH1 cannot fully substitute for the lack of the Fpg and the MutY functions in preventing spontaneous mutations in *E.coli*. Nevertheless, these results represent independent evidence for the 8-oxoG repair activity of hOGH1 and the functional similarities between hOGH1 and Fpg from *E.coli*.

Purification of hOGH1 expressed in E.coli

The *HOGH1* ORF (1035 bp) was amplified by PCR (primers IV/V) and subcloned into pUC18 for expression in *E.coli*. The sequence was checked to verify that no mutations had been introduced by the PCR. The double mutant *fpg nth* was used for expression to avoid interference and contamination by host enzymes in the assaying during purification. The hOGH1 protein was traced by assaying for faPy DNA glycosylase activity and purified to apparent homogeneity from 4 litres of cell culture by a four-step procedure involving Affigel Blue, DNA cellulose, MonoQ and MonoS chromatography. The final preparation produced a single protein band of 38 kDa upon SDS–PAGE (Figure 4).

Excision of faPy and 8-oxoG by hOGH1

The purified hOGH1 protein was found to remove faPy residues from a ^{3}H-labelled faPy-poly(dG–dC) substrate with efficiency similar to that of *E.coli* Fpg (Figure 5). The removal of 8-oxoG was analysed on duplex oligodeoxyribonucleotides containing a single 8-oxoG residue opposite each of the four normal DNA bases (Figure 6A). Enzymatic hOGH1 cleavage was evident only with the 8-oxoG:C substrate, although a faint band in the corresponding position was also observed for 8-oxoG:T. The band position was consistent with AP-site cleavage via β-elimination (AP-lyase), as would be expected. However, hOGH1 was apparently removing 8-oxoG from all the different substrates as revealed by including an AP-endonuclease (*E.coli* endonuclease IV,

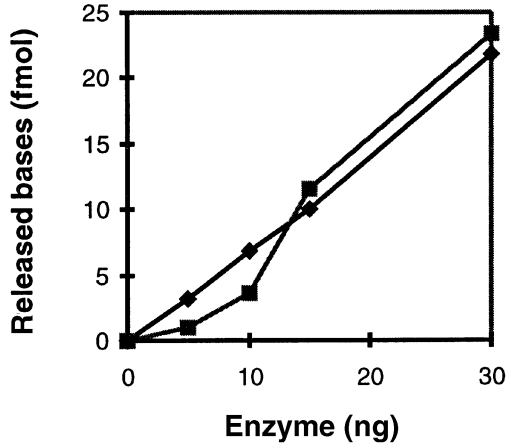

Fig. 5. FaPy DNA glycosylase activity of hOGH1. Release of faPy from ^{3}H-labelled faPy-poly(dG–dC) DNA by increasing amounts of purified human hOGH1 (■) and Fpg from *E.coli* (◆).

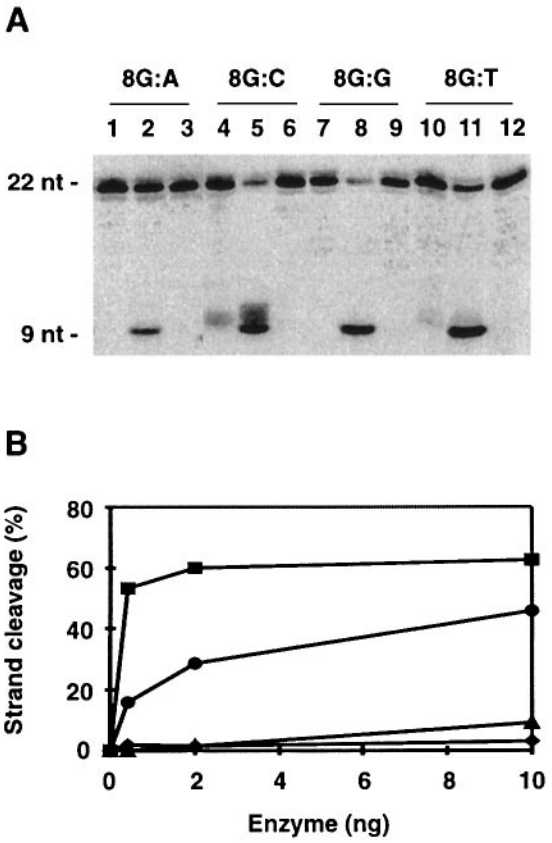

Fig. 6. 8-OxoG DNA glycosylase activity of hOGH1. (**A**) Purified hOGH1 (5 ng; lanes 1, 2, 4, 5, 7, 8, 10 and 11) was incubated with 10 fmol of a 22 bp duplex oligodeoxyribonucleotide containing a single 8-oxoG residue opposite A (lanes 1–3), C (lanes 4–6), G (lanes 7–9), or T (lanes 10–12). *E.coli* Nfo (5 ng) was included in lanes 2, 3, 5, 6, 8, 9, 11 and 12.The cleavage products were analysed by 20% denaturing PAGE and PhosphorImager scanning. (**B**) Increasing amounts of hOGH1 were incubated with 80 fmol 8-oxoG:C (■), 8-oxoG:T (●), 8-oxoG:G (▲) or 8-oxoG:A (◆) in the presence of 5 ng *E.coli* Nfo and the extent of strand cleavage was quantified by 20% PAGE and PhosphorImager scanning.

Nfo) in the assay to detect base removal by the formation of AP-sites. Nfo cleaves DNA specifically 5′ of AP-sites in DNA, leaving 3′-OH ends producing fragments

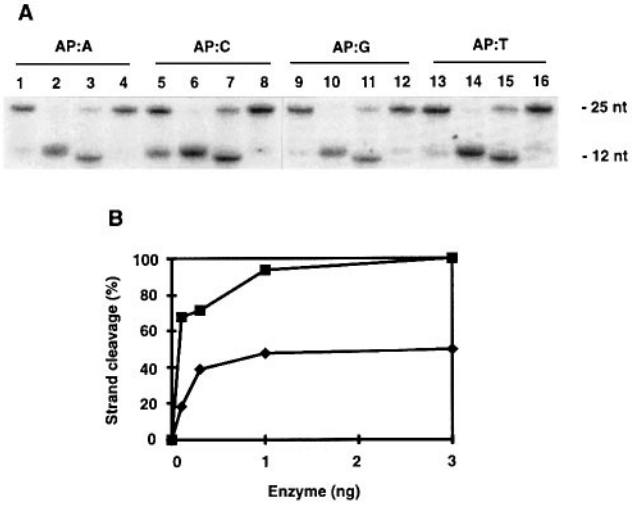

Fig. 7. Complementary strand dependent AP-lyase activity of hOGH1. (**A**) Purified hOGH1 (10 ng; lanes 1, 5, 9 and 13), *E.coli* Nth (2.5 ng; lanes 2, 6, 10 and 14), *E.coli* Nfo (2.5 ng; lanes 3, 7, 11 and 15) or no enzyme (lanes 4, 8, 12 and 16) was incubated with 80 fmol of a duplex 25-mer oligodeoxyribonucleotide containing a single AP-site opposite A (lanes 1–4), C (lanes 5–8), G (lanes 9–12) or T (lanes 13–16). (**B**) Eighty femtomoles of a duplex 25-mer oligodeoxyribonucleotide containing a single AP-site opposite C was incubated with various amounts of purified hOGH1 (◆) or *E.coli* Nth (■). Strand cleavage was quantified by 20% denaturing PAGE and PhosphorImager scanning.

migrating faster than those resulting from AP-lyase cleavage at the 3′-side (Figure 6A). No conversion was observed with Nfo alone, excluding the possibility that Nfo would be acting at AP-sites that might have been induced during substrate preparation. Control experiments with hOGH1 and heat-inactivated Nfo also gave results similar to those presented for hOGH1 alone (data not shown). These results indicate that hOGH1 will remove 8-oxoG from all basepair contexts, but has significant AP-lyase activity only when 8-oxoG is removed opposite C. However, experiments with increased amounts of substrate and reduced hOGH1 enzyme concentrations (excess Nfo) showed that 8oxoG:C is the best and 8oxoG:A the poorest substrate for hOGH1 (Figure 6B), as previously reported for Fpg (Tchou *et al*., 1991; Boiteux *et al.*, 1992). The yeast OGG1 was reported to have strong associated AP-lyase activity and also a clear preference for 8-oxoG opposite C and no detectable activity for 8-oxoG opposite A (Nash *et al*., 1996; van der Kemp *et al*., 1996).

The hOGH1 enzyme only cleaves AP-DNA with C in the complementary strand

The AP-lyase activity associated with hOGH1 was further analysed on DNA constructed to contain a single AP-site opposite each of the four normal bases in the complementary strand. Both *E.coli* Nth and Nfo introduced strand breaks on all substrates with the expected migration of their respective cleavage products (Figure 7A). In contrast, hOGH1 only cleaved the AP:C DNA. The band position was consistent with a β-elimination mechanism similar to that observed for Nth. These results indicate that hOGH1 cleaves AP-DNA with the same specificity as for the 8-oxoG-DNA. Reactions with higher concentrations of enzyme did not produce any significant AP-lyase activity

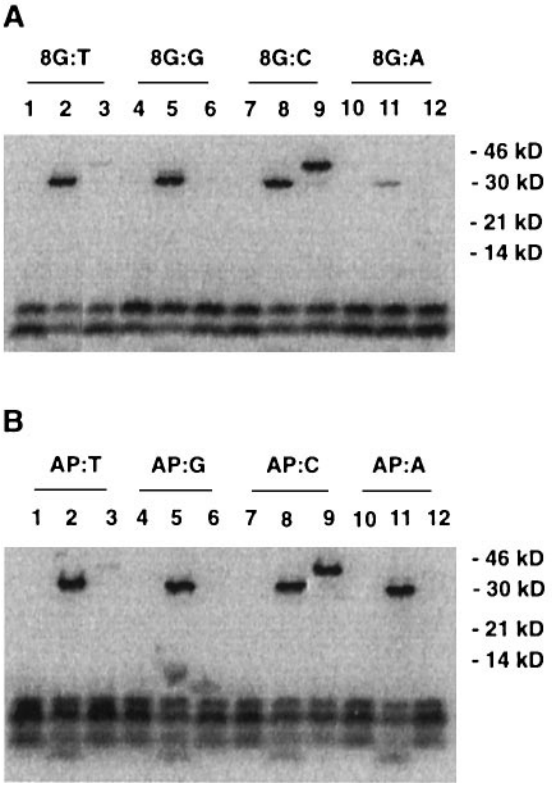

Fig. 8. Probing for covalent hOGH1 DNA intermediates by $NaCNBH_3$ reduction. Eighty moles of a duplex ^{32}P-labelled oligodeoxyribonucleotide containing a single 8-oxoG residue (**A**) or AP-site (**B**) opposite T (lanes 1–3), G (lanes 4–6), C (lanes 7–9) and A (lanes 10–12) was incubated with purified hOGH1 (5 ng; lanes 3, 6, 9 and 12), *E.coli* Fpg (5 ng; lanes 2, 5, 8 and 11) or buffer (lanes 1, 4, 7 and 10). Protein–DNA complexes were separated from DNA on 10% Tricine–SDS–PAGE.

for AP:A or AP:G whereas a weak activity (<5% cleavage) was observed for AP:T (data not shown). High amounts of enzyme did not produce more than ~50% cleavage of the AP:C substrate (Figure 7B). It is possible that hOGH1 only reacts at one configuration of the AP-site, for instance the α- or the β-hemiacetals, but elucidation of this point requires further investigation.

Formation of Shiff base intermediates of hOGH1 with 8-oxoG:C and AP:C DNA substrates

DNA glycosylases with associated AP lyase activity normally use an amino group as a nucleophile, resulting in a covalent imino enzyme–DNA substrate complex intermediate (Dodson *et al*., 1994). Such complexes can be trapped with $NaCNBH_3$ (Dodson *et al*., 1993) and monitored as DNA band shifts on SDS denaturing polyacrylamide gels. Experiments of this type confirmed the results from the DNA cleavage experiments implying that hOGH1 only has significant AP-lyase cleavage activity on 8-oxoG:C and AP:C DNA (Figure 8). For both the 8-oxoG-DNA and AP-DNA a weak band was observed also for substrates with T in the complementary strand. However, the amount of trapped hOGH1/AP:T intermediate is <1% after 2 h of incubation, whereas a similar amount of enzyme produced ~40% base excision in 30 min (Figure 6B). Comparative experiments with *E.coli* Fpg on the same substrates show weak activity on 8-oxoG:A and strong activity on the other 8-oxoG substrates (Figure 8A). However, on the AP-DNA a similar formation of Schiff base intermediates was observed, irrespective of the base in the opposite strand (Figure 8B). These results are consistent with previous data showing that Fpg has a weak activity on 8-oxoG:A DNA but similar activities for 8-oxoG removal in other basepair contexts (Tchou *et al*., 1991). However, Fpg cleavage at AP-sites is independent of the base residing in the opposite strand (Figure 8B).

Discussion

The guanine modification 8-oxoG probably represents the most important type of oxidation product induced in DNA, because of its mispairing properties, and because of the large number of residues formed per cell generation (Fraga *et al*., 1990; Lindahl, 1993; Ames *et al*., 1995). At least two different repair activities have previously been described for removal of 8-oxoG in human cells (Bessho *et al*., 1993a). One is a DNA glycosylase, whereas the other is a DNA endonuclease that cleaves phosphodiester bonds 3′ to 8-oxoG residues in DNA. It has been proposed that the glycosylase may be identical to the human DNA glycosylase (Mpg) for the repair of alkylation damage (Bessho *et al.*, 1993b). In this work, we have cloned the cDNA and characterized a human DNA glycosylase, hOGH1, removing 8-oxoG and faPy residues from DNA. The enzyme is homologous to the recently reported OGG1 enzyme from yeast (Nash *et al.*, 1996; van der Kemp *et al.*, 1996) and clearly different from the Mpg DNA glycosylase in human cells. Functional expression of hOGH1 in an 8-oxoG repair deficient mutator strain of *E.coli*, shows that the human enzyme to a large extent will suppress the formation of spontaneous mutations and can substitute for the repair defect of the mutant. Since hOGH1 is localized to the nucleus in human cells, it is conceivable that the enzyme has a similar antimutator effect on the human genome.

The hOGH1 enzyme was purified to apparent physical homogeneity after expression in *E.coli* and was shown to be a protein of M_r 38 000, in agreement with the molecular weight of 38.8 kDa predicted from the cDNA sequence. The efficiency with which the hOGH1 enzyme removes faPy lesions is similar to that of the bacterial Fpg enzyme (Figure 5). On the 8-oxoG-DNA substrates, hOGH1 is most effective on the 8-oxoG:C DNA, as would be expected from the previous studies of OGG1 from yeast. When strand cleavage is used to monitor enzymatic activity, an almost complete specificity is observed for 8-oxoG:C DNA (Figure 6). However, when the formation of an AP-site is measured, appreciable 8-oxoG base excision can also be detected for the other substrates, particularly for 8-oxoG:T, but also for 8-oxoG:G and to a lower extent for 8-oxoG:A. Such activity is potentially harmful as removal of 8-oxoG from mispairs might contribute to fix mutations in DNA. Since mispairs with A are formed with high frequencies during replication, it is as expected that removal of 8-oxoG across A is the least efficient reaction, similar to what was observed for Fpg and yeast OGG1. However, the hOGH1 enzyme has a second level of discrimination against mispairs since strand incisions are formed efficiently only when the correct base is situated in the opposite strand. This implies

