DNA Damage Levels and Biochemical Repair Capacities Associated with XRCC1 Deficiency

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Received June 15, 2005; Revised Manuscript Received August 22, 2005

ABSTRACT: Base excision repair (BER) is the major corrective pathway for most spontaneous, oxidative, and alkylation DNA base and sugar damage. X-ray cross-complementing 1 (XRCC1) has been suggested to function at nearly every step of this repair process, primarily through direct protein−protein interactions. Using whole cell extract (WCE) repair assays and DNA damage measurement techniques, we examined systematically the quantitative contribution of XRCC1 to specific biochemical steps of BER and single-strand break repair (SSBR). Our studies reveal that XRCC1-deficient Chinese hamster ovary WCEs exhibit normal base excision activity for 8-oxoguanine (8-OH-dG), 5-hydroxycytosine, ethenoadenine, and uracil lesions. Moreover, XRCC1 mutant EM9 cells possess steady-state levels of endogenous 8-OH-dG base damage similar to those of their wild-type counterparts. Abasic site incision activity was found to be normal in XRCC1-deficient cell extracts, as were the levels of abasic sites in isolated chromosomal DNA from mutant cells. While one- and five-nucleotide gap filling was not affected by XRCC1 status, a significant ∼2−4-fold reduction in nick ligation activity was observed in EM9 WCEs. Our results herein suggest that the primary biochemical defect associated with XRCC1 deficiency is in the ligation step of BER/SSBR, and that XRCC1 plays no significant role in endogenous base damage and abasic site repair, or in promoting the polymerase gap-filling step.

More than two decades ago, researchers generated and isolated Chinese hamster Ovary (CHO)[1] mutant cells that exhibit hypersensitivity to specific classes of physical and/or chemical DNA-damaging agents (see, for instance, refs *1* and *2*). The mutant strain EM9, derived from the wild-type CHO AA8 line, was distinguished by its >10-fold hypersensitivity to the alkylating agent ethyl methanesulfonate (EMS) (*3*). In addition to EMS, EM9 cells exhibit increased sensitivity to other alkylating agents [e.g., methyl methanesulfonate (MMS), ethyl nitrosourea, and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine], as well as to X-rays. Moreover, following treatment with either EMS or ionizing radiation, the rate of rejoining of DNA single-strand breaks (SSBs) was found to be reduced severalfold. EM9 cells also display a very high level of sister chromatid exchange (SCE) when grown in the presence of bromodeoxyuridine or chlorodeoxyuridine (CldUrd) (*4*). While the precise reason for the elevated level of chromosomal aberrations is unclear, it has been suggested that halogen-substituted pyrimidine molecules give rise to SSBs (via processes of DNA repair), which when unrepaired promote lethal and/or recombinogenic outcomes (*5*).

Many of the mutant CHO cell lines have proven to be valuable tools for the molecular cloning of human DNA repair genes via functional complementation (reviewed in refs *6* and *7*). In particular, the human X-ray cross-complementing 1 (XRCC1) gene was isolated as a clone that corrects the CldUrd sensitivity of EM9 cells (*8* and references therein). This gene also complemented the EMS sensitivity, SSB repair (SSBR) deficiency, and elevated SCEs associated with the EM9 mutant strain. While the open reading frame of the XRCC1 clone revealed no obvious sequence homology with any protein in the databases at the time, the EM9 cellular phenotypes clearly indicated that XRCC1 functions prominently in the repair of DNA SSBs. It is important to note that Western blot analysis has indicated that EM9 cells possess no detectable XRCC1 protein (*9*).

Subsequent studies revealed that XRCC1 plays a major role in stabilizing the strand break repair protein, DNA ligase 3 (Lig3), in vivo (*10*−*12*). It was later determined that XRCC1 and ligase 3α (Lig3α)—the mitotic, nuclear form of the enzyme—interact directly through a C-terminal BRCT domain found in each protein (refs *13* and *14* and references therein). Since the discovery that XRCC1 is a key contributor

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[1] Abbreviations: BER, base excision repair; XRCC1, X-ray cross-complementing 1; WCE, whole cell extract; SSBR, single-strand break repair; 8-OH-dG, 8-oxoguanine; CHO, Chinese hamster ovary; EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; SSB, single-strand break; SCE, sister chromatid exchange; CldUrd, chlorodeoxyuridine; Lig3, ligase 3; AP, apurinic/apyrimidinic; EM9-V, EM9 vector-complemented; EM9-WT, EM9 wild-type human XRCC1-complemented; ARP, aldehyde reactive probe; OGG1, 8-OH-dG DNA glycosylase; NEIL1, endonuclease VIII-like 1; TCR, transcription-coupled repair; 5-OH-dC, 5-hydroxycytosine; etheno-dA, ethenoadenine; dU, uracil; NTH1, endonuclease III homologue 1; APG, alkyl purine glycosylase; UNG, uracil glycosylase; APE1, AP endonuclease 1; POL*β*, polymerase *β*; PARP-1, poly(ADP-ribose) polymerase 1; PNKP, polynucleotide kinase/phosphatase; dRp, 5′-deoxyribose 5-phosphate.

10.1021/bi051161o CCC: $30.25
Published on Web 10/08/2005

FIGURE 1: Deoxyribose oligonucleotide substrates. The name and nucleotide sequence are shown. All oligonucleotides are written in the 5′ to 3′ orientation, except 34G, which is written upside-down, with the 5′ end to the right (depicted complementary to the 34 damage-containing substrates). The underlined X denotes the position of the modified base, where OxG is 8-oxoguanine, OHC is 5-hydroxycytosine, EthA is ethenoadenine, and U is uracil. F is the abasic site analogue, tetrahydrofuran. HO is the 3′-hydroxyl end, and P is 5′-phosphate. The one-nucleotide gap and the nick substrate oligonucleotides are marked.

to Lig3 protein stability and function, it has been demonstrated that XRCC1 physically and/or functionally interacts with other proteins of the base excision repair (BER) and SSBR pathways (*15*, *16*). To ascertain which of these interactions (detailed later) are quantitatively critical to the phenotypes of XRCC1-deficient EM9 cells, we measured systematically for the first time the enzymatic efficiency of specific steps of BER/SSBR using defined oligonucleotide substrates and whole cell extract (WCE) assays. Moreover, we employed previously established techniques to measure steady-state levels of certain BER DNA damage substrates. The investigations indicate that nick ligation is the major biochemical deficiency associated with XRCC1 absence, and that XRCC1 is not a major contributor to endogenous base damage or apurinic/apyrimidinic (AP) site repair in vivo, or in facilitating the gap-filling polymerization step of BER/SSBR.

MATERIALS AND METHODS

Oligonucleotides and Cell Lines. Undamaged and AP site-containing oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Base-modified oligonucleotides were obtained from Synthegen (Houston, TX). Wild-type parental AA8 and XRCC1-defective EM9 CHO cell lines were kindly provided by L. Thompson (Lawrence Livermore National Laboratory, Livermore, CA). The EM9 vector-complemented (EM9-V) and the EM9 wild-type human XRCC1-complemented (EM9-WT) lines were created as previously described (*17*). All cells were cultured at 5% CO_2 in DMEM (Gibco, Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, 1% glutamine, and 1% penicillin−streptomycin.

