Interaction of Human AP Endonuclease 1 with Flap Endonuclease 1 and Proliferating Cell Nuclear Antigen Involved in Long-Patch Base Excision Repair

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ABSTRACT: To understand the mechanism involved in the coordination of the sequential repair reactions that lead to long-patch BER, we have investigated interactions between proteins involved in this pathway. We find that human AP endonuclease 1 (APE1) physically interacts with flap endonuclease 1 (FEN1) and with proliferating cell nuclear antigen. An oligonucleotide substrate containing a reduced abasic site, which was pre-incised with APE1, was employed to reconstitute the excision step of long-patch BER with purified human DNA polymerase β and FEN1. We demonstrate that addition of APE1 to the excision reaction mixture slightly (1.5−2-fold) stimulates the removal of the displaced flap by FEN1. These results suggest the possibility that long-patch BER is coordinated and directed by protein−protein interactions.

Base excision repair (BER)[1] operating in human cells provides an effective mechanism for protecting genomic DNA from the accumulation of altered bases. BER is initiated by a DNA glycosylase which removes the modified base, and then an apurinic/apyrimidinic endonuclease (AP endonuclease) binds to the abasic site and hydrolyzes the phosphodiester bond 5′ to it (*1*). This single-strand DNA break cannot be repaired directly by DNA ligase because it contains a 5′-sugar phosphate (dRP) (*2*). The removal of the dRP can be accomplished by DNA polymerase β (Pol β), which adds one nucleotide to the 3′-end of the nick and removes the dRP by catalyzing a β-elimination reaction (*3*, *4*). The nick is finally sealed by DNA ligase, and the entire repair reaction results in the removal and replacement of only one nucleotide (the single-nucleotide BER pathway) (*5*). Alternatively, Pol β may add several nucleotides to the 3′-end of the nick and displace the 5′-dRP as part of a flap of two to six nucleotides, which is later excised by flap endonuclease 1 (FEN1). The strand break is finally sealed by DNA ligase (*6*, *7*). This repair reaction results in a longer (mainly two to six nucleotides) repair patch (the long-patch BER pathway). Long-patch BER may also be accomplished by Pol δ or Pol ϵ (*6*, *8*, *9*) through the proliferating cell nuclear antigen (PCNA)-dependent mechanism. Although AP sites arising as a result of either spontaneous base loss or the removal of damaged bases by DNA glycosylase can be processed via both BER pathways, the single-nucleotide BER pathway is responsible for the majority (up to 80−90%) of AP site repair. However, ∼10−20% of AP sites and any AP sites resistant to the β-elimination reaction catalyzed by Pol β are processed by the long-patch BER mechanism (*5*, *10*, *11*).

If a DNA strand break arising as an intermediate product during BER remains unprotected from the cellular milieu, it may lead to a double-strand break and/or an elevated level of recombination. It is likely that cells can prevent the exposure of repair intermediates by masking the repair site and handing repair intermediates from one repair protein to another ("passing the baton" mechanism) through a series of protein-protein interactions (*12*). Such an orchestration of repair events is thought to be an important part of single-nucleotide BER (*1*, *12*, *13*). Several research groups recently demonstrated that DNA glycosylases can remain bound to the AP site after removal of the damaged DNA base. The glycosylase can later be displaced by AP endonuclease (APE1), which incises the AP site and remains bound to the nicked DNA after incision (*14*, *15*). When bound to DNA, APE1 was shown to interact with human Pol β, thus providing coupling to the next step of BER (*16*). At the next step, Pol β interacts with DNA ligase I or, through the XRCC1 protein, with DNA ligase III. The ligase then finalizes DNA repair by sealing the strand break (*17*−*19*). However, the nature of the protein-protein interactions involved in coordinating the Pol β-dependent long-patch repair pathway is not clear. We have previously shown that FEN1 functionally interacts with Pol β and that this interaction stimulates FEN1-dependent long-patch excision (*20*), but we were unable to demonstrate any direct physical interaction between these proteins. In this paper, we further investigate the mechanism for coordination of Pol β-dependent long-patch BER and the role of protein-protein interactions in directing this pathway. Specifically, we demonstrate that APE1 physically interacts with FEN1 and PCNA.

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[1] Abbreviations: BER, base excision repair; AP sites, apurinic/apyrimidinic sites, abasic sites; APE1, apurinic/apyrimidinic endonuclease 1; PCNA, proliferating cell nuclear antigen; FEN1, flap endonuclease; Pol β and Pol δ/ϵ, DNA polymerases β and δ/ϵ, respectively; dRP, 5′-deoxyribose phosphate; BSA, bovine serum albumin; WCE, whole cell extract.

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MATERIALS AND METHODS

Materials. Synthetic oligodeoxyribonucleotides purified by high-performance liquid chromatography were obtained from the Midland Certified Reagent Co. [α-^{32}P]ddATP and [γ-^{32}P]-ATP (3000 Ci/mmol) were purchased from Amersham Pharmacia Biotech. Terminal deoxynucleotidyltransferase and polynucleotide kinase were from Promega. Recombinant human Pol β was overexpressed and purified as described previously (*21*). Human APE1 and UDG with 84 amino acids deleted from the amino terminus were purified as described previously (*22*, *23*). Normal human lymphoid cells (AG9387) were obtained from the Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ). Cells were grown in the medium recommended by the supplier. Whole cell extracts (WCE) were prepared from 3−5 g of cells as described previously (*24*), and dialyzed overnight against buffer containing 25 mM Hepes/KOH (pH 7.9), 2 mM dithiothreitol (DTT), 12 mM $MgCl_2$, 0.1 mM EDTA, 17% glycerol, and 0.1 M KCl. Extracts were aliquoted and stored at −80 °C.

Preparation of FEN1−Sepharose. The plasmid containing human FEN1 gene was kindly provided by M. R. Lieber (Washington University School of Medicine, St. Louis, MO). Histidine-tagged FEN1 protein was purified on Ni^{2+}-charged His-Bind Resin (Novagen, Cambridge, MA) as recommended by the manufacturer. Two milligrams of purified FEN1 or bovine serum albumin (BSA) was covalently coupled to 1 mL of CNBr-activated Sepharose (Amersham Pharmacia Biotech) as described by the supplier.

FEN1−APE1 Interaction. FEN1−Sepharose (25 μL) was washed three times with 300 μL of binding buffer [50 mM Hepes (pH 7.9), 2 mM $MgCl_2$, 0.02% Triton X-100, and 0.1 mM EDTA] containing 10 mg/mL BSA, mixed with 25 μL of the same buffer containing 25 ng (0.74 pmol) of APE1, and incubated for 1 h at 4 °C. After incubation, FEN1−Sepharose was washed three times with 0.35 mL of binding buffer, and beads were finally washed with 20 μL of binding buffer and eluted with 20 μL of binding buffer containing 0.3 M NaCl and then with 20 μL of binding buffer containing 0.6 M NaCl. Samples (10 μL) of the last wash and elution were loaded on a 10% SDS−polyacrylamide gel followed by transfer to nitrocellulose membrane and immunoblot analysis with antibodies against APE1. Control experiments with BSA−Sepharose were performed exactly as described for FEN1−Sepharose.

PCNA−APE1 Interaction. Histidine-tagged PCNA was purified on Ni^{2+}-charged His-Bind Resin (Novagen) as recommended by the manufacturer. One milligram of purified PCNA was covalently coupled to 0.5 mL of CNBr-activated Sepharose (Amersham Pharmacia Biotech) as described by the supplier. Experiments with PCNA−Sepharose were performed exactly as described for FEN1−Sepharose.

Immunoprecipitations. Human whole cell extract (0.5 mg) was precleared by incubation for 30 min at 4 °C with 2.5 μL of preimmune serum and 25 μL (50% slurry) of protein A−Sepharose beads. After addition of 0.1 μg of affinity-purified antibodies and incubation for 1 h at 4 °C, 25 μL of protein A−Sepharose beads were added and incubation was continued overnight. Immunoprecipitates were washed five times with 0.35 mL of binding buffer and eluted into 50 μL of SDS−PAGE sample buffer. Proteins were separated on a 10% SDS−polyacrylamide gel followed by transfer to nitrocellulose membrane and immunoblot analysis with the indicated antibodies.

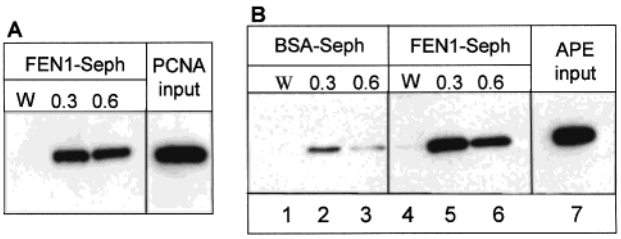

FIGURE 1: FEN1 protein interacts with APE1. (A) PCNA protein specifically binds to FEN1−Sepharose. Purified PCNA (25 ng, 0.86 pmol) in 25 μL of binding buffer was mixed with 25 μL of FEN1−Sepharose and the mixture incubated for 1 h at 4 °C. The resin was extensively washed with binding buffer and then consequently eluted with 20 μL of the following buffers: binding buffer (W), binding buffer containing 0.3 M NaCl (0.3), and binding buffer containing 0.6 M NaCl (0.6). Ten microliters of each sample was subjected to electrophoresis via a 10% SDS−polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against PCNA antibodies. Ten nanograms (0.34 pmol, ∼40% of the input) of PCNA was loaded on the last lane (PCNA input). (B) APE1 protein specifically binds to FEN1−Sepharose. Purified APE1 (25 ng, 0.74 pmol) in 25 μL of binding buffer was mixed with 25 μL of BSA−Sepharose (left) or 25 μL of FEN1−Sepharose (right) and incubated for 1 h at 4 °C. The resin was processed as described above. Wash (W), eluates (0.3 and 0.6), and 10 ng (0.29 pmol, ∼40% of the input) of APE1 (APE input) were subjected to electrophoresis via a 10% SDS−polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against APE1 antibodies.

Substrate Labeling. A 33-mer oligonucleotide containing a uracil residue at position 17 (5′-TACCGCGGCCGGC-CGAUCAAGCTTATTGGGTAC) was labeled at the 3′-end with terminal deoxynucleotidyltransferase and [α-^{32}P]ddATP. Labeling at the 5′-end was performed with T4 polynucleotide kinase and [γ-^{32}P]ATP. Unincorporated labeled nucleotides were removed on a Sephadex G-25 spin column.

Preparation of DNA Substrates for the Excision Assay. To prepare the oligonucleotide duplex, labeled oligonucleotide containing a uracil residue was annealed with its complementary strand. The equimolar solution of both oligonucleotides in TE and 100 mM KCl was incubated at 90 °C for 3−5 min, and the solution was allowed to cool slowly to 25 °C. Prior to assembly of the excision reaction mixture, the DNA substrate (500 ng, 50 pmol) was pretreated with uracil-DNA glycosylase (200 ng, 6.25 pmol) in 10 mM Hepes (pH 7.9), 1 mM EDTA, and 100 mM KCl. The reaction mixture was incubated at 37 °C for 1 h. The resulting AP site was reduced by addition of sodium borohydride to a concentration of 0.1 M, and after incubation on ice for 10 min, the reaction buffer was exchanged with TE by filtering through a Sephadex G-25 spin column. To generate substrate containing pre-incised AP sites, the AP-containing substrate was treated with 100 ng (3 pmol) of APE1 in a buffer containing 45 mM Hepes (pH 7.8), 70 mM KCl, 7.5 mM $MgCl_2$, 0.5 mM EDTA, and 1 mM DTT for 30 min at 37 °C. To remove APE1, the reaction mixture was treated with a phenol/chloroform mixture and filtered through a Sephadex G-25 spin column.

Excision Reaction Using Oligonucleotide DNA Substrates. The excision reaction was reconstituted in a reaction mixture (10 μL) that contained 45 mM Hepes (pH 7.8), 70 mM KCl,

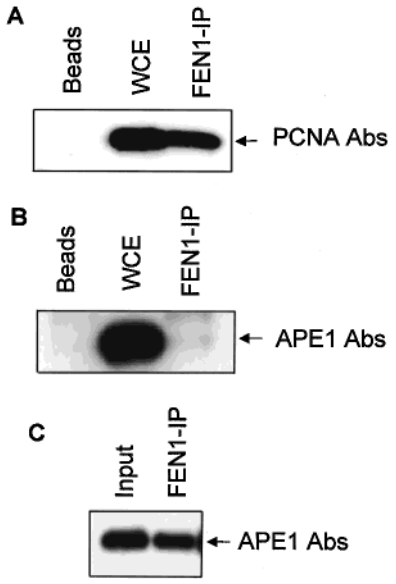

FIGURE 2: Immunoprecipitation of PCNA and APE1 from whole cell extracts. Human whole cell extracts were subjected to immunoprecipitation with protein A−Sepharose beads (Beads) or FEN1 antibodies (FEN1-IP) as described in Materials and Methods. Samples of WCE (30 μg) and immunoprecipitates were fractionated on a 10% polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against PCNA (A) or against APE1 antibodies (B). (C) Co-immunoprecipitation of purified PCNA and APE1 by FEN1 antibodies. One hundred nanograms (2.4 pmol) of FEN1 and 100 ng (3 pmol) of APE1 were incubated with FEN1 antibodies and precipitated with protein A−Sepharose beads. Proteins were eluted with 50 μL of a SDS sample buffer. Five nanograms of APE1 (input) or 20 μL of the eluates was subjected to electrophoresis via a 10% polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against APE1 antibodies.

7.5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, 2 mM ATP, 2 mg/mL BSA, 20 μM dATP, 20 μM dGTP, 20 μM dCTP, and 20 μM dTTP, and a ^{32}P-labeled oligonucleotide substrate (5−10 ng, 0.5−1 pmol). The reaction was initiated by adding FEN1 and Pol β, at the amount indicated in the figure legends. After incubation for the indicated time at 37 °C, the reaction was stopped by addition of 10 μL of gel loading buffer (95% formamide, 20 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanol). Following incubation at 80 °C for 2 min, the reaction products were separated by electrophoresis in a 20% polyacrylamide gel containing 8 M urea in 89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA (pH 8.0). All experiments were repeated at least three to five times, and representative gels are shown.

RESULTS

Purified APE1 Interacts with FEN1. We have recently demonstrated that FEN1 functionally interacts with Pol β during the excision step of long-patch BER by stimulation of strand displacement synthesis (*20*). However, using a FEN1 affinity column, we were unable to detect a FEN1−Pol β physical interaction (data not shown), whereas in control experiments, we detected the FEN1−PCNA interaction (Figure 1A) previously observed by others (*25*, *26*). We have suggested that the functional interaction between FEN1 and Pol β could be supported by other BER protein(s) interacting with both FEN1 and Pol β. Since Pol β had previously been shown to communicate with APE1 (*27*), we tested whether APE1 interacts with FEN1. To identify physical interactions between FEN1 and APE1, purified APE1 was incubated with FEN1−Sepharose in the presence of a 10000-fold excess of BSA. After extensive washes with low-salt binding buffer, the bound protein was eluted with the same buffer containing 0.3 M NaCl and then 0.6 M NaCl. We found that 40−50% of the input APE1 remained bound to FEN1−Sepharose. Approximately 70% of the bound APE1 was eluted with 0.3 M NaCl, and the rest was eluted with 0.6 M NaCl (Figure 1B, lanes 5 and 6). In contrast, when identical experiments were performed using BSA−Sepharose, only 2−5% of the APE1 was found in the elution fractions (Figure 1B, lanes 2 and 3). Thus, we conclude that APE1 specifically binds to FEN1−Sepharose because of a physical interaction with FEN1.

Co-Immunodepletion of FEN1 and APE1. Both APE1 and PCNA interact with FEN1. However, although the FEN1−PCNA complex was easily precipitated from whole cell extract with FEN1 antibodies (Figure 2A), we found very little if any of the APE1−FEN1 complex in a WCE (Figure 2B); though, in a control experiment with purified proteins, we were able to detect APE1 precipitation with FEN1 antibodies (Figure 2C). There are at least two possible explanations for this observation: either all FEN1 is involved in complex with PCNA, or a large excess of free PCNA in WCE competes for FEN1 with APE1 during immunopre-

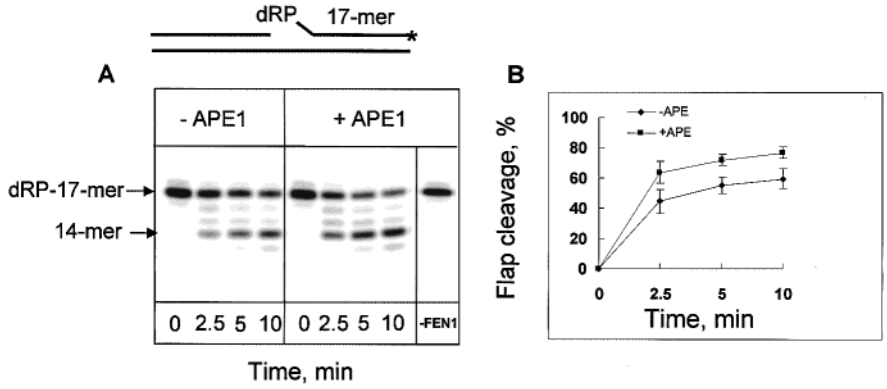

FIGURE 3: Flap excision by FEN1 is more efficient in the presence of APE1. The reaction conditions and product analysis are described in Materials and Methods. The 3′-end-labeled (∗) oligonucleotide duplex (400 fmol) containing an untreated AP site preincised with APE1 was incubated with 25.6 fmol of Pol β and 23.8 fmol of FEN1 with (+APE, 30 fmol) or without (−APE) APE1. Substrate DNA incubated for 10 min without FEN1 (−FEN1) was loaded on the last lane. A representative gel (A) and PhosphorImager quantification of the results of a series of experiments (B) are shown.

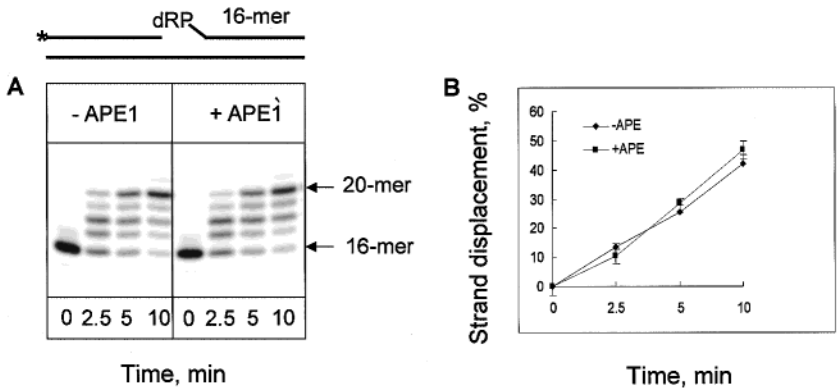

FIGURE 4: APE1 does not stimulate DNA synthesis by Pol β. Reaction conditions and product analyses are described in Materials and Methods. The 5′-end-labeled (*) oligonucleotide duplex substrate containing an AP site preincised with APE1 (400 fmol) was incubated with 25.6 fmol of Pol β and with (+APE, 30 fmol) or without (−APE) APE1. The length of the extension products is indicated. A representative gel (A) and PhosphorImager quantification of the results of a series of experiments (B) are shown.

cipitation. Indeed, using quantitative Western blot analysis, we found that approximately 1 pmol of APE1, 5 pmol of FEN1, and 100 pmol of PCNA are present in a WCE per milligram of extract protein (data not shown).

Stimulation of Flap Excision in the Presence of APE1. To investigate whether the FEN1−APE1 interaction might influence the rate of long-patch BER, we reconstituted the excision step of the BER reaction with purified Pol β and FEN1 proteins. We measured the efficiency of excision using a 3′-end-labeled substrate containing a reduced AP site preincised with APE1 (Figure 3A). Since the AP lyase activity of Pol β cannot remove the reduced sugar phosphate, Pol β must add a few nucleotides to the 3′-end of the nick to generate a flap structure that is subsequently removed by FEN1, and this results in a 14-mer product (Figure 3A). Since the substrate was already preincised with APE1, it did not require APE1 for further processing. Thus, addition of APE1 to the reaction mixture could only affect DNA synthesis by Pol β or the rate of flap excision by FEN1. We found that in the absence of APE1 the excision reaction catalyzed by FEN1 and Pol β was less efficient than when APE1 was present. Although the stimulatory effect was only 1.5−2.0-fold, it was very reproducible over multiple series of reactions that were performed (Figure 3B). In the absence of FEN1, APE1 had no effect on the substrate DNA (Figure 3A, right panel, last lane). We thus concluded that, under conditions when equal amounts of Pol β were used, addition of APE1 stimulates the excision reaction performed by FEN1.

No Effect of APE1 on DNA Synthesis by Pol β. It was previously demonstrated that, when bound to DNA, APE1 interacts with Pol β (*16*). To exclude the possibility that addition of APE1 stimulated strand displacement by Pol β and thereby indirectly affected the excision of the flap by FEN1, we examined the strand displacement reaction using a 5′-end-labeled substrate containing a preincised AP site. We carried out the reactions with equal amounts of Pol β with or without APE1 and found no effect of APE1 on the rate of strand displacement mediated by Pol β (Figure 4).

APE1 Interacts with PCNA. PCNA is involved in multiple interactions with many DNA repair proteins, including DNA ligase I (*28*, *29*) and FEN1 (*26*, *30*). On the basis of these interactions of PCNA, it was suggested that it might play a role in the coordination of DNA repair events by loading DNA repair proteins onto DNA and providing physical

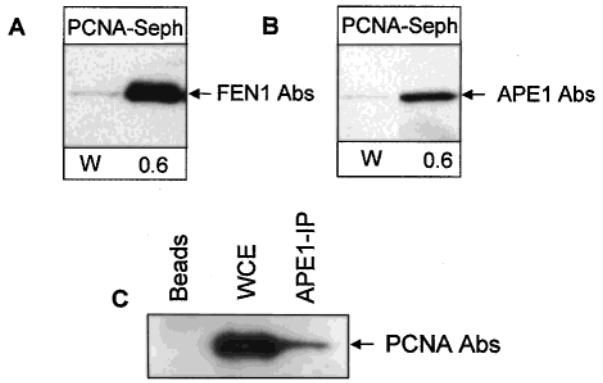

FIGURE 5: APE1 specifically binds to PCNA−Sepharose. Twenty-five microliters of PCNA−Sepharose was mixed with 25 μL of binding buffer containing 200 ng (4.8 pmol) of FEN1 (A) or 200 ng (5.9 pmol) of APE1 (B) and incubated for 1 h at 4 °C. The resin was extensively washed with binding buffer and then consequently eluted with 20 μL of the following buffers: binding buffer (W) and binding buffer containing 0.6 M NaCl (0.6). Ten microliters of each sample was subjected to electrophoresis through a 10% polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against FEN1 antibodies (A) or APE1 antibodies (B). (C) Human whole cell extracts were subjected to immunoprecipitation with protein A−Sepharose beads (Beads) or APE1 antibodies (APE1-IP) as described in Materials and Methods. Samples of the WCE (30 μg) and immunoprecipitates were fractionated on a 10% polyacrylamide gel, transferred onto PVDF membrane, and immunoblotted against PCNA antibodies.

contact between proteins that are not otherwise directly interacting (*31*−*33*). It was interesting to test the possibility that APE1 might directly interact with PCNA. We used PCNA−Sepharose resin and in control experiments first demonstrated that FEN1 binds to the PCNA−Sepharose in a low-salt binding buffer and could be eluted with buffer containing 0.6 M NaCl (Figure 5A). In similar experiments using APE1, we found that APE1 was bound to PCNA−Sepharose (Figure 5B). Although APE1 binding was about 4-fold less efficient than FEN1 binding, it was still at least 3-fold above the level of APE1 retained on BSA−Sepharose (Figure 1B). Thus, we conclude that APE1 binds to PCNA−Sepharose (albeit with an efficiency lower than that of FEN1), because of a physical interaction with PCNA. Although there is very little APE1 in comparison to PCNA in a WCE, we were able to detect co-immunoprecipitation of a small amount (∼1−2%) of PCNA and APE1 with APE1 antibodies (Figure 5C).

DISCUSSION

There are two distinct BER subpathways involving subsets of repair proteins and operating independently (*6*, *8*, *9*, *34*). Both BER subpathways are initiated by a DNA glycosylase that removes an altered base, and then the AP endonuclease generates a DNA strand break 5′ to the abasic site. Subsequently, two different scenarios for further repair of the incised abasic site may take place. One subpathway driven by Pol β removes the 5′-sugar phosphate and fills the gap (*3*, *4*). The other subpathway, which involves displacement of the 5′-sugar phosphate as a part of a flap oligonucleotide, is thought to be supported by either Pol β (*6*, *7*) or Pol δ/ϵ (*6*, *8*, *9*). Although both Pol β (*6*, *35*) and Pol δ/ϵ (*33*, *36*, *37*) can operate in a long-patch repair reaction reconstituted with purified proteins, we previously demonstrated that in mammalian cell extracts Pol β is the DNA polymerase that initiates both short- and long-patch repair of uracil and reduced AP sites in DNA (*7*, *38*, *39*). We also demonstrated recently that FEN1 stimulates strand displacement by Pol β and that these proteins interact functionally during long-patch BER (*20*). In this report, we describe a FEN1−APE1 physical interaction. The purified proteins efficiently interact on FEN1−Sepharose or can be co-immunoprecipitated. However, since we were unable to coprecipitate the FEN1−APE1 complex from the WCE, the majority of FEN1 protein is (most probably) in a complex with PCNA and is not complexed with APE1 in whole cell extracts. Nevertheless, the strong in vitro interaction suggests that these proteins may interact during the course of repair. What is the potential biological role for this interaction? Under our conditions, we observed a moderate (1.5−2-fold) stimulation of the excision reaction, suggesting that the interaction between FEN1 and APE1 can only slightly accelerate long-patch BER. However, the role of this interaction may not be to stimulate the flap excision reaction but rather to provide coordinated loