A novel mechanism for preventing mutations caused by oxidation of guanine nucleotides

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MutT-related proteins, including the *Escherichia coli* MutT and human MutT homologue 1 (MTH1) proteins, degrade 8-oxo-7,8-dihydrodeoxyguanosine triphosphate (8-oxo-dGTP) to a monophosphate, thereby preventing mutations caused by the misincorporation of 8-oxoguanine into DNA. Here, we report that human cells have another mechanism for cleaning up the nucleotide pool to ensure accurate DNA replication. The human Nudix type 5 (NUDT5) protein hydrolyses 8-oxo-dGDP to monophosphate with a K_m of 0.77 μ M, a value considerably lower than that for ADP sugars, which were originally identified as being substrates of NUDT5. NUDT5 hydrolyses 8-oxo-dGTP only at very low levels, but is able to substitute for MutT when it is defective. When NUDT5 is expressed in *E. coli mutT*⁻ cells, the increased frequency of spontaneous mutations is decreased to normal levels. Considering the enzymatic parameters of MTH1 and NUDT5 for oxidized guanine nucleotides, NUDT5 might have a much greater role than MTH1 in preventing the occurrence of mutations that are caused by the misincorporation of 8-oxoguanine in human cells.

EMBO reports 4, 479–483 (2003)

doi:10.1038/sj.embor.embor838

INTRODUCTION

The formation of oxidized guanine nucleotides is a major cause of spontaneous mutations, and may contribute to carcinogenesis and ageing in mammals (Kasai *et al.*, 1986; Ames & Gold, 1991; Shibutani *et al.*, 1991; Sekiguchi & Tsuzuki, 2002). Studies on *Escherichia coli* mutator mutants have shown that cells have elaborate mechanisms to prevent mutations caused by the oxidation of the guanine base, both in DNA and in the free-nucleotide form. In DNA, 8-oxoguanine residues are removed by the enzyme encoded by the *mutM* gene, whereas the *mutY* gene product

removes adenine from an adenine:8-oxoguanine mismatch (Au *et al.*, 1988; Michaels *et al.*, 1992). Thus, two proteins, MutM and MutY, act consecutively at sites of oxidized guanine residues in DNA to prevent the occurrence of mutations (Tchou *et al.*, 1993). Alternatively, mutations due to the misincorporation of 8-oxo-dGTP can be prevented by the *mutT* gene product, which hydrolyses 8-oxo-dGTP (Maki & Sekiguchi, 1992). Mutations in *mutT* specifically cause A:T to C:G transversions (Yanofsky *et al.*, 1966), and this mutational specificity occurs through the combined actions of the MutM and MutY proteins (Tajiri *et al.*, 1995).

8-Oxoguanine-related mutagenesis may account for many spontaneous mutations in mammalian cells. Enzyme activities similar to those of the *E. coli* proteins mentioned above have been identified in mammalian cells (Yeh *et al.*, 1991; Bessho *et al.*, 1993) and, among them, the MutT-related protein has been studied the most extensively (Sekiguchi & Tsuzuki, 2002). On the basis of the partial amino-acid sequence determined from a purified 18-kDa protein that has 8-oxo-dGTPase activity, complementary DNA and the gene for the human enzyme were isolated, and the gene was called *MTH1* (Sakumi *et al.*, 1993; Furuichi *et al.*, 1994). As the expression of human *MTH1* cDNA in *E. coli mutT*⁻ cells significantly suppressed the frequency of spontaneous mutations in these cells, the *MTH1* protein may have the same antimutagenic ability as MutT. However, the levels of the increase in the frequency of spontaneous mutations due to the loss of MutT-related functions vary considerably between *E. coli* and mammalian cells. The frequency of spontaneous mutations detected in mouse *Mth1*^{-/-} cells is approximately twice that detected in *Mth1*^{+/+} cells (Tsuzuki *et al.*, 2001), whereas the mutation frequency in *E. coli mutT*⁻ cells is 1,000 times greater than that of wild-type cells (Yanofsky *et al.*, 1966; Akiyama *et al.*, 1989; Tajiri *et al.*, 1995). This difference may be due to the ability of the two types of enzyme to cleave 8-oxo-dGTP. The K_m values of MutT and MTH1 for 8-oxo-dGTP cleavage are 0.48 and 12.5, respectively (Maki & Sekiguchi, 1992; Mo *et al.*, 1992). Considering these facts, mammalian cells must have another mechanism that is able to efficiently eliminate 8-oxoguanine-containing nucleotides from the precursor pool.

8-Oxo-dGMP, which is formed by the action of MTH1, cannot be used for DNA synthesis, as the cellular guanylate kinase enzyme is completely inactive for 8-oxoguanine-containing nucleotides (Hayakawa *et al.*, 1995). However, 8-oxo-dGDP,

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Received 4 February 2003; revised 25 March 2003; accepted 31 March 2003

Published online 25 April 2003

Fig. 1 | NUDT5 and MutT-related proteins. (A) Comparison of amino-acid sequences of *Escherichia coli* MutT and human MTH1 and NUDT5 proteins (Sekiguchi & Tsuzuki, 2002). The 23-residue modules that were previously identified in MutT-related proteins are shown in the lower panel, in which amino-acid residues conserved in all three proteins are indicated by bold letters. Amino acids that could not be replaced by any other amino acid without losing 8-oxo-dGTPase activity are indicated by dots above the columns (Fujii *et al.*, 1999; Shimokawa *et al.*, 2000). (B) SDS-polyacrylamide gel electrophoresis analysis of purified NUDT5 proteins. Lane 1, molecular weight markers. Lane 2, amino-terminal His-tagged NUDT5. Lane 3, carboxy-terminal His-tagged NUDT5. Lane 4, C-terminal His-tagged NUDT5, treated with Factor Xa. Lane 5, an authentic NUDT5 protein, purified on an anti-NUDT5 IgG affinity column. Lane 6, Ni-NTA-agarose bound fraction from M15 cells transfected with vector only (negative control). In lanes 2–5, 0.5 µg of protein was loaded. For lane 6, the same amount of protein from the cell extracts as that used for the purification of 0.5 µg of N-terminal His-tagged NUDT5 was loaded. 8-oxo-dGTP, 8-oxo-7,8-dihydrodeoxyguanosine triphosphate.

which is produced by the direct oxidation of dGDP, and also by the enzymatic cleavage of 8-oxo-dGTP, is readily phosphorylated by nucleoside diphosphate kinase to generate 8-oxo-dGTP. Considering these facts, it seems important for mammalian cells to be able to degrade 8-oxo-dGDP to the monophosphate. We report here that human NUDT5, which was originally known as ADP-sugar pyrophosphatase (Gasmi *et al.*, 1999; Yang *et al.*, 2000), prevents mutations caused by the oxidation of guanine nucleotides by specifically degrading 8-oxo-dGDP to 8-oxo-dGMP.

RESULTS AND DISCUSSION

To identify an enzyme that degrades 8-oxo-dGDP to the monophosphate, we made use of the GenBank human EST (expressed sequence tag) database (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). On the basis of the 23-amino-acid sequence that is conserved in MutT-related proteins (Bessman *et al.*, 1996; Fujii *et al.*, 1999), cDNA clones were isolated using the BLAST programme. Among several candidates, NUDT5 was found to have the highest level of similarity to MutT-related proteins. Thirty (23.2%) and 27 (17.3%) amino-acid residues of NUDT5 are identical to those of MutT and MTH1, respectively. Amino-acid residues that are conserved in these three proteins were found to be located almost exclusively in the 23-residue conserved sequence, which is essential for the hydrolysis of a phosphodiester bond in Nudix and in diphosphoinositol derivatives (Bessman *et al.*, 1996; Fujii *et al.*, 1999; Nakabeppu, 2001). A comparison of amino-acid sequences from *E. coli* MutT and human MTH1 and NUDT5 proteins is shown in Fig. 1A. It should be noted that in the highly conserved regions, two of the amino-acid residues of NUDT5 (A96 and L98) differ from those of MutT and MTH1. The glycine residue (G37) of MutT, which corresponds to A96 of NUDT5, is essential for 8-oxo-dGTPase activity, as changes of this residue to any of the other 19 amino acids resulted in loss of enzyme activity (Shimokawa *et al.*, 2000). These amino-acid residues may be required for the substrate specificities of the enzymes.

NUDT5 was expressed as a His-tagged protein in *E. coli* M15 cells and was purified (Fig. 1B). Assays for enzyme activities were carried out using 5 µM 8-oxo-dGDP or 8-oxo-dGTP, and the products were analysed by high-performance liquid chromatography (HPLC). As shown in Fig. 2, NUDT5 efficiently degrades 8-oxo-dGDP to its monophosphate form. Under the same conditions, hydrolysis of 8-oxo-dGTP was never detected. Similar results were obtained with NUDT5 from which the His tag had been removed, which was made by treatment with Factor Xa, and with an authentic NUDT5 protein, which was purified on an anti-NUDT5 IgG affinity column (data not shown). The corresponding fraction derived from M15 cells carrying vector only (Fig. 1B, lane 6) had no hydrolytic activity.

The kinetic parameters of the NUDT5 enzyme (K_m and V_{max}) were measured for the hydrolysis of several nucleotides (Table 1). The K_m for the hydrolysis of 8-oxo-dGDP is ten times lower than that for dGDP, which is the second best substrate for the enzyme. 8-Oxo-dGTP is only hydrolysed by NUDT5 at very low levels under these conditions, but when a large amount of NUDT5 was used in the reaction, cleavage of 8-oxo-dGTP was detected, for which the apparent K_m was 63 µM. It should be noted that NUDT5 has a K_m of 0.77 µM for 8-oxo-dGDP, which is considerably lower than those for ADP sugars (32 µM for ADP-ribose, and higher values for other ADP sugars), which have previously been identified as substrates (Yang *et al.*, 2000). The value of NUDT5 for 8-oxo-dGDP is almost equal to that of MutT for 8-oxo-dGTP (0.48 µM). On the basis of these results, we conclude that 8-oxo-dGDP is a specific substrate for the NUDT5 protein.

To determine the biological significance of the cleavage of 8-oxo-dGDP, we expressed an *NUDT5* cDNA in *mutT*-deficient *E. coli* mutant cells. As the *mutT* mutator specifically results in an A:T to C:G transversion (Yanofsky *et al.*, 1966; Tajiri *et al.*, 1995), we used an *E. coli* tester strain (CC101T) to detect this type of

Fig. 2 | Effect of NUDT5 on 8-oxo-dGDP and 8-oxo-dGTP. (A) Reference nucleotides. (B) 8-Oxo-dGDP without treatment. (C) Treatment of 8-oxo-dGDP with NUDT5. (D) 8-Oxo-dGTP without treatment. (E) Treatment of 8-oxo-dGTP with NUDT5. In these experiments, 5 μ M 8-oxo-dGDP or 5 μ M 8-oxo-dGTP were incubated with a purified preparation of 0.16 μ M human amino-terminal His-tagged NUDT5 protein, under the conditions described in the Methods section (C,E), or were left untreated (B,D). The treated and untreated forms were then analysed by high-performance liquid chromatography. Arrowheads indicate peaks for 8-oxo-dGMP (single arrowhead), 8-oxo-dGDP (double arrowhead) and 8-oxo-dGTP (triple arrowhead). 8-Oxo-dGTP, 8-oxo-7,8-dihydrodeoxyguanosine triphosphate; 8-oxo-dGDP, 8-oxo-7,8-dihydrodeoxyguanosine diphosphate.

mutation (Furuichi *et al.*, 1994). Numerous papillae were formed in cells that carried the vector plasmid without cDNA, and this formation of papillae was almost completely suppressed when we introduced a plasmid carrying the human *NUDT5* cDNA into these cells (Fig. 3A,B). More quantitative data were obtained in a fluctuation test using a procedure described in Capizzi & Jameson (1973) and Fujii *et al.* (1999). The mutation rate in *mutT*⁻ cells is almost 1,000-fold higher than that in wild-type cells, and this increased mutation rate was reduced to the wild-type level by the introduction of *NUDT5* cDNA into *mutT*⁻ cells (Fig. 3C). These results show that human NUDT5 can function in *E. coli* to clean up the nucleotide pool. As a significantly high level of nucleoside triphosphatase activity is present in *E. coli* CC101T (*mutT*⁻) cell extracts (data not shown), suppression of the *mutT* mutator activity by NUDT5 can be explained by the successive reactions of nucleotide triphosphatase and NUDT5 (see Fig. 4).

We note that NUDT5 and MutT/MTH1 have opposite preferences for substrates; NUDT5 cleaves 8-oxo-dGDP, but not 8-oxo-dGTP, whereas MutT and MTH1 degrade 8-oxo-dGTP, but not 8-oxo-dGDP. As these nucleotides are interconvertible within a cell, NUDT5 can replace MutT function. These situations are illustrated in Fig. 4. 8-Oxo-dGDP can be phosphorylated to 8-oxo-dGTP by nucleoside diphosphate kinase, and 8-oxo-dGTP is cleaved to 8-oxo-dGDP by nucleoside triphosphatase (Mo *et al.*, 1992). In *E. coli* cells, MutT protein, which has a potent 8-oxo-dGTPase activity, is almost solely responsible for reducing the mutagenic nucleotide level, on the basis of the finding that *mutT*⁻ mutants show a 1,000-fold higher frequency of spontaneous mutations, as compared with wild-type cells. In human cells, however, two types of enzyme seem to function; MTH1

Table 1 | Substrate specificity of human NUDT5

Substrate	K_m	V_{max}	$V_{max}/K_m (\times 100)$
8-oxo-dGDP	0.77	1.000	130.000
dGDP	7.10	3.100	42.000
dADP	11.00	0.700	6.400
dTDP	13.00	0.340	2.600
dCDP	130.00	0.120	0.092
8-oxo-dGTP	63.00	0.059	0.094
dGTP	120.00	0.550	0.460

The relative velocities for the hydrolysis of nucleoside diphosphates and triphosphates were determined in timecourse experiments for eight concentrations of substrates. V_{max} was expressed as the ratio of that for 8-oxo-dGDP. 8-oxo-dGTP, 8-oxo-7,8-dihydrodeoxyguanosine triphosphate; 8-oxo-dGDP, 8-oxo-7,8-dihydrodeoxyguanosine diphosphate.

Fig. 3 | Suppression of A:T to C:G transversions by the expression of *NUDT5* complementary DNA. (A) *Escherichia coli* CC101T (*mutT*⁻) cells containing plasmid pQE30 (vector). (B) *E. coli* CC101T (*mutT*⁻) cells containing plasmid pQE30::NUDT5 (cDNA). (C) Mutation rates, as determined by *lacZ* reversion, in three independent experiments. *mutT*⁺ and *mutT*⁻ cells are of *E. coli* strains CC101 and CC101T, respectively, and carry either pQE30 (vector) or pQE30::NUDT5 (cDNA).

specifically hydrolyses 8-oxo-dGTP, and NUDT5 cleaves 8-oxo-dGDP. Taking into account the parameters for these enzymatic reactions, NUDT5 may have a greater role than does MTH1. It should be noted that 8-oxo-dGDP is a potent inhibitor of the MTH1 reaction (Bialkowski & Kasprzak, 1998; Fujikawa *et al.*, 1999). Thus, NUDT5 has another role in promoting the MTH1 reaction, in removing its inhibitor, 8-oxo-dGDP. In this respect, it is important to know the levels of 8-oxo-dGDP and 8-oxo-dGTP in the nucleotide pools, as well as their intracellular localization.

Recent studies of Mth1-deficient mice revealed that MTH1 is involved, to some extent, in the suppression of spontaneous tumorigenesis (Tsuzuki *et al.*, 2001). More definite conclusions about the biological significance of NUDT5 and MTH1 proteins in maintaining the integrity of genetic information might be obtained by producing mice deficient for NUDT5, as well as those lacking both proteins. Studies on this line are in progress.

METHODS

Cloning and purification of NUDT5. Human *NUDT5* cDNA from a lymph node cDNA library (Takara) was amplified by PCR using two primers, P5 (5'-CGTTTGACCATGGAGAGCCAAGAACCAACG-3') and P3 (5'-CTTAGTCTAGAAGGCCTGGTGGTCTCGTTT-3'), which are specific for *NUDT5* cDNA (Gasmi *et al.*, 1999; Yang *et al.*, 2000). The PCR product was subcloned into the *NcoI*-*XbaI* site of the plasmid pTT100 (Furuichi *et al.*, 1994), which is a derivative of pTrc99A (Amersham Pharmacia) and lacks the *lacI* gene, giving pTT100::NUDT5. Constructs that place His tags at the N and C termini of the NUDT5 protein were made by amplifying the inserts from pTT100::NUDT5 using primers encoding a Factor Xa

Fig. 4 | A model for the exclusion of 8-oxoguanine-containing deoxyribonucleotides from the DNA precursor pool in mammalian cells. 8-Oxo-dGTP and 8-oxo-dGDP, which are produced by the oxidation of dGTP and dGDP, respectively, are interconverted by the actions of nucleoside diphosphate kinase (a) and nucleoside triphosphatase (b). 8-Oxo-dGTP is misincorporated into DNA by DNA polymerase (c) to produce mutations. NUDT5 degrades 8-oxo-dGDP to 8-oxo-dGMP, a unusable form for DNA synthesis, and MTH1 cleaves 8-oxo-dGTP to 8-oxo-dGMP. As the activity of MTH1 is inhibited by 8-oxo-dGDP, NUDT5 works in two ways: first, to reduce the amount of substrate for 8-oxo-dGTP synthesis and, second, to promote the cleavage of 8-oxo-dGTP by MTH1. 8-oxo-dGTP, 8-oxo-7,8-dihydrodeoxyguanosine triphosphate; 8-oxo-dGDP, 8-oxo-7,8-dihydrodeoxyguanosine diphosphate.

protease-recognition site (Ile-Glu-Gly-Arg). To place the His tag at the N terminus, the primers P5Xa (5'-ATTGAAGGGAGAATGGAGAGCCAAGAACCAAC-3') and P3 were used, and the amplified insert was cloned into the pQE30 UA vector (Qiagen). For the C-terminal His-tagged construct, primers P5 and P3Xa (5'-CCAGGAATTCCCTTCCCTCTATAAATTTCAAGAAGGGCACT-3') were used, and the PCR product was cloned into the *NcoI*-*EcoRI* site of the pQETriSystem vector (Qiagen). Cells were transformed with these plasmids. As overproduction of NUDT5 considerably reduced the colony-forming ability of cells, isopropyl-1-thio-β-D-galactopyranoside (IPTG) was not added to the medium during these experiments. To purify NUDT5 protein, transformants of the M15 strain (Qiagen) were cultured in 200 ml of LB broth containing 50 μg ml⁻¹ ampicillin at 23 °C until they gave a spectrophotometric absorbance reading of 0.5 at 600 nm. Cell pellets were suspended in buffer A (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 5 mM dithiothreitol (DTT), 0.1 mM EDTA) containing 20 mM imidazole and were lysed by sonication. The lysate was applied to an Ni-NTA agarose column (Qiagen) after clarification by centrifugation. After washing with buffer A containing 100 mM imidazole, NUDT5 was eluted in buffer A containing 250 mM imidazole.

Proteins were concentrated using Microcon YM3 columns (Amicon) in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 5 mM DTT, 10% glycerol. Without IPTG induction, 1 mg of N-terminal and 0.4 mg of C-terminal His-tagged proteins were obtained. To remove the His tag, a purified preparation of C-terminal His-tagged NUDT5 was treated with Factor Xa (Qiagen) at 20 °C for 2 h. The protein was purified using Factor Xa-removal resin (Qiagen) and Ni-NTA agarose. Authentic NUDT5 protein was partially purified from cells expressing pTT100::NUDT5 using an *N*-hydroxysuccinimide (NHS)-activated Sepharose high-performance (HP) column (Amersham Pharmacia), which binds polyclonal anti-NUDT5 IgG, which was prepared by immunization of rabbits with purified His-tagged NUDT5.

Enzymatic reactions. 8-Oxo-dGTP, 8-oxo-dGDP and 8-oxo-dGMP were prepared as described in Fujikawa *et al.* (1999). The reaction mixture for hydrolysis assays contained 20 mM Tris-HCl, pH 8.0, 80 µg ml⁻¹ BSA, 8 mM MgCl₂, 40 mM NaCl, 5 mM DTT, 2% glycerol, and the protein sample to be examined. The reaction was carried out at 30 °C for 30 min and terminated by adding SDS to 0.1%. The samples were separated by HPLC using a TSK-GEL DEAE-2SW column (Tosoh) in an isocratic flow of 0.1 M sodium phosphate, pH 6.0, 40% acetonitrile, at a flow rate of 0.9 ml min⁻¹. Nucleotides were quantified by measuring the area of ultraviolet-light absorbance using a Whatmann HPLC detection system and the Millennium programme. The relative velocity for hydrolysis was determined in timecourse experiments with substrate concentrations in the range 0.1–10 µM for 8-oxo-dGDP, 2–200 µM for dGDP, dADP and dTDP, and 10–1,200 µM for dCDP, 8-oxo-dGTP and dGTP. *K_m* and relative *V_{max}* values were obtained from Lineweaver–Burk plots of the data.

ACKNOWLEDGEMENTS

We thank Y. Takagi and Y. Nakatsu for discussions.

REFERENCES

- Akiyama, M., Maki, H., Sekiguchi, M. & Horiuchi, T. (1989) A specific role of MutT protein: to prevent dG.dA mispairing in DNA replication. *Proc. Natl Acad. Sci. USA*, **86**, 3949–3952.
- Ames, B.N. & Gold, L.S. (1991) Endogenous mutagens and the causes of aging and cancer. *Mutat. Res.*, **250**, 3–16.
- Au, K.G., Cabrera, M., Miller, J.H. & Modrich, P. (1988) *Escherichia coli* mutY gene product is required for specific A-G→C-G mismatch correction. *Proc. Natl Acad. Sci. USA*, **85**, 9163–9166.
- Bessho, T., Tano, K., Kasai, H., Ohtsuka, E. & Nishimura, S. (1993) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J. Biol. Chem.*, **268**, 19416–19421.
- Bessman, M.J., Frick, D.N. & O'Handley, S.F. (1996) The MutT proteins or 'Nudix' hydrolases, a family of versatile, widely distributed, 'housecleaning' enzymes. *J. Biol. Chem.*, **271**, 25059–25062.
- Bialkowski, K. & Kasprzak, K.S. (1998) A novel assay of 8-oxo-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase (8-oxo-dGTPase) activity in cultured cells and its use for evaluation of cadmium(II) inhibition of this activity. *Nucleic Acids Res.*, **26**, 3194–3201.
- Capizzi, R.L. & Jameson, J.W. (1973) A table for the estimation of the spontaneous mutation rate of cells in culture. *Mutat. Res.*, **17**, 147–148.
- Fujii, Y., Shimokawa, H., Sekiguchi, M. & Nakabeppu, Y. (1999) Functional significance of the conserved residues for the 23-residue module among MTH1 and MutT family proteins. *J. Biol. Chem.*, **274**, 38251–38259.
- Fujikawa, K., Kamiya, H., Yakushiji, H., Fujii, Y., Nakabeppu, Y. & Kasai, H. (1999) The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. *J. Biol. Chem.*, **274**, 18201–18205.
- Furuichi, M., Yoshida, M.C., Oda, H., Tajiri, T., Nakabeppu, Y., Tsuzuki, T. & 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.
- Gasmi, L., Cartwright, J.L. & McLennan, A.G. (1999) Cloning, expression and characterization of YSA1H, a human adenosine 5'-diphospho-sugar pyrophosphatase possessing a MutT motif. *Biochem. J.*, **344**, 331–337.
- Hayakawa, H., Taketomi, A., Sakumi, K., Kuwano, M. & Sekiguchi, M. (1995) Generation and elimination of 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate, a mutagenic substrate for DNA synthesis, in human cells. *Biochemistry*, **34**, 89–95.
- Kasai, H., Crain, P.F., Kuchino, Y., Nishimura, S., Ootsuyama, A. & 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.
- Maki, H. & Sekiguchi, M. (1992) MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, **355**, 273–275.
- Michaels, M.L., Cruz, C., Grollman, A.P. & 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.
- Mo, J.Y., Maki, H. & 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.
- Nakabeppu, Y. (2001) Molecular genetics and structural biology of human MutT homolog, MTH1. *Mutat. Res.*, **477**, 59–70.
- Sakumi, K., Furuichi, M., Tsuzuki, T., Kakuma, T., Kawabata, S., Maki, H. & 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.
- Sekiguchi, M. & Tsuzuki, T. (2002) Oxidative nucleotide damage: consequences and prevention. *Oncogene*, **21**, 8895–8904.
- Shibutani, S., Takeshita, M. & Grollman, A.P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.
- Shimokawa, H., Fujii, Y., Furuichi, M., Sekiguchi, M. & Nakabeppu, Y. (2000) Functional significance of conserved residues in the phosphohydrolase module of *Escherichia coli* MutT protein. *Nucleic Acids Res.*, **28**, 3240–3249.
- Tajiri, T., Maki, H. & 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., Michaels, M.L., Miller, J.H. & Grollman, A.P. (1993) Function of the zinc finger in *Escherichia coli* Fpg protein. *J. Biol. Chem.*, **268**, 26738–26744.
- Tsuzuki, T. *et al.* (2001) Spontaneous tumorigenesis in mice defective in the *MTH1* gene encoding 8-oxo-dGTPase. *Proc. Natl Acad. Sci. USA*, **98**, 11456–11461.
- Yang, H. *et al.* (2000) Cloning and characterization of a new member of the Nudix hydrolases from human and mouse. *J. Biol. Chem.*, **275**, 8844–8853.
- Yanofsky, C., Cox, E.C. & Horn, V. (1966) The unusual mutagenic specificity of *E. coli* mutator gene. *Proc. Natl Acad. Sci. USA*, **55**, 274–281.
- Yeh, Y.C., Chang, D.Y., Masin, J. & Lu, A.L. (1991) Two nicking enzyme systems specific for mismatch-containing DNA in nuclear extracts from human cells. *J. Biol. Chem.*, **266**, 6480–6484.