Preparation of WCE. Upon attaining ~80% confluence, cells were trypsinized, washed with phosphate-buffered saline, and then harvested by centrifugation at 2000 rpm for 2 min. Cell pellets were resuspended in 1 mL of lysis buffer [50 mM Tris (pH 7.4), 1 mM EDTA, 1 mM DTT, 10% glycerol, and 0.5 mM PMSF] followed by sonication. Upon clarification by centrifugation, supernatants, defined here as WCE, were retained and stored at −80 °C in small aliquots. Protein concentrations were measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA), and confirmed by standard SDS−polyacrylamide gel electrophoresis and Coomassie blue staining.

DNA Repair Assays. Substrates were 5′-^{32}P-end-labeled using T4 polynucleotide kinase essentially as described previously (*18*). In the DNA glycosylase and AP site incision assays, the damage-containing strand (Figure 1) was labeled. In the gap-filling and nick ligation experiments, oligonucleotide 15P (Figure 1) was labeled. Following heat inactivation of the kinase, unlabeled complementary DNAs were mixed at an equimolar concentration in the following arrangements: 34U*:34G, 34OxG*:34G, 34EthA*:34G, 34OHC*:34G, 34F*:34G, 15P*:pG18:34G, and 15P*:pC19:34G (where the asterisk denotes the labeled oligonucleotide). For each of the WCE repair assays (see below), the appropriate duplex substrate at 100 nM was used unless otherwise instructed. Assays were typically performed at varying protein concentrations first, and then at a set protein concentration for varying lengths of time.

For base excision activity, reactions were performed at 37 °C for 2 h in 50 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.5 mM EDTA, and 5% glycerol. AP site incision, gap-filling, and nick ligation assays were executed at the indicated times and WCE concentrations using the same reaction buffer, except EDTA was replaced with 5 mM $MgCl_2$. Reactions were carried out at 37 °C, unless otherwise specified. In the one-nucleotide (nt) gap-filling assays, dCTP was included at a final concentration of 0.5 mM. ATP was added to a final concentration of 1 mM in the nick ligation assays. In all cases, at the completion of the reaction, an equal volume of stop dye was added (95% formamide, 20 mM EDTA, bromophenol blue, and xylene cyanole), samples were heated to 95 °C for 5 min, and an aliquot was separated on a denaturing polyacrylamide gel essentially as described previously (*18*). Quantitation of the substrate and product (defined within) was carried out using standard Molecular Dynamics PhosphorImager analysis. Percent conversion = product/(product plus substrate), followed by subtraction of the background in the substrate alone lane.

AP Site Measurements. The number of AP sites was determined using a commercially available DNA damage quantification kit (Dojindo Molecular Technology, Gaithersburg, MD). In brief, a Get pure DNA kit (Dojindo

Molecular Technology) was used to extract and purify genomic DNA from the indicated CHO cell line according to the manufacturer's specifications. Purified DNA (1 μg) was then incubated for 1 h at 37 °C with an aldehyde reactive probe (ARP) reagent (*N*′-aminooxymethyl-carbonyl-hydrazino-D-biotin), which reacts specifically with the ring-open aldehyde form of an AP site (*19*), as described in the damage quantification kit protocol (Dojindo Molecular Technology). After the overnight fixation step, biotin-tagged AP sites were quantified via colorimetric detection with peroxidase-conjugated streptavidin using a Bio-Rad Benchmark Plus microplate spectrophotometer and Microplate Manager version 5.2 (Bio-Rad Laboratories).

8-Oxoguanine (8-OH-dG) Measurements. For base damage measurements, genomic DNA was extracted using the salting out procedure described in ref *20*. Three independently cultured batches of each cell line (AA8, EM9, EM9-V, and EM9-WT) were used. Briefly, cell pellets were resuspended in lysis buffer [0.5 M Tris-HCl (pH 8), 20 mM EDTA, 10 mM NaCl, 1% SDS, and 0.5 mg/mL proteinase K] and incubated overnight at 37 °C. One-fourth volume of saturated NaCl was then added, and samples were centrifuged at 500*g* for 45 min to remove protein precipitants. DNA in the supernatant was precipitated with ethanol, and next washed with 70% ethanol. After treatment with 100 μg/mL RNase for 3 h at 37 °C, one-fifth volume of 11 M sodium acetate was added to the DNA solution. Final DNA pellets were resuspended in water prior to quantification by UV spectroscopy.

The concentration of DNA in aqueous samples was determined by UV spectroscopy (1 absorbance unit = 0.05 mg of DNA/mL). 8-OH-dG-15N5 (Cambridge Isotope Laboratories, Cambridge, MA) was used as an internal standard for quantification of 8-OH-dG in hydrolyzed DNA samples by LC−MS. For enzymatic hydrolysis, 50 μg aliquots of DNA were dissolved in 50 μL of a 10 mM Tris-HCl solution (pH 7.5) supplemented with 1.25 μL of 1 M sodium acetate containing 45 mM $ZnCl_2$ (final pH of 6.0). Aliquots of *Penicillium citrinum* nuclease P1 (3 units; Sigma Chemical Co., St. Louis, MO), snake venom phosphodiesterase (0.002 unit; Worthington Biochemical Corp., Lakewood, NJ), alkaline phosphatase (16 units; Roche Diagnostics Corp., Indianapolis, IN), and 8-OH-dG-15N5 (5 pmol) as an internal standard were added, and the samples were incubated at 37 °C for 24 h (*21*). The samples were then centrifuged at 13200*g* for 4 min at room temperature, and the supernatant was filtered using ultrafiltration membranes (Biomax5 from Millipore, Bedford, MA) with a molecular mass cutoff of 5 kDa by centrifugation at 8000*g* for 30 min at 4 °C.

LC−MS analysis was performed using a liquid chromatograph-mass selective detector (1100B Series, Agilent Technologies, Rockville, MD) with the atmospheric pressure ionization-electrospray (API-ES) process in the positive ionization mode. Experimental conditions were as described elsewhere (*22*). Aliquots of filtered enzymatic hydrolysates of DNA were injected on the column without any further treatment. For identification and quantification of 8-OH-dG, selected-ion monitoring was used to monitor the characteristic ions of 8-OH-dG (*m*/*z* 168 and 306) and 8-OH-dG-15N5 (*m*/*z* 173 and 311) during LC−MS analyses of DNA hydrolysates (*23*). For the LC−MS analyses, acetonitrile (HPLC grade) was from Burdick and Jackson (Muskegon, MI) and HPLC-grade water was from J. T. Baker (Phillipsburg, NJ).

