Recombinant Schizosaccharomyces pombe Nth1 Protein Exhibits DNA Glycosylase Activities for 8-oxo-7,8-dihydroguanine and Thymine Residues Oxidized in the Methyl Group

Shin-Ichiro YONEKURA1, Nobuya NAKAMURA1, Takashi DOI1, Hiroshi SUGIYAMA2, Kazuo YAMAMOTO3, Shuji YONEI1 and Qiu-Mei ZHANG1*

DNA glycosylase/S. pombe Nth1/ Oxidative base damage/8-oxo-7,8-dihydroguanine/5-formyluracil/5-hydroxymethyluracil.

Bacteria and eukaryotes possess redundant enzymes that recognize and remove oxidatively damaged bases from DNA through base excision repair. DNA glycosylases remove damaged bases to initiate the base excision repair. The exocyclic methyl group of thymine does not escape oxidative damage to produce 5-formyluracil (5-foU) and 5-hydroxymethyluracil (5-hmU). 5-foU is a potentially mutagenic lesion. A homolog of E. coli endonuclease III (SpNth1) had been identified and characterized in Schizosaccharomyces pombe. In this study, we found that SpNth1 recognizes and removes 5-foU and 5-hmU from DNA with similar efficiency. The specific activities for the removal of 5-foU and 5-hmU were comparable with that for thymine glycol. The expression of SpNth1 reduced the hydrogen peroxide toxicity and the frequency of spontaneous mutations in E. coli nth nei mutant. It was also revealed that SpNth1 had DNA glycosylase activity for removing 8-oxo-7,8-dihydroguanine (8-oxoG) from 8-oxoG/G and 8-oxoG/A mismairs. These results indicated that SpNth1 has a broad substrate specificity and is involved in the base excision repair of 8-oxoG and thymine residues oxidized in the methyl group in S. pombe.

INTRODUCTION

Reactive oxygen species (ROS) are continuously generated as by-products of aerobic metabolism. Exogenous stimuli such as ionizing radiation and chemical carcinogens also produce ROS in cells. A prominent target for oxidation by ROS is the cellular DNA. The spectrum of ROS-induced damage to DNA is broad and includes various types of oxidative modifications to purine and pyrimidine bases,1–3 which might be involved in various biological processes including mutagenesis, carcinogenesis and aging.4,5 To overcome the deleterious effects of oxidative base damage in DNA, bacteria and eukaryotes have evolved base excision repair (BER). 8-oxo-7,8-dihydroguanine (8-oxoG) in DNA is primarily repaired by the BER initiated by MutM in E. coli and 8-oxoG-DNA glycosylase (Ogg1) in budding yeast and mammalian cells.6–10 Unrepaired 8-oxoG in the template directs the incorporation of A and sometimes G during DNA replication, yielding G:C→T:A and G:C→C:G transversions.3,11,12 In such a situation, MutY removes the A or G and provides a second chance for restoring the normal DNA sequence by removing 8-oxoG in a subsequent round of DNA replication.12,13 Endonucleases III (Nth) and VIII (Nei) of E. coli are also DNA glycosylases with associated AP lyase activity that catalyze both the cleavage of the glycosyl bond to release damaged bases and the incision of the phosphodiester backbone at the resulting AP sites via β- or β- and δ-elimination reactions.7,14,15 These two DNA glycosylases are primarily responsible for removal of oxidized pyrimidines such as thymine glycol (Tg), 5-hydroxycytosine and dihydrothymine from DNA.2,3,7,14 In mammalian cells, NTH1 and NEIL1–3 are homologs of E. coli Nth and Nei, respectively.16–18 In S. pombe, the nth1 gene encodes a DNA glycosylase (SpNth1) that functions in the repair of oxidative base damage in DNA.19–21 SpNth1 efficiently removes Tg, 5-hydroxycytosine, 5-hydroxy-6-hydrothymine, 5,6-
dihydroxycytosine and 5-hydroxycytosine from DNA.\textsuperscript{19,20} When the methyl group of thymine suffers oxidative damage, 5-hydroxycytosine is produced and spontaneously decomposes to generate two kinds of methyl group-oxidized thymine, 5-formyluracil (5-foU) and 5-hydroxymethyluracil (5-hmU).\textsuperscript{21} Recent studies revealed that 5-foU is potentially mutagenic.\textsuperscript{23,24} Several enzymes recognize and remove 5-foU and 5-hmU from DNA; MutM, Nei and Nth in \textit{E. coli}, Ntg1 and Ntg2 in \textit{S. cerevisiae}, and hNTH1, NEIL1 and SMUG in mammalian cells.\textsuperscript{25–29}

