Role of XRCC1 in the Coordination and Stimulation of Oxidative DNA Damage Repair Initiated by the DNA Glycosylase hOGG1*

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XRCC1 participates in DNA single strand break and base excision repair (BER) to preserve genetic stability in mammalian cells. XRCC1 participation in these pathways is mediated by its interactions with several of the acting enzymes. Here, we report that XRCC1 interacts physically and functionally with hOGG1, the human DNA glycosylase that initiates the repair by BER of the mutagenic oxidized base 8-oxoguanine. This interaction leads to a 2- to 3-fold stimulation of the DNA glycosylase activity of hOGG1. XRCC1 stimulates the formation of the hOGG1 Schiff-base DNA intermediate without interfering with the endonuclease activity of APE1, the second enzyme in the pathway. On the contrary, the stimulation in the appearance of the incision product seems to reflect the addition of the effects of XRCC1 on the two first enzymes of the pathway. The data presented support a model by which XRCC1 will pass on the DNA intermediate from hOGG1 to the endonuclease APE1. This results in an acceleration of the overall repair process of oxidized purines to yield an APE1-cleaved abasic site, which can be used as a substrate by DNA polymerase β. More importantly, the results unveil a highly coordinated mechanism by which XRCC1, through its multiple protein-protein interactions, extends its orchestrating role from the base excision step to the resealing of the repaired DNA strand.

A major threat to genetic stability is the damaging of DNA by either endogenous or exogenous compounds. This is underscored by the cancer-prone phenotype of human cells defective in DNA repair processes. Exposure of the cellular DNA to reactive oxygen species (ROS), generated either by the normal metabolism of the cell or by chemical and physical exogenous agents, is at the origin of lesions that can have genotoxic or mutagenic consequences. To avoid the effects of ROS and, therefore, to maintain the integrity of their genetic information, organisms have evolved multiple DNA repair mechanisms (1).

Because of its capacity to pair with an adenine during replication, 7,8-dihydro-8-oxoguanine (8-oxoG), an oxidized derivative of guanine, is arguably the major mutagenic lesion in DNA. Indeed, in Escherichia coli, the inactivation of the genes involved in the repair of this oxidized base leads to the strongest spontaneous mutator phenotypes, characterized by the exclusive increase in G to T transversions. Like for other ROS-induced modifications of DNA, 8-oxoG is mainly repaired by the base excision repair (BER) pathway. This pathway is initiated by the recognition and excision of the oxidized guanine by a DNA glycosylase, OGG1 being the major one in yeast and mammalian cells (2). In human cells, the resulting abasic (apurinic/apyrimidinic (AP)) site can be cleaved by a second enzymatic activity of the hOGG1 polypeptide, namely an AP lyase activity. If such a reaction takes place, the nick produced has a 3′-open aldehyde residue that is supposed to be removed by the 3′-deoxyribose phosphatase activity of APE1, the major AP endonuclease. However, recent data suggest that, once the base is removed by hOGG1, the AP site is cleaved by APE1, bypassing the AP lyase step (3, 4). This would not only make the initial steps of the repair more efficient, but also avoid the exposure of 3′-blocked single strand DNA nicks to the cellular milieu that could lead to cell death or chromosomal instability. The resulting nick would then be used as substrate by DNA polymerase β gap filling and deoxyribose-5-phosphate (dRP) lyase activities.