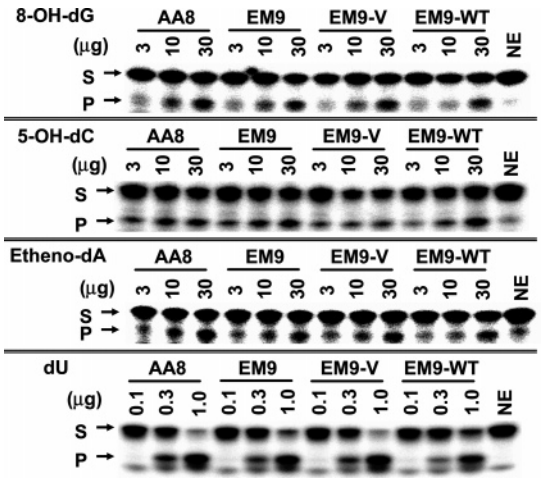

FIGURE 2: DNA base excision activities. A representative gel is shown for repair activity toward the four indicated base lesions: 8-OH-dG, 5-OH-dC, etheno-dA, and dU. WCEs examined are denoted AA8, EM9, EM9-V, and EM9-WT. NE represents the no enzyme, DNA alone control. Extracts were incubated at increasing amounts (indicated in micrograms per 20 μL) for 2 h at 37 °C. Initial intact substrate (S) and cleaved DNA product (P) are indicated. Quantification of multiple experimental runs is reported in Table 1. We note that in several cases, most notably the dU assays, additional contaminant DNA bands are seen, but are also present in the NE control lanes (where appropriate, these bands were subtracted out as background).

RESULTS

Repair of DNA Base Damage

BER involves five primary steps: (1) base excision, (2) AP site incision, (3) termini "cleanup", (4) gap filling, and (5) nick ligation (*24*). Recent work has demonstrated an in vitro physical and functional (i.e., stimulatory) interaction of XRCC1 with the 8-OH-dG DNA repair glycosylase OGG1 (*25*). Additionally, XRCC1 has been reported to interact weakly under certain conditions with the human endonuclease VIII-like 1 (NEIL1) protein (*26*), a glycosylase suggested to operate preferentially in transcription-coupled repair (TCR) of oxidative base lesions (*27*). However, there currently is no biological evidence for a role of XRCC1 in the repair of DNA base damage.

To assess this possibility, we prepared WCEs from wild-type CHO AA8 cells and its XRCC1-deficient EM9 counterpart, as well as from EM9 cell lines stably complemented with either the pcDNA3 vector (EM9-V) or a recombinant pcDNA3 plasmid expressing wild-type human XRCC1 (EM9-WT). Using oligonucleotide duplexes containing a single, site-specific base damage (Figure 1), we then assessed the ability of mutant and wild-type cell extracts to excise the lesion and cleave at the resulting AP site. The four base modifications, 8-OH-dG, 5-hydroxycytosine (5-OH-dC), ethenoadenine (etheno-dA), and uracil (dU), were chosen because they represent the major endogenous substrates for the DNA glycosylases OGG1, endonuclease III homologue 1 (NTH1), alkyl purine glycosylase (APG), and uracil glycosylase (UNG), respectively (*28*−*30*). As shown in Figure 2 (and quantitatively reported in Table 1), all cell

Table 1: DNA Base Damage Repair Efficiency of AA8, EM9, EM9-V, and EM9-WT WCEs[a]

cell extract	8-OH-dG	5-OH-dC	etheno-dA	dU
AA8	6.1 ± 1.2 (1)	3.5 ± 0.5 (1)	7.2 ± 1.6 (1)	334 ± 111 (1)
EM9	7.0 ± 1.4 (1.2)	3.7 ± 0.5 (1)	7.1 ± 1.9 (1)	311 ± 81 (0.9)
EM9-V	7.1 ± 1.7 (1.2)	3.8 ± 0.6 (1.1)	9.6 ± 3.2 (1.3)	359 ± 99 (1.1)
EM9-WT	6.3 ± 1.3 (1.1)	3.6 ± 0.5 (1)	5.7 ± 2.0 (0.8)	270 ± 60 (0.8)

[a] Specific activities (i.e., femtomoles of base damage excised/incised per hour per microgram of cell extract) and efficiency relative to AA8 (in parentheses) are reported. Activity numbers represent the average and standard deviation of at least three independent experimental measurements.

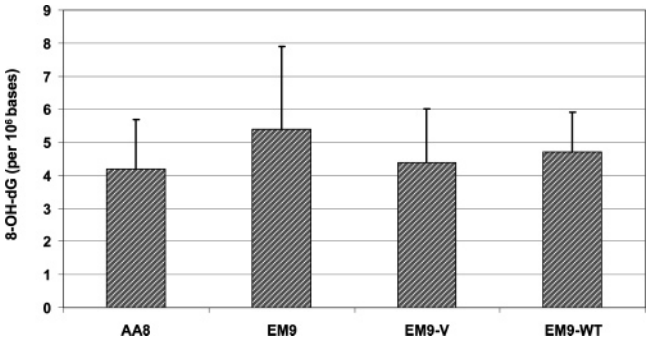

FIGURE 3: Endogenous levels of 8-OH-dG. Damage measurements were performed as described in Materials and Methods. Shown are the average and standard deviation of values obtained from three to five independent genomic DNA isolates. Numbers represent 8-OH-dG residues per 10^6 bases.

extracts exhibited statistically similar excision activity for each of the base lesions.

In addition to the WCE assays, 8-OH-dG base damage content was quantified in genomic DNA isolated from AA8, EM9, EM9-V, and EM9-WT cells. The hypothesis was that if XRCC1 functions prominently in facilitating base damage removal in vivo, then the level of such lesions would be elevated in an XRCC1-deficient background. Using LC−MS (see Materials and Methods), we specifically measured the steady-state level of 8-OH-dG, a major oxidative DNA damage product that is a prominent substrate for both OGG1 and NEIL1 (*31*, *32*). This analysis revealed that both wild-type and mutant cells maintain similar endogenous levels of this lesion in their chromosomal DNA (Figure 3).

Repair of AP Sites

Prior work had reported a direct interaction between XRCC1 and the major mammalian AP endonuclease, APE1, and had found that XRCC1-deficient cell extracts exhibit a 1.2−1.7-fold reduced AP site incision activity (*33*). However, earlier work had found no difference in total AP endonuclease activity between EM9 and wild-type AA8 cell lines (*34*). To examine this repair function further, and to scrutinize the potential biological significance of this association, we determined the AP site incision efficiency of mutant and wild-type XRCC1 WCEs, and measured the steady-state levels of AP sites in EM9-V and EM9-WT genomic DNA. As shown in Figure 4 (panels A and B), we found no significant difference in the ability of vector- or wild-type-complemented cell extracts to convert an AP site-containing duplex substrate to the site-specifically, incised product. More importantly, as determined via an ARP-based colorimetric assay, both EM9-V and EM9-WT cell lines were found to contain a statistically similar steady-state level of AP sites in their chromosomal DNA (Figure 4C); identical results were obtained with AA8 and EM9 (data not shown).

