Mammalian 8-Oxoguanine DNA Glycosylase 1 Incises 8-Oxoadenine Opposite Cytosine in Nuclei and Mitochondria, while a Different Glycosylase Incises 8-Oxoadenine Opposite Guanine in Nuclei*

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Cells are continuously exposed to oxidative species, which cause several types of oxidative DNA lesions. Repair of some of these lesions has been well characterized but little is known about the repair of many DNA lesions. The oxidized adenine base, 7,8-dihydro-8-oxoadenine (8-oxoA), is a relatively common DNA lesion, which is believed to be mutagenic in mammalian cells. This study investigates repair of 8-oxoA in nuclear and mitochondrial mammalian extracts. In nuclei, 8-oxoA:C and 8-oxoA:G base pairs are recognized and cleaved; in contrast, only 8-oxoA:AC base pairs are cleaved in mitochondria. High stability of the DNA helix increased the efficiency of incision of 8-oxoA, and the efficiency decreased at DNA bends and condensed regions of the helix. Using liver extracts from mice knocked out for 8-oxoguanine DNA glycosylase 1 (OGG1), we demonstrated that OGG1 is the only glycosylase that incises 8-oxoA, when base-paired with cytosine in mitochondria and nuclei, but a different enzyme incises 8-oxoA when base-paired with guanine in the nucleus. Consistent with this result, a covalent DNA-protein complex was trapped using purified human OGG1 or human nuclear homologue; NEIL, Nei-like endonuclease; OGG1, 8-oxoguanine DNA glycosylase 1; pol, DNA polymerase.

Unrepaired oxidative DNA damage can be mutagenic and lead to impaired cellular functions. The implication of oxidative DNA damage in a broad range of pathophysiological processes including carcinogenesis, neurological disorders, and aging has been suggested (reviewed in Refs. 1 and 2). A significant source of oxidative DNA damage is oxidative phosphorylation, an essential part of cellular energy metabolism, which takes place in mitochondria. Reactive oxygen species such as superoxide and hydrogen peroxide are byproducts of oxidative phosphorylation, which can oxidize lipids, proteins, and nucleic acids. Several oxidative DNA lesions are mutagenic and contribute to genetic instability (reviewed in Refs. 1 and 3).

The repair of thymine glycol, uracil, and 7,8-dihydro-8-oxoguanine (8-oxoG)1 has been investigated extensively (reviewed in Ref. 4). Many other DNA lesions including 7,8-dihydro-8-oxoadenine (8-oxoA) have not been studied in great detail. Previous studies show that 8-oxoA and 8-oxoG occur at similar frequencies in mammalian DNA (5, 6) and that 8-oxoA is as mutagenic as 8-oxoG (7). 8-OxoA leads to A → G transitions and A → C transversions (8). Increased levels of 8-oxoA have been detected in human tumor tissue (9), suggesting that it may be involved in carcinogenesis. Finally, the level of 8-oxoA seems to increase with age. The level of 8-oxoA was 2-fold higher in liver from 12-month-old rats than in 1-month-old rats (5). Similar observations have been made for 8-oxoG in rat liver mitochondria (10). Additionally, Tuo et al. (11) recently reported that 8-oxoA incision is deficient in Cockayne syndrome group B cells, and these cells accumulate 8-oxoA after γ-irradiation.

Although it has been shown that 8-oxoA is repaired in mammalian cells (6), no repair pathway for 8-oxoA has been identified. However, it was shown that purified human 8-oxoG glycosylase 1 (OGG1) cleaves 8-oxoA:C base pairs in vitro (12, 13). Oxidized and alkylated base lesions are predominantly repaired by the base excision repair pathway (reviewed in Ref. 4). Base excision repair is initiated by a DNA glycosylase, which recognizes and removes the modified nucleotide by cleavage of the glycosylic bond between the damaged base and deoxyribose. Many glycosylases incise the damaged base by flipping the modified nucleotide out of the helix and into the active site pocket for glycosylic bond cleavage and base release (14–16). The glycosylase leaves an apurinic/apyrimidinic (AP) site in the DNA, which is processed by AP endonuclease or by the AP lyase activity of the glycosylase. OGG1, endonuclease III homologue 1 (NTH1), and the Nei-like proteins (NEIL1 and NEIL2) are all bifunctional glycosylases that possess AP lyase activity (17–20). DNA strand cleavage by OGG1 is rate-limiting in base excision repair, and AP endonuclease 1 (APE1) is therefore believed to hydrolyze the AP site after OGG1 removes the damaged base (21, 22). After DNA strand cleavage the one nucleotide gap can be filled by DNA polymerase β (pol β) and ligated (22). In this study, we have employed oligonucleotide duplexes containing a single 8-oxoadenine together with mam-

