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Involvement of XRCC1 and DNA Ligase III Gene Products in DNA Base Excision Repair*

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DNA ligase III and the essential protein XRCC1 are present at greatly reduced levels in the xrcc1 mutant CHO cell line EM-C11. Cell-free extracts prepared from these cells were used to examine the role of the XRCC1 gene product in DNA base excision repair *in vitro*. EM-C11 cell extract was partially defective in ligation of base excision repair patches, in comparison to wild type CHO-9 extracts. Of the two branches of the base excision repair pathway, only the single nucleotide insertion pathway was affected; no ligation defect was observed in the proliferating cell nuclear antigen-dependent pathway. Full complementation of the ligation defect in EM-C11 extracts was achieved by addition to the repair reaction of recombinant human DNA ligase III but not by XRCC1. This is consistent with the notion that XRCC1 acts as an important stabilizing factor of DNA ligase III. These data demonstrate for the first time that xrcc1 mutant cells are partially defective in ligation of base excision repair patches and that the defect is specific to the polymerase β-dependent single nucleotide insertion pathway.

DNA base excision repair (BER)[1] counteracts the mutagenic and cytotoxic effects of various kinds of base alterations that do not significantly distort the secondary structure of the double helix. A common intermediate of this pathway is the abasic (AP) site, that arises as a consequence of removal of altered bases by DNA-*N*-glycosylases or as spontaneous detachment of normal bases from the deoxyribose-phosphate backbone. It has been calculated that 2000–10000 AP sites arise each day in a mammalian cell under physiological conditions (1). Therefore, the task of BER is engaging and important, and data obtained in *Escherichia coli* and transgenic mice show that this process is essential for survival (2–4). We have recently shown that, in addition to the polymerase β-dependent single nucleotide insertion pathway previously investigated in mammalian cells (5), a distinct proliferating cell nuclear antigen (PCNA)-dependent pathway is also present that incorporates a repair patch size of 7–14 nucleotides extending 3′ to the site of the lesion (6). Our knowledge of the enzymology of the two pathways has several gaps. In particular, the enzymology of the ligation step is poorly defined. A role for the XRCC1 protein has been suggested on the basis of the sensitivity of xrcc1 mutant cell lines (the CHO derivatives EM9 and EM-C11) to agents that introduce DNA base damage (7, 8) and because of their reduced rate of single-strand break rejoining following exposure to ionizing radiation or alkylating agents (9, 10). Consistent with a role for XRCC1 in DNA ligation and BER is its observed interaction with DNA ligase III and DNA polymerase β (7, 11, 12). Here, we have examined directly the role of XRCC1 and DNA ligase III in mammalian BER using a cell-free system. We report for the first time that (i) xrcc1 mutant cells are partially defective in ligation of BER patches and (ii) the defect involves only the polymerase β-dependent single nucleotide insertion pathway and not the PCNA-dependent pathway.

EXPERIMENTAL PROCEDURES

Cell Culture—The CHO-9 cell line and its mutant derivative EM-C11 (10) were cultured in F-10/Dulbecco's modified Eagles's medium, 1:1, with 10% fetal calf serum. The CHO AA8 cell line and its mutant derivative EM9 were cultured in α-minimal essential medium, as described previously (13).

