Repair of Formamidopyrimidines in DNA Involves Different Glycosylases

ROLE OF THE OGG1, NTH1, AND NEIL1 ENZYMES*

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The oxidatively induced DNA lesions 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA) are formed abundantly in DNA of cultured cells or tissues exposed to ionizing radiation or to other free radical-generating systems. In vitro studies indicate that these lesions are miscoding, can block the progression of DNA polymerases, and are substrates for base excision repair. However, no study has yet addressed how these lesions are metabolized in cellular extracts. The synthesis of oligonucleotides containing FapyG and FapyA at defined positions was recently reported. These constructs allowed us to investigate how these lesions are metabolized in cellular extracts. The synthesis of oligonucleotides containing FapyG and FapyA at defined positions was recently reported.

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FapyA (8) and FapyG (9) are miscoding in vitro, both directing the preferential misincorporation of adenine opposite the lesions by a bacterial DNA polymerase (Klenow exo-). Experiments using the methyalted analogue of FapyG, i.e. 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (Me-FapyG), have suggested that formamidopyrimidines might also constitute blocks to DNA polymerases (10, 11). It is noteworthy, however, that FapyG and FapyA are chemically distinct from Me-FapyG. FapyG and FapyA are formed from purines in DNA by oxidative attack or UV radiation, whereas Me-FapyG is the product of alkylation damage to guanine in DNA. Consequently, any results obtained with Me-FapyG should not be directly extrapolated to FapyG or FapyA.

Oxidative DNA damage is primarily repaired by the base excision repair pathway. Base excision repair is initiated by a DNA glycosylase that recognizes the modified base and hydrolyzes the N-glycosidic bond. Repair then proceeds through the coordinated actions of an abasic site endonuclease, DNA polymerase, and DNA ligase (12). Numerous DNA glycosylases have been identified in mammals, and although they show distinct substrate specificities, there is a considerable degree of overlap, most notably among DNA glycosylases that repair oxidative DNA damage (for review, see Ref. 13). Studies with purified enzymes from various sources have identified several glycosylases that can release FapyG and FapyA from irradiated DNA, such as the bacterial Fpg, NtI, and Nth; the yeast, plant, and mammalian oxoguanine DNA glycosylase (OGG1); and the recently identified human and mouse homologues of Escherichia coli endonuclease VIII (Nei) (NEIL1) (14, 15). However, to date, no study has directly addressed repair of FapyG and FapyA in cellular extracts from mammalian cells.

Synthetic single lesion oligonucleotide constructs have been instrumental for the understanding of repair pathways for modified DNA lesions.
bases (16). A method for the synthesis of oligonucleotides containing FapyG and FapyA at defined sites was recently developed (17, 18). Here, we have reported the first study of the repair of FapyG and FapyA in these oligonucleotides, using nuclear and mitochondrial extracts of wild type and mice deficient in the two major glycosylases for the repair of oxidative DNA damage, OGG1 and NTH1. We determined the endogenous levels of FapyA, FapyG, and 8-oxoG in genomic DNA from wild type mice and found that Fapy levels are significantly higher than 8-oxoG. Our results showed that FapyG is repaired mainly by OGG1, both in nucleus and in mitochondria. NTH1 is the major glycosylase for the repair of FapyA. We found significantly higher levels of FapyG and FapyA in DNA from the knock-out mice, emphasizing the biological significance of OGG1 and NTH1 in the repair of these lesions.

**EXPERIMENTAL PROCEDURES**

**Materials**—T4 polydeoxyribonuclease kinase was from Stratagene (La Jolla, CA). [γ-32P]ATP (3000 Ci/mmol) was from PerkinElmer Life Sciences. G25 spin columns were from Amersham Biosciences. Nuclease P1 (from Penicillium citrinum) was from Calbiochem. Snake venom phosphodiesterase was obtained from Sigma. Alkaline phosphatase was purchased from Roche Diagnostics. 8-Hydroxy-2'-deoxyguanosine-15N5 (8-oxo-dG-15N5) and (19). All experiments were approved by the GRC Animal Care and Use Committee and performed in accordance with Ref. 43.