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1 The abbreviations used are: 8-oxoG, 8-oxo-7,8-dihydroxyguanine; 8-oxoA, 8-oxo-7,8-dihydroxyadenine; AP, apurinic/apyrimidinic; APE1, AP endonuclease 1; NTH1, endonuclease III homologue 1; MHY, MutY homologue; NEIL, Nei-like endonuclease; OGG1, 8-oxoguanine DNA glycosylase 1; pol β, DNA polymerase β; DTT, dithiothreitol; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-[hydroxyethyl]propane-1,3-diol.
malian nuclear and mitochondrial extracts to characterize the repair of 8-oxoA.

MATERIALS AND METHODS

Oligonucleotides—Oligonucleotides used in this study were from DNA Technology (Denmark), and their sequences are presented in Table I. The oligonucleotides were 5′-end labeled using T4 polynucleotide kinase (MBI Fermentas) and γ-32P-ATP (Amersham Biosciences), as described previously (23).

Incorporation Assay—A 30-nucleotide oligomer (10 nmol) containing a single 8-oxoA opposite uracil was pretreated with 100 units of uracil DNA glycosylase (MBI Fermentas) for 1 h at 37 °C (buffer conditions as described by the manufacturer). The reaction was stopped by addition of EDTA to a final concentration of 30 mM and the DNA was purified on a MicroSpin™ G-25 column (Amersham Biosciences). The DNA backbone was cleaved at the resulting AP site by the addition of 100 units of APE1 (Trevigen) for 1 h at 37 °C (buffer conditions as described by the manufacturer), and the reaction was stopped by adding EDTA to a final concentration of 30 mM. The reaction buffer was exchanged on a MicroSpin™ G-25 column. Incorporation reactions (10 μl) were performed with prepared oligonucleotide (100 pmol), 0.5 units of human polymerase β (Trevigen), 50 mM Tris-HCl (pH 8.8), 10 mM MgCl2, 10 mM KCl, 1.0 mM DTT, 1% glycerol, 0.4 mg/ml bovine serum albumin, and 10 pmol each of dATP, dGTP, dTTP, and dCTP where one deoxynucleotide was substituted with the corresponding [α-32P]dNTP for each reaction. The samples were incubated for 2 h at 37 °C and the reactions were stopped by adding 2 μl of FA-loading dye (80% formamide, 10 mM EDTA, pH 8.0, 1 mg/ml xylene cyanol FF, 1 mg/ml bromphenol blue). The samples were heated for 2 min at 95 °C and analyzed on a 20% denaturing polyacrylamide gel containing 7 M urea. The radioactively labeled DNA was heated for 2 min at 95 °C and analyzed on a 20% denaturing polyacrylamide gel containing 7 M urea. The radioactively labeled DNA was visualized using a Personal Molecular ImageTr™ (Bio-Rad). Cells and Culture Conditions—The SV40-transformed wild type human fibroblasts, GM06637D (Coriell Institute for Medical Research) were grown in minimal essential medium supplemented with 1× amino acids, 1× vitamins, 10% fetal bovine serum, 100 units/ml penicillin and streptomycin (Invitrogen).