The importance of the coordination of the various steps in the BER pathway is further suggested by the finding of multiple interactions between the proteins of the pathway. In particular, the polypeptide coded by the XRCC1 gene seems to play a central role in coordinating the activities of the enzymes participating in the BER pathway. Abundant evidence has emerged for a critical role of XRCC1 in various DNA repair processes in mammalian cells (5). This gene was identified by its capacity to complement the radiosensitivity of mutant Chinese hamster ovary cell lines (6). Cells lacking a functional XRCC1 protein are not only hypersensitive to ionizing radiation but also to oxidizing and alkylating agents. Moreover, they display genetic instability, reflected in a elevated spontaneous frequency of chromosomal aberrations and deletions (5). Xrc1-1/mice display embryonic lethality (7). All these phenotypes point to an essential role for XRCC1 in the maintenance of the DNA repair capacity of the cells. Recent data has associated the presence of XRCC1 polymorphisms to the occurrence of tumors (8–12).
The coordinating role for XRCC1 in BER has been proposed, because this protein, even though devoid of any known enzymatic activity, interacts with all the enzymes involved in BER downstream of the DNA glycosylase, namely APE1 (13), DNA polymerase β (polβ) (14, 15), and DNA ligase 3 (Lig3) (16). The interactions can result in a stimulation or stabilization of the corresponding enzyme, as it is the case for APE1 or Lig3, respectively. The complete structure of XRCC1 is not known. However, it is known to possess three distinct globular domains (17). An N-terminal domain (NTD) has been characterized by NMR and provides the interface with the DNA polβ (18). Two BRCT domains (BRCT1 and 2) mediate the interactions with the PARP proteins (19, 20) and Lig3 (21), respectively. The two linker regions between the globular domains have been denominated hinges and have been predicted to be unstructured (17).

In single strand break repair (SSBR), the interactions with key enzymes of the pathway, poly(ADP-ribose) polymerase (PARP-1), polynucleotide kinase (PNK), polβ, and Lig3 (22), mediate XRCC1 role. In both SSBR and BER pathways, XRCC1 could be recruited to the DNA by the enzyme recognizing the lesion (APE1 for AP sites and PARP-1 for SSBs) and then could itself capture the downstream activities to the repair intermediates. Likewise, XRCC1, through this scaffolding role, could coordinate the rate and sequence of the enzymatic activities to avoid the exposure of recombinogenic strand breaks, generated as intermediates in both pathways, that could lead to chromosomal rearrangements. Here we explore the role of XRCC1 in the very first step of BER of 8-oxoG initiated by the human DNA glycosylase hOGG1.

EXPERIMENTAL PROCEDURES

Oligonucleotide Substrates and Enzymes—The 34-mer oligodeoxynucleotide containing an 8-oxoguanine was labeled at the 5′-end using γ-32P-ATP (3000 Ci/mmol, Amersham Biosciences) and T4 polynucleotide kinase (New England Biolabs). The 5′-phosphorylated strand was hybridized with a complementary sequence containing a cytosine (C) opposite to the lesion yielding the duplex 8-oxoG:C. The recombinant hOGG1 and APE1 proteins were purified as described (23, 24). XRCC1 was further purified by fixation to an heparin column. The XRCC1 pET16b expression vector was kindly donated by K. Caldecott. His-tagged recombinant XRCC1 was expressed in _Escherichia coli_ BL21 as described before (25). After XRCC1 induction, cells from 4 liters of culture (A600 = 1.3) were harvested by centrifugation, resuspended, and sonicated in 150 ml of ice-cold lysis buffer (50 mM Tris-HCl, 500 mM NaCl, 10% glycerol, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), pH 8) with antipain (2.5 μg/ml), aprotinin (2.5 μg/ml), and leupeptin (2.5 μg/ml), and lysosyme (1 mg/ml). The lysate was centrifuged at 100,000 × g for 1 h at 4 °C, and the supernatant was precipitated with 50% saturation ammonium sulfate. The precipitated proteins containing XRCC1 were resuspended in 10 μl of lysis buffer, desalted on a PD10 column (Amersham Biosciences) equilibrated in the same buffer with 0.05% Triton X-100 added, and loaded on 1 ml of nickel column (Amersham Biosciences). The column was washed with lysis buffer (plus Triton X-100) containing 40 mM imidazole, and XRCC1 was eluted at 300 mM imidazole. Lysis buffer was exchanged using PD10 against A100 buffer (buffer A: 50 mM NaPO4, 0.5% glycerol, 0.05% Triton X-100, 1 mM DTT, 0.1 mM EDTA, pH 7.2, + 0.1 mM NaBH4), and incubated in 20 μl of a HeLa cell extract prepared as described previously (26). Values correspond to the mean of two independent experiments.

