Repair Pathways for Processing of 8-Oxoguanine in DNA by Mammalian Cell Extracts*

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The repair pathways involved in the removal of 8-oxo-7,8-dihydroguanine (8-oxoguanine) in DNA by mammalian cell extracts have been examined. Closed circular DNA constructs containing a single 8-oxoguanine at a defined site were used as substrates to determine the patch size generated after in vitro repair by mammalian cell extracts. Restriction analysis of the repair incorporation in the vicinity of the lesion indicated that up to 75% of the 8-oxoguanine was repaired via the single nucleotide replacement mechanism in both human and mouse cell extracts. Approximately 25% of the 8-oxoguanine lesions were repaired by the long patch repair pathway. Repair incorporation 3’ to the lesion, characteristic for nucleotide excision repair, was not significant. Elimination of the DNA polymerase β (polβ)-dependent single nucleotide base excision repair pathway in extracts prepared from polβ-deficient mouse cells resulted in extension of the repair gap to 4–5 nucleotides 3’ to the lesion in 50% of the repair events, suggesting the increased involvement of the long patch repair pathway. However, about one-half of the 8-oxoguanine repair was still accomplished through replacement of only one nucleotide in the polβ-deficient cell extracts. These data indicate the existence of an alternative polβ-independent single nucleotide repair pathway for processing of 8-oxoguanine in DNA.

In living cells reactive oxygen species are formed continuously as a consequence of normal cellular metabolism and are also generated by a number of external factors. The reaction of active oxygen species with DNA results in numerous forms of base damage, and 8-oxoguanine is one of the most abundant lesions generated. An increased level of 8-oxoguanine has been observed after treatment of cells with UV, ionizing irradiation or chemical mutagens that generate oxygen radicals (for review, see Ref. 1). Although many different lesions are formed in DNA after oxidative stress, 8-oxoguanine is one of the most significant and the most studied lesions. 8-Oxoguanine in a syn conformation can base pair with adenine and induce transversion mutations (2, 3). This demonstrated mutagenic potential is thought to be involved in cancer and aging (4–8). To avoid deleterious consequences, 8-oxoguanine must be efficiently removed from DNA. Based on previous studies in bacterial cells it has been established that base excision repair (BER) is the major pathway for the removal of this lesion (8). Recently human 8-oxoguanine-DNA glycosylase (hOGG1) has been cloned and purified by several groups (9–11), thus providing an enzymatic basis for the involvement of BER in removal of this lesion in mammalian cells.

There are two pathways for base excision repair involving different subsets of proteins and operating independently (12–14). Both pathways are initiated by DNA glycosylases that recognize and remove the damaged base leaving an abasic site (AP site). The AP site is recognized by AP endonuclease, which introduces a DNA strand break 5’ to the baseless sugar, and then DNA polymerase β (polβ) catalyzes β elimination of the 5’ sugar phosphate residue and fills the one nucleotide gap. The nick is then sealed by DNA ligase (14). In polβ-deficient cell extracts this repair mechanism is disrupted and the PCNA-dependent pathway then becomes the major mode of base excision repair (16, 17). The proliferating cell nuclear antigen (PCNA)-dependent pathway, in addition to DNA glycosylase and AP endonuclease, also involves flap endonuclease, PCNA, DNA polymerase δ, and DNA ligase (14). Neither of these enzymes can remove a 5’ sugar phosphate and generate a 1-nucleotide gap. To remove the 5’ sugar phosphate, DNA polymerase first adds several nucleotides to the 3’ end of the nick and exposes the 5’ sugar phosphate as part of a single-stranded flap structure. This flap structure is recognized and excised by flap endonuclease, and the DNA is finally ligated by DNA ligase (14). These repair events result in a 2–5-nucleotide-long repair patch (13). In this reaction, PCNA and probably replication factor C assist in loading the DNA polymerase onto DNA (18) and also stimulate the endonuclease flap endonuclease (19).