Isolation of Mitochondria from Cells in Culture—Isolation of mitochondria was accomplished using a combination of differential centrifugation and Percoll gradient centrifugation employing a protocol modified from Croteau et al. (24). Briefly, actively growing cells were washed once with ice-cold phosphate-buffered saline, pelleted, and resuspended in M-SHE buffer (0.21 mM mannitol, 0.07 mM sucrose, 10 mM Hepes (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.15 mM spermine, 0.75 mM spermidine, 5 mM DTT, 2 μM phenylmethylsulfonyl fluoride, 2 μM protease inhibitor mixture set III (Calbiochem)). The cell suspension was loaded onto a Percoll gradient centrifugation, and protein concentration was determined by the Lowry method (25). Preparation of Nuclear Extract from Cells in Culture—Nuclear extracts were prepared in parallel with the mitochondrial isolation, according to modified versions of two protocols (25, 26). Briefly, the nuclear pellet (obtained during preparation of mitochondria) was treated with 10 mM Hepes (pH 7.9), 400 mM NaCl, 1.5 mM MgCl2, 0.1 mM EDTA (pH 8.0), 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, 0.1% Nonidet P-40, and 2 μg/ml protease inhibitor mixture set III and subsequently subjected to centrifugation. The supernatant containing the mitochondria was subjected to differential and Percoll gradient centrifugation, and protein concentration was determined by the Lowry method (50).

Mice—Male C57BL/6J mice with or without a targeted mutation of the OGG1 gene (27) were housed under standard conditions. For isolation of nuclear and mitochondria fractions from the liver, the organ was removed from the animal and washed free of blood with M-SHE buffer. Mouse liver mitochondria were then isolated as previously described by Souza-Pinto et al. (23). Mouse liver nuclear extracts were prepared using the nuclear fraction obtained during the isolation of mitochondria, according to Kawakami et al. (28) with modifications described by Souza-Pinto et al. (26).

RESULTS

Incorporation of Nucleotides Opposite 8-OxoA—Fig. 1 shows the structures of 8-oxoA and 8-oxoG. These nucleotides are quite similar in structure, as there are only minor differences in the 6-ring of the heterocyclic base. This suggests that they may be repaired by a similar mechanism in vivo. The 8-oxoG repair is strongly dependent on base pairing (17), which may also be the case for 8-oxoA. Thus, we set out to test this hypothesis. First, we examined which nucleotides may occur in vivo. For this purpose the low fidelity human pol β was employed in order not to exclude any infrequent base pairs. As seen in Fig. 2, pol β incorporated dGTP, dCTP, and dTTP at a reduced AP-site opposite 8-oxoA with similar efficiencies (Fig. 2, lanes 2–4), whereas dATP was incorporated with lower efficiency (Fig. 2, lane 1). Therefore, incision of 8-oxoA opposite all 4 bases was tested in the following experiments.

Base Pairing-dependent Removal of 8-OxoA—Nuclei and mitochondria were isolated from wild type human cells (GM06637D) in culture. To ensure that mitochondrial extracts...
were rich in mitochondrial proteins and free of nuclear contamination, the extracts were analyzed for the presence of an abundant nuclear matrix protein, CDC47, and a mitochondrial protein, cytochrome oxidase IV, by Western blotting. Fig. 3 shows a typical Western blot assessing the quality of the mitochondrial extract; nuclear extract was included as a control. Only minimum amounts of CDC47 were detected in the mitochondrial extract (Fig. 3), demonstrating that only traces of nuclear contamination were detected in the mitochondrial extracts.