In Vitro BER Assay—The procedures for preparation of plasmids carrying a single AP site have been described previously (6) (see Fig. 2). Briefly, pGEM3Zf(+) single-stranded DNA was annealed with an oligonucleotide (22 base pairs (bp)) carrying a single uracil. Control pGEM T plasmids were prepared with an oligonucleotide carrying a thymine in the same position. Closed circular double-stranded DNA was obtained by incubating with T4 DNA polymerase, single strand binding protein, and T4 DNA ligase. The AP site was generated by incubation of pGEM U with *E. coli* uracil DNA glycosylase. The *in vitro* BER assay, described in Frosina *et al.* (6, 14) was employed as described below. Briefly, 300 ng of the single lesion substrate pGEM X or the control substrate pGEM T were incubated with 20 μg of extract protein for the indicated times at 30 °C in the presence of [^{32}P]dTTP or [^{32}P]dCTP. [^{32}P]dTTP was the label of choice when the single nucleotide insertion pathway was under investigation, as the single AP site is located opposite dAMP. [^{32}P]dCTP was the label of choice when the PCNA-dependent pathway was under investigation because, within the *Acc*I-*Hin*dIII fragment located 3′ to the lesion, cytosine is the most represented base (6 out of 17 bases). After the repair reaction, the DNA reaction product was purified, treated with the appropriate restriction endonucleases, and separated by polyacrylamide gel electrophoresis in the presence of 7 M urea for 1–1.5 h at 30 mA. The gel was subsequently dried and subjected to autoradiography. In most experiments, reactions were run in multiple volumes with the amounts of all components multiplied accordingly. The most frequent restriction treatment was with *Sma*I and *Hin*dIII endonucleases. If BER was complete, this yielded a fragment of 33 bp with the repaired AP site centrally located (bp 16). However, the presence of 16–33-mer fragments signified unligated BER reaction products. The length of this fragment (whether 16 bp or longer) was diagnostic of in which BER pathway ligation had failed. Unligated 16 mers indicated that the defective ligation step was in the polymerase β-dependent single nucleotide insertion pathway,

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[1] The abbreviations used are: BER, base excision repair; AP sites, abasic sites; PCNA, proliferating cell nuclear antigen; bp, base pair(s); CHO, Chinese hamster ovary.

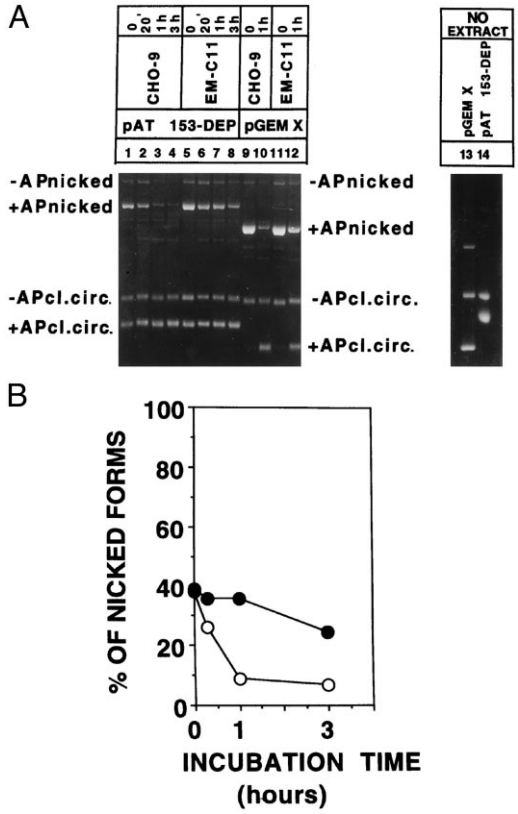

FIG. 1. **Persistence of incised plasmid forms after incubation with EM-C11 extracts.** *A*, a mixture of 300 ng of depurinated closed circular pAT 153 and undamaged pBR 322 plasmids (*lanes 1–8*) or pGEM X substrate with a single AP site and undamaged pBR 322 plasmids (*lanes 9–12*) were incubated with 150 μg of protein of CHO-9 (*lanes 1–4*, *9*, and *10*) or EM-C11 (*lanes 5–8*, *11*, and *12*) extracts under standard repair conditions for the indicated times. Nicked and closed circular forms were resolved by agarose gel electrophoresis. *Lanes 13* and *14* show the initial state of plasmids (absence of extracts) analyzed on a separate gel. *B*, quantitation of data. Residual nicked depurinated pAT 153 plasmids (%) after incubation with (○——○) CHO-9 extract or (●——●) EM-C11 extract.

whereas unligated 17–33 mers indicated that the ligation defect was in the PCNA-dependent pathway. The length of the fragment was determined by size markers represented by 5′-end-labeled oligonucleotides of sequence identical to those of the expected excised fragments.

Immunodetection of XRCC1, DNA Ligase III, and DNA Ligase I—Immunodetection of XRCC1 with the anti-XRCC1 monoclonal antibody 33-2-5 was conducted as described previously (8). Immunodetection of DNA ligase III and DNA ligase I was conducted as for XRCC1, but using as primary antibodies the polyclonal antibodies TL-25 and TL-6 (1/500 dilution), respectively.