Repair of SSBs

Gap Filling. While the interaction between XRCC1 and DNA polymerase β (POLβ) has been characterized in great detail (*35*−*37* and references therein), the specific biochemical impact of this interaction remains unclear. Since POLβ is the major gap-filling DNA polymerase of mammals (*38*), we sought to determine specifically whether short-patch nucleotide replacement, a central step of BER, is defective in XRCC1-deficient cell extracts. Using the one-nucleotide gap substrate described in Figure 1 (i.e., 15P, pG18, and 34G), or a five-nucleotide gap oligonucleotide substrate (not shown), we evaluated gap filling by quantifying the percent of 5′-labeled primer extended (ligation was prevented by omission of ATP). With the one-nucleotide gap substrate, we found that XRCC1-deficient WCEs displayed a similar polymerization capacity (i.e., one-nucleotide extended product) as wild-type extracts (Figure 5); comparable extension results were also obtained with the five-nucleotide gap substrate (data not shown). Moreover, positioning an abasic residue (i.e., the AP site analogue F) in place of the phosphate at the 5′-terminus within the one-nucleotide gap did not noticeably affect the comparative polymerization outcomes of wild-type and mutant cell extracts (data not shown).

Nick Ligation. Previous work has demonstrated that XRCC1 functions to stabilize DNA Lig3α protein in vivo (*12*). More recent work indirectly concluded that XRCC1-deficient EM-C11 extracts exhibit a defect in the ligation step of AP site-initiated repair, with short-patch BER being specifically affected (and not the long-patch PCNA-dependent BER pathway) (*39*). We sought here to extend this previous analysis, which could not distinguish between the different steps of BER unambiguously, and examine explicitly the nick ligation capacity of wild-type and XRCC1-deficient cell extracts using a defined DNA substrate (Figure 1). Our studies reveal that EM9 and EM9-V cell extracts display a 1.5−2.7-fold decrease in nick ligation activity specifically in comparison to AA8 cell extracts (Figure 6).

While the initial studies were performed at room temperature (panels A and B), subsequent analysis at 37 °C produced a similar result, with the following nick ligation rates (femtomoles of nicked substrate converted to ligated product per minute): 17 ± 1.7 for AA8, 4.3 ± 0.1 for EM9, 9.0 ± 0.3 for EM9-V, and 16 ± 2.1 for EM9-WT (Figure 6C). The reason(s) for the difference between EM9 and EM9-V is not known, but may in part stem from slight inaccuracies in protein concentration measurements or altered protein expression that may have arisen during the geneticin-selection step with EM9-V. Nonetheless, the observed ligation defect was corrected by complementation with the wild-type XRCC1 cDNA (EM9-WT), indicating that this deficiency is a direct result of the absence of XRCC1 in the EM9 cells (*9*). Finally, consistent with XRCC1 functioning

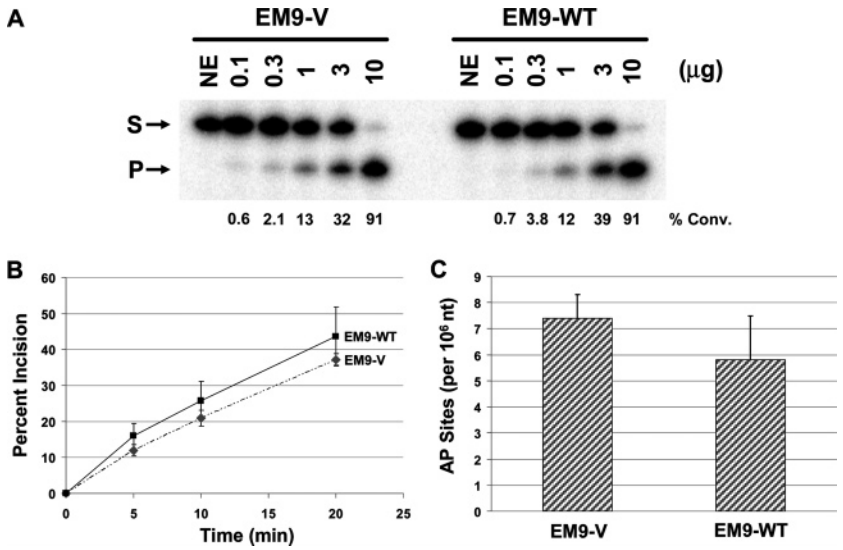

FIGURE 4: AP site incision efficiency. (A) WCE concentration-dependent incision. Shown is a representative gel of reactions performed at the indicated concentrations of EM9-V or EM9-WT WCE (micrograms per 10 μL). NE represents the no enzyme control. Percentage of intact substrate (S) converted to incised product (P) is denoted under each lane. (B) Incision kinetics. The indicated extract (1 μg/10 μL) was incubated for the specified time, and the percent of substrate converted to product was determined. Plotted are the average and standard deviation of at least three data points. (C) AP site levels in isolated chromosomal DNA. Genomic DNA was purified and analyzed as described in Materials and Methods. The number of AP sites measured per 10^6 nucleotides is shown for EM9-V and EM9-WT. Values represent the average and standard deviation of five independent measurements.

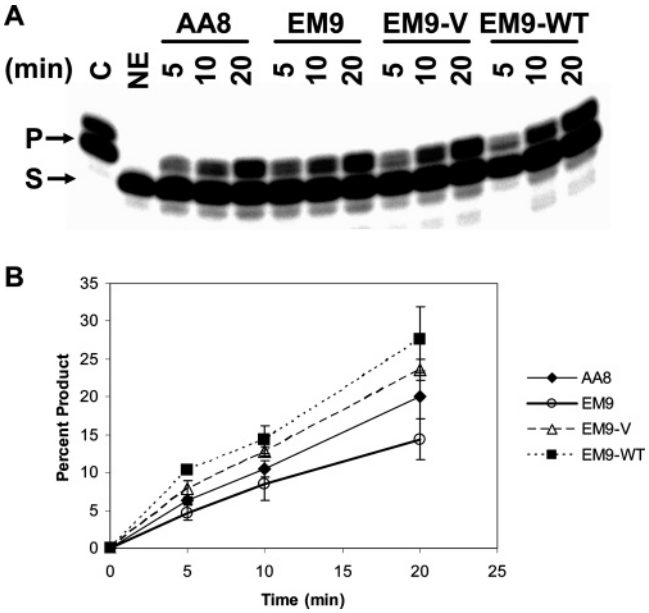

FIGURE 5: Single-nucleotide gap filling. (A) Representative gel of a typical time course reaction set. The indicated WCE (AA8, EM9, EM9-V, or EM9-WT at 0.5 μg/10 μL) was incubated with a one-nucleotide gap substrate for the specified time (minutes). Initial substrate (S) and the one-nucleotide polymerase extended product (P) are denoted. C denotes the purified recombinant DNA POLβ positive control. NE represents no enzyme control. (B) Quantitation of multiple time course reactions. Shown are the average and standard deviation of three independent data points for the indicated WCEs. The percent extended product formed is plotted against time.

most prominently in short-patch BER and not interacting with flap endonuclease 1 (*39*, *40*), the primary 5′-flap excision enzyme (*41*), XRCC1-deficient cell extracts exhibited normal 26-nucleotide flap processing activity (data not shown).