Purified yeast and murine OGG1 incise 8-oxoA when the lesion is base-paired with cytosine (12, 13). In this study, the incision activity of human OGG1 (hOGG1) was examined using 8-oxoA:C, 8-oxoA:G, 8-oxoA:A, and 8-oxoA:T containing substrates (oligonucleotide 1, Table I) (Fig. 4A). The results show that 8-oxoA:C was incised by purified hOGG1 (Fig. 4A, lane 6), but no incision was observed when 8-oxoA:A was base-paired with G, A, or T (Fig. 4A, lanes 2, 4, and 8). The hOGG1 activity was specific for the oxidized adenine as no incision was observed for an A:C mismatch (oligonucleotide 2, Table I; Fig. 4A, lane 5). Nuclear and mitochondrial extracts from human cells were also challenged with 8-oxoA-containing DNA substrates. Assay conditions were optimized by varying the amount of protein, Mg\(^{2+}\), and incubation time (data not shown). The results of assays using the optimized conditions are shown in Fig. 4, B and C. The nuclear extract produced a 10-nucleotide incision product when 8-oxoA:A was paired with cytosine or guanine (Fig. 4B, lanes 9 and 12). No incision was observed with a lesion-free oligonucleotide (oligonucleotide 2, Table I) (Fig. 4B, lanes 7 and 10) or with substrates containing 8-oxoA paired with adenine or thymine (Fig. 4B, lanes 3 and 6). The mitochondrial extract only cleaved substrates containing 8-oxoA paired with cytosine (Fig. 4C, lane 9), but did not cleave an undamaged oligonucleotide (Fig. 4C, lane 7) or substrates containing 8-oxoA paired with adenine, thymine, or guanine (Fig. 4C, lanes 3, 6, and 12).

**Influence of DNA Helix Structure on 8-OxoA Repair**—Repair of 8-oxoA most likely involves a nucleotide flipping mechanism. Local secondary DNA structure may influence the efficiency of flipping and therefore affect glycosylase activity. This idea was tested by measuring 8-oxoA incision in different sequence contexts. To avoid partial or complete strand dissociation, these experiments were carried out with 60-nucleotide oligomers (oligonucleotides 5, 6, and 7) whose sequences are shown in Table I. The 10 bases surrounding the 8-oxoA in oligonucleotide 5 are guanine or cytosine, which creates high thermal stability ($T_m = 80.5^{\circ}C$). In oligonucleotide 6, the sequence surrounding the 8-oxoA lesion includes all 4 nucleotides and the substrate has lower thermal stability ($T_m = 77.0^{\circ}C$) than oligonucleotide 5. Oligonucleotide 7 has 5 adenines’ 5’ to the lesion and 5 thymines’ 3’ to the lesion. The adenine-thymine tract creates a stable molecular structure similar to B-DNA with a 4% contraction in the axial rise per residue and a bend at the junction between A:T and T:A base pairs (31) thus, the 8-oxoA base is located at the DNA bend (32). The melting temperature of alternating AT is lower than that of homopolymeric AT (31). This means that the actual $T_m$ of oligonucleotide 7 is higher than the calculated melting temperature ($T_m = 73.6^{\circ}C$). Fig. 5 shows that nuclear and mitochondrial extracts incised oligonucleotide 5 with the highest efficiency (Fig. 5A, lanes 1 and 5), oligonucleotide 6 with lower efficiency (Fig. 5A, lanes 2 and 6), and oligonucleotide 7 with the lowest efficiency (Fig. 5A, lanes 3 and 7) of the three DNA substrates. For the nuclear extract, 49 ± 7, 37 ± 2, or 22 ± 2% of oligonucleotides 5, 6, or 7, was cleaved, respectively (Fig. 5B). The percent cleavage values for the mitochondrial extract were similar (Fig. 5B), although a smaller difference in efficiency was seen between oligonucleotides 5 and 6 (31 ± 6 and 26 ± 3%, respectively). The mitochondrial extract incised a 61% lower fraction of oligonucleotide 7 (12 ± 2% incision) than oligonucleotide 5 (31 ± 6%).

**8-OxoA Repair Defect in ogg1−/− Mice**—The OGG1 gene encodes mitochondrial and nuclear isoforms of OGG1, which remove 8-oxoG from DNA in their respective organelles (33, 34). It was considered possible that OGG1 incised 8-oxoA from the substrates in the assays described above. This possibility was tested using ogg1−/− mice. Nuclear and mitochondrial extracts were prepared using liver from wild type and ogg1−/− mice and the extracts were tested for incision activity using 8-oxoA:C and 8-oxoA:G oligonucleotides (oligonucleotide 1, Table I). Wild type mouse liver nuclear extract incised 8-oxoA:C and 8-oxoA:G base pairs (Fig. 6A, lanes 3 and 5). In contrast, the ogg1−/− nuclear extract did not incise 8-oxoA:C, but did incise 8-oxoA:G (Fig. 6A, lanes 7 and 8) to approximately the same extent as the wild type nuclear extract. The activity was DNA damage-specific (Fig. 6A, lanes 1 and 6). These results suggest that OGG1 is the only nuclear repair enzyme, which incises 8-oxoA when base paired with cytosine in mouse liver.
nuclei. In addition, a different repair enzyme in mouse nuclei incises 8-oxoA when it is base-paired with G.