Recombinant Human Polypeptides—Recombinant human XRCC1 protein was expressed as a C-terminal histidine-tagged polypeptide in *E. coli* and purified as described previously (8). Recombinant human DNA ligase III protein was expressed as an N-terminal histidine-tagged polypeptide and purified as described elsewhere (11). T4 DNA ligase was purchased from Boehringer Mannheim. The effect of these proteins on the various steps of BER was investigated by preincubating the cell extracts (usually 20 μg of protein) with the appropriate amount of recombinant protein(s) at 30 °C for 20 min in a standard repair reaction mixture. Thereafter, plasmid substrate was added and the repair reaction started.

Incision Experiments—A mixture of 300 ng of depurinated closed circular pAT 153 plasmids carrying an average of 0.5 AP sites/circle and 300 ng of undamaged closed circular pBR 322 plasmids was incubated with CHO-9 or EM-C11 cell extracts (150 μg of protein) for the indicated times, and reactions were stopped by addition of EDTA to 20 mM final concentration and freezing on dry ice. Alternatively, a mixture of 300 ng of pGEM X, containing a single AP site, and 300 ng of undamaged pBR 322 was used as substrate. After the repair reaction, plasmid DNA was extracted once with phenol-chloroform, and nicked and closed circular forms were resolved by 1% agarose gel electrophoresis. The percent of

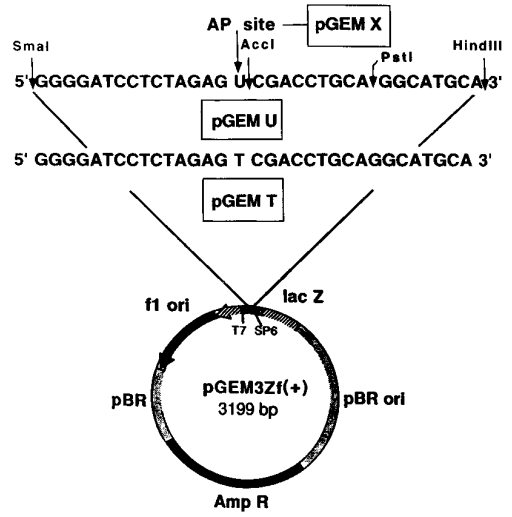

FIG. 2. **Schematic of pGEM X (carrying a single AP site) and pGEM-T (control) plasmid substrates.** The location of the single AP site (indicated with *X*) and the relevant restriction sites are shown.

incised DNA was calculated for each experimental point, by scanning densitometry of a photographic negative. The higher (1.6) fluorescence of nicked DNA as compared with closed circular DNA was taken into account.

RESULTS

EM-C11 Cell-free Extracts Are Defective in Processing Nicked BER Intermediates—Initial experiments examined the incision of depurinated plasmid substrates by wild type CHO-9 and xrcc1 mutant EM-C11 extracts (Fig. 1). Heat-depurinated pAT 153 plasmids (Fig. 1*A*, *lanes 1–8* and *14*) were mixed with undamaged pBR 322 control plasmids and incubated with CHO-9 or EM-C11 extracts for 0, 20, 60, and 180 min. Damaged plasmids carried 0.5 AP site/circle and were rapidly and specifically incised upon addition of either CHO-9 or EM-C11 extract, as indicated by the zero time points in which the reaction was stopped immediately after the addition of cell extract (*lanes 1* and *5*). The incised plasmid forms were subsequently converted to closed circular forms during continued incubation. Some nonspecific degradation was also observed, as suggested by a slight decrease in the overall amount of DNA (15). Incised, form II plasmids persisted for longer periods after incubation with EM-C11 extracts (Fig. 1, *A*, *lanes 5–8*, and *B*, *solid circles*), when compared with plasmids incubated with wild type CHO-9 extracts (Fig. 1, *A*, *lanes 1–4*, and *B*, *open circles*). Essentially the same results were obtained when pGEM X plasmids carrying a single AP site (Fig. 2) were used as substrates (Fig. 1*A*, *lanes 9–13*). Unlike pBR 322 plasmids, damaged pGEM X plasmids were quickly and efficiently incised by both wild type and mutant extracts. After 60 min of incubation at 30 °C, 47% incised plasmids persisted in reactions containing EM-C11 extracts, whereas only 26% persisted in those containing wild type CHO-9 extract (Fig. 1*A*, compare *lanes 10* and *12*). Taken together these experiments suggest that EM-C11 extracts possess a partial defect in processing nicked DNA after incision by AP endonucleases.