The role of BER pathways has been characterized for the repair of uracil and AP sites (12–14, 20–22), but there are no studies on the detailed pathways for the removal of 8-oxoguanine. The processing of 8-oxoguanine may be different from that of uracil or AP sites for several reasons. First, 8-oxoguanine-DNA glycosylase unlike uracil-DNA glycosylase has an intrinsic AP lyase activity, and this raises the possibility for the existence of an alternative BER pathway independent of the AP lyase activity of DNA polymerase β. Second, it has recently been observed that 8-oxoguanine is recognized and processed by the human nucleotide excision repair (NER) system, proposing a role for NER in removal of this lesion (23). To examine the pathways involved in the repair of 8-oxoguanine, we have used

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The abbreviations and trivial names used are: 8-oxoguanine, 8-oxo-7,8-dihydroguanine; NER, nucleotide excision repair; BER, base excision repair; AP sites, apurinic/apyrimidinic sites, abasic sites; PCNA, proliferating cell nuclear antigen; bp, base pair(s); polβ, DNA polymerase β; polδ, DNA polymerase δ; hOGG1, human 8-oxoguanine-DNA glycosylase; bp, base pair; XP-A, xeroderma pigmentosum group A.

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Figure 1. DNA substrate containing a single 8-oxoguanine residue. A, nucleotide sequence and restriction sites of the 8-oxoguanine-containing strand (8-oxo) of the closed circular double-stranded DNA construct. The restriction fragments generated by hydrolyses with HindIII, HaeIII, and EagI restriction endonucleases are shown as solid bars. The direction of BER incorporation is indicated at the right corner. B, the overlap of repair incorporation in the 48-mer restriction fragment during single nucleotide patch (a) and long patch (b) base excision repair of 8-oxoguanine (bold). The repair patch is shown as a dotted line. The overlapped region is indicated by the vertical lines.

In this paper we report that the single nucleotide repair patch BER pathway is the primary mechanism for processing of 8-oxoguanine by mammalian cell extracts. We also present evidence for the existence of a previously unidentified, polβ-independent single nucleotide repair patch pathway for removal of 8-oxoguanine in DNA.

Materials and Methods

Cells—Human lymphoid AG9387 cells (apparently normal) and GM 2250 (xeroderma pigmentosum group A (XP-A)) cells were obtained from the Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ). Cells were grown in the medium recommended by the Repository. DNA polymerase β knockout mouse fibroblasts MB19tsA and isogenic wild-type cell line MB16tsA were obtained from Dr. S. H. Wilson and were grown as described (24).

Construction of Closed Circular M13 DNA Containing a Single 8-Oxoguanine or Uracil Residue—All oligonucleotides used were purchased from Midland Certified Reagent Company (Midland, TX) and gel-purified. The synthetic DNA duplex corresponding to the sequence 5'-59ATATACCGCGCCGCGATCAAGCTTATT-3' was cloned into the Smal site of M13mp18 DNA. The recombinant single-stranded DNA, named M13in, was purified, and the insert orientation was verified by sequencing. Double-stranded closed circular DNA containing a single lesion was constructed by priming 30 nM of single-stranded M13in DNA with a 6-fold molar excess of the oligonucleotide 5'-p59ATATACCGCGCGATCAAGCTTATT-3' for the single-stranded DNA construct, and with 5'-p59ATATACCGCGCCGCGATCAAGCTTATT-3' for the control plasmid. The 250-μl reaction mixture containing 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, 7 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM EDTA, 100 μM ATP, 500 μM dATP, dCTP, dGTP, and TTP, 40 units of T4 DNA polymerase, 35 Weiss units of T4 DNA ligase, and 100 μg of T4 gene 32 protein (all enzymes from Boehringer Mannheim) was incubated for 4 h at 37 °C. Closed circular DNA was isolated by CsCl/ EtBr density gradient centrifugation, purified by butanol extraction, desalted, and concentrated by centrifugation in a Centricon-10 microcentrator (Amicon). DNA substrates were stored at −20 °C in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

A DNA construct containing a single 8-oxoguanine lesion at a defined position. We have explored the role of polβ- and PCNA-dependent BER pathways as well as that of the NER pathway in the removal of this lesion in mammalian cell extracts. The repair synthesis incorporation was measured in the vicinity of the lesion, and the number and directionality of nucleotides replaced were assessed to determine whether the repair occurs via the single nucleotide polβ-dependent pathway, via the longer patch, PCNA-dependent BER pathway, or through the NER system. The repair incorporation was also analyzed in extracts prepared from polβ-knockout cells to explore the role of the polβ-independent pathways in the removal of 8-oxoguanine in DNA.