DISCUSSION

Studies have demonstrated interactions of XRCC1 with the following BER/SSBR proteins: OGG1, APE1, POLβ, poly(ADP-ribose) polymerase 1 (PARP-1), polynucleotide kinase/phosphatase (PNKP), and DNA Lig3α (*10*, *25*, *33*, *35*, *36*, *42*−*44*). In this capacity, XRCC1 has been suggested to operate as a major nonenzymatic, assembly/scaffold protein, facilitating each step of the BER/SSBR process. However, several of the reported interactions have only been demonstrated by in vitro biochemical approaches. To obtain a more detailed understanding of the biological contributions of XRCC1, cell extracts were prepared from both wild-type and mutant CHO cell lines and assayed for repair competence at the major steps of BER/SSBR: that is, base excision, AP site incision, gap filling, and nick ligation. In addition, total genomic DNA was isolated from XRCC1-deficient and -proficient cells and evaluated for in vivo steady-state base damage and AP site content.

Our finding that XRCC1-deficient cell extracts and cells (i) exhibit normal base excision activity for 8-OH-dG, 5-OH-dC, etheno-dA, and dU (Figure 2 and Table 1) and (ii) possess normal endogenous levels of 8-OH-dG (Figure 3) contradicts the idea of XRCC1 being a major contributor to global-genome base damage repair. However, a more specialized role for XRCC1 in facilitating base lesion removal, for instance, during TCR or upon DNA damage induction, cannot be excluded. Our extract results for AP site incision (Figure 4A,B) and measurements of AP site steady-state levels in wild-type and mutant XRCC1 cell lines (Figure 4C) also rule out a major role for XRCC1 in normal AP site processing in vivo. This conclusion is consistent with two groups (including ours) independently being unable to reproduce the stable interaction reported for Ape1 and XRCC1 (*40*, *45*). Thus, the current data contend that the most prominent function(s) of XRCC1 takes place after AP site incision in BER, or at direct (i.e., agent-induced) SSBs (*44*, *46*, *47*). This nonenzymatic function has been suggested to be orchestrated in part through a physical interaction with PARP-1, a major strand break recognition/binding protein, and poly(ADP-ribosyl)ation (*42*, *43*, *48*). In fact, a role for

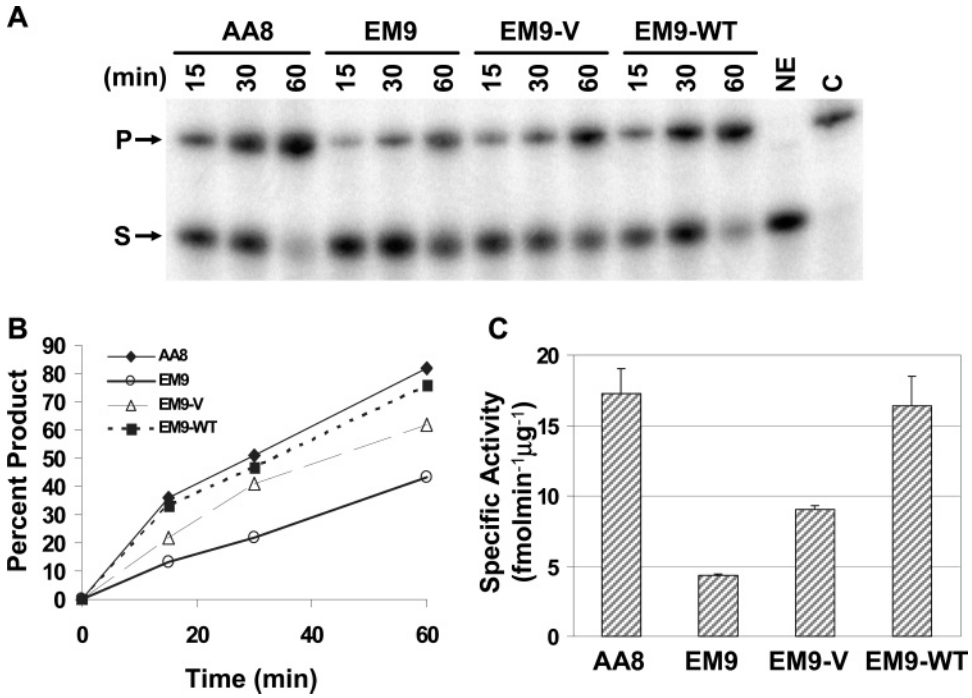

FIGURE 6: Nick ligation activity. (A) Representative gel of a typical time course reaction set. The indicated WCE (AA8, EM9, EM9-V, or EM9-WT at 10 μg/10 μL) was incubated with the nick substrate for the specified time (minutes) at room temperature. Initial substrate (S) and ligated product (P) are denoted. C represents the recombinant T4 polynucleotide kinase control. NE represents the no enzyme control. (B) Plot of the average of two independent time point reactions performed at room temperature. The average percent of full-length product is plotted against time. WCE analyzed is denoted. (C) Nick ligation efficiency at 37 °C. Reactions were performed as described above, except at 1 μg/10 μL WCE for 10, 20, and 30 min. Specific activities (femtomoles of substrate converted to product per minute per microgram of extract) represent the average and standard deviation of three independent data points.

XRCC1 in direct, specific DNA binding in vivo remains in question (*17*, *46*, *49*, *50*).

Several groups have demonstrated that XRCC1 and POLβ physically interact (*35*, *36*). Indeed, Mullen and colleagues have mapped in detail the protein−protein interface using NMR spectroscopy and biochemical/molecular biology techniques (*37*, *51*, *52*). Recently, this interaction was found to be important biologically, as site-specific mutations in XRCC1 which disrupt its physical association with POLβ are unable to fully complement the hydrogen peroxide or MMS sensitivities of XRCC1 mutant CHO cell lines (*17*, *53*). Nevertheless, the precise biochemical role of this interaction remains unclear, although (as seen with Lig3α; see below) it is not to stabilize the POLβ protein in vivo (*17*, *54*). We report here that XRCC1 does not regulate gap-filling (one nucleotide or five nucleotides) efficiency (Figure 5 and unpublished observations), a major function of DNA POLβ (*38*).

Attempts were also made to evaluate the role of XRCC1 in 5′-deoxyribose 5-phosphate (dRp) excision, another prominent task of DNA POLβ in BER (*55*), using wild-type and mutant WCE assays. While firm conclusions cannot be drawn because of the overall inefficient removal of these termini (*56*, *57*), qualitative judgment of the results suggests that XRCC1 does not influence dRp excision rates significantly (unpublished observations). Consistent with the assertions given above, preliminary reconstitution experiments with purified recombinant proteins and defined DNA substrates have not revealed a major effect of XRCC1 on the gap-filling and dRp lyase activities of POLβ in vitro (J. Fan and D. M. Wilson, III, unpublished observations). Thus, current evidence suggests that the XRCC1−POLβ interaction functions primarily during BER/SSBR to promote efficient assembly of a repair complex containing DNA Lig3α (*50*, *53*).

In XRCC1-deficient CHO cell lines (EM9 and EM-C11), Lig3 protein levels have been consistently found to be 4−6-fold lower compared to their wild-type counterparts (see, for instance, ref *12*). However, assays with extracts from XRCC1-deficient cells indicate an only ~50% decline in total DNA ligase activity (ref *11* and Figure 6). Since DNA Lig1 protein levels were found to be unchanged in XRCC1 mutant extracts (*12*), this apparent discord probably reflects the ability of DNA Lig1 to operate as a partial backup enzyme in sealing SSB intermediates (*58*), at least in the in vitro assays. Nevertheless, the incomplete compensation by DNA Lig1, and thus the reduced overall ligation activity displayed by XRCC1 mutant cells, is likely a key factor in the poor SSBR, elevated SCEs, and increased DNA-damaging agent hypersensitivities exhibited by XRCC1-deficient cell lines.

In summary, while many physical interactions have been reported for XRCC1 (*16*), data in total favor the idea that the primary biochemical deficiency associated with XRCC1 absence is defective DNA ligation, in terms of both ligase protein levels and repair complex assembly, particularly in the context of mammalian BER/SSBR substrate processing. Other studies argue that XRCC1 also plays a prominent role in facilitating the repair of 3′-phosphate-containing oxidative strand breaks or 3′-topoisomerase I-mediated DNA lesions, which block repair synthesis and ligation (*44*, *59*−*61*). Such results are consistent with the many cell biology studies that indicate a general defect in the kinetics of SSBR in XRCC1 mutant cells (see, for instance, refs *8*, *44*, and *62*−*64*).

Nonetheless, it is important to emphasize that analyses also suggest unique, specialized roles for XRCC1 outside of classical SSBR. In particular, the *Drosophila melanogaster* XRCC1 gene product apparently lacks the C-terminal BRCT domain found in the mammalian proteins that stabilizes and promotes the activity of DNA Lig3α (*65*). This observation strongly suggests additional roles for eukaryotic XRCC1 proteins outside of simply mediating BER/SSBR nick ligation. Specifically, studies have identified specialized functions for XRCC1 during the S phase or in direct connection with DNA replication (*14*, *40*, *65*), as well as in repairing DNA double-strand breaks (*62*, *66*). These biochemical functions undoubtedly contribute to the pronounced cellular defects displayed by XRCC1 mutant cells, such as the elevated SCEs (*15*, *67*). Moreover, recent evidence has revealed an interaction of XRCC1 with the histidine triad superfamily protein aprataxin, a protein defective in the neurodegenerative disease ataxia-oculomotor apraxia 1. Investigations have suggested that this interaction contributes to a global response to genotoxic stress (*68*−*71*). Future studies will need to more explicitly define the molecular details of these cell cycle-specific and/or pathway-targeted repair roles of human XRCC1.

Around the time of submission, a report appeared indicating that XRCC1 interacts with DNA glycosylases APG, NTH1, and NEIL2 (*72*). While we emphasize that the studies herein do not exclude the possibility that XRCC1 coexists in a multiprotein complex with certain DNA glycosylases or Ape1 in vivo (*33*, *72*, *73*), our data argue against a significant role for XRCC1 in facilitating or promoting the early steps of BER of endogenous DNA damage. Moreover, we note that the in vitro studies suggesting a function for XRCC1 in stimulating base damage removal (i.e., glycosylase activity) or AP site incision have often required an excess of XRCC1 protein over the repair enzyme in question (in some cases, >1000-fold molar excess). At this time, a more thorough analysis of the base damage content of XRCC1-deficient cells and the biological role of XRCC1 in base lesion removal seems warranted.

ACKNOWLEDGMENT

We thank Dr. Miral Dizdaroglu (National Institute of Standards and Technology, Gaithersburg, MD) for assistance with 8-OH-dG analysis. We thank Drs. Jinshui Fan, Mohammad Hedayati, and Nadja Souza-Pinto (NIA) for constructive input on the manuscript.

REFERENCES

1. Thompson, L. H., Rubin, J. S., Cleaver, J. E., Whitmore, G. F., and Brookman, K. (1980) A screening method for isolating DNA repair-deficient mutants of CHO cells, *Somatic Cell Genet. 6*, 391−405.
2. Busch, D. B., Cleaver, J. E., and Glaser, D. A. (1980) Large-scale isolation of UV-sensitive clones of CHO cells, *Somatic Cell Genet. 6*, 407−418.
3. Thompson, L. H., Brookman, K. W., Dillehay, L. E., Carrano, A. V., Mazrimas, J. A., Mooney, C. L., and Minkler, J. L. (1982) A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister-chromatid exchange, *Mutat. Res. 95*, 427−440.
4. Dillehay, L. E., Thompson, L. H., Minkler, J. L., and Carrano, A. V. (1983) The relationship between sister-chromatid exchange and perturbations in DNA replication in mutant EM9 and normal CHO cells, *Mutat. Res. 109*, 283−296.
5. Dillehay, L. E., Thompson, L. H., and Carrano, A. V. (1984) DNA-strand breaks associated with halogenated pyrimidine incorporation, *Mutat. Res. 131*, 129−136.
6. Thompson, L. H. (1998) Chinese hamster cells meet DNA repair: An entirely acceptable affair, *BioEssays 20*, 589−597.
7. Thacker, J., and Zdzienicka, M. Z. (2003) The mammalian XRCC genes: Their roles in DNA repair and genetic stability, *DNA Repair 2*, 655−672.
8. Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A., and Carrano, A. V. (1990) Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange, *Mol. Cell. Biol. 10*, 6160−6171.
9. Shen, M. R., Zdzienicka, M. Z., Mohrenweiser, H., Thompson, L. H., and Thelen, M. P. (1998) Mutations in hamster single-strand break repair gene XRCC1 causing defective DNA repair, *Nucleic Acids Res. 26*, 1032−1037.
10. Caldecott, K. W., McKeown, C. K., Tucker, J. D., Ljungquist, S., and Thompson, L. H. (1994) An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III, *Mol. Cell. Biol. 14*, 68−76.
11. Ljungquist, S., Kenne, K., Olsson, L., and Sandstrom, M. (1994) Altered DNA ligase III activity in the CHO EM9 mutant, *Mutat. Res. 314*, 177−186.
12. Caldecott, K. W., Tucker, J. D., Stanker, L. H., and Thompson, L. H. (1995) Characterization of the XRCC1-DNA ligase III complex in vitro and its absence from mutant hamster cells, *Nucleic Acids Res. 23*, 4836−4843.
13. Nash, R. A., Caldecott, K. W., Barnes, D. E., and Lindahl, T. (1997) XRCC1 protein interacts with one of two distinct forms of DNA ligase III, *Biochemistry 36*, 5207−5211.
14. Moore, D. J., Taylor, R. M., Clements, P., and Caldecott, K. W. (2000) Mutation of a BRCT domain selectively disrupts DNA single-strand break repair in noncycling Chinese hamster ovary cells, *Proc. Natl. Acad. Sci. U.S.A. 97*, 13649−13654.
15. Caldecott, K. W. (2003) XRCC1 and DNA strand break repair, *DNA Repair 2*, 955−969.
16. Fan, J., and Wilson, D. M., III (2005) Protein−protein interactions and posttranslational modifications in mammalian base excision repair, *Free Radical Biol. Med. 38*, 1121−1138.
17. Wong, H. K., and Wilson, D. M., III (2005) XRCC1 and DNA polymerase β interaction contributes to cellular alkylating-agent resistance and single-strand break repair, *J. Cell. Biochem. 95*, 794−804.
18. McNeill, D. R., Narayana, A., Wong, H. K., and Wilson, D. M., III (2004) Inhibition of Ape1 nuclease activity by lead, iron, and cadmium, *Environ. Health Perspect. 112*, 799−804.
19. Nakamura, J., Walker, V. E., Upton, P. B., Chiang, S. Y., Kow, Y. W., and Swenberg, J. A. (1998) Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions, *Cancer Res. 58*, 222−225.
20. Tuo, J., Muftuoglu, M., Chen, C., Jaruga, P., Selzer, R. R., Brosh, R. M., Jr., Rodriguez, H., Dizdaroglu, M., and Bohr, V. A. (2001) The Cockayne Syndrome group B gene product is involved in general genome base excision repair of 8-hydroxyguanine in DNA, *J. Biol. Chem. 276*, 45772−45779.
21. Jaruga, P., Theruvathu, J., Dizdaroglu, M., and Brooks, P. J. (2004) Complete release of (5′S)-8,5′-cyclo-2′-deoxyadenosine from dinucleotides, oligodeoxynucleotides and DNA, and direct comparison of its levels in cellular DNA with other oxidatively induced DNA lesions, *Nucleic Acids Res. 32*, e87.
22. Jaruga, P., Birincioglu, M., Rodriguez, H., and Dizdaroglu, M. (2002) Mass spectrometric assays for the tandem lesion 8,5′-cyclo-2′-deoxyguanosine in mammalian DNA, *Biochemistry 41*, 3703−3711.
23. Dizdaroglu, M., Jaruga, P., and Rodriguez, H. (2001) Measurement of 8-hydroxy-2′-deoxyguanosine in DNA by high-performance liquid chromatography−mass spectrometry: Comparison with measurement by gas chromatography−mass spectrometry, *Nucleic Acids Res. 29*, E12.
24. Fortini, P., Pascucci, B., Parlanti, E., D'Errico, M., Simonelli, V., and Dogliotti, E. (2003) The base excision repair: Mechanisms and its relevance for cancer susceptibility, *Biochimie 85*, 1053−1071.
25. Marsin, S., Vidal, A. E., Sossou, M., Menissier-de Murcia, J., Le Page, F., Boiteux, S., de Murcia, G., and Radicella, J. P. (2003) Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1, *J. Biol. Chem. 278*, 44068−44074.