No homologs of MutM, Ogg1 or Nei have been identified in \textit{S. pombe}. \textit{S. pombe} has only one member of the Nth superfamily.\textsuperscript{19–21,30} How such organisms as \textit{S. pombe} are able to deal with the deleterious 8-oxoG and 5-foU that assault their genome remains unsolved. One possibility is that the single DNA glycosylase is able to recognize a broad spectrum of base damage. Recent studies have revealed that \textit{E. coli} Nth and human hNTH1 are able to recognize and remove 8-oxoG preferentially from 8-oxoG/G and 8-oxoG/A mispairs as efficiently as oxidized pyrimidines.\textsuperscript{31} In this study, it was demonstrated that SpNth1 efficiently removed 5-foU and 5-hmU from DNA. The expression of SpNth1 reduced the hydrogen peroxide toxicity and the frequency of spontaneous mutations in \textit{E. coli ntni} mutant. Furthermore, SpNth1 also cleaved the 8-oxoG-containing duplex oigonucleotides.

**MATERIALS AND METHODS**

**Enzymes and oligonucleotides**

Restriction enzymes and T4 polynucleotide kinase were obtained from Toyobo (Osaka, Japan). Thrombin was purchased from Amersham Pharmacia Biotech. (Uppsala, Sweden). \textit{E. coli} Nth protein was purified according to Zhang \textit{et al.}\textsuperscript{25} The 8-oxoG-containing oligonucleotide was obtained from Trevigen (Gaithersburg, MD). Oligonucleotides containing Tg, 5-foU or 5-hmU were prepared and purified as previously described.\textsuperscript{25,26} The presence of 5-foU and 5-hmU in oligonucleotide was verified by high-performance liquid chromatography/mass spectrometry (HPLC/MS) analyses. Oligonucleotides complementary to the damage-containing oligonucleotide were obtained from Takara Shuzo (Kyoto Japan). The nucleotide sequences of the oligonucleotides are shown in Fig. 1.

**E. coli strains and growth**

Bacteria used in this study were derivatives of \textit{E. coli} K12. Luria-Bertoni (LB) broth was used for culturing bacteria throughout this study. LB agar plates contained 2\% agar. When necessary, ampicillin (100 μg/ml), kanamycin (50 μg/ml) or chloramphenicol (30 μg/ml) was added to the medium.

**Construction of expression plasmid for SpNth1**

The \textit{S. pombe nthl} gene is composed of a 1,065-bp open reading frame that has no spliced region. Hence, genomic DNA from \textit{S. pombe} Y31 was prepared and used as the template for the polymerase chain reaction (PCR). One primer contained an \textit{EcoRI} site followed by the sequence around the putative start codon (5'-CCCGGAATTCATGAGTAAAGAC-3') and the other contained a \textit{SalI} site followed by the sequence around the stop codon (5'-ACCGCTGCATGAGTAAAGAC-3'). The amplified fragment was digested with \textit{EcoRI} and \textit{SalI}, and then the \textit{EcoRI} \textit{SalI} fragment containing the whole coding region of the \textit{nthl} gene of \textit{S. pombe} was sub-cloned into \textit{EcoRI/SalI}-digested \textit{E. coli} expression vector (pGEX-4T-1). The resulting plasmid was designated pGN2. The sequence was checked to verify that no mutations had been introduced by the PCR.

**Expression and purification of the SpNth1 protein**

Plasmid pGN2 was introduced into \textit{E. coli} BL21. The overnight culture was diluted 100-fold into fresh LB medium supplemented with 100 μg/ml of ampicillin and grown at 30°C until the optical density at 600 nm reached 0.6. After addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG), the culture was further incubated at 30°C for 4 hr. The cells were harvested by centrifugation at 8,000 × g for 10 min and resuspended in buffer A [50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 2 mM EDTA and 0.02% Triton X-100]. All subsequent procedures were carried out at 4°C. A cell extract was prepared by sonication of the cell suspension, followed by centrifugation at 30,000 × g for 30 min. The supernatant was applied to a glutathione (GSH)-Sepharose 4B column (Amersham Pharmacia Biotech.). After washing with buffer A, the glutathione S-transferase (GST)-SpNth1 fusion protein was eluted with 15 mM GSH in buffer A and the yellowish brown-colored fractions were pooled and dialyzed against buffer B [10 mM phosphate buffer (pH 7.6), 100 mM NaCl, 5% glycerol, 14.3 mM β-mercaptoethanol]. The active fractions were fractionated on a Resource S column (Amersham Pharmacia Biotech.) by a linear gradient.