In this paper we report that the single nucleotide repair patch BER pathway is the primary mechanism for processing of 8-oxoguanine by mammalian cell extracts. We also present evidence for the existence of a previously unidentified, polβ-independent single nucleotide repair patch pathway for removal of 8-oxoguanine in DNA.

DNA Repair Synthesis Assay—Whole cell extracts were prepared from 3–5 g of cells by the method of Manley et al. (25). Standard 50-μl repair reactions contained 100–200 ng of single 8-oxoguanine-containing DNA, 45 mM Hepes-KOH (pH 7.8), 70 mM KCl, 7.5 mM MgCl₂, 1 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 40 mM phosphocreatine (d-Tris salt, Sigma), 2.5 μg of creatine phosphokinase (type I, Sigma), 3.4% glycerol (Fluka), 20 μg of bovine serum albumin, 50 μM dCTP, dATP, and TTP, 5 μM dGTP, 2 μCi of [α-32P]dGTP (3000 Ci/mmol, Amersham), and 100 μg of a whole cell extract protein. When the reaction was completed with both labeled dGTP and dCTP, the concentration of cold dCTP was also reduced to 5 μM. Reactions were carried out at 30 °C for 3 h. After the reaction, plasmid DNA was purified from the reaction mixture by phenol-chloroform extraction and ethanol precipitation and treated with 20 units of the indicated restriction endonuclease for 3–12 h in a buffer supplied by the manufacturer. Reactions were evaporated in a Speedvac (Savant), and the pellet was dissolved in 20 μl of formamide-dye (0.1% xylene cyanol-0.1% bromophenol blue and 50% formamide), incubated for 5 min at 90 °C, and electrophoresed on a 20% polyacrylamide gel containing 7% urea, 89 mM Tris borate (pH 8.8), and 2 mM EDTA. The amount of radioactivity was quantified on a PhosphorImager (Molecular Dynamics).

Results

Closed Circular Double-stranded DNA Containing a Single 8-Oxoguanine Residue—An oligonucleotide containing restriction sites suitable for analysis of DNA repair incorporation in the vicinity of the lesion was cloned in M13mp18 DNA. Single 8-oxoguanine-containing DNA was constructed by priming recombinant single-stranded DNA with an 8-oxoguanine-containing oligonucleotide, and covalently closed double-stranded DNA was purified by cesium chloride gradient centrifugation. The 7280-bp substrate DNA contained a unique 8-oxoguanine lesion and restriction sites suitable for analysis of repair patch size (Fig. 1A). To confirm the presence of the lesion, we demonstrated that our DNA construct is sensitive to Fpg protein, the bacterial enzyme that recognizes 8-oxoguanine in DNA and cleaves the 5’ and 3’ phosphodiester bonds next to the lesion (26). The digestion of the substrate DNA with HindIII generates two fragments: a 59-mer fragment containing the lesion and a 7171-bp fragment representing the rest of the DNA. The fragments were then end-labeled with Escherichia coli DNA polymerase I. The filling of the protruding ends of the 59-mer fragment generated a 63-bp duplex DNA fragment labeled in both strands and containing 8-oxoguanine in only one of them. The labeled DNA was then treated with Fpg protein. The
cleavage of the 63-mer fragment at the site of 8-oxoguanine should generate a 15-mer labeled fragment. The result of the described experiment is shown in Fig. 2A. There are no Fpg-sensitive sites in the control DNA, and the expected 15-mer product is observed only in 8-oxoguanine-containing DNA. Because both DNA strands were labeled and only one of them contains the lesion, the radioactivity of the 15-mer fragment (46% of the total amount of radioactivity in the 63- and 15-mer fragments) indicates that at least 92% of the construct DNA contains single 8-oxoguanine at the defined position.