26. Wiederhold, L., Leppard, J. B., Kedar, P., Karimi-Busheri, F., Rasouli-Nia, A., Weinfeld, M., Tomkinson, A. E., Izumi, T., Prasad, R., Wilson, S. H., Mitra, S., and Hazra, T. K. (2004) AP Endonuclease-Independent DNA Base Excision Repair in Human Cells, *Mol. Cell 15*, 209−220.
27. Dou, H., Mitra, S., and Hazra, T. K. (2003) Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2, *J. Biol. Chem. 278*, 49679−49684.
28. Krokan, H. E., Drablos, F., and Slupphaug, G. (2002) Uracil in DNA: Occurrence, consequences and repair, *Oncogene 21*, 8935−8948.
29. Ide, H., and Kotera, M. (2004) Human DNA glycosylases involved in the repair of oxidatively damaged DNA, *Biol. Pharm. Bull. 27*, 480−485.
30. Hang, B. (2004) Repair of exocyclic DNA adducts: Rings of complexity, *BioEssays 26*, 1195−1208.
31. Dizdaroglu, M., Jaruga, P., Birincioglu, M., and Rodriguez, H. (2002) Free radical-induced damage to DNA: Mechanisms and measurement, *Free Radical Biol. Med. 32*, 1102−1115.
32. Dizdaroglu, M. (2003) Substrate specificities and excision kinetics of DNA glycosylases involved in base-excision repair of oxidative DNA damage, *Mutat. Res. 531*, 109−126.
33. Vidal, A. E., Boiteux, S., Hickson, I. D., and Radicella, J. P. (2001) XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein−protein interactions, *EMBO J. 20*, 6530−6539.
34. La Belle, M., Linn, S., and Thompson, L. H. (1984) Apurinic/apyrimidinic endonuclease activities appear normal in the CHO-cell ethyl methanesulfonate-sensitive mutant, EM9, *Mutat. Res. 141*, 41−44.
35. Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E., and Lindahl, T. (1996) Reconstitution of DNA base excision-repair with purified human proteins: Interaction between DNA polymerase β and the XRCC1 protein, *EMBO J. 15*, 6662−6670.
36. Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. (1996) XRCC1 polypeptide interacts with DNA polymerase β and possibly poly(ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro, *Nucleic Acids Res. 24*, 4387−4394.
37. Marintchev, A., Gryk, M. R., and Mullen, G. P. (2003) Site-directed mutagenesis analysis of the structural interaction of the single-strand-break repair protein, X-ray cross-complementing group 1, with DNA polymerase beta, *Nucleic Acids Res. 31*, 580−588.
38. Wilson, S. H. (1998) Mammalian base excision repair and DNA polymerase β, *Mutat. Res. 407*, 203−215.
39. Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. (1997) Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair, *J. Biol. Chem. 272*, 23970−23975.
40. Fan, J., Otterlei, M., Wong, H. K., Tomkinson, A. E., and Wilson, D. M., III (2004) XRCC1 co-localizes and physically interacts with PCNA, *Nucleic Acids Res. 32*, 2193−2201.
41. Lieber, M. R. (1997) The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair, *BioEssays 19*, 233−240.
42. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. (1998) XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage, *Mol. Cell. Biol. 18*, 3563−3571.
43. El Khamisy, S. F., Masutani, M., Suzuki, H., and Caldecott, K. W. (2003) A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage, *Nucleic Acids Res. 31*, 5526−5533.
44. Whitehouse, C. J., Taylor, R. M., Thistlethwaite, A., Zhang, H., Karimi-Busheri, F., Lasko, D. D., Weinfeld, M., and Caldecott, K. W. (2001) XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair, *Cell 104*, 107−117.
45. Takanami, T., Nakamura, J., Kubota, Y., and Horiuchi, S. (2005) The Arg280His polymorphism in X-ray repair cross-complementing gene 1 impairs DNA repair ability, *Mutat. Res. 582*, 135−145.
46. Marintchev, A., Mullen, M. A., Maciejewski, M. W., Pan, B., Gryk, M. R., and Mullen, G. P. (1999) Solution structure of the single-strand break repair protein XRCC1 N-terminal domain, *Nat. Struct. Biol. 6*, 884−893.
47. Lan, L., Nakajima, S., Oohata, Y., Takao, M., Okano, S., Masutani, M., Wilson, S. H., and Yasui, A. (2004) In situ analysis of repair processes for oxidative DNA damage in mammalian cells, *Proc. Natl. Acad. Sci. U.S.A. 101*, 13738−13743.
48. Okano, S., Lan, L., Caldecott, K. W., Mori, T., and Yasui, A. (2003) Spatial and temporal cellular responses to single-strand breaks in human cells, *Mol. Cell. Biol. 23*, 3974−3981.
49. Mani, R. S., Karimi-Busheri, F., Fanta, M., Caldecott, K. W., Cass, C. E., and Weinfeld, M. (2004) Biophysical characterization of human XRCC1 and its binding to damaged and undamaged DNA, *Biochemistry 43*, 16505−16514.
50. Parsons, J. L., Dianova, I. I., Allinson, S. L., and Dianov, G. L. (2005) DNA Polymerase β Promotes Recruitment of DNA Ligase IIIα-XRCC1 to Sites of Base Excision Repair, *Biochemistry 44*, 10613−10619.
51. Marintchev, A., Robertson, A., Dimitriadis, E. K., Prasad, R., Wilson, S. H., and Mullen, G. P. (2000) Domain specific interaction in the XRCC1-DNA polymerase β complex, *Nucleic Acids Res. 28*, 2049−2059.
52. Gryk, M. R., Marintchev, A., Maciejewski, M. W., Robertson, A., Wilson, S. H., and Mullen, G. P. (2002) Mapping of the interaction interface of DNA polymerase β with XRCC1, *Structure 10*, 1709−1720.
53. Dianova, I. I., Sleeth, K. M., Allinson, S. L., Parsons, J. L., Breslin, C., Caldecott, K. W., and Dianov, G. L. (2004) XRCC1-DNA polymerase β interaction is required for efficient base excision repair, *Nucleic Acids Res. 32*, 2550−2555.
54. Kubota, Y., and Horiuchi, S. (2003) Independent roles of XRCC1's two BRCT motifs in recovery from methylation damage, *DNA Repair 2*, 407−415.
55. Matsumoto, Y., and Kim, K. (1995) Excision of deoxyribose phosphate residues by DNA polymerase β during DNA repair, *Science 269*, 699−702.
56. Nakamura, J., and Swenberg, J. A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues, *Cancer Res. 59*, 2522−2526.
57. Sobol, R. W., Prasad, R., Evenski, A., Baker, A., Yang, X. P., Horton, J. K., and Wilson, S. H. (2000) The lyase activity of the DNA repair protein β-polymerase protects from DNA-damage-induced cytotoxicity, *Nature 405*, 807−810.
58. Sleeth, K. M., Robson, R. L., and Dianov, G. L. (2004) Exchangeability of mammalian DNA ligases between base excision repair pathways, *Biochemistry 43*, 12924−12930.
59. Sossou, M., Flohr-Beckhaus, C., Schulz, I., Daboussi, F., Epe, B., and Radicella, J. P. (2005) APE1 overexpression in XRCC1-deficient cells complements the defective repair of oxidative single strand breaks but increases genomic instability, *Nucleic Acids Res. 33*, 298−306.
60. Plo, I., Liao, Z. Y., Barcelo, J. M., Kohlhagen, G., Caldecott, K. W., Weinfeld, M., and Pommier, Y. (2003) Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions, *DNA Repair 2*, 1087−1100.
61. El Khamisy, S. F., Saifi, G. M., Weinfeld, M., Johansson, F., Helleday, T., Lupski, J. R., and Caldecott, K. W. (2005) Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1, *Nature 434*, 108−113.
62. Nocentini, S. (1999) Rejoining kinetics of DNA single- and double-strand breaks in normal and DNA ligase-deficient cells after exposure to ultraviolet C and γ radiation: An evaluation of ligating activities involved in different DNA repair processes, *Radiat. Res. 151*, 423−432.
63. Nakamura, J., Asakura, S., Hester, S. D., de Murcia, G., Caldecott, K. W., and Swenberg, J. A. (2003) Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single strand break repair in base excision repair deficient cells in real time, *Nucleic Acids Res. 31*, e104.
64. Brem, R., and Hall, J. (2005) XRCC1 is required for DNA single-strand break repair in human cells, *Nucleic Acids Res. 33*, 2512−2520.
65. Taylor, R. M., Moore, D. J., Whitehouse, J., Johnson, P., and Caldecott, K. W. (2000) A cell cycle-specific requirement for the XRCC1 BRCT II domain during mammalian DNA strand break repair, *Mol. Cell. Biol. 20*, 735−740.
66. Audebert, M., Salles, B., and Calsou, P. (2004) Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining, *J. Biol. Chem. 279*, 55117−55126.