EM-C11 Cell-free Extracts Exhibit a DNA Ligation Defect during Mammalian BER—To better investigate the putative BER defect in xrcc1 mutant EM-C11 cell extracts, repair replication experiments were conducted with pGEM X plasmid substrate which harbors a single AP site at a defined location. The AP site is centrally located (16th bp) within a *Sma*I-*Hin*dIII 33-mer restriction fragment and was obtained by incubating pGEM U with *E. coli* uracil-DNA glycosylase (Fig. 2). After incubation with cell extract and subsequent recovery

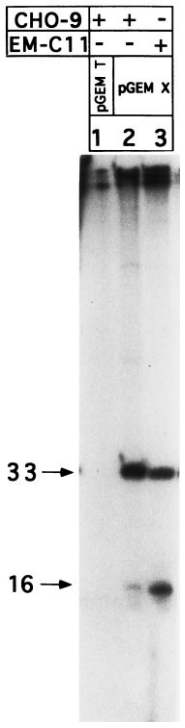

FIG. 3. **DNA ligation defect in the single nucleotide insertion BER pathway in EM-C11 extracts.** 300 ng of pGEM X plasmids carrying a single AP site were incubated for 60 min under standard repair conditions (see "Experimental Procedures") with 20 μg of protein of CHO-9 extract (*lane 2*) or 20 μg of protein of EM-C11 cell extract (*lane 3*). After the repair reaction, DNA was purified and treated with *Sma*I-*Hin*dIII restriction endonucleases. The excised 33-bp fragment was resolved by denaturing polyacrylamide gel electrophoresis. As a control, 300 ng of undamaged pGEM T plasmids were incubated with 20 μg of CHO-9 cell extract under the same conditions (*lane 1*).

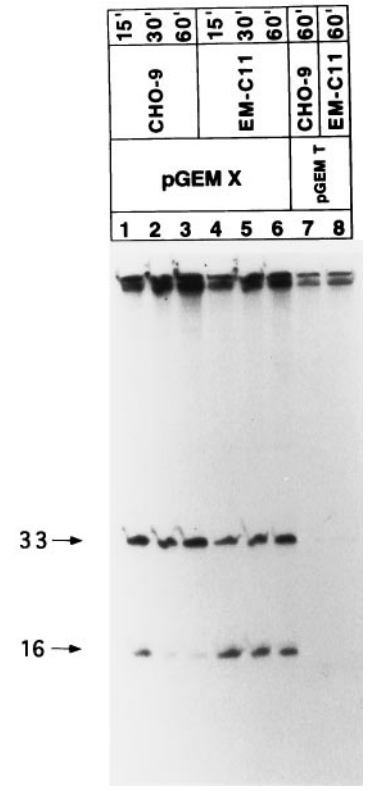

FIG. 4. **Kinetics of repair reaction.** 300 ng of pGEM X plasmids carrying a single AP site were incubated for 15, 30, and 60 min with 20 μg of protein of CHO-9 extract (*lanes 1–3*) or 20 μg of protein of EM-C11 cell extract (*lanes 4–6*). DNA was purified and treated as in the experiment of Fig. 3. As a control, 300 ng of undamaged pGEM T plasmids were incubated with 20 μg of CHO-9 or EM-C11 cell extract under the same conditions (*lanes 7* and *8*).

from the BER reaction, pGEM X BER products were treated with *Sma*I and *Hin*dIII and separated by denaturing polyacrylamide gel electrophoresis. Whereas the presence of radiolabeled 33-mer fragments reflects completed BER events, the presence of 16–32-mer fragments indicates the persistence of unligated intermediates of BER. Furthermore, whereas the persistence of 16 mers reflects unligated intermediates arising from the single nucleotide insertion pathway, the persistence of 17–32 mers reflects unligated intermediates arising from multiple nucleotide insertion events. As previously shown, these occur via polymerase β (5)- or PCNA-dependent (6) synthesis, respectively.