Repair of 8-Oxoguanine in Human Whole Cell Extracts—The repair of 8-oxoguanine was measured in the DNA repair incorporation assay. DNA containing 8-oxoguanine or control DNA, prepared by the same method but containing a normal G:C base pair at the same position, were incubated with human whole cell extracts in the presence of all cofactors needed for DNA repair, including [α-32P]dGTP. After incubation, the DNA was purified from the reaction mixture, cleaved with HindIII restriction endonuclease, and analyzed by gel electrophoresis. The reaction with HindIII released a 59-bp fragment that contained an 8-oxoguanine residue in substrate DNA. The analysis of the gel shown in Fig. 2B indicated that after 3-h incubation with human cell extracts some nonspecific incorporation accumulates in both damaged and undamaged DNA constructs (the band at the very top of the gel). However, if normalized to the fragment length, the incorporation into the 59-bp HindIII fragment was 150-fold more than nonspecific incorporation into the rest of the plasmid. The incorporation into the 59-bp HindIII fragment was highly damage-specific; i.e. very little or no incorporation was seen in the corresponding fragment of the control DNA. The incorporation of dGMP into the 59-bp HindIII fragment also indicates that repair of 8-oxoguanine was completed by the cell extract, because we did not observe any labeled intermediates of incision that were not ligated. To estimate the size of the repair patch produced in human extracts, we performed a simultaneous digestion of repaired DNA with HindIII and HaeIII restriction endonucleases. This hydrolysis generates a 48-bp fragment that contains the 8-oxoguanine residue in substrate DNA, a 4-mer fragment that includes nucleotides 2–5, and a 7-mer fragment including nucleotides 7–12 3’ to the lesion. A single nucleotide repair patch would be detected by dGMP incorporation in the 48-mer HindIII-HaeIII fragment, and any further extension of the repair patch would result in the additional labeling of the 4- or 7-mer fragment (Fig. 1A, scheme). The PhosphorImager quantification of the relative incorporation in the restriction fragments revealed that >75% of the total incorporation into the 59-mer HindIII fragment was detected in the 48-mer fragment, and 25% of the incorporation was found in the 4- and 7-mer bands (Fig. 3). When the incorporation was normalized to the number of potential sites for dGMP incorporation (one in the 48-mer, two in the 4-mer and one in the 7-mer fragments), we conclude that ~84% of the repair incorporation was detected in the 48-mer fragment. Similar experiments were performed in which incorporation of both dCMP and dGMP was measured in the same repair reaction. The nucleotide sequence 3’ to the 8-oxoguanine contains 8 consequent guanines and cytosines from nucleotides 48–55 (Fig. 1A). In these experiments incorporation into each of these nucleotides 3’ to the 8-oxoguanine was monitored. When these results were normalized to the number of potential sites for dGMP and dCMP incorporation, >85% of the incorporation was found in the 48-mer fragment, indicating that a single nucleotide repair patch is the major pathway for the repair of 8-oxoguanine in human cell extracts. All data were pooled and are summarized in Table I.

Repair of 8-Oxoguanine in polβ-deficient Cell Extracts—To elucidate the role of the polβ-independent pathways in the repair of 8-oxoguanine from DNA, we measured the repair of this lesion in extracts prepared from polβ-knockout mouse cells (polβ−/−) and from isogenic wild-type cells (polβ+/+). The substrate DNA containing a single 8-oxoguanine residue was incubated with these extracts, purified, and then digested with HindIII restriction endonuclease. Similar incorporation was found in the 59-mer fragment cleaved from the 8-oxoguanine-containing DNA repaired in normal and polβ-deficient cell extracts (Fig. 4, lanes 1 and 2). Surprisingly, nonspecific incorporation was much higher in the polβ−/− cell extracts than in polβ+/+ cell extracts (Fig. 4, compare bands at the top of the gel). The analysis of repair patch size by hydrolysis with HindIII and HaeIII restriction endonucleases was performed as described above for human cell extracts. The simultaneous incorporation of labeled dGMP and dCMP was monitored in these experiments. After repair of 8-oxoguanine-containing DNA in cell extracts prepared from normal mouse fibroblasts, ~80–90% of the incorporation was localized in the 48-mer HindIII-HaeIII fragment (Fig. 4, lane 3). This indicates that, as was shown above for human cell extracts, mouse cell extracts
Repair of 8-Oxoguanine