that repair completion is triggered only when the correct base will be incorporated during repair synthesis, whereas strand continuity is preserved for mismatch removal. This situation is particularly pronounced for the removal of 8-oxoG opposite T where base excision is quite efficient and strand cleavage is hardly detectable. Maintaining the strand continuity, however, will permit error-free postreplication repair by strand exchanges, at least until the AP-site will be further processed by an AP-endonuclease. However, in a cellular context with all the other proteins present being involved in repair and replication, the AP-site may well be protected from further action by an AP-endonuclease.

The most unexpected finding of the hOGH1 characterization was the specificity observed for the AP-lyase activity. The AP-DNA constructed from the removal of uracil residues is treated with the same specificity as the 8-oxoG-DNA substrates with respect to strand cleavage, indicating that the AP-lyase reaction occurs independently of the 8-oxoG removal (Figures 7 and 8). This is also supported by the results showing that 8-oxoG opposite C is removed about twice as fast as the AP-site is cleaved (Figure 6A). The preferential cleavage of AP:C DNA suggests that hOGH1 interacts with the base in the opposite strand or discriminates between structural differences of AP-sites opposite different bases. NMR studies have shown that the structure of the abasic sites in DNA depends on the nature of the opposite base (Manoharan *et al.*, 1988). Preference for opposite base has not previously been observed for the strong AP-lyase activity associated with other repair DNA glycosylases involved in repair of oxidative DNA damage, e.g. *E.coli* Fpg and Nth (Boiteux, 1993), or human hNTH1 (Aspinwall *et al.*, 1997; L.Luna, M.Bjøras and E.Seeberg, unpublished).

DNA glycosylases with associated AP-lyase activities are shown to act through a strong nucleophile such as a lysine residue for activation of the C-1′ sugar carbon (Dodson *et al.*, 1994; Nash *et al.*, 1996). The lysine proposed to be involved in such activation is at the border of the hairpin structure in the helix–hairpin–helix motif (K^{241} in yeast OGG1; Figure 1). This lysine is also conserved in hOGH1 (K^{249}). Trapping of hOGH1 DNA covalent complexes by cyanoborhydride proves that an imino complex is involved in the enzymatic reaction of hOGH1 with 8-oxoG:C or AP:C DNA. However, there is no evidence for the formation of an imino complex for 8-oxoG base excision from the other substrates. The hOGH1 enzyme therefore appears to have a lyase-independent glycosylase activity which could be similar to those of the DNA glycosylases removing alkylated base residues from DNA. These enzymes are thought to act by a weaker nucleophile such as an activated water molecule (Dodson *et al.*, 1994; Labahn *et al.*, 1996; Nash *et al.*, 1996; Yamagata *et al.*, 1996). The possibility still exists that the imino intermediate is reversed after base removal such that strand break formation will not occur (Dodson *et al.*, 1994). However, significant protein–DNA imino complexes should then be trapped by the cyanoborhydride inhibition. We therefore conclude that the enzyme has a dual reaction mode; one bifunctional glycosylase/lyase reaction operating when 8-oxoG is positioned opposite C, and a monofunctional glycosylase mechanism operating when 8-oxoG is within mismatches.

The human hOGH1 enzyme represents still another member of the family of DNA glycosylases containing the helix–hairpin–helix motif (Seeberg *et al.*, 1995; Thayer *et al.*, 1995). Specificity of the base removal reaction appears to be strongly correlated with the protein sequence within this motif. Another motif shared by several members of this family, but not hOGH1 or OGG1, is the iron–sulfur cluster involved in DNA binding (Fisher *et al.*, 1992). A schematic alignment of the different members of this enzyme family from *E.coli*, yeast and mammalian cells is outlined in Figure 9. It can be predicted that the three-dimensional structure of all members of this protein family may be similar, although unknown functional and enzyme-specific domains may still be present in the N-terminal and/or the C-terminal parts of the enzymes. Some proteins participating in nucleotide excision repair have more than one function in the cells and this may also be the case for base excision repair proteins.

Materials and methods

Bacterial strains and enzymes

E.coli strain BK3010 (*nth fpg*) was constructed by phage T4 transduction of *fpg::kan* (BH10) into BW415 (*nth*). Strain BK3002 (*fpg mutY::*TetR*)* was generated by phage T4 transduction of the *mutY::*TetR marker of CSH117 (Coli Genetic Stock Centre) into BH20 (*fpg). E.coli* endonuclease III (Nth) and Fpg proteins were kindly provided by S.Boiteux. *E.coli* endonuclease IV (Nfo) was purified after expression from the expression plasmid pQE-Nfo by the 6×His tag purification system (Qiagen).

Oligodeoxyribonucleotides for PCR

Primers used were as follows: I,: 5′-CCCAAGCTTCTGGTTCTGGGTAGGCGG-3′; II, 5′-CGCGGATCCGCTCCACCATGCCAGTGAT-3′; III, 5′-CGCGGATCCGTGATACAGTTGAGCCAGGG-3′; IV, 5′-GGAATTCCATGCCTGCCCGCGCGCTT-3′; V, 5′-CGGGATCCATACAAATGTTTCTA-3′; VI, 5′-CGCGGATCCCCTTCCGGCCCTTTGGA-3′.

PCR amplification

Standard PCR was performed in 50 µl reaction mixtures containing 1.5 mM $MgCl_2$, 200 µM dNTPs, *Taq* polymerase (2 units) or *Pfu* polymerase (Promega), standard buffer (Promega or New England Biolabs) and 0.4 pmol of each primer. Two different amplification cycles were employed. For the PCR of *HOGH1* with primers I/II (525 bp) and I/III (377 bp), 3 min denaturation at 94°C was used followed by 35 cycles of amplification at 94°C (1 min), 55°C (1 min) and 72°C (45 sec), and the reaction was completed by extension for 10 min at 72°C. For the amplification of *HOGH1* with primers IV/V (1232 bp) and IV/VI (1034 bp), 3 min denaturation at 94°C was used followed by 32 cycles at 94°C (1 min), 50°C (1 min) and °C (1 in, 45 sec), and the reaction was completed by extension for 10 min at 72°C.

Hybridization screening and DNA sequence analysis

Five human cDNA libraries were used to screen for the presence of the *HOGH1* cDNA: hippocampus (cDNA library constructed in a modified pCDSp6T7 cloning vector; Bjørås *et al*., 1996); liver (catalogue no. HL 1115b, Clontech); fetal liver (catalogue no. HL 1064b, Clontech); blood (catalogue no. 938202, Stratagene); and brain (catalogue no. 936213, Stratagene). Only the blood library contained detectable levels of *HOGH1* cDNA and a probe was generated by PCR with primers I/II. The probe was used to search for a full-length cDNA by plaque hybridization. Southern blot hybridization was carried out at 68°C in a buffer of 3× SSC, 10× Denhardt's solution, 0.1% SDS, 10% dextran sulfate and 100 µg/ml denatured salmon sperm DNA. Probes were labelled using the Megaprime DNA labelling system or the *redi*prime DNA labelling system (Amersham). Positive clones (Lambda ZAP Express) were rescued into the plasmid vector pBK-CMV using the Exassist/XLOLR *in vivo* excision system (Stratagene). Sequencing was performed by the dideoxynucleotide chain-termination method using Sequenase Version 2.0 (US Biochemical Corp.). Geneting software (Lillestrom, Norway) and the GCG sequence analysis software package (University of Wisconsin, Madison, WI) were used to store, manipulate and analyse DNA sequences. Database searches were performed by the BLAST algorithm

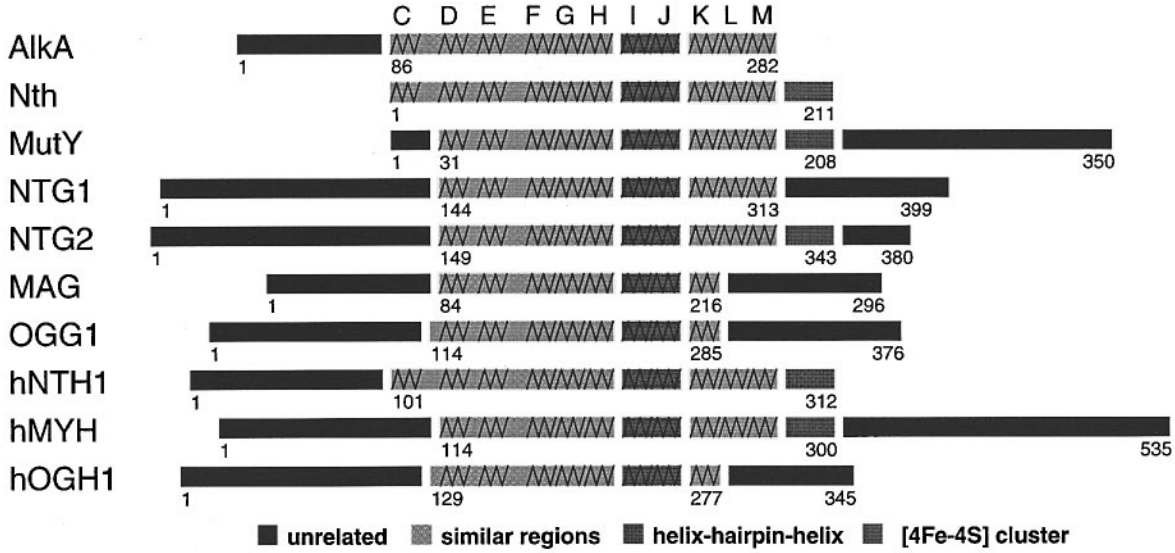

Fig. 9. Schematic alignment of DNA glycosylases from *E.coli*, yeast and human cells belonging to the helix–hairpin–helix superfamily. Based on direct and indirect sequence alignments with the *E.coli* AlkA and Nth sequences of known structure (Kuo *et al*., 1992; Labahn *et al*., 1996; Yamagata *et al.*, 1996).

(Altschul *et al.*, 1990) and by SALSA (T.Rognes and E.Seeberg, unpublished). The PSORT software (Nakai and Kanehisa, 1992) was used to predict intracellular localization.

Translation of the HOGH1 cDNA in vitro

In vitro transcription/translation was carried out using the TNT coupled reticulocyte lysate system from Promega. The plasmid vector used, pBK-CMV-*HOGH1*-83, contained the T7 RNA polymerase promoter for transcription in the correct orientation relative to the ORF, and the T3 RNA polymerase promoter for transcription starting downstream in the opposite orientation. Transcription–translation mixtures contained [^{35}S]methionine (1000 Ci/mmol, 10 mCi/ml, Amersham Corp.) and extracts were separated on a 10% SDS–polyacrylamide gel for PhosphorImager scanning (Molecular Dynamics) to detect labelled proteins.

Cellular localization of hOGH1–green fluorescent protein fusions

Primers IV and VI were used to introduce *Eco*RI and *Bam*HI restriction sites at the beginning and end of the *HOGH1* cDNA respectively. The amplified fragment *HOGH1*-IV/VI was inserted into a pEGFP-N1 vector (Chlontech). The construct was designated p*HOGH1*-EGFP-12 and was sequenced to verify that the construct was in-frame with the ATG of the EGFP–N1 fusion protein and the intactness of the putative nuclear localization signal. Transient transfection of HeLa cells were done with the $CaPO_4$ method (Profection, Promega) according to the manufacturer's recommendations and confocal microscopy of HeLa cells transfected with p*HOGH1*-EGFP-12 was carried out after 16 h as described by Nilsen *et al*. (1997).

Assays for faPy DNA glycosylase activity

All enzyme activities were assayed in a reaction buffer containing 70 mM MOPS, pH 7.5, 1 mM EDTA, 5% glycerol and 1 mM dithiothreitol and the mixtures were incubated at 37°C for 30 min. *N*-[^{3}H]methyl-*N*′-nitrosourea (18 Ci/mmol) was used to prepare poly(dG–dC) DNA containing faPy residues (5000 dpm/μg DNA) as described by Boiteux *et al*. (1984). FaPy DNA glycosylase activity was measured in a total volume of 50 μl containing 0.4 μg faPy–DNA substrate.

Assays for enzyme cleavage of 8-oxoG and AP-site containing DNA fragments

Duplex DNA fragments containing a single 8-oxoG residue at position 10 (ATCACCGGC[8-oxoG]CCACACGAGCTG) and duplex DNA containing a single AP-site at position 13 (GCTCATGCGCAG[·]CAGCCGTACTCG) were constructed as described by Eide *et al.* (1996); the 5′-ends were ^{32}P-labelled by T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (3000 Ci/mmol; Amersham). Reaction mixtures contained 10–80 fmol substrates and enzymes as indicated in a total volume of 5 μl. Samples were incubated for 30 min at 37°C unless otherwise indicated. The products of the reaction were analysed by 20% denaturing DNA sequencing gels and PhosphorImaging.

Sodium cyanoborhydride-mediated cross-linking of enzyme to oligonucleotides

Eighty femtomoles of 5′-^{32}P-end-labelled double-stranded oligonucleotides containing either a single 8-oxoG or AP-site opposite A, C, G or T were prepared as previously described (Eide *et al*., 1996). Purified *E.coli* Fpg or hOGH1 were incubated with oligonucleotides in the presence of 40 mM $NaCNBH_3$, 20 mM HEPES, pH 7.5, 50 mM KCl, 5 mM EDTA in a total volume of 12.5 μl. After incubation at room temperature for 2 h, 3 μl of 5× SDS–PAGE loading buffer was added to each sample. Samples were boiled for 5 min at 90°C, separated on 10% Tricine–SDS gel and analysed by PhosphorImaging.

Purification of the hOGH1 enzyme

Primers IV and V were used to introduce *Eco*RI and *Bam*HI restriction sites at the beginning and end, respectively, of the *HOGH1* cDNA coding region. The amplified fragment was ligated into a pUC18 expression vector (pUC18-*HOGH1*-30) and the construct was sequenced. *E.coli* BK3010 was transformed by pUC18-*HOGH1*-30 and grown in LB medium at 37°C to a density of 8×10^{8} cells/ml; IPTG (1 mM) was then added and the incubation continued for 2 h. Extracts were prepared by a combination of plasmolysis and lysozyme treatment as previously described (Seeberg, 1978). Assays for faPy activity was used to monitor hOGH1 purification. The cell extract (Fraction I) was applied to an Affigel Blue (Bio-Rad) column (2 cm×8 cm) equilibrated with buffer A (0.1 M Tris, pH 8.0, 1 mM EDTA, 20% glycerol, 10 mM mercaptoethanol) and the activity was step eluted with 1 M KCl in buffer A. Active fractions were pooled (Fraction II), desalted on Sephadex G-25M (Column PD-10, Pharmacia) equilibrated with buffer A containing 0.1 M KCl, and applied to a calf thymus DNA cellulose column (HR 5/5, Pharmacia). The column was eluted with a linear gradient of 0.1–0.4 M KCl. Active fractions eluting at 0.2 M KCl were pooled (Fraction III) and desalted on Sephadex G-25M equilibrated with buffer A containing 20 mM KCl. Fraction III was applied to a MonoQ column (HR 5/5; Pharmacia) and the flowthrough (Fraction IV) was collected and equilibrated with buffer B (50 mM MES, pH 6.0, 1 mM EDTA, 20% glycerol, 10 mM mercaptoethanol) by Sephadex G-25M and applied to a MonoS column (HR 5/5; Pharmacia). The MonoS column was eluted with a linear gradient from 0 to 0.5 M NaCl and the purified enzyme eluted at 0.4 M NaCl (Fraction V).

Mutation analysis

The *mutY fpg* double mutant BK3002 harbouring plasmid pUC18 or pUC18-*HOGH1*-30 and wild-type strain AB1157 (wild type) were grown overnight at 37°C in LB medium. Appropriate dilutions were plated on LB plates with ampicillin (100 μg/μl) for the plasmid-containing strains,

or without ampicillin for AB1157, to monitor viable cell count. Undiluted aliquots were plated on LB agar with rifampicin (100 μg/μl) and ampicillin for plasmid-containing strains, and only rifampicin for AB1157, to measure spontaneous mutations to rifampicin resistance. Colonies were counted after 24 h incubation at 37°C.

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References

Altschul,S.F., Gish,W., Miller,E., Myers,W. and Lipman,D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.

Ames,B.N., Gold,L.S. and Willet,W.C. (1995) The causes and prevention of cancer. *Proc. Natl Acad. Sci. USA*, **92**, 5258–5265.

Aspinwall,R., Rothwell,D.G., Roldan-Arjona,T., Anselmino,C., Ward, C.J., Cheadle,J.P., Sampson,J.R., Lindahl,T., Harris,P.C. and Hickson,I.D. (1997) Cloning and characterization of a functional human homolog of *Escherichia coli* endonuclease III. *Proc. Natl Acad. Sci. USA*, **94**, 109–114.

Bessho,T., Tano,K., Kasai,H., Ohtsuka,E. and Nishimura,N. (1993a) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J. Biol. Chem.*, **268**, 19416–19421.

Bessho,T., Roy,R., Yamamoto,K., Kasai,H., Nishimura,S., Tano,K. and Mitra,S. (1993b) Repair of 8-hydroxyguanine in DNA by mammalian *N*-methylpurine-DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **90**, 8901–8904.

Bjørås,M., Gjesdal,O., Erickson,J., Torp,R., Levy,L.M., Ottersen,O.P., Degree,M., Storm-Mathisen,J., Seeberg,E. and Danbolt,N.C. (1996) Cloning and expression of a neuronal rat brain glutamate transporter. *Mol. Brain Res.*, **36**, 163–168.

Boiteux,S. (1993) Properties and biological functions of the NTH and FPG proteins of *Escherichia coli*: two DNA glycosylases that repair oxidative DNA damage. *J. Photochem. Photobiol.*, **19**, 87–96.

Boiteux,S. and Laval,J. (1983) Imidazole open ring 7-methylguanine: an inhibitor of DNA synthesis. *Biochem. Biophys. Res. Commun.*, **110**, 552–558.

Boiteux,S., Belleney,J., Roques,B.P. and Laval,J. (1984) Two rotameric forms of open ring 7-methylguanine are present in alkylated polynucleotides. *Nucleic Acids Res.*, **12**, 5429–5439.

Boiteux,S., O'Connor,T.R. and Laval,J. (1987) Formamidopyrimidine-DNA glycosylase of *Escherichia coli*: cloning and sequencing of the *fpg* structural gene and overproduction of the protein. *EMBO J.*, **6**, 3177–3183.

Boiteux,S., Gajewski,J., Laval,J. and Dizdaroglu,M. (1992) Substrate specificity of the *Escherichia coli* Fpg protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, **31**, 106–110.

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.

Demple,B. and Harrison,L. (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annu. Rev. Biochem.*, **63**, 915–948.

Dodson,M.L., Schrock,R.D. and Lloyd,S. (1993) Evidence for an imino intermediate in the T4 endonuclease V reaction. *Biochemistry*, **32**, 8284–8290.

Dodson,M.L., Michaels,M.L. and Lloyd,R.S. (1994) Unified catalytic mechanism for DNA glycosylases. *J. Biol. Chem.*, **269**, 32709–32712.

Eide,L., Pirovano,M., Bjørås,M., Alseth,I., Berdal,K.G. and Seeberg,E. (1996) Base excision of oxidative purine and pyrimidine DNA damage by a DNA glycosylase from yeast whose sequence is similar to endonuclease III from *E. coli*. *Proc. Natl Acad. Sci. USA*, **93**, 10735–10740.

Fisher,C.L., O'Handley,S.F., Cunningham,R.P. and Tainer,J.A. (1992) Atomic structure of the DNA repair [4Fe-4S] enzyme endonuclease III. *Science*, **258**, 434–440.

Fraga,C.G., Shigenaga,M.K., Park,J.W. and Ames,B.N. (1990) Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proc. Natl Acad. Sci. USA*, **87**, 4533–4537.