In human wild type mitochondria, 8-oxoA was only incised when base-paired with cytosine (Fig. 4C). Therefore, extracts from wild type and \textit{ogg1}−/− mouse liver mitochondria were assayed with an 8-oxoA:C-containing DNA substrate (oligonucleotide 1, Table I). The wild type mouse liver mitochondrial extract incised the 8-oxoA:C-containing substrate efficiently (Fig. 6B, lane 6), but no incision activity was observed with \textit{ogg1}−/− mouse liver mitochondrial extract (Fig. 6B, lane 4). However, the \textit{ogg1}−/− mitochondrial extract was active toward a 30-nucleotide oligomer containing uracil at position 12 (oligonucleotide 4, Table I). The amount of incision activity for this uracil-containing DNA was similar in wild type and \textit{ogg1}−/− mitochondrial extracts (Fig. 6B, lanes 7 and 8). Thus, the \textit{ogg1}−/− extracts are competent for repair of some DNA lesions involving uracil. These data suggest that OGG1 is the only repair enzyme that cleaves 8-oxoA:C in mouse liver mitochondria.

**Trapping of Glycosylases**—DNA glycosylases/AP lyases cleave the phosphodiester bond 3’ to the AP site via a β-elimination reaction. The reaction involves the formation of a transient Schiff base intermediate that can be reduced by NaCNBH₃ or NaBH₄, which traps the enzyme in a covalent enzyme-DNA complex (35, 36). Purified human and yeast OGG1 can be trapped on DNA substrates containing 8-oxoG base-paired with thymine, guanine, and cytosine, where the enzyme-DNA complex forms with the highest efficiency using 8-oxoG:C (29). This method was used to trap enzyme-DNA complexes using purified hOGG1, nuclear, or mitochondrial extracts and DNA substrates containing 8-oxoA base-paired with adenine, guanine, cytosine, and thymine (oligonucleotide 1, Table I). Fig. 7A shows that trapped complexes are formed with purified hOGG1 and an 8-oxoA:C base pair (Fig. 7A, lane 6) or an 8-oxoG:C base pair (Fig. 7A, lane 9). Western analysis demonstrated that the purified hOGG1 contained two differentially sized species (data not shown). This explains the occurrence of two bands of reduced electrophoretic mobilities in lanes 6 and 9. No trapping was observed between hOGG1 and the three other 8-oxoA base pairs (Fig. 7A, lanes 2, 4, and 8). The binding/trapping reaction was not observed with an A:C mismatch (Fig. 7A, lane 5). Similar results were observed with human nuclear and mitochondrial extracts (Fig. 7, B and C, respectively). In addition, the electrophoretic mobility of the trapped protein-DNA complexes were identical whether the lesion was an 8-oxoA:C base pair (Fig. 7, B, lane 6, and C, lane 6) or an 8-oxoG:C base pair (Fig. 7, B, lane 9, and C, lane 9). This strongly suggests that the protein-DNA complex is a trapped Schiff base intermediate formed by purified hOGG1 or endogenous nuclear or mitochondrial hOGG1 in the cellular extracts. These results also support the conclusion that hOGG1 incises an 8-oxoA:C base pair. No enzyme-DNA complex was detected with the 8-oxoA:G substrate and the nuclear extract (Fig. 7B, lane 8). This result suggests that an alternative pathway not involving hOGG1 incises 8-oxoA when base-paired with guanine in nuclei. This alternative pathway could involve a monofunctional DNA glycosylase, which cannot be trapped by NaCNBH₃ or NaBH₄. Alternatively, a bifunctional glycosylase might catalyze this reaction, but the concentration of the enzyme might be too low to be detected in the trapping assay.

**Involvement of APE1**—APE1 is an abundant enzyme that incises abasic sites formed during base excision repair. It is likely that APE1 is present and active in the extracts of human cells used in the above experiments. This possibility was tested by examining the effect of Mg²⁺ on the incision efficiency of nuclear and mitochondrial extracts, because previous studies show that APE1 activity is Mg²⁺-dependent (37). The addition of Mg²⁺ stimulated 8-oxoA incision in mitochondrial and nuclear extracts (Fig. 8A, lanes 2, 5, and 8). Mg²⁺ was present during preparation of the nuclear extract, however, incision activity was not strongly inhibited by EDTA compared with incision activity without Mg²⁺ (Fig. 8A, lanes 3 and 4 and 6 and 7).

When OGG1 cleaves a DNA substrate with an 8-oxoG or 8-oxoA DNA lesion, the product has a slightly slower electrophoretic mobility than the product of cleavage by APE1. This fact was used to confirm whether APE1 or OGG1 cleaves the DNA substrate in human cell extracts. DNA substrates with 8-oxoA:C and 8-oxoA:G base pairs were incubated with nuclear extract (Fig. 8B, lanes 5–8) or mitochondrial extract (Fig. 8B, lanes 3 and 4) without or with purified APE1 (Fig. 8B, lanes 4, 6, and 8). Exogenous APE1 did not change the mobility of the reaction products, but APE1 did increase the mobility of the reaction product produced by purified hOGG1 (Fig. 8B, lanes 1 and 2). These results confirm that APE1 is active in the removal of 8-oxoA in human nuclear and mitochondrial extracts (Fig. 8B).

**DISCUSSION**

This study investigates repair of 8-oxoA in mammalian DNA by challenging human and mouse liver nuclear and mitochondrial extracts with 8-oxoA containing DNA substrates. The results provide evidence that OGG1 incises 8-oxoA:C containing DNA in mammalian cells, as \textit{ogg1}−/− mouse liver nuclear and mitochondrial extracts did not incise DNA in which 8-oxoA was base-paired with cytosine. The \textit{ogg1}−/− mitochondrial extract was competent for incision of uracil, indicating that it was not subject to a general dysfunction. The \textit{ogg1}−/− extracts

**TABLE I**

| Oligonucleotide number | Sequence                                                                 |
|------------------------|--------------------------------------------------------------------------|
| 1                      | 5′-ATATACCGGCA[ACCGCCGATCAAGCTATT-3′]                                    |
| 2                      | 3′-TATATGCGCGCNGCGCGCGCTAGTTCGAATAA-5′                                    |
| 3                      | 5′-ATATACCGGCA[ACCGCCGATCAAGCTATT-3′]                                    |
| 4                      | 3′-TATATGCGCGCNGCGCGCGCTAGTTCGAATAA-5′                                    |
| 5                      | 5′-GCGGACCGCTGGGATCCAGATATACCGGC[ACCGCCGATCAAGCTATT-3′]                   |
| 6                      | 3′-CCGGTTCGACCGACTCTAGGGATGGTCTAGTAAACTAGGCGCG-3′                        |
| 7                      | 5′-GCGGACCGCTGGGATCCAGATATACCGGC[ACCGCCGATCAAGCTATT-3′]                   |

**Sequences of oligonucleotides used in this study**

1. 5′-CGCCGTGCGGACCCTAGGTCTATATTTTTTCAAAAAGCTAGTTCGAATAACCTAGGCCGC-5′
2. 5′-CGCCGTGCGGACCCTAGGTCTATATGGCGCCGGCCGGCTAGTTCGAATAACCTAGGCCGC-5′
3. 5′-CGCCGTGCGGACCCTAGGTCTATATGGCGCCGGCCGGCTAGTTCGAATAACCTAGGCCGC-5′
4. 5′-GCGGACCGCTGGGATCCAGATATACCGGC[ACCGCCGATCAAGCTATT-3′]                   |
and extracts from wild type mice and human cells have a nuclear activity that incises 8-oxoA base-paired with guanine. This novel activity may play a role as a monofunctional glycosylase that facilitates repair of 8-oxoA:G containing DNA in mammalian cells.

γ-Irradiation and oxidative stress produce 8-oxoA in DNA (5, 38), and 8-oxoA induces misincorporation during DNA synthesis and mutations in mammalian cells (8). This study shows that human pol γ incorporates dTTP, dGTP, and dCTP opposite 8-oxoA in vitro, which is in agreement with in vivo studies of mouse cells previously performed by Kamiya et al. (8).

Extracts of mammalian cells do not incise 8-oxoA:T or 8-oxoA:A base pairs, but nuclear extracts incise 8-oxoA base-paired with guanine or cytosine with incision of the 8-oxoA:C base pair twice as efficient as incision of 8-oxoA:G. Mitochondrial extracts only incise 8-oxoA:C base pairs. These results suggest that two distinct enzymes with activity for 8-oxoA are expressed in mammalian cells and one of the activities is localized to the nucleus but not to the mitochondria. Previous studies show that OGG1 deficiency results in dysfunctional repair of 8-oxoG and accumulation of 8-oxoG in mitochondrial and nuclear DNA of mammalian cells (27, 34). Furthermore, repair of 8-oxoA is deficient in Cokayne syndrome group B cells, leading to accumulation of 8-oxoA in genomic DNA (11). It is also possible that unrepaired 8-oxoA:G base pairs in mitochondrial DNA accumulate in normal cells, because of the absence of a repair activity that incises this DNA lesion.

This study shows that OGG1 can be trapped in a covalent complex with DNA substrates containing an 8-oxoA:C base pair. Similar results were obtained with human nuclear and mitochondrial extracts, suggesting that 8-oxoA:C is recognized by OGG1 in the extracts and a Schiff base intermediate is formed during the cleavage reaction. Data discussed above suggests that an hOGG1-independent pathway incises 8-oxoA incision by hOGG1, nuclear or mitochondrial extracts from human cells. A, purified hOGG1 (20 ng) was incubated for 4 h at 37 °C with oligonucleotides containing either 8-oxoA (A*) or no base modification (A) (oligonucleotides 1 and 2, Table I) annealed to a complementary strand with thymine (lanes 1 and 2), adenine (lanes 3 and 4), cytosine (lanes 5 and 6), or guanine (lanes 7 and 8) opposite the lesion. An oligonucleotide with an 8-oxoG:C base pair (oligonucleotide 3, Table I) was used as a control (lane 9). Incision activity was detected as a 10-nucleotide oligomer incision product.

FIG. 5. Influence of the DNA helix structure and stability on 8-oxoA incision. A, oligonucleotides (Table I) containing 8-oxoA:C in different sequence contexts, oligonucleotide 5 (lanes 1 and 5), oligonucleotide 6 (lanes 2 and 6) and oligonucleotide 7 (lanes 3 and 7), were incubated with 30 μg of nuclear extract (lanes 1-3) or 25 μg of mitochondrial extract (lanes 5-7) for 4 h at 37 °C. As a control oligonucleotide 5 was incubated without extract (lane 4). B, mean ± S.D. was calculated using data from 3 extracts, which were assayed in triplicate, and data are presented as a bar diagram.
8-oxoA:G base pairs. Klungland et al. (27) reported an 8-oxoG:G repair activity in whole cell extracts from ogg1/H11002/H11002 mice. This OGG1-independent activity is likely to be the hNTH1 glycosylase (39) or one of the recently described human Nei homologs, NEIL 1 and NEIL 2 (19, 20, 40). Another candidate could be the MutY homologue (MYH) (41, 42). However, MYH activity has been observed both in nuclei and mitochondria (43, 44).