**Preparation of Mouse Liver Mitochondrial and Nuclear Extracts**—Knock-out mice were kindly provided by Arne Klangsland (University of Oslo, Norway) (OGGI−/−) and Rhod Elder (Patterson Cancer Institute, Manchester, UK) (NTH1−/− and (OGGI/NTH1)−/−) and bred at the Gerontology Research Center (GRC) Animal Facility. Wild type littermates (WT) were used. Mice were sacrificed by cervical dislocation, and the livers were immediately removed and processed. Nuclear and mitochondrial extracts were prepared as described earlier (19). All experiments were approved by the GRC Animal Care and Use Committee and performed in accordance with Ref. 43.

**Oligonucleotides**—The sequences of the oligonucleotides used here are shown in TABLE ONE. FapyG- or FapyA-containing oligonucleotides were synthesized as described previously (17, 18). The oligonucleotides were characterized by electrospray ionization mass spectrometry, and the spectra showing the expected fragment masses are presented in Supplemental Fig. 1. The oligonucleotide containing 8-oxoG was obtained from Midland Certified Reagent Co. (Midland, TX). Thymine glycol (Tg)-containing substrate was generated as described in Ref. 20. All oligonucleotides were 5'-end-labeled using T4 polynucleotide kinase and [γ-32P]ATP, as described in Ref. 21. FapyG, 8-oxoG, and Tg oligonucleotides were annealed to the complementary strand in 10 mM Tris-HCl, pH 7.8, 1 mM EDTA, 100 mM KCl by heating the samples at 90 °C for 5 min and slowly cooling to room temperature. FapyA oligonucleotide was annealed as described above, except that heating was at 70 °C for 5 min.

**DNA Glycosylase Assays**—Incision of FapyG was performed in a reaction mixture (20 μl) containing 20 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 2 mM dithiothreitol, 100 mM NaCl, 0.2 μg/μl bovine serum albumin, 10% glycerol, and 10 fmol of 32P-labeled oligonucleotide. The reactions were incubated at 37 °C for 2 h and stopped with the addition of 5 μg of proteinase K and 1 μl of 10% SDS followed by 30 min of incubation at 55 °C. To promote complete strand cleavage at the abasic sites, 100 μl NaOH was added and incubated at 37 °C for 15 min. An equal amount of formamide loading dye was added, and the samples were incubated at 90 °C for 5 min and resolved by electrophoresis on a 20% polyacrylamide/7 m urea gel. Gels were visualized by PhosphorImager and analyzed using the ImageQuant™ software (Amersham Biosciences). The percentage of incision was calculated as the amount of radioactivity present in the product band relative to the total radioactivity and converted into fmol of substrate incised. Incision of 8-oxoG-containing oligonucleotide was described as above, except that 89 fmol of substrate were used. Incision of FapyA- and Tg-containing oligonucleotides was carried out using 150 and 250 fmol of substrate, respectively. The experiments were processed as described above, without incubation with NaOH.

**DNA Trapping Assay**—DNA trapping assays were performed as described for the glycosylase assay, with the addition of 50 mM NaBH4. After incubation at 37 °C for 2 h, the reactions were terminated by adding 5 μl of 5× SDS-PAGE sample buffer, and the samples were heated at 95 °C for 5 min. Trapped protein-DNA complexes were separated in 12% SDS-PAGE. The gels were visualized using PhosphorImager.

**Preparation of Total DNA from Mouse Liver**—Genomic DNA was isolated using a modification of the salting-out method. Half of freshly removed livers were chopped and homogenized in 2 ml of buffer containing 210 mM mannitol, 70 mM sucrose, 10 mM Hepes-KOH, pH 7.4, 2 mM EGTA, 1 mM EDTA, 2 mM dithiothreitol, 0.15 mM spermine, and 0.75 mM spermidine. The homogenates were then diluted in buffer containing 10 mM Tris-HCl, pH 8.2, 2 mM EDTA, 400 mM NaCl, 1% SDS, and 2 mg/ml proteinase K and incubated at 55 °C for 1 h to promote cell lysis. One-forth volume of saturated NaCl was added, and the samples were incubated at 55 °C for 1 h. After a brief incubation in ice, proteins were precipitated by centrifugation at 16,100 × g for 15 min. The supernatant was recovered, and the DNA was precipitated with 2.5 volumes of 96% ethanol overnight at −20 °C. The precipitated DNA was collected by centrifugation, suspended in 5 ml of 10 mM Tris, 1 mM EDTA and incubated with RNaseA (0.1 mg/ml) at 37 °C for 1 h. The samples

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**TABLE ONE**

Oligonucleotides used in this study

| Name       | Sequence*                                      |
|------------|-----------------------------------------------|
| FapyG      | 5'-AGCCGTTTCAACGTGCAGTTGTCAGCAGTCCCATGTT-3'  |
|            | 3'-TCGGCAAGTTGACGTCACAGTCTTGACGGATCA-5'       |
| FapyA      | 5'-CGTTCAGCAGTCATFAACACGAGCTCCCAT-3'          |
|            | 3'-GCAAAGTTGACGCTGATGTGTCGAGGGTA-5'           |
| 8-oxoG     | 5'-ATATACCAGGGCCCGCCGATCAAGCTTATT-3'          |
|            | 3'-TATATGGCCGAGCCCGGCTAGTCCGAAT-5'            |
| Thymine glycol | 5'-GAACGACAGTGACAGCAGACAGAAGCA-3'       |
|            | 3'-CTGCTGCTCCTAAGGTGCATGACGTGTCCGT-5'         |

* The important features are underlined in bold. FG, FapyG; FA, FapyA; OG, 8-oxoG.
Repair of Fapy Lesions in Mitochondria and Nucleus

were then subjected to another protease K (0.5 mg/ml) digestion in lysis buffer at 55 °C for 3 h. After precipitation in ice and centrifugation as above, DNA was precipitated from the supernatant with ethanol, dried, and suspended in water.

Analysis by Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry (GC/MS—LC/MS) was used to identify and quantify 8-oxoG as its nucleoside 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) in DNA samples isolated from WT and knock-out mice. Fifty μg of DNA were supplemented with 5 pmol of 8-oxo-dG-15N5 as an internal standard. DNA samples were hydrolyzed with nuclease P1, snake venom phosphodiesterase, and alkaline phosphatase for 24 h and then analyzed by LC/MS as described in Ref. 22. For identification and quantification, selected-ion monitoring was used to monitor the characteristic ions of 8-oxo-dG (m/z 168 and 306) and 8-oxo-dG-15N5 (m/z 173 and 311) (23).

The identification and quantification of FapyG and FapyA in DNA was performed by GC/MS following hydrolysis by E. coli Fpg (isolated as described in Ref. 24). Fifty μg of DNA were supplemented with 8 pmol of FapyA-13C,15N2 and 8 pmol of FapyG-13C,15N2, as internal standards and then hydrolyzed with 2 μg of Fpg. Following ethanol precipitation, DNA pellets and supernatant fractions were separated by centrifugation. Supernatant fractions were lyophilized, trimethylsilylated, and analyzed by GC/MS as described (24). For identification and quantification, selected-ion monitoring was used to monitor the characteristic ions of trimethylsilylated FapyA and FapyG and their stable isotope-labeled analogues (25).

RESULTS

Endogenous Levels of FapyG and FapyA in Mouse Liver DNA—Formamidopyrimidine levels in cellular DNA have previously been determined only in mice subjected to γ-irradiation (4). We determined background levels of 8-oxoG, FapyA, and FapyG in liver DNA from 4–6-month-old WT mice (Fig. 1). Levels of 8-oxoG and FapyA were quite similar, ~1 lesion/10⁶ nucleotides. FapyG levels, on the other hand, were significantly higher than 8-oxoG and FapyA, at about 6.5 lesions/10⁶ nucleotides. Jaruga et al. (26) also found FapyG levels to be higher than 8-oxoG levels in rat liver DNA.

Excision of FapyG in Liver Mitochondria—Mitochondria are the major cellular source of reactive oxygen species, and the mitochondrial DNA has been shown to be subjected to higher oxidative damage than nuclear DNA (27). We investigated how FapyG and FapyA are repaired in mammalian mitochondria using mouse liver mitochondrial extracts (MLME) from WT and knock-out mice for OGG1 and NTH1, the two major DNA glycosylases responsible for repairing oxidative base lesions. These extracts were free of nuclear contamination, as assessed by Western blot (Supplemental Fig. 2) against Lamin B2, an abundant nuclear matrix protein. Cytochrome oxidase subunit IV was used as a marker for mitochondrial content.

MLME extracts from WT mice incised the FapyG-containing oligonucleotide in a protein concentration-dependent manner (Fig. 2A and Supplemental Fig. 3), with the reaction reaching saturation between 25 and 100 μg of extract. MLME from NTH1−/− mice showed only slightly less incision at lower protein concentrations but similar incision efficiency with more than 25 μg of extract. Extracts from OGG1−/− mice had significantly less incision of the FapyG-containing substrate, and extracts from (OGG1/NTH1)−/− mice showed very limited incision, only detectable at very high protein concentrations.

DNA glycosylases that catalyze β-elimination reaction form Schiff base intermediates with the substrate, which can be covalently trapped in the presence of NaBH₄ (28). Using this approach, we detected the formation of substrate-enzyme complexes in MLME from WT, OGG1−/−, and NTH1−/− mice but not in extract from the double knock-out (Fig. 2B). We observed two closely migrating bands in the WT extracts. OGG1 and NTH1 knock-out extracts showed only one band each, corresponding to the two bands observed in the WT. The band in NTH1−/− extract co-migrated with recombinant OGG1 (lane 6). These results suggest that both OGG1 and NTH1 can form covalent intermediates with a FapyG-containing substrate. However, the results presented in Fig. 2A suggest that OGG1 is the major DNA glycosylase for FapyG in mouse liver mitochondria and that NTH1 plays only a secondary role. The small residual incision observed with extracts from the double knock-out mice suggests that another DNA glycosylase may play a minor role in FapyG repair in liver mitochondria.

We previously showed that OGG1 is likely the only DNA glycosylase for removal of 8-oxoG from mtDNA (29). Since steady-state levels of FapyG in DNA in vivo are higher than those of 8-oxoG (Fig. 1), we compared the kinetics of base excision by WT MLME for FapyG- and 8-oxoG-containing substrates (Fig. 2C). Because of technical limitations, we had to use different amounts of each substrate; thus, incision activity is presented as the percentage of substrate cleaved instead of fmol of product generated. Under saturating conditions (excess substrate), FapyG incision activity was about 2-fold higher than that of 8-oxoG cleavage of both lesions was greatly decreased in extracts from OGG1 knock-out mice (Fig. 2D). However, OGG1−/− extracts still retained some FapyG incision, once again indicating that another DNA glycosylase may participate in FapyG repair in the absence of OGG1.
Excision of FapyA in Liver Mitochondria—We next investigated the repair of FapyA lesions in mitochondrial extracts. FapyA-containing oligonucleotides were incised by WT MLME in a protein-dependent manner (Fig. 3A and Supplemental Fig. 4). Similar incision activity was observed for extracts from OGG1−/−/− mice. In contrast, NTH1−/− and (OGG1/NTH1)−/− extracts showed a significant decrease in incision activity with protein concentrations higher than 50 μg. There was no difference in activity between the two latter extracts. This suggests that NTH1 is the major glycosylase for FapyA removal in mitochondrial extracts and that OGG1 most likely does not participate in this process.

In agreement with the incision results, we observed trapped DNA/enzyme complexes in WT and OGG1−/−/− but not in NTH1−/− and (OGG1/NTH1)−/− extracts (Fig. 3B). FapyA incision in NTH1−/− and (OGG1/NTH1)−/− was comparable with WT at lower protein concentrations, indicating that another DNA glycosylase may partially compensate for the absence of NTH1. However, we did not detect any additional trapped band in NTH1−/− extracts with sodium borohydride (lines 2 and 4).

Tgs are repaired in mouse liver mitochondria solely by NTH1 (30). Thus, we compared excision efficiency of FapyA and Tg. WT extracts incised the FapyA-containing substrate with similar efficiency as that of a substrate containing Tg (Fig. 3C). In support of a major role for NTH1 in the removal of these two lesions in liver mitochondria, incision activity for both lesions was greatly reduced in NTH1−/− extracts, although Tg incision activity was lower than FapyA incision (Fig. 3D).

Detection of NEIL1 in Liver Mitochondria—The observation that mitochondrial extracts from (OGG1/NTH1)−/− still retained a limited excision activity toward FapyG- or FapyA-containing substrates suggested the existence of another DNA glycosylase recognizing those lesions. Recently, Fpg mammalian homologues have been identified, and three new DNA glycosylases, NEIL (for nei-like)-1, 2, and 3, have been cloned (14, 31–34). The substrate specificity of human and mouse NEIL1 proteins indicate that FapyA and FapyG are preferred substrates (14, 15). NEIL1 has been identified as the backup glycosylase for oxidized pyrimidines in nuclear extracts from NTH1−/− mice (34). Thus, NEIL1 is a likely candidate for the residual excision activity we detected in mitochondria from the double-knock-out mice. However, to date, none of the NEIL glycosylases have been localized to mitochondria. We investigated whether liver mitochondria contain NEIL1 by Western blot, with recombinant human NEIL1 (hNEIL1) as a positive control (Fig. 4). NEIL1 was detected, at similar levels, in both WT and (OGG1/NTH1)−/− MLME, indicating that this glycosylase is present in mouse mitochondria and thus could account for the residual excision activity we observed in the absence of OGG1 and NTH1.

Excision of FapyG/FapyA in Liver Nuclei—We next investigated the excision of FapyG- and FapyA-containing substrates by liver nuclear
extracts (MLNE) from the same knock-out mice used for preparation of mitochondria. FapyG was excised with similar efficiency by nuclear extracts from WT and NTH1∗/− (Fig. 5A and supplemental Fig. 5). A significant reduction in incision was observed with MLNE from OGG1∗/−, demonstrating a major role for OGG1 in FapyG repair in the nucleus. Although we did not observe any difference in incision activity in WT and NTH1∗/−, the small decrease in extracts from (OGG1/NTH1)∗/−− mice when compared with OGG1∗/− alone (Fig. 5A) may imply that, similar to what we observed in the mitochondrial extracts, NTH1 plays a minor role in FapyG removal in the nucleus in the absence of OGG1.

FapyA was also efficiently incised by WT MLNE (Fig. 5B), but incision was very limited in extracts from NTH1∗/−. No additional decrease in activity was observed in (OGG1/NTH1)∗/− extracts than in WT (Fig. 5B, triangle symbols), suggesting a possible up-regulation of one or more DNA glycosylases in the OGG1∗/− background.

FapyA is an efficient substrate for both NTH1 and NEIL1, and our previous data established a role for NTH1 in FapyA repair in vivo. To determine whether NEIL1 could also contribute to the repair of FapyA in vivo, we measured FapyA incision in whole cell extracts from heart, liver, and spleen of WT and knock-out mice (Fig. 5C) because the relative expression of these two glycosylases is known to vary significantly among different mouse tissues (34). In heart, NEIL1 is expressed at a significantly higher level than NTH1; in liver, NTH1 is more abundant than NEIL1, and in spleen, similar mRNA levels for both glycosylases have been detected (34). FapyA incision by whole cell extracts from liver, heart, and spleen from WT and knock-out mice showed a striking correlation with the mRNA levels determined by Takao et al. (34); incision activity was significantly decreased in liver whole cell extracts lacking NTH1 (from NTH1∗/− and (OGG1/NTH1)∗/−− mice) but only slightly lower in heart from these animals. Spleen whole cell extracts from mice lacking NTH1

FIGURE 3. Incision of FapyA-containing double strand oligonucleotides by MLME from WT and knock-out animals. A, 150 fmol of 32P-labeled FapyA substrate were incubated with increasing concentrations of MLME (0–100 µg) from WT and knock-out mice. B, a trapping assay was carried out with 150 fmol of FapyA substrate and 50 µg of MLME. Fpg (lane 5) was used as a positive control. C, oligonucleotides containing FapyA (150 fmol) or Tg (250 fmol) were incubated with 0–100 µg of MLME from WT mice, and incision was measured as done previously. D, FapyA and Tg incision in MLME from WT and NTH1∗/−− mice was measured with 100 µg of MLME in each reaction, as in panel C. The results presented are mean ± S.E. from 2–3 separate experiments. Background cleavage of the substrate was measured in parallel and subtracted from the values obtained with MLME.

FIGURE 4. Detection of NEIL-1 in mouse liver mitochondrial extracts. 100 µg of MLME from WT and (OGG1/NTH1)∗/−− mice (lanes 2 and 3, respectively) were resolved in a 12% Tris-glycine gel and transferred to a polyvinylidene difluoride membrane. The membrane was probed with anti-NEIL1 (Oncogene). 50 ng of recombinant NEIL-1 (a kind gift of Dr. Sankar Mitra) was used as a positive control (lane 1). hNEIL1, human NEIL1.
(NTH1<sup>−/−</sup> and (OGG1/NTH1)<sup>−/−</sup>) had about 50% of WT incision. Not surprisingly, these results suggested that the contribution of each glycosylase for the repair of FapyA (and perhaps FapyG) depends largely on their relative expression levels.

Detection of Oxidized DNA Bases in Genomic DNA from Knock-out Mice—One prediction ensuing from the above results is that mice lacking OGG1 and/or NTH1 would accumulate Fapy lesions in their DNA.

DISCUSSION

We have shown here that FapyG accumulates in mouse liver DNA at much higher levels than 8-oxoG, which is considered a biomarker of oxidative DNA damage. Since Fapy lesions have been shown to be mis-coding and/or blockers of polymerases (9, 35, 36), their efficient repair is necessary for stability of both the nuclear and the mitochondrial genomes. The vast majority of the previous studies on the repair of
Repair of Fapy Lesions in Mitochondria and Nucleus

formamidopyrimidines used either a Me-FapyG analogue or γ-irradiated DNA, which contains numerous oxidatively induced DNA modifications. In this study, we investigated the repair of Fapy lesions by mitochondrial and nuclear extracts using oligonucleotides containing a single FapyG or FapyA. We have shown that both lesions are efficiently repaired in mouse liver mitochondria and nuclei. FapyG is excised with higher efficiency than 8-oxoG in mitochondrial extracts from WT mice, and FapyA is repaired with the same efficiency as thymine glycol lesions. Furthermore, we have shown that FapyG, FapyA, and 8-oxoG accumulate in liver DNA from knock-out mice lacking OGG1 and NTH1, underscoring the biological role of these glycosylases in the repair of formamidopyrimidines. To our knowledge, this is the first study demonstrating the specific removal of formamidopyrimidines from DNA using mammalian cell extracts.

We found that FapyG is preferentially removed by OGG1, both in mitochondria and in nuclei. In a minor role, NTH1 can also participate in FapyG repair, especially if opposite G (not shown), a base composition that could be formed by the misincorporation of Gs opposite FapyG during DNA replication occurs. Equivalently, purified E. coli endonuclease III removed both FapyA and FapyG from DNA, although FapyA was excised more rapidly (37). The small contribution of NTH1 to FapyG repair was also confirmed by the presence of a sodium borohydride-trapped DNA-enzyme complex in extracts from OGG1−/− liver mitochondria not seen in extracts from (OGG1/NTH1)−/− animals and by the elevated levels of FapyG in liver genomic DNA from NTH1−/− mice. On the other hand, we did not find any evidence that OGG1 can act upon FapyG in mitochondrial or nuclear extracts. This is in agreement with the observation that purified human OGG1 (hOGG1) does not release FapyA lesions from γ-irradiated DNA (38).

Although a significant percentage of the incision activity against FapyG and FapyA was lost in extracts from the double knock-out mice lacking OGG1 and NTH1, we consistently observed some residual incision activity, both in mitochondrial and in nuclear extracts, suggesting that another glycosylase could contribute to the incision activity. The newly identified human and mouse NEIL1 proteins efficiently excise FapyG and FapyA from γ-irradiated DNA in vitro (14, 15), making them likely candidates for this residual activity. It has been previously suggested that NEIL1 (33) and NEIL2 (31) localize exclusively to the nucleus. However, our results have shown for the first time that NEIL1 is also found in mouse liver mitochondria and thus could account for the residual incision observed in the double knock-out extracts.

It is of importance to determine the relative contribution of each DNA glycosylase to the repair of one particular lesion in the whole cell, where all enzymes are present and likely competing for the substrate. We addressed this question with regards to FapyA incision using whole cell extracts from tissues with different expressions of NTH1 and NEIL1. Our results suggested that the relative expression level of each protein strongly impacts their contribution to the overall repair efficiency. Both NEIL1 (14) and NTH1 (39) mRNA levels increase in early-mid S phase, suggesting a role for both glycosylases in replication-associated repair. However, their tissue-specific expression patterns are quite distinct, with NTH1 being highly expressed in lung and testis and NEIL1 being highly expressed in heart and spleen. Thus, it is possible that these two glycosylases display overlapping biological roles in different tissues. Another possibility is that their biological roles are differentiated on the basis of post-translational modifications. For example, OGG1 serine-phosphorylation by protein kinase C modulates its intracellular localization (40), whereas its serine/threonine phosphorylation by cyclin-dependent kinase 4 (cdk4) modulates its catalytic properties (41).

In this study, we found that mouse liver mitochondria contain NEIL1, as originally suggested by Hazra et al. (14) and Takao et al. (34) can function as a backup activity for 8-oxoG and thymine glycol repair. However, we previously showed that in mouse liver mitochondria, 8-oxoG is repaired solely by OGG1 protein (29) and that thymine glycol lesions are repaired solely by NTH1 (30). We confirmed our previous results in this study, and additionally, we showed that in the absence of OGG1 or NTH1, mitochondrial extracts can still excise some FapyG and FapyA, respectively. Together, our results suggested that NEIL1 may function as a backup glycosylase for Fapy in mouse mitochondria but not for 8-oxoG and Tg removal. More recent studies failed to detect 8-oxoG excision by recombinant human (14, 42) and mouse NEIL1 (15) and showed a much lower efficiency for the excision of Tg in comparison with FapyG/FapyA by mouse NEIL1 (15). These results with purified enzymes supported our conclusion that NEIL1 does not participate in the repair of 8-oxoG and Tg in mouse mitochondria.

This study has shown for the first time that formamidopyrimidines are efficiently repaired in cellular extracts and that OGG1 and NTH1 play major roles in this process. The results have also indicated that NEIL1 may participate in base excision repair of formamidopyrimidines, particularly in tissues in which it is highly expressed. Our results strongly suggested that Fapy lesions are physiological substrates of these enzymes and that these lesions may be important oxidized DNA modifications leading to mutagenicity and cytotoxicity.

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