\textbf{Fig. 1.} Nucleotide sequences of oligonucleotides. X, F, H and 8 represent Tg, 5-foU, 5-hmU and 8-oxoG, respectively.
of 100 to 500 mM NaCl. The active fractions of GST-SpNth1 (~150 mM NaCl) were pooled and treated with threonin at 4°C for 24 hr to remove the GST fragment. There was no difference in DNA glycosylase activity between GST-SpNth1 and purified SpNth1 for Tg-, 5-foU- and 5-hmU-containing oligonucleotides. Hence, we used GST-SpNth1 for the following biochemical studies. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The concentration of protein was determined using a BCA Protein Assay Reagent Kit (Pierce, Rockford, IL) with bovine serum albumin as the standard.

**DNA cleavage assay**

The oligonucleotides containing 5-foU, 5-hmU, Tg or 8-oxoG was labeled at the 5'-end with [γ-32P]ATP by T4 polynucleotide kinase and then annealed with the complementary oligonucleotide (Fig. 1). [γ-32P] ATP (>148 TBq/mmol) was obtained from ICN Biomedicals (Costa Mesa, CA). The DNA cleavage assay was performed at 37°C for 60 min in a reaction mixture (10 μl) containing 32P-labeled oligonucleotide substrates (100 fmol) and GST-SpNth1 protein in 25 mM Tris-HCl (pH 7.5) containing 2.5 mM EDTA, 1 mM Dithiothreitol, 50 mM NaCl and 5% glycerol. After addition of a stop solution (95% formamide, 0.1% BPB, 0.1% xylene cyanol and 20 mM EDTA), the samples were heated at 95°C for 5 min and cooled to room temperature. The samples were applied to 20% denaturing polyacrylamide gels containing 7 M urea. After electrophoresis at 1,300 V, the gels were dried and autoradiographed using Fuji RX film at –80°C. The densitometry analysis of the gel image was performed using NIH image software.

**Survival assay**

Overnight cultures of E. coli SY5, SY5 nth::Cm nei::Kan29, SY5 nth::Cm nei::Kan/pGEX-4T-1 and SY5 nth::Cm nei::Kan/pGN2 were appropriately diluted and plated onto LB plates containing hydrogen peroxide (0.05 mM). After incubation at 37°C for 20 hr, the number of viable colonies on the plates was counted to estimate the surviving fraction.

**Spontaneous mutation assay**

A single colony of E. coli SY5, SY5 nth::Cm nei::Kan29, SY5 nth::Cm nei::Kan/pGEX-4T-1 and SY5 nth::Cm nei::Kan/pGN2 was inoculated into LB medium and cultured at 37°C for 18 hr with vigorous aeration. A 0.1-ml aliquot of overnight culture was plated on an LB plate containing 100 μg/ml of rifampicin and then incubated at 37°C for about 20 hr. Mutant colonies on the plate were counted. The mutation frequency was expressed as number of mutants/108 viable cells.

**RESULTS**

Cleavage of Tg-, 5-foU and 5-hmU-containing oligonucleotides by SpNth1

SpNth1 is a DNA glycosylase with AP lyase activity that catalyzes both the cleavage of the glycosylic bond to release damaged bases and the subsequent incision of the phosphodiester backbone at the resulting AP site.19,20 In this study, active GST-fused SpNth1 was collected by GSH affinity chromatography, followed by further purification using Resource S and cation exchange column chromatography. We performed a DNA cleavage assay to examine whether SpNth1 has DNA glycosylase/AP lyase activity for 5-foU and 5-hmU in DNA. We measured single turnover rates. Increasing amounts of SpNth1 were incubated with double-stranded oligonucleotides containing 5-foU or 5-hmU, followed by polyacrylamide gel electrophoresis. The results are shown in Fig. 2. It was evident that SpNth1 cleaved the 5-foU- and 5-hmU-containing oligonucleotides via β-elimination reaction as E. coli Nth (Fig. 2A).

5-foU arises from the oxidation of the methyl group of thymine.21 Hence, the 5-foU/A base-pairs are formed in situ. On the other hand, 5-foU in the template DNA directs the incorporation of mismatched bases such as G opposite the lesion during DNA synthesis in vitro12,23 and in vivo.24,25 Therefore, we examined the cleavage efficiency of SpNth1 for 5-foU paired with A and G. The results are shown in Fig. 2. SpNth1 cleaved the oligonucleotides containing 5-foU paired with A and G with similar efficiency (Figs. 2B and 2C). The cleavage activity of SpNth1 for 5-foU-containing oligonucleotide was comparable with that for Tg-containing oligonucleotide (Fig. 2B).

5-hmU is another frequent and stable oxidation product in DNA. It arises from direct oxidation of the methyl group of thymine and the oxidation and deamination (or deamination and oxidation) of 5-methylcytosine in DNA.27,33 SpNth1 cleaved the duplex oligonucleotides containing 5-hmU/A and 5-hmU/G with nearly equal efficiency (Fig. 2C). The specific activities of SpNth1 for 5-foU/A and 5-hmU/A-containing oligonucleotides were 1.28 and 1.21 fmol/ng protein/hr, respectively, while 1.26 fmol/ng protein/hr for Tg/A-containing oligonucleotide (data not shown).

Cleavage of 8-oxoG-containing duplex oligonucleotides by SpNth1

The purified GST-SpNth1 could form the trapped complex with the 8-oxoG-containing duplex DNA (data not shown). To make it more evident that SpNth1 indeed has a DNA glycosylase/AP lyase activity for 8-oxoG, double-stranded oligonucleotide containing 8-oxoG/G or 8-oxoG/C was incubated with the purified GST-SpNth1, followed by polyacrylamide gel electrophoresis. The results are shown in Fig. 3. The cleavage products increased with the
amount of protein, indicating that the activity of SpNth1 was sufficient to remove 8-oxoG from 8-oxoG/G. Similar activity of SpNth1 was estimated for oligonucleotide containing 8-oxoG/A (data not shown). The specific activities of SpNth1 for cleavage of 8-oxoG/G- and Tg/A-containing oligonucleotides were 0.21 and 1.23 fmol/ng protein/hr, respectively. On the other hand, SpNth1 had a weak activity for 8-oxoG/C-containing oligonucleotides (Fig. 3B). The specific activity for 8-oxoG/C was 0.09 fmol/ng protein/hr with SpNth1, while 0.03 fmol/ng protein/hr with *E. coli* Nth.

Complementation of hydrogen peroxide sensitivity by the expression of SpNth1 in *E. coli* SY5 nth nei mutant

In order to determine whether SpNth1 is required for the cellular response to DNA damage in vivo, we introduced the SpNth1-bearing plasmid (pGN2) into *E. coli* SY5 nth nei mutant. The protective effect of SpNth1 against the cytotoxicity of hydrogen peroxide was examined with the *E. coli*

---

Fig. 2. Cleavage of the double-stranded oligonucleotides containing 5-foU or 5-hmU by the purified GST-SpNth1. (A) Double-stranded oligonucleotides (100 fmol) containing 5-foU/G (Oligo 3/5) or 5-hmU/G (Oligo 6/8) were incubated at 37°C for 60 min with GST-SpNth1 (lanes 1–3) and *E. coli* Nth (lanes 6–8) at 0 (lanes 1 and 8) and 100 (lanes 2, 3, 6 and 7) ng. Lane 4 and 5 represent the markers of 8- and 9-mer for lanes 1–3. (B) Double-stranded oligonucleotides (100 fmol) containing 5-hmU/A (Oligo 6/7), 5-hmU/G (Oligo 6/8), 5-foU/A (Oligo 3/4), 5-foU/G (Oligo 3/5) of Tg/A (Oligo 1/2) were incubated at 37°C for 60 min with GST-SpNth1 at 0 (lanes 1, 6, 11, 16 and 21), 12.5 (lanes 2, 7, 12, 17 and 22), 25 (lanes 3, 8, 13, 18 and 23), 50 (lanes 4, 9, 14, 19 and 24), 100 (lanes 5, 10, 15, 20 and 25), 150 (lane 26) ng. (C) The cleavage activity was plotted versus the amount of GST-SpNth1. The intensity of each band was analyzed using NIH image software. The values represent the mean ± standard deviation (n = 3). P values were obtained to determine the statistical significance of differences in cleavage activities at 50 and 100 ng of SpNth1 for a given substrate. The P values were >0.05, indicating no significant difference at the 95% confidence level. - –, 5-foU/G; - , 5-foU/A; - , 5-hmU/G; - , 5-hmU/A.
Repair of 8-oxoG, 5-foU and 5-hmU by S. pombe Nth1

J. Radiat. Res., Vol. 48, No. 5 (2007); http://jrr.jstage.jst.go.jp

SY5 nth nei mutant\(^{29}\) with or without pGN2. The results are shown in Fig. 4. The nth nei mutant was highly sensitive to hydrogen peroxide. The introduction of pGN2 conferred resistance to hydrogen peroxide on E. coli SY5 nth nei, while the vector pGEX-4T-1 did not.

**Complementation of spontaneous mutations by the expression of SpNth1 in E. coli nth nei**

Next, we examined whether spontaneous mutations are suppressed by the expression of SpNth1 in E. coli SY5 nth nei. Table 1 represents that E. coli SY5 nth nei mutant

**Table 1.** Spontaneous mutation to rifampicin resistance in E. coli SY5 nth nei mutant with plasmid carrying S. pombe nth1 gene

| Strain          | Rifampicin-resistant mutants/10\(^8\) cells |
|-----------------|------------------------------------------|
| SY5             | 9 ± 4                                    |
| SY5 nth nei     | 50 ± 3\(^a\)                              |
| SY5 nth nei/pGEX-4T-1 | 51 ±16\(^a\)                           |
| SY5 nth nei/pGN2 | 17 ± 6\(^b\)                              |

The values are the mean of 5 separate experiments \pm standard deviation. \(^a\)P value <0.05 indicates a significant difference from the value for SY5 at the 95% confidence level. \(^b\)P value >0.05 indicates no significant difference at the 95% confidence level.
showed enhanced spontaneous mutation compared with the wild-type strain, as previously reported.29) The expression of SpNth1 significantly reduced the mutation frequency, while the vector did not show such complementation (Table 1).

**DISCUSSION**

The potential role of SpNth1 in repair of oxidatively damaged base in DNA was investigated. It was evident that SpNth1 is capable of suppressing the hydrogen peroxide-sensitive phenotype of *E. coli nth nei* mutant (Fig. 4). SpNth1 significantly restored the frequency of spontaneous mutations in the *nth nei* mutant under aerobic conditions (Table 1). Furthermore, the present experiments revealed that SpNth1 efficiently cleaved oligonucleotides containing Tg, 5-foU and 5-hmU (Fig. 2). Therefore, these results could imply that SpNth1 is capable of removing oxidatively damaged pyrimidine bases with high efficiency.

The sensitivity to hydrogen peroxide significantly increased in *E. coli nth nei* mutant, compared with in wild-type strain.14,25) Single mutations in the *nth* or *nei* gene did not affect the hydrogen peroxide sensitivity.25) Therefore, these gene products could be a back-up enzyme to repair oxidative base damage in DNA in *E. coli*. The *nth* mutants of *S. pombe* showed no increase in sensitivity to hydrogen peroxide and menadione.34) This might be due to a second *Nth* and/or *Nei* homologs in *S. pombe* as in *E. coli*. However, we failed to find any homologs of *Nth* and *Nei* in the sequence database. Previous studies also suggested that *S. pombe* possesses a member of the *Nth* family.19–21,30) Further approaches are needed to identify functional homologs of *Nth* and *Nei* and clarify their roles in repair of oxidative base damage.

In this study, it was evident that SpNth1 has DNA glycosylase activity that excises 5-foU and 5-hmU (Fig. 2). 5-foU is potentially mutagenic.23,24) Nth, Nei and MutM recognize and remove 5-foU from DNA to prevent mutations,25–29) On the other hand, the biological consequences of the formation of 5-hmU in DNA remain uncertain. When 5-hmU arises in DNA via the oxidation of thymine, it makes a normal base-pair with A. Recently, Rusmintratip and Sowers33) and Hori *et al.*27) suggested another pathway of 5-hmU formation: oxidation and deamination of 5-methylcytosine (5-mC). The oxidation of 5-mC to 5-hmU causes the transitions from C:G to T:A. The transition is the most frequent base substitution found in human cancer.33,35) Accordingly, bacteria and mammalian cells must remove the 5-hmU from DNA. In *E. coli*, MutM, Nth and Nei play critical roles in the repair of 5-hmU/G mispairs to avoid C:G to T:A transitions.27) The present experiments revealed that SpNth1 efficiently cleaved oligonucleotides containing 5-hmU as 5-foU and Tg, which is the first demonstration of repair mechanisms for oxidative damage of thymine in the methyl group in *S. pombe*. The activity of *E. coli* Nth for removing 5-hmU from 5-hmU/G was much greater than from 5-hmU/A.25) However, SpNth1 cleaved the 5-hmU/A-containing duplex oligonucleotide with nearly equal efficiency to the 5-hmU/G oligonucleotide (Fig. 2). The difference in substrate recognition between *E. coli* Nth and SpNth1 remains unsolved.

8-oxoG is abundantly produced by oxidation of G in cellular DNA.2,3) 8-oxoG is the most important damaged base because of its property of mispairing with A or G during DNA replication, forming the base substitutions G:C→T:A and G:C→C:G.3,11–13) In all organisms tested so far from *E. coli* to humans, 8-oxoG in DNA is repaired primarily by the BER pathway, and initiated by a specific DNA glycosylase2,3,6–10) homologous to either MutM in *E. coli* or Ogg1 in mammalian cells. However, neither homolog of MutM nor Ogg1 has been identified in *S. pombe*. How, then, is this organism able to deal with 8-oxoG in the genome? One possibility is that SpNth1 is able to recognize a broader spectrum of base damage than the orthologs of Nth in *E. coli*, *S. cerevisiae* and human cells.

8-oxoG is likely to be generated through two pathways. One is the direct oxidation of G in DNA, and the other is the incorporation of oxidatively modified dGMP, through 8-oxo-dGTP, by DNA polymerases during DNA replication. Thus, the misincorporation of 8-oxoG from the nucleotide pool would be a major source of 8-oxoG in DNA.50) MutM and MutY proteins cannot recognize the 8-oxoG incorporated into nascent strand opposite A or G. Hence, another DNA glycosylase activity must be efficient in removing 8-oxoG from 8-oxoG/A or 8-oxoG/G pairs. Previously, Hazra *et al.*31) characterized a second Ogg activity in *S. cerevisiae* and human cells. These enzymes remove 8-oxoG incorporated into the nascent strand opposite G or A. Nei had similar activity to the enzymes in being able to remove 8-oxoG when paired with G or A, and thereby to prevent mutations.30) Furthermore, Matsumoto *et al.*31) demonstrated that *E. coli* Nth and hNTH1 recognizes and removes 8-oxoG preferentially from 8-oxoG/G or 8-oxoG/A pairs. In this study we demonstrated that SpNth1 is an 8-oxoG DNA glycosylase that catalyzes the cleavage of the glycosyl bond to release 8-oxoG preferentially from 8-oxoG/G and 8-oxoG/A mispairs in DNA (Fig. 3). SpNth1 may also operate in the repair of misincorporated 8-oxoG opposite G or A in the template to prevent mutations in *S. pombe*. Recently Kino and Sugiyama30) found that imidazolone, an oxidized deriv-ative of 8-oxoG, is a major lesion of guanine leading to G:C to C:G transversions. Therefore, it is of interest to examine whether SpNth1, Nth and human NTH1 are able to remove the lesion from DNA to reduce the frequency of G:C to C:G transversions.

SpNth1 cleaved, if slightly, the 8-oxoG/C-containing oligonucleotide, while *E. coli* Nth did not (Fig. 3B). The results suggested that SpNth1 might play a role, if weakly, in repairing 8-oxoG/C in DNA. There must exist another repair enzyme for 8-oxoG produced in situ. Nucleotide excision
repair pathway may operate on oxidatively damaged bases such as 8-oxoG in DNA. Further investigations are needed to elucidate the repair mechanisms for 8-oxoG/C in DNA in *S. pombe*.

**ACKNOWLEDGEMENTS**

This work was supported by Grants-in-Aid for Scientific Research and Grant-in-Aid for 21st Century COE Research Kyoto University (A-14) from the Ministry of Education, Science, Sports and Culture of Japan. We are grateful to the Central Research Institute of Electric Power Industry (Tokyo) and Takeda Science Foundation (Osaka) for supporting Q.-M. Zhang.

**REFERENCES**

1. Cadet, J., Delatour, T., Douki, T., Gasparutto, D., Pouget, J.-P., Ravanat, J.-L. and Sauvaigo, S. (1999) Hydroxyl radicals and DNA base damage. Mutat. Res., 424: 9–21.
2. Wallace, S. S. (2002) Biological consequences of free radical-damaged DNA bases. Free Radical Biol. Med., 33: 1–14.
3. Bjelland, S. and Seeberg, E. (2003) Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutat. Res., 531: 37–80.
4. Ames, B. N., Shigenaga, M. K. and Hagen, T. M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Nat. Acad. Sci. USA, 90: 7915–7922.
5. Jackson, A. L. and Loeb, L. A. (2001) The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat. Res., 477: 7–21.
6. Michaels, M. L., Pham, L., Cruz, C. and Miller, J. H. (1991) MutM, a protein that prevents G:C→T:A transversions, is a 5-methylaminomethyluracil-DNA glycosylase. Nucieic Acids Res., 19: 3629–3632.
7. Gros, L., Saparbaev, M. K. and Laval, J. (2002) Enzymology of the repair of free radicals-induced DNA damage. Oncogene, 21: 8905–8925.
8. 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 codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. Proc. Nat. Acad. Sci. USA, 93: 5197–5202.
9. Hazra, T. K., Hill, J. W., Izumi, T. and Mitra, S. (2001) Multiple DNA glycosylases for repair of 8-oxoguanine and their potential in *in vivo* functions. Prog. Nucieic Acids Res. Mol. Biol., 68: 193–205.
10. Rosenquist, T. A., Zharkov, D. O. and Grollman, A. P. (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. Proc. Nat. Acad. Sci. USA, 94: 7429–7434.
11. Hsu, G. W., Ober, M., Carell, T. and Reese, L. S. (2004) Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase. Nature, 431: 217–221.
12. Zhang, Q.-M., Ishikawa, N., Nakahara, T. and Yonei, S. (1998) *Escherichia coli* MutY protein has a guanine-DNA glycosylase that acts on 7,8-dihydro-8-oxoguanine:guanine mispair to prevent spontaneous GC-CG transversions. Nucieic Acids Res., 26: 4669–4675.
13. 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. Nat. Acad. Sci. USA, 89: 7022–7025.
14. Wallace, S. S. (1997) Oxidative damage to DNA and its repair. In Scandalios, J. G. (ed.), Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, NY, pp. 49–90.
15. Bailly, V. and Verly, W. G. (1987) *Escherichia coli* endonuclease III is not an endonuclease but a β-elimination catalyst. Biochem. J., 242: 565–572.
16. 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. Nat. Acad. Sci. USA, 94: 109–114.
17. Hazra, T. K., Izumi, T., Boldogh, I., Imhoff, B., Kow, Y. W., Jaruga, P., Dizdaroglu, M. and Mitra, S. (2002) Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. Proc. Nat. Acad. Sci. USA, 99: 3523–3528.
18. Takao, M., Kanno, S., Kobayashi, K., Zhang, Q.-M., Yonei, S., van der Horst, G. T. J. and Yasui, A. (2002) A back-up glycosylase in *Nhl1* knock-out mice is a functional Nei (endonuclease VIII) homologues. J. Biol. Chem., 277: 42205–42213.
19. Roldan-Arjona, T., Anselmino, C. and Lindahl, T. (1996) Molecular cloning and functional analysis of a *Schizosaccharomyces pombe* homologue of *Escherichia coli* endonuclease III. Nucieic Acids Res., 24: 3307–3312.
20. Karahalil, B., Roldan-Arjona, T. and Dizdaroglu, M. (1998) Substrate specificity of *Schizosaccharomyces pombe* Nth protein for products of oxidative DNA damage. Biochemistry, 37: 590–595.
21. Alseth, I., Korvald, H., Osman, F., Seeberg, E. and Bjørås, M. (2004) A general role of the DNA glycosylase Nth1 in the abasic sites cleavage step of base excision repair in *Schizosaccharomyces pombe*. Nucieic Acids Res., 32: 5119–5125.
22. Tofighi, S. and Frenkel, K. (1989) Effect of metals on nucleoside hydroperoxide, a product of ionizing radiation in DNA. Free Radical Biol. Med., 7: 131–143.22.
23. Bjelland, S., Anensen, H., Knavelsrud, I. and Seeberg, E. (2001) Cellular effects of 5-formyluracil in DNA. Mutat. Res., 486: 147–154.
24. 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.
25. Zhang, Q.-M., Miyabe, I., Matsumoto, Y., Kino, K., Sugiyama, H. and Yonei, S. (2000) Identification of repair enzymes for 5-formyluracil in DNA. Nth, Nei and MutM proteins of *Escherichia coli*. J. Biol. Chem., 275: 35471–35477.
26. Zhang, Q.-M., Hashiguchi, K., Kino, K., Sugiyama, H. and Yonei, S. (2003) Ntg1 and Ntg2 proteins as a 5-formyluracil-DNA glycosylases/AP lyases in *Saccharomyces cerevisiae*. Int. J. Radiat. Biol., 79: 341–349.
27. Hori, M., Yonei, S., Sugiyama, H., Kino, K., Yamamoto, K.,
and Zhang, Q.-M. (2003) Identification of high excision capacity for 5-hydroxymethyluracil mispaired with guanine in DNA of Escherichia coli MutM, Nei and Nth DNA glycosylases. Nucleic Acids Res., 31: 1191–1196.

28. Masaoka, A., Matsubara, M., Hasegawa, R., Tanaka, T., Kurisu, S., Terato, Ohyama, Y., Karino, N. and Ide, H. (2003) Mammalian 5-formyluracil-DNA glycosylase. 2. Role of SMUG1 uracil-DNA glycosylase in repair of 5-formyluracil and other oxidized and deaminated base lesions. Biochemistry, 42: 5003–5012.

29. Zhang, Q.-M., Yonekura, S., Takao, M., Yasui, A., Sugiyama, H. and Yonei, S. (2005) DNA glycosylase activities for thymine residues oxidized in the methyl group are functions of the hNEIL1 and hNTH1 enzymes in human cells. DNA Repair, 4: 71–79.

30. Denver, D. R., Swenson, S. L. and Lynch, M. (2003) An evolutionary analysis of the helix-hairpin-helix superfamily of DNA repair glycosylases. Mol. Biol. Evol., 20: 1603–1611.

31. Matsumoto, Y., Zhang, Q.-M., Takao, M., Yasui, A. and Yonei, S. (2001) Escherichia coli Nth and human hNTH1 DNA glycosylases are involved in removal of 8-oxoguanine from 8-oxoguanine/guanine mispairs in DNA. Nucleic Acids Res., 29: 1975–1981.

32. 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.

33. Rusmintratip, L. and Sowers, C. (2000) An unexpectedly high excision capacity for mispaired 5-hydroxymethyluracil in human cell extracts. Proc. Nat. Acad. Sci. USA, 97: 14183–14187.

34. Osman, F., Björkás, M., Alseth, I., Morland, I., McCreary, S., Seeberg, E. and Tsaneva, I. (2003) A new Schizosaccharomyces pombe base excision repair mutant, nth1, reveals overlapping pathways for repair of DNA base damage. Mol. Microbiol., 48: 465–480.

35. Spruck, C. H. II, Rideout, W. M. III and Jones, P. A. (1993) DNA methylation and cancer. In Jost, J. P. and Saluz, H. P. (eds.), DNA Methylation: Molecular Biology and Biological Significance. Birkhauser Verlag, Basel, pp. 487–509.

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

37. Hazra, T. K., Izumi, T., Maitl, L., Floyd, R. A. and Mitra, S. (1998) The presence of two distinct 8-oxoguanine repair enzymes in human cells: their potential complementary roles in preventing mutation. Nucleic Acids Res., 26: 5116–5122.

38. Hazra, T. K., Izumi, T., Venkataraman, R., Kow, Y. W., Dizdaroglu, M. and Mitra, S. (2000) Characterization of a novel 8-oxoguanine-DNA glycosylase activity in Escherichia coli and identification of the enzyme as Endonuclease VIII. J. Biol. Chem., 275: 27762–27767.

39. Kino, K. and Sugiyama, H. (2001) Possible cause of G:C→C:G transversions mutation by guanine oxidation product, imidazolone. Chem. Biol. 8: 369–378.

40. Rybanska, I. and Pirsel, M. (2003) Involvement of the nucleotide excision repair proteins in the removal of oxidative DNA base damage in mammalian cells. Neoplasma, 50: 389–395.

Received on April 19, 2007
1st Revision received on May 24, 2007
2nd Revision received on June 15, 2007
Accepted on June 16, 2007
J-STAGE Advance Publication Date: July 18, 2007