67. Thompson, L. H., and West, M. G. (2000) XRCC1 keeps DNA from getting stranded, *Mutat. Res. 459*, 1−18.
68. Gueven, N., Becherel, O. J., Kijas, A., Chen, P., Howe, O., Rudolph, J. H., Gatti, R., Date, H., Onodera, O., Taucher-Scholz, G., and Lavin, M. F. (2004) Aprataxin, a novel protein that protects against genotoxic stress, *Hum. Mol. Genet. 13*, 1081−1093.
69. Luo, H., Chan, D. W., Yang, T., Rodriguez, M., Chen, B. P., Leng, M., Mu, J. J., Chen, D., Songyang, Z., Wang, Y., and Qin, J. (2004) A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment, *Mol. Cell. Biol. 24*, 8356−8365.
70. Clements, P. M., Breslin, C., Deeks, E. D., Byrd, P. J., Ju, L., Bieganowski, P., Brenner, C., Moreira, M. C., Taylor, A. M., and Caldecott, K. W. (2004) The ataxia-oculomotor apraxia 1 gene product has a role distinct from ATM and interacts with the DNA strand break repair proteins XRCC1 and XRCC4, *DNA Repair 3*, 1493−1502.
71. Date, H., Igarashi, S., Sano, Y., Takahashi, T., Takahashi, T., Takano, H., Tsuji, S., Nishizawa, M., and Onodera, O. (2004) The FHA domain of aprataxin interacts with the C-terminal region of XRCC1, *Biochem. Biophys. Res. Commun. 325*, 1279−1285.
72. Campalans, A., Marsin, S., Nakabeppu, Y., O'Connor, T. R., Boiteux, S., and Radicella, J. P. (2005) XRCC1 interactions with multiple DNA glycosylases: A model for its recruitment to base excision repair, *DNA Repair 4*, 826−835.
73. Akbari, M., Otterlei, M., Pena-Diaz, J., Aas, P. A., Kavli, B., Liabakk, N. B., Hagen, L., Imai, K., Durandy, A., Slupphaug, G., and Krokan, H. E. (2004) Repair of U/G and U/A in DNA by UNG2-associated repair complexes takes place predominantly by short-patch repair both in proliferating and growth-arrested cells, *Nucleic Acids Res. 32*, 5486−5498.

BI051161O