Grollman,A.P. and Moriya,M. (1993) Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet.*, **9**, 246–249.

Haribabu,B., Hook,S.S., Selbert,M.A., Goldstein,E.G., Tomhave,E.D., Edelman,A.M., Snyderman,R. and Means,A.R. (1995) Human calcium–calmodulin dependent protein kinase I: cDNA cloning, domain structure and activation by phosphorylation at threonine-177 by calcium–calmodulin dependent protein kinase I kinase. *EMBO J.*, **14**, 3679–3686.

Imlay,J.A., Chin,S.M. and Linn,S. (1988) Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro*. *Science*, **240**, 640–642.

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, London, UK, pp. 99–116.

Kasai,H., Crain,P.F., Kuchino,Y., Nishimura,S., Ootsuyama,A. and Tanooka,H. (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis*, **7**, 1849–1851.

Kuo,C.F., McRec,D.E., Fisher,C.L., O'Handley,S.F., Cunningham,R.P. and Tainer,J.A. (1992) Atomic structure of the DNA repair [4Fe-45] enzyme endonuclease III. *Science*, **258**, 434–440.

Labahn,J., Scharer,O.D., Long,A., Ezaz-Nikpay,K., Verdine,G.L. and Ellenberger,T.E. (1996) Structural basis for the excision repair of alkylation-damaged DNA. *Cell*, **86**, 321–329.

Lindahl,T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.

Maki,H. and Sekiguchi,M. (1992) MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, **355**, 273–275.

Manoharan,M., Ransom,S.C., Mazumder,A. and Gerlt,J.A. (1988) The characterization of abasic sites in DNA heteroduplexes by site specific labeling with ^{13}C. *J. Am. Chem. Soc.*, **110**, 1620–1622.

Michaels,M.L. and Miller,J.H. (1992) The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine. *J. Bacteriol.*, **174**, 6321–6325.

Michaels,M.L., Pham,L., Nghiem,Y., Cruz,C. and Miller,J.H. (1990) MutY, an adenine glycosylase active on G–A mispairs, has homology to endonuclease III. *Nucleic Acids Res.*, **18**, 3841–3845.

Michaels,M.L., Cruz,C., Grollman,A.P. and Miller,J.H. (1992) Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. *Proc. Natl Acad. Sci. USA*, **89**, 7022–7025.

Moriya,M., Ou,C., Bodepudi,V., Johnson,F., Takeshita,M. and Grollman,A.P. (1991) Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in *E. coli*. *Mutat. Res.*, **254**, 281–288.

Nakai,K. and Kanehisa,M. (1992) A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics*, **14**, 897–911.

Nash,H., Bruner,S.D., Scharer,O.D., Kawate,T., Addona,T.A., Spooner,E., Lane,W.S. and Verdine,G. (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision repair protein superfamily. *Curr. Biol.*, **6**, 968–980.

Nilsen,H., Otterlei,M., Haug,T., Solum,K., Nagelhus,T.A., Skorpen,F. and Krokan,H. (1997) Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the *UNG* gene. *Nucleic Acids Res.*, **25**, 750–755.

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-oxodGTP, a mutagenic substrate for DNA synthesis. *J. Biol. Chem.*, **268**, 23524–23530.

Seeberg,E. (1978) Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated *uvrA*$^+$ and *uvrB*$^+$/*uvrC*$^+$ gene products. *Proc. Natl Acad. Sci. USA*, **75**, 2569–2573.

Seeberg,E., Eide,L. and Bjørås,M. (1995) The base excision repair pathway. *Trends Biochem. Sci.*, **20**, 391–397.

Shibutani,S., Takeshita,M. and Grollman,A.P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxoG. *Nature*, **349**, 431–434.

Slupska,M.M., Baikalov,C., Luther,W.M., Chiang,J.-H., Wei,Y.-F. and Miller,J.H. (1996) Cloning and sequencing a human homolog (*h*MYH) of the *Escherichia coli MutY* gene whose function is required for the repair of oxidative DNA damage. *J. Bacteriol.*, **178**, 3885–3892.

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.

Tchou,J. and Grollman,A.P. (1993) Repair of DNA containing the oxidatively-damaged base, 8-oxoguanine. *Mutation Res.*, **299**, 277–287.

Tchou,J., Kasai,H., Shibutani,S., Chung,M.H., Laval,J., Grollman,A.P. and Nishimura,S. (1991) 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc. Natl Acad. Sci. USA*, **88**, 4690–4694.

Thayer,M.M., Ahern,H., Xing,D., Cunningham,R.P. and Tainer,J.A. (1995) Novel DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure. *EMBO J.*, **14**, 4108–4120.

van der Kemp,P.A., Thomas,D., Barbey,R., de Oliveira,R. and Boiteux,S. (1996) Cloning and expression in *Escherichia coli* of the OGG1 gene of *Saccharomyces cerevisiae*, which encodes a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine. *Proc. Natl Acad. Sci. USA*, **93**, 5197–5202.

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.

Yamagata,Y. *et al.* (1996) Three-dimensional structure of a DNA repair enzyme, 3-methyladenine DNA glycosylase II, from *Escherichia coli*. *Cell*, **86**, 311–319.

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