Unusual DNA structures such as condensed and rigid T-tracks contribute to DNA bending and are abundant elements in genomic DNA. They often exhibit unusual properties in vitro and in chromatin in vivo (45) and play important roles in processes such as transcription (46). Data presented here indicate that helix stability is an important factor in the efficiency of 8-oxoA incision. Thus, when 8-oxoA is located in a condensed helix structure in a bend or in a sequence with relatively low helix stability, the efficiency of incision is reduced. Previous experiments indicate that accessibility to the DNA backbone is reduced in DNA with these structures.2 Furthermore, DNA

2 T. Stevnsner, unpublished data.

FIG. 6. Incision of 8-oxoA with nuclear and mitochondrial extracts from ogg1 knock-out mice. A, wild type mouse liver nuclear extract (60 µg) was incubated for 4 h at 32 °C with either 8-oxoAC (A*-G) (lanes 2 and 3) or 8-oxoAG (A*-C) (lanes 4 and 5) containing oligonucleotides (oligonucleotide 1, Table I). ogg1-/- mouse liver nuclear extract (30 µg) was incubated with 8-oxoAC (lane 7) or 8-oxoAG (lane 8). An oligonucleotide with an A:C mismatch (oligonucleotide 2, Table I) was incubated under identical conditions with either 30 µg of wild type (lane 1) or ogg1-/- nuclear extract (lane 6). B, the 8-oxoAC (A*-G) containing oligonucleotide 1, Table I) was incubated for 4 h at 32 °C with 100 µg of wild type (lane 6) or ogg1-/- mouse liver mitochondrial extract (lane 4), respectively. An A:C mismatch (oligonucleotide 2, Table I) was incubated under the same conditions as a control without extract (lane 1), 100 µg of wild type (lane 5), or ogg1-/- (lane 2) mouse liver mitochondria extract, respectively. An oligonucleotide containing an ura:G base pair (oligonucleotide 4, Table I) was incubated with 100 µg of wild type (lane 7) or ogg1-/- (lane 8) extract for 2 h at 32 °C.

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concentration may influence the nucleotide flipping mechanism of OGG1; as noted above, OGG1 recognizes the DNA lesion and the base on the complementary strand opposite the DNA lesion, and may therefore use a double flipping mechanism (14, 16).

Data presented here also show that incision of 8-oxoA is stimulated by Mg$^{2+}$, which indicates the probable involvement of APE1 (37). This is consistent with incision of 8-oxoA by a monofunctional glycosylase or by a glycosylase with a weak AP lyase activity such as OGG1. The involvement of APE1 in 8-oxoA repair in human extracts was confirmed by showing that the products produced by nuclear and mitochondrial extracts comigrate with the products of APE1 cleavage.

No nuclear or mitochondrial activity that incises 8-oxoA:T, was detected. However, incision at 8-oxoA:C by OGG1 is efficient. This suggests that when a 8-oxoA:C base pair is formed by misincorporation of cytosine opposite 8-oxoA instead of by misincorporation of 8-oxoA opposite cytosine, the incision of 8-oxoA by OGG1 could fix a mutation and decrease genomic stability.

Genomic stability depends on proper DNA repair in nuclear and mitochondrial DNA. Studies of mitochondria suggest that they play a role in aging, and show that oxidative damage accumulates in rat mitochondria but not in nuclei in an age-dependent manner (10). A high level of oxidative DNA damage may lead to mitochondrial dysfunction, which may induce cellular apoptosis (reviewed in Ref. 47). Mitochondrial DNA repair may also play an important role in cancer, as mitochondrial repair activity decreases in cancer cells, but nuclear DNA repair does not (48). Mitochondrial dysfunction is also likely to be involved in the onset of neurodegenerative diseases as a consequence of defective oxidative phosphorylation that also accompanies normal aging (49) (reviewed in Ref. 47). Mitochondrial DNA repair activity decreases in cancer cells, but nuclear DNA repair does not (48). Mitochondrial dysfunction is also likely to be involved in the onset of neurodegenerative diseases as a consequence of defective oxidative phosphorylation that also accompanies normal aging (49) (reviewed in Ref. 47).

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