The experiment in Fig. 3 shows that the single lesion is repaired efficiently by the CHO-9 cell extract (*lane 2*) during a 1-h incubation in the presence of [^{32}P]dTTP; almost all the repair incorporation is associated with the 33-mer fragment cut by *Sma*I-*Hin*dIII endonucleases, thus showing that all repair events were completed. In contrast, we did not observe any incorporation within the *Sma*I-*Hin*dIII region of control, undamaged pGEM T plasmids (*lane 1*). When the pGEM X plasmid substrate was incubated with the xrcc1 mutant EM-C11 extract, incomplete BER was observed, as indicated by the persistence of an unligated repair intermediate and reduced formation of fully repaired 33 mer (*lane 3*). The unligated intermediates comigrated with a size marker 16 bp long. This suggests that the unligated intermediates terminate immediately 3′ of the repair site and were thus generated by BER incorporation of a single nucleotide (5).

The kinetics of the repair reaction with the wild type and mutant extracts is shown in Fig. 4. Reactions were performed as in the experiment in Fig. 3 and stopped after 15, 30, and 60 min. With the CHO-9 extract (*lanes 1–3*), all repair events were completed after 30 min of incubation with conversion to the full-length 33-mer fragment. In contrast, incomplete ligation was observed with EM-C11 extract (*lanes 4–6*) at both 30 and 60 min. The unligated intermediates represented on average one-third of the total radioactivity incorporated during BER. A limited degree of variability in the amount of unligated fragments using different batches of EM-C11 extracts could be observed. The total repair incorporation stimulated by the EM-C11 extract was similar to that stimulated by the wild type CHO-9 extract.

The DNA Ligase Defect in EM-C11 Is Specific to the Single Nucleotide Insertion Pathway of BER—The absence of unligated BER intermediates of a length greater than 16 bp suggests that the defect in EM-C11 is specific to the single nucleotide insertion pathway and does not involve the PCNA-dependent pathway acting 3′ to the lesion. To confirm this latter notion, the repair reaction was run in the presence of [^{32}P]dCTP as labeling nucleotide and an *Acc*I-*Hin*dIII restriction fragment (located 3′ to the AP site) was analyzed after the repair incubation (Fig. 5, *lanes 5* and *6*). Only BER events involving multiple nucleotide incorporation (7–14 nucleotides, according to previous estimations) (6) are detected by this approach, in which completed events are indicated by the presence of a radiolabeled 17 mer. No fragments smaller than 17 bp were present, thus indicating that no defect in DNA ligation during PCNA-dependent BER pathway was present in EM-C11 cell extracts. Furthermore, when reaction products formed in the presence of [^{32}P]dCTP were incubated with *Sma*I and *Hin*dIII, only fully repaired fragments of 33 bp were detected (*lanes 1* and *2*). This is in marked contrast to the "*Sma*I-

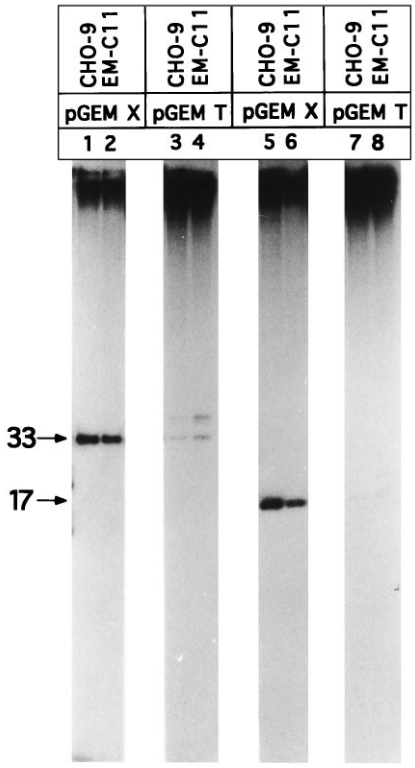

FIG. 5. **The DNA ligation step of the PCNA-dependent BER pathway is unaffected in EM-C11 extracts.** pGEM X (*lanes 1*, *2*, *5*, and *6*) or pGEM T (*lanes 3*, *4*, *7*, and *8*) plasmids were incubated with 20 μg of protein of CHO-9 (*lanes 1*, *3*, *5*, and *7*) or EM-C11 (*lanes 2*, *4*, *6*, and *8*) extract for 60 min at 30 °C. After the repair reaction, plasmids were purified and treated with *Sma*I-*Hin*dIII (*lanes 1–4*) or *Acc*I-*Hin*dIII (*lanes 5–8*) restriction endonucleases.