**In Vitro Transcription/Translation of XRCC1**—In _in vitro_ transcription/translation of XRCC1 was performed using a TNT-coupled lysate system (Promega) according to the manufacturer’s instructions. The reaction containing [35S]methionine and the pBS-XRCC1 expression vector was incubated for 90 min at 30 °C. Reaction products were analyzed directly by SDS-PAGE and used for the GST-pull-down experiments with _E. coli_-expressed GST-hOGG1.

**GST Pull-down Experiments**—An hOGG1 open-frame reading was cloned into plasmid pBC to transiently express a GST-hOGG1 fusion in mammalian cells. The GST-XRCC1 fusion was expressed from the plasmid described by Masson et al. (20) in HeLa cells expressing an _myc_-tagged hOGG1. These cells were obtained by transformation of HeLa cells with a pCDNA3.1 _myc_ vector (Invitrogen) in which the hOGG1 cDNA was cloned (pPrT7). The GST pull-down experiments were performed as described (13, 20). For immunodetection, blots were incubated with rabbit polyclonal anti-XRCC1 (20), rabbit polyclonal anti-hOGG1 (27), or monoclonal anti-GST (Amersham Biosciences) and subsequently with the appropriate secondary antibody coupled to horseradish peroxidase (Amersham Biosciences). The bands were detected by enhanced chemiluminescence (ECL, Amersham Biosciences).

For the GST pull-down of the _in vitro_ synthesized 35S-XRCC1, the GST or GST-hOGG1 proteins were expressed in _E. coli_ and fixed to glutathione-Sepharose beads (Amersham Biosciences). Equal amounts of GST or GST-hOGG1 proteins (−25 μg) coupled to glutathione-Sepharose beads were incubated in the presence of the labeled XRCC1 in 250 μl of binding buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.3 mM DTT, 0.5% Nonidet P-40, and a mixture of protease inhibitors) for 1 h at 37 °C. The beads were subsequently washed several times with binding buffer, and the bound proteins were separated on a 12% SDS-PAGE. The gels were dried and scanned using a Storm PhosphorImager (Amersham Biosciences).

**8-oxoG DNA Glycosylase and AP-Lyase Assays**—In a standard reaction (10 μl final volume), 50 fmol of the 8-oxoG:C-labeled duplex was incubated in glycosylase reaction buffer (50 mM HEPES, 2 mM EDTA, 50 mM NaCl, 5% glycerol, 0.1 mg/ml bovine serum albumin (BSA), pH 7.6) with various amounts of hOGG1, XRCC1, and or APE1. Reactions were carried out at 37 °C for the time indicated and stopped on ice with NaOH for the glycosylase assays (0.1 N final concentration) or NaBH4 for the AP-lyase experiment (50 mM final concentration). The reactions were incubated 5 min at 37 °C and stopped by boiling, followed by heating for 5 min at 95 °C. The products of the reactions were resolved by denaturing 20% PAGE (19:1 acrylamide: bisacrylamide). Gels were scanned, and band intensities were quantified using a Storm PhosphorImager.

**APE1 Cleavage Assay**—In a standard reaction (16 μl final volume), 50 fmol of the 8-oxoG:C-labeled duplex was incubated in APE1 reaction buffer (25 mM Tris-HCl, 1 mM MgCl2, 0.4 mg/ml BSA, pH 8) with various amounts of hOGG1, APE1, and or XRCC1. Reactions were carried out at 37 °C during different times and stopped by adding 6 μl of formamide dye, followed by heating for 5 min at 95 °C. The products of APE1 reactions were analyzed as described for the hOGG1 glycosylase assay.

**Borohydride Trapping Assay**—In this assay, for elucidation of the interacting proteins, _E. coli_ lysates were incubated in the presence of the labeled XRCC1 in 250 μl of glycosylase reaction buffer containing 10 fmol of 8-oxoG:C-labeled duplex and various amounts of hOGG1, APE1, and or XRCC1. NaBH4 at a final concentration of 50 mM was then added, and incubation continued for 10 min at 37 °C. The reaction was terminated by addition of Laemmli buffer (1× final) and heated at 95 °C for 5 min. Trapped complexes were separated by SDS-PAGE. The gels were dried and scanned using a Storm PhosphorImager.