The results of restriction analysis of an in vitro repaired 8-oxoguanine-containing DNA were quantified with Phosphorimager, and the incorporation was normalized to the number of potential sites for incorporation. An average of three independent experiments is shown.

| Restriction fragment | Human cell extracts | Mouse cell extracts |
|----------------------|---------------------|---------------------|
|                      | Polβ⁺ | Polβ⁻ |
| 48-mer               | 84.0 ± 2.5 | 85.0 ± 5.3 | 65.4 ± 5.8 |
| 4-mer                | 12.0 ± 2.5 | 8.2 ± 4.8  | 20.4 ± 7.5 |
| 7-mer                | 4.0 ± 1.2  | 6.8 ± 1.5  | 14.2 ± 1.9 |

**Fig. 4.** Restriction analysis of patch size after repair of 8-oxoguanine-containing DNA in normal and polβ-deficient mouse cell extracts. Repair reactions with 200 ng of the substrate DNA were performed as described with 100 µg of protein of whole cell extracts prepared from DNA polymerase β-knockout mouse cell line MB19tsA (polβ⁻) and isogenic wild-type cells MB18tsA (polβ⁺). Reactions were carried out at 30 °C for 3 h. After the reaction DNA was recovered from the reaction mixture and cut with HindIII and HaeIII restriction endonucleases. Repair incorporation into individual fragments was analyzed after electrophoresis on a 20% polyacrylamide gel.

repair 8-oxoguanine predominantly via the single nucleotide repair patch pathway. In the polβ⁻ cell extracts the repair gap most commonly was extended by >1 nucleotide, and more incorporation in the 4- and 7-mer restriction fragments was found (Fig. 4, compare lanes 3 and 4). However, the 48-mer HindIII-HaeIII fragment from DNA repaired in polβ⁻ extracts contained up to 65% of the incorporated radioactivity, suggesting that a substantial part of the repair in these extracts was accomplished through a single nucleotide gap. The distribution of incorporation in the restriction fragments observed in several independent experiments with polβ⁻ and polβ⁺ mouse extracts is summarized in Table I. A high level of incorporation into the 48-mer restriction fragment indicates that an alternative, previously unidentified, polβ-independent repair pathway generating a 1-nucleotide repair patch is involved in the repair of 8-oxoguanine in a polβ-deficient cell extracts.

**Fig. 5.** Nucleotide excision repair is not involved in in vitro repair of 8-oxoguanine. Analysis of dGMP incorporation into the HindIII-EagI fragment located 5' to the 8-oxoguanine. Substrate DNA was incubated for 3 h at 30 °C with 100 µg of whole cell extract protein prepared from the normal human lymphoblastoid cell line AG9387 (A, lanes 1 and 2), xeroderma pigmentosum group A lymphoblastoid cell line GM2250 (A, lanes 3 and 4), DNA polymerase β-knockout mouse cell line MB19tsA, and isogenic wild-type MB18tsA cells (B, lanes 1–4, respectively). The DNA was recovered from the reaction mixture and cut with HindIII and EagI restriction endonucleases. Repair incorporation into individual fragments was analyzed after electrophoresis on 15% polyacrylamide gel.

region includes 20–24 nucleotides 5’ and 5–9 nucleotides 3’ to the damaged site (27, 28). Thus, the 46-mer HindIII-EagI fragment of the 8-oxoguanine-containing plasmid should be labeled during in vitro NER (Fig. 1A). The dGMP and dCMP incorporation into this fragment, when normalized to the total incorporation into the 59-mer HindIII-HindIII fragment, will reflect the relative contribution of NER to the repair of 8-oxoguanine in the cell extract. There are two overlapping EagI restriction sites close to the 8-oxoguanine, so cleavage with HindIII and EagI generates two independent sets of fragments. One of these (46-mer HindIII-EagI and 13-mer EagI-HindIII) was used to monitor incorporation 5’ to the lesion. We found that only ~10% of the total incorporation into the 59-mer fragment was attributable to incorporation into the 46-mer HindIII-EagI fragment (Fig. 5A, lane 2). If this incorporation represents NER activity on the 8-oxoguanine lesion, it should decrease after repair in cell extracts derived from NER-deficient mutants. To test this, incorporation into the 46-mer HindIII-EagI fragment after repair of 8-oxoguanine-containing DNA in an XP-A cell extract was analyzed. We reproducibly observed a 30–40% decrease in incorporation into the 59-mer HindIII fragment after the repair reaction in the XP-A extracts (Fig. 5A, compare lanes 1 and 3), but there were no changes in the relative incorporation into the 46-mer HindIII-EagI fragment compared with the incorporation into the 59-mer HindIII fragment.

We also investigated whether the role for NER would increase when the major BER pathway is disrupted. The experiments with normal and polβ-deficient mouse cell extracts were performed exactly as described above for human cell extracts. No changes in the relative incorporation in the 46-mer HindIII-EagI fragment in the polβ-deficient cell extracts were found compared with the normal cell extracts (Fig. 5B, compare lanes 2 and 4). These data suggest that the role of NER in repair of 8-oxoguanine is not increased in polβ-deficient cell extracts.

**DISCUSSION**

We have examined the involvement of different repair pathways in the processing of 8-oxoguanine in mammalian cell extracts using restriction analysis of in vitro repaired DNA. We
made the assumption that the gap size during long patch repair events is at least 4–5 nucleotides and that repair incorporation is independent of the local nucleotide sequence at each guanine and cytosine 3’ to the lesion. Using restriction analysis we have mapped the repair incorporation in the first 11 nucleotides 3’ to the 8-oxoguanine. The data on repair incorporation into restriction fragments in the vicinity of 8-oxoguanine are summarized in Table I. These data are already normalized to the number of potential sites for the dGMP and dCMP incorporation. However, to be interpreted in terms of a relative contribution of the different pathways to repair of 8-oxoguanine, these data should be further adjusted because the 48-mer restriction fragment is labeled not only during the filling of a 1-nucleotide gap but also during repair of longer gaps (Fig. 1B). Thus, to calculate the absolute contribution of a single nucleotide repair pathway, the long patch component of incorporation into the 48-mer restriction fragment should be subtracted. When the incorporation detected in the 4-mer fragment was subtracted from the total incorporation detected in the 48-mer fragment, we found that 72 ± 2.5% of a repair events were accomplished solely through a single nucleotide repair patch. We thus conclude that in whole cell extracts this pathway is the primary pathway for the repair of 8-oxoguanine in DNA. The other pathways contribute to the repair of the remaining 28 ± 2.5% of the 8-oxoguanine. The final normalized data on the contribution of BER pathways to the processing of 8-oxoguanine by human cell extracts are shown in Fig. 6. These data are in good agreement with a previous finding from this laboratory indicating that multiple pathways are involved in the repair of 8-oxoguanine in randomly damaged plasmid DNA (29).

Recently, the involvement of NER in the removal of 8-oxoguanine has been reported (23). We have examined the relative roles of BER and NER in the repair of this lesion in mammalian cell extracts. Although the in vitro repair system we have used in our experiments is optimal for both BER and NER, we were unable to see any significant involvement of NER in the removal of 8-oxoguanine either in whole cell extracts or in cell extracts prepared from polβ-knockout cells, in which the major pathway for base excision repair is disrupted. The excision assay used by Reardon et al. (23) was specifically designed to monitor only NER activity, so the sensitivity of their method was high in measuring even minor repair of 8-oxoguanine. In our assay NER and BER compete for the same substrate, and because the BER system is the primary pathway for repair of 8-oxoguanine, it is more effective in processing 8-oxoguanine. Nevertheless, our experiments do not rule out a role for NER in the repair of oxidative DNA damage. NER may serve as a backup system when the major BER pathways are inactivated. Also, NER is important for transcription-coupled repair, and transcription-coupled repair of thymine glycol in DNA has been demonstrated recently (30). We observed a reproducible 30–40% reduction of total repair incorporation in the XP-A extract (Fig. 5A). The XPA protein binds preferentially to UV-damaged DNA and is involved in DNA damage recognition and processing during NER (28). The XPA protein also has an affinity for osmium tetroxide-treated DNA (31), and reduced repair of oxidative DNA damage in XP-A cell extracts has previously been documented by several groups, including this laboratory (29, 32). So far no role for the XPA protein in BER has been ascribed. It is possible that the XPA protein is involved in the recognition of 8-oxoguanine in DNA and assists other BER proteins in processing of this lesion.

There is strong evidence from studies in mammalian cell extracts that DNA polymerase β is the major polymerase participating in single nucleotide gap filling during BER (24, 33–35). DNA polymerase β has an intrinsic activity that removes the 5’ sugar phosphate by β elimination (36, 37). Thus, this enzyme plays a key role in the single nucleotide patch repair pathway, because it both removes the 5’ sugar phosphate generated by DNA glycosylase and AP endonuclease and also fills in the resulting 1-nucleotide gap. In the polβ-deficient cell extracts the repair of uracil and AP sites is accomplished by a PCNA-dependent mechanism and results in a 2–5-nucleotide-long repair patch (16, 17). We found that removal of 8-oxoguanine in polβ-deficient cell extracts was only partially accomplished through a long patch repair mechanism, and approximately one half of the repair events involves a single nucleotide repair patch mechanism (65% incorporation into the 48-mer restriction fragment minus 20% incorporation contributed by long patch repair). During long patch repair, flap endonuclease activity releases the 5’ sugar phosphate residue as a part of a 2–5-base oligonucleotide and is not able to generate a one nucleotide gap (38, 39). We conclude that in addition to long patch repair, another mechanism is involved in the repair of 8-oxoguanine in polβ-deficient cell extracts. Purified human OGG1 protein can catalyze a β elimination reaction (9–11) and most probably like its yeast counterpart yOGG1 can also catalyze removal of a 3’ sugar phosphate from an incised AP site (15). This suggests that hOGG1 alone, or in combination with other enzymes, can generate a 1-nucleotide gap even in the absence of polβ, and it is likely that a polymerase other than polβ can fill the resulting 1-nucleotide gap. Fortini et al. (17) recently reported that a polβ-independent single nucleotide repair patch mechanism can participate in the repair of uracil in DNA in mammalian cell extracts. In their experiments, Fortini et al. (17) studied the repair of a plasmid DNA containing a single uracil residue at a defined position. They found that some repair in polβ-deficient cell extracts may be accomplished through a single nucleotide patch repair path. The polβ-independent single nucleotide patch repair pathway for removal of uracil and the one that we report in this paper for 8-oxoguanine may differ, because different enzymes are involved. In the substrate used by Fortini et al. (17), uracil-DNA glycosylase removes the uracil from DNA and creates an AP site. In contrast to hOGG1, uracil-DNA glycosylase does not have intrinsic AP lyase activity, and the AP site is presumably further processed by AP endonuclease, which generates a nick with a 5’ sugar phosphate. In the absence of the AP lyase activity of polβ, the slow rate single nucleotide patch repair observed by Fortini et al. (17) may be supported by other weak β eliminators or caused by the spontaneous β elimination of the 5’ sugar phosphate during incubation in the whole cell extract. In the case of 8-oxoguanine repair, we observed very efficient involvement of a single nucleotide patch pathway. It was responsible for up to 50% of repair events in a polβ-deficient cell extracts. These data suggest that AP lyase activity of hOGG1 is actively involved into repair of 8-oxoguanine. Based on our results, we propose that this polβ-independent single nucleotide repair patch pathway includes 8-oxoguanine-DNA glyco-
sylase, DNA polymerase (δ or ε), and DNA ligase as a core enzymes. There is a possibility that additional activity is involved in the removal of the 3′ sugar phosphate in the nick generated by hOGG1. We also cannot exclude that some other proteins that are specifically induced only in a polβ-deficient cell line. R. M. Anson, R. Stierum, and S. Mazur are thanked for critical reading of the manuscript.

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