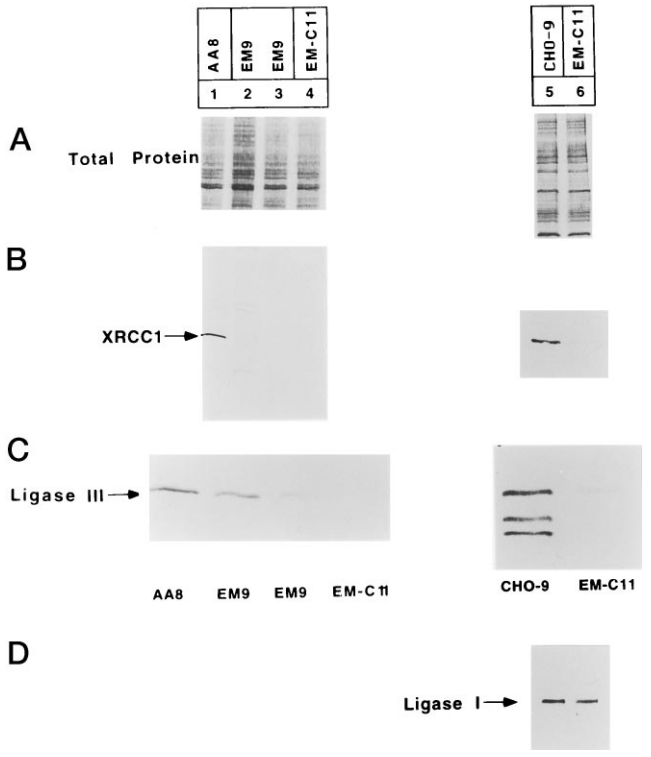

FIG. 6. **XRCC1, DNA ligase III, and DNA ligase I levels in xrcc1 mutant cell extracts.** Western blotting experiments were performed with 33-2-5 monoclonal antibodies raised against human XRCC1 protein (*panel B*), TL25 polyclonal antibodies raised against human DNA ligase III (*panel C*) or TL6 polyclonal antibodies against human DNA ligase I (*panel D*). All antibodies cross-react with the respective hamster proteins. Total protein staining by Coomassie Blue (*lanes 1–4*) or Amido Black (*lanes 5* and *6*) is in *panel A*. Crude cell extracts were used for *lanes 1–4* and BER extracts for *lanes 5* and *6*.

*Hin*dIII" 16 mers present when dTTP was used as radionuclide (Figs. 3, *lane 3*, and 4, *lanes 4–6*), further demonstrating that the DNA ligation defect was specific to the single nucleotide incorporation pathway. That the repair patches observed in these experiments were exclusively due to BER was again indicated by the presence of only a small amount of incorporation in undamaged control pGEM T plasmids (*lanes 3*, *4*, *7*, and *8*). Experiments which measured the repair patch size generated by EM-C11 extracts showed that more than 90% repair synthesis 3′ to the lesion was confined to the *Acc*I-*Pst*I fragment, thus indicating a predominant repair patch size of less than 10 nucleotides. Repair synthesis in the PCNA-dependent pathway carried out by the EM-C11 extracts was less efficient in comparison to that conducted by the parental CHO-9 extract (Fig. 5, compare *lanes 6* and *5*). Residual repair replication in EM-C11 cell extracts was 48%.

In summary, experiments in Figs. 3–5 indicate that xrcc1 mutant EMC11 extracts possess a DNA ligation defect that is specific to the single nucleotide incorporation pathway of BER, previously shown to involve DNA polymerase β (5).