**MBP Pull-down Assays**—For each of the eight polypeptide-coding sequences, two oligonucleotides were designed for PCR, the first one introducing a EcoRI restriction site on the 5′ extremity and the second a XhoI restriction site on the 3′ end. The oriented amplified sequences were cloned into the pGEX-4T3 and pGEX-4T4 vectors. The recombinant GST fusion proteins were expressed from the plasmid described by Boardman (New England Biolabs) in fusion with a maltose-binding protein (MBP) expressing sequence.

The plasmid pMAL-c2x, harboring the different XRCC1 domains, was introduced in _B. subtilis_ Rosetta cells (Invitrogen). Cultures and inductions were performed according to the manufacturer’s instructions (Novagen). _B. subtilis_ pellets of induced cells were resuspended and sonicated at A = 30 in MBP lysis buffer (20 mM NaPO4, 1 mM EDTA, 1 mM DTT, 0.1% Nonidet P-40, pH 7.2) supplemented with 150 mM NaCl, antipain (2.5 μg/ml), aprotinin (2.5 μg/ml), leupeptin (2.5 μg/ml), and lysosyme (1 mg/ml). The lysis solutions were centrifuged at 4 °C during 30 min at 10,000 × g, and the supernatants were used in MBP pull-down assays as described above.

For each interaction, 30 μl of amylase resin (New England Biolabs), equilibrated in MBP lysis buffer containing 150 mM NaCl, was incubated, for 20 min at 4 °C in a shaker, with an excess (1 ml) of protein extract prepared as described above. The amylase resin was recovered.
pressed an myc-
with the GST-hOGG1 fusion from the cell extracts (Fig. 1B). The presence of hOGG1 associated to GST-XRC1 was confirmed by the detection of the 8-oxoG DNA glycosylase activity on the glutathione-Sepharose beads (data not shown). The co-purification from human cell extracts of XRC1 and hOGG1 shows that these two proteins are associated in mammalian cells, either directly or through interaction with other proteins.

Simultaneously, yeast AH109 cells expressing the two-hybrid bait construct pGBKKT7-hOGG1 were used for screening a human HeLa cDNA library. From $9.2 \times 10^6$ transformants, 250 clones displayed the double adenine/histidine prototrophy, but only 23 clones were obtained possessing an associated $\beta$-galactosidase activity (Fig. 1C). Sequencing of the cDNA candidates revealed that two of them shared exact homology with the XRC1 open reading frame (amino acids 141–633), suggesting a direct interaction between hOGG1 and XRC1. Biochemical evidence for such direct interaction between the two proteins emerged from the specific association of an in vitro synthesized ^32P-XRC1 with a GST-hOGG1 expressed in E. coli (Fig. 1D).

Taken together, the data presented demonstrate that XRC1 interacts with hOGG1 through direct protein-protein contact.

**XRCC1 Stimulates the DNA Glycosylase Activity of hOGG1—**To assess whether the physical interaction between XRC1 and hOGG1 had an impact in the latter's protein activity, we performed enzymatic tests on an oligonucleotide substrate carrying a single 8-oxoG. To analyze the hOGG1 8-oxoG DNA glycosylase activity, the reactions were stopped by the addition of 0.1 N NaOH to chemically cleave all the AP sites generated by incubation with hOGG1 in the presence or absence of varying amounts of XRC1. The addition of an excess of XRC1 to the hOGG1 reaction mix results in a 2-fold increase in the appearance of cleaved products (Fig. 2, A and B). XRC1 by itself has no cleavage activity on the 8-oxoG-harboring substrate (Fig. 2A, last lane); therefore, ruling out a contamination of the preparation with a DNA glycosylase capable of removing 8-oxoG from DNA. Furthermore, the stimulation is XRC1-specific, because an equivalent amount of bovine serum albumin (BSA) has no effect on the enzymatic activity (data not shown), and all reactions were carried out in the presence of an excess of BSA to avoid nonspecific stabilizing effects. To examine whether XRC1 also stimulates the AP lyase activity of hOGG1, equivalent aliquots of the same reaction mixtures used above were analyzed without addition of NaOH (Fig. 2B). Under conditions where XRC1 maximally stimulated hOGG1 DNA glycosylase activity, no AP lyase activity was detectable. These results are consistent with the reported dissociation between the DNA glycosylase and AP lyase activities of OGG1 (28).

In the course of the reaction carried out by hOGG1 on 8-oxoG-harboring substrates, a Schiff base intermediate is formed (29). This reaction intermediate can be trapped by the formation of a DNA-protein covalent complex in the presence of a reducing agent and detected by denaturing PAGE. We used this approach to further characterize the effect of XRC1 on the hOGG1 enzymatic activity. Fig. 2C shows that the addition of XRC1 to the reaction resulted in a 2-fold increase of the sodium borohydride trapping of hOGG1 with 8-oxoG:C-con-
containing duplex oligonucleotide. This stimulation is observed with XRCC1 in only a 6-fold excess with respect to hOGG1, compared with the over 200-fold excess needed to detect the DNA glycosylase stimulation. Because the Schiff base is formed after or simultaneously to the excision of the base, these results suggest that XRCC1 stimulates either the binding of hOGG1 to the damaged DNA or the glycosylase step itself rather than the enzyme turnover.

**APE1 and XRCC1 Have Additive Effects on hOGG1 Activity**

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**FIG. 2.** XRCC1 stimulates the DNA glycosylase hOGG1 activity. A, effect of XRCC1 on hOGG1 8-oxoG DNA glycosylase activity. 5'-End-labeled 34-mer duplex containing a 8-oxoG (5 nM) was incubated with (+) or without (−) hOGG1 and XRCC1 at the indicated concentrations. After 10-, 30-, and 60-min incubations at 37 °C, reactions were stopped by treatment with 0.1 N NaOH to cleave the remaining AP sites. The products of the reactions were separated by denaturing 20% PAGE. The presence of the two product bands corresponds to the different cleavage products generated by the NaOH treatment of AP sites, namely a 3'-phosphate (lower band) and an 3'-open aldehyde or a 3'-OH group (upper band). B, quantification of hOGG1 activities, filled and open symbols correspond to the DNA glycosylase and AP lyase activities, respectively, of 0.5 nM hOGG1 (squares), 0.5 nM hOGG1 plus 140 nM XRCC1 (triangles), and 0.5 nM hOGG1 plus 280 nM XRCC1 (circles). C, effect of XRCC1 on borohydride trapping of hOGG1. 5'-End-labeled 34-mer duplex containing a 8-oxoG (1 nM) was incubated with (+) or without (−) hOGG1 and XRCC1 at the indicated concentrations. After incubation at 37 °C, 50 mM sodium borohydride was added. The reactions were pursued for 5 min at 37 °C, stopped in 1× SDS sample buffer and heated for 5 min at 100 °C. The trapped complexes were separated from free substrate by SDS-PAGE 10%.

**FIG. 3.** Effects of XRCC1, APE1, and hOGG1 on an 8-oxoG-harboring substrate. A, additive effect of XRCC1 and APE1 on hOGG1 DNA glycosylase activity. Experiments were conducted as indicated in Fig. 2A. B, additive action of XRCC1 and APE1 on the reaction initiated by hOGG1. 5'-End-labeled 34-mer duplex containing a 8-oxoG (1 nM) was incubated with combinations of hOGG1 (1 nM), XRCC1 (90 nM), and/or APE1 (21 pM) during different times at 37 °C in the presence of 5 mM MgCl2 and stopped by addition of 10 mM EDTA as described under "Experimental Procedures." The percentage of incised oligonucleotide was quantified after migration on denaturing 20% PAGE. C, effect of XRCC1 and APE1 on sodium borohydride trapping of hOGG1. Experiments were conducted as indicated in Fig. 2C.

**XRCC1-hOGG1 Interactions**
performed in the absence of magnesium and terminated by the addition of NaOH, this assay reveals all the AP sites formed, reflecting the extent of base excision by hOGG1, independently of the incision activities of the proteins.

The final product of the reaction, including an active APE1, was analyzed by similar experiments performed in the presence of magnesium (Fig. 3B). In this case, no NaOH was added at the end the reaction. The presence of APE1 increased the yield of the cleaved product as a result of both the stimulation of the glycosylase activity and the processing of the AP sites left by hOGG1. The addition of XRCC1 to the reaction further stimulated the appearance of cleaved substrate. However, the addition of XRCC1 to hOGG1 alone did not increase the extent of AP lyase product of hOGG1. Parallel experiments carried out using a 3'-labeled substrate indicated that the final product in the presence of all three proteins is indeed that of the cleavage of the AP site by APE1, leaving a 5'-DRP (data not shown), consistent with the bypass of the AP lyase activity of hOGG1. Therefore, addition of both proteins, APE1 and XRCC1, further stimulated the accumulation of the end product.

Borohydride trapping experiments were used to characterize the XRCC1 stimulation mechanism of the overall reaction leading to an APE1-incised DNA (Fig. 3C). In this assay, APE1 and XRCC1 by themselves produce seemingly contradictory effects on the hOGG1 activity. As it had been shown by Hill et al. (3), the presence of APE1 inhibits the trapping of hOGG1 with the 8-oxoG-containing DNA. Our results show that XRCC1 enhanced the formation of the covalent complex (Fig. 2B). The simultaneous presence of all three proteins results in a lower yield of covalent complex (Fig. 3C).

The Same Region of XRCC1 Is Required for Its Interaction with Both hOGG1 and APE1—XRCC1 has been shown to interact with several other proteins. In particular, XRCC1 interacts and is associated in cells with polβ, PARPs 1 and 2, Lig3, APE1, and PNK. Except for the latter two, the interface domains of XRCC1 in those interactions have been determined. In the case of polβ, it is the N-terminal domain (NTD) of XRCC1 that interacts with the DNA polymerase (30). The two PARP proteins share the BRCT1 domain as a contact region (19), whereas Lig3 interacts with the BRCT2 domain (21). The XRCC1 peptide coded by the plasmids obtained in the two-hybrid screen using hOGG1 as a bait encompassed the region between residues 141 and the C terminus of the protein. This rules out the NTD as a required interacting domain with hOGG1. Consistently, the GST pull-down experiments described in Fig. 1B were performed with a fusion limited to residues 141–572 of XRCC1, showing that neither the NTD nor the BRCT2 domains are required for the interaction between hOGG1 and XRCC1 in mammalian cells. To further define the interface domain of XRCC1 with the glycosylase and with APE1, maltose-binding protein (MBP) pull-down experiments were performed (Fig. 4). For this approach, MBP either alone or fused to different XRCC1 fragments was expressed in E. coli and fixed to an amylose resin. hOGG1 or APE1 purified from E. coli was put into contact with the protein bound to the resin. After two washes in 100 mM NaCl buffer (see “Experimental Procedures”), the proteins associated to the resin were separated by SDS-PAGE, transferred to a membrane, and immunoblotted with antibodies against hOGG1 or APE1. B, regions of XRCC1 expressed fused to MBP. Interacting domains with hOGG1 and APE resistant (+) or not (+/-) to high ionic stringency are indicated.

APE1 was detected when either the NTD or the BRCT2 domains were tested (lanes 4 and 5). Interestingly, the use of higher stringency conditions suggests that the interaction of hOGG1 and APE1 with BRCT1 is weak, whereas the interactions involving fusions carrying the hinge region between the NTD and the BRCT1 domains are resistant to higher salt conditions (data not shown). These results demonstrate that APE1 and hOGG1 share the same binding domain of XRCC1 (Fig. 5A).

**DISCUSSION**

The mammalian *XRCC1* gene has been identified as necessary for the maintenance of the genetic stability and for resistance to genotoxic agents such as ionizing radiation and alkylating compounds (5). Its role in SSBR has been well established (22). Although devoid of known enzymatic activities, the XRCC1 protein participates in several of the SSBR reactions through its interactions with the enzymes carrying them out. Indeed, XRCC1 participates to the repair activities at basically all the steps of the pathway, from the recognition of the lesion by PARPs 1/2 (20) to the ligation by Lig3 (16), including the processing of DNA ends by PNK (31) and gap filling by polβ (14). Because the re-synthesis and ligation steps of SSBR can be shared by the BER pathway, XRCC1 has also been implicated in the repair of base lesions through BER. It was recently shown that, in mammalian cells, XRCC1 is involved in the initiation of the repair of AP sites by interacting with and stimulating the activity of the major human AP endonuclease (13). Here, these observations have been extended to the initiation of BER of an oxidative DNA lesion such as 8-oxoG. The physical interaction between the 8-oxoG DNA glycosylase hOGG1 and XRCC1 results in an enhancement of the base excision activity of hOGG1. The stimulation of the excision of 8-oxoG is obtained in conditions where no AP lyase activity is detected, underscoring the dissociation of the two
enzymatic activities of hOGG1. A similar situation was found
for the acceleration of the 8-oxoG repair by APE1 (3, 4). These
results, together with the requirement of the polβ dRP lyase
activity for an efficient repair of 8-oxoG in cell extracts (32),
strengthen the model by which in the cell hOGG1 would act as
a monofunctional DNA glycosylase.

The overall stimulation by XRCC1 and APE1, of the steps
leading to the incised DNA that can be used as a substrate by
polβ, is likely to be the consequence of the additive stimulation by
XRCC1 of the excision activity of hOGG1 and the sequential
stimulation of the glycosylase by APE1 and of APE1 by XRCC1.
The relatively modest direct (2- to 3-fold) stimulation of the DNA
glycosylase activity by a large excess of XRCC1 does not seem to
be the main consequence of the interaction described in this
work. Interestingly, a much lower concentration of XRCC1 is
needed to enhance the formation of the Schiff base intermediate
generated in the presence of a reducing agent. The simplest
interpretation of this result is that XRCC1 stabilizes hOGG1 on
the AP site generated by the DNA glycosylase activity. However,
the increase in the intermediate is readily reversed in the pres-
ence of APE1. This suggests that hOGG1 is maintained on the
AP site generated by the excision of the oxidized base until APE1
is able to bind to the DNA, allowing a coordinated transfer of
the DNA substrate from the DNA glycosylase product to the AP
endonuclease. Although there is no evidence for a direct hOGG1-
APE1 physical interaction, and band-shift and cross-link assays
failed to reveal complexes, including all three proteins (data not
shown), it is likely that the transfer of the DNA repair interme-
diate is mediated by XRCC1 (Fig. 5B, step 3). The skipping of a
toxic cleaved intermediate (3, 4) and the passing of the DNA from
hOGG1 to APE1 orchestrated by XRCC1 would not only make
the initial steps of 8-oxoG repair more efficient but also avoid the
damaged DNA to engage in potentially toxic rearrangement re-
actions (33).

The finding of an interaction between XRCC1 and hOGG1
implies that XRCC1 is the sole known protein participating at
every step of the BER pathway. As in the case of SSBR, this
involvement of XRCC1 seems to be the result of its capacity to
physically interact with each of the enzymes (34). The mapped
domains of XRCC1 mediating the interactions with its BER
partners corresponded to each of the globular domains, opening
the possibility that all four proteins, namely XRCC1, polβ,
PARP (1 or 2), and Lig3 could form a single complex. The
results presented here show that APE1 and hOGG1 both in-
teract with XRCC1 via the central region of the latter protein
(Fig. 5A). Together with the borohydride trapping experiments
showing that APE1 displaces hOGG1 from the AP site, these
data suggest that there is a sequential occupation of the hinge
of pol
in the different domains of XRCC1 yield distinct cellular phe-
formed and as such participate in all the pathway. Mutations
interaction of XRCC1 with Lig3, this complex could be pre-
be formed by recruitment of the ligase after the initiation steps
XRCC1-Lig3 complex, mediated by the BRCT2 domain, could
DNA termini could be sensed by the XRCC1-APE1 complex.
requirement of PARP in situations in which the DNA termini
XRCC1. Recent data has associated a polymorphism at amino
region, suggesting the possibility of an altered affinity of the
tumoral XRCC1 for its BER partners. The interaction of BRCT1 with the first two enzymes of BER of 8-oxoG could provide a way to exclude the PARP-1 from participating at these steps and therefore to channel the reac-
tion through the short patch pathway (15) (Fig. 5B, step 4).
Indeed, experiments performed on cell extracts (37, 38) suggest
the repair of oxidized purines, AP sites, and single strand
breaks.

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