In Vitro Complementation of the EM-C11 Defect—Fig. 6 shows that XRCC1 and DNA ligase III levels are greatly reduced in EM9 and EM-C11 crude cell extracts, and in EM-C11 cell extracts used for BER in this study. Western blotting experiments were conducted using 33-2-5 monoclonal antibodies raised against human XRCC1 protein (*panel B*) or TL-25 polyclonal antibodies raised against human DNA ligase III (*panel C*). Both antibodies cross-react with the respective hamster proteins.[2] Total protein detected by staining with Coomassie Blue (*lanes 1–4*) or Amido Black (*lanes 5* and *6*) is shown in *panel A*. The XRCC1 gene product is present in control AA8 and CHO-9 cell extracts (*lanes 1* and *5*), but is greatly reduced or absent in EM-C11 (*lane 4* and *6*) and EM9 (*lanes 2* and *3*, two independent cell cultures). Similarly, levels of DNA ligase III were reduced 4–6-fold in EM9 and EM-C11 cell extracts (*lanes 2–4* and *6*). A slight decrease in DNA ligase I level was also observed in EM-C11 extracts (*panel D*) compared with the parental CHO-9 extract as detected by immunoblotting with TL 6 anti-DNA ligase I antibodies.

The BER defects observed in the EM-C11 extract might be due to the reduced levels of XRCC1 and ligase III proteins. We examined the role of these polypeptides in the BER defect by *in vitro* complementation experiments (Fig. 7). Purified proteins were preincubated with EM-C11 whole cell extract for 20 min at 30 °C, and plasmid substrates were subsequently added to begin the repair reaction. The BER defect in EM-C11 cell-free extract (41% of BER products remaining as unligated fragments; *lanes 1* and *6*) was fully complemented by addition of purified DNA ligase III (no unligated fragments; *lane 3*) or a mixture of recombinant human XRCC1 and DNA ligase III (*lane 4*) or purified DNA ligase from bacteriophage T4 (1.5 units; *lane 7*). In contrast, only a very inefficient complementation of the ligation defect was provided by recombinant XRCC1 alone (33% of BER products remaining as unligated fragments; *lane 2*). No unspecific incorporation was detected with pGEM T control plasmids (*lanes 5* and *8*).

DISCUSSION

We report here that EM-C11 cell extracts, which possess greatly reduced levels of XRCC1 and DNA ligase III polypeptides, are defective in the DNA ligation step of BER. Of the two

[2] K. Caldecott, unpublished data.

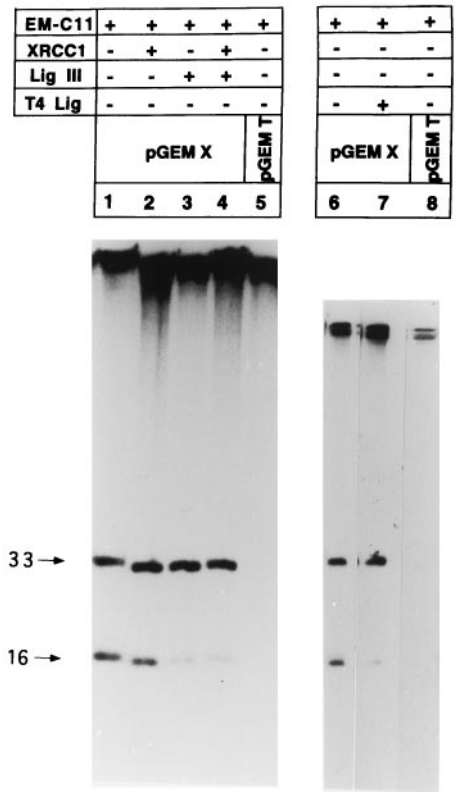

FIG. 7. **Complementation of the ligation defect in EM-C11 extract by recombinant polypeptides.** 300 ng of pGEM X plasmids carrying a single AP site were incubated under standard repair conditions with 20 μg of protein of EM-C11 extract (*lanes 1* and *6*) or 20 μg of protein of EM-C11 extract preincubated for 20 min at 30 °C with either 1 μg of XRCC1 protein (*lane 2*), or 1 μg of DNA ligase III (*lane 3*), or a mixture of 1 μg of XRCC1 and 1 μg of ligase III proteins (*lane 4*) or 1.5 units of DNA ligase from bacteriophage T4 (*lane 7*). After the repair reaction, DNA was purified and treated with *Sma*I-*Hin*dIII restriction endonucleases. As a control, 300 ng of undamaged pGEM T plasmids were incubated with 20 μg of EM-C11 cell extract under the same conditions (*lanes 5* and *8*).

branches of the BER pathway (6), only the single nucleotide insertion pathway was affected by the XRCC1 deficiency. No ligation defect was detectable in the PCNA-dependent pathway, thus indicating that ligase activities other than XRCC1-DNA ligase III are involved in this pathway. The ligation defect in EM-C11 extracts within the single nucleotide insertion pathway was partial, with approximately one-third of repair events remaining unligated after 1 h in comparison to CHO-9 wild type extracts. This indicates that the residual DNA ligase III is sufficient to complete some of the BER ligation events, or that other DNA ligases can partially compensate, at least *in vitro*, for the DNA ligase III defect. That the latter possibility is significant is supported by the finding that efficient complementation was achieved by addition of bacteriophage T4 DNA ligase. *In vivo*, one possible backup activity is DNA ligase I, since a partial defect in cell-free BER was observed for the DNA ligase I mutant, 46BR (16) and a specific interaction between DNA polymerase β and DNA ligase I in a multiprotein BER complex has been recently found in bovine testis extracts (17). DNA ligase I could also be the major sealing activity in the PCNA-dependent pathway. The latter pathway is probably less efficient than the one nucleotide insertion pathway and can only partially compensate the defect of EM-C11.

Levels of DNA ligase III protein and activity are severely reduced in EM-C11 cell extract (8) (Fig. 6). The cell-free BER defect in the EM-C11 extract was fully corrected by addition of recombinant DNA ligase III but not by recombinant XRCC1, indicating that it can be accounted for by the DNA ligase III deficiency. The lack of significant effect of adding recombinant XRCC1 to the BER reaction suggests that this protein is not required enzymatically for BER, at least *in vitro*. Rather, taken together, these results support the notion that XRCC1 is required to maintain normal cellular levels of DNA ligase III, a role presumably reflecting a dependence of the latter polypeptide on interaction with XRCC1 for physical stability (8). A very small reduction in DNA ligase I was observed in EM-C11 extracts as compared with parental CHO-9 extracts. This is unlikely to reflect any direct influence of XRCC1 protein on DNA ligase I, since these proteins do not appear to associate (11), but rather may reflect the decreased proliferation rate of EM-C11.

In contrast to EM-C11, a DNA ligation defect was not detected in BER supported by EM9 cell extracts (12). This discrepancy may similarly result from differences in levels of residual DNA ligase III, since levels of DNA ligase III were lower in cell extracts from EM-C11 cells than from EM9 cells (Fig. 6) (8). It is possible that the less severe DNA ligase III deficiency in EM9 is more readily complemented in the cell-free BER assay by the promiscuity of other DNA ligases. Such nonspecific complementation may not be possible *in vivo* due to the sequestration of different DNA ligases into different protein complexes separated spatially, and possibly temporally, within the nucleus.

Although levels of DNA ligase III are the only cause of the BER defect observed in this cell-free system, it remains to be determined whether this is also the case *in vivo*, or whether XRCC1 has additional roles other than maintaining the level of DNA ligase III. Consistent with the latter possibility, we and others have recently reported that XRCC1 directly interacts with DNA polymerase β, and also possibly with poly(ADP)-ribose polymerase in addition to DNA ligase III (7, 11, 12, 18). On this basis, it has been proposed that XRCC1 might function as a scaffold protein physically linking together components of the BER machinery (12), or that XRCC1 may act as a molecular chaperone to actively target polymerase β and/or DNA ligase III to DNA repair events *in vivo* (11). Alternatively, XRCC1 may possess a novel catalytic activity. One such role suggested for XRCC1 is to promote single nucleotide incorporation and so prevent excessive repair synthesis by polymerase β. It was reported that XRCC1 mutant EM9 extracts display elevated repair patch size during polymerase β-dependent BER (12). Such a defect was not apparent in our studies, since this would have manifested as elevated levels of incorporation in experiments measuring repair replication downstream of the AP site, which we did not see (Fig. 5, *lanes 5* and *6*). Indeed, a decrease of 52% in repair replication located 3′ to the the lesion was observed in our experiments. The latter finding suggests the possible involvement of XRCC1 protein in the PCNA-dependent pathway but clearly, whether and how XRCC1 participates in repair replication downstream of the AP site requires further study.

In summary, we report here for the first time that XRCC1 mutant cell extracts are defective in the ligation step of BER and that this defect is specific to the single nucleotide insertion pathway catalyzed by DNA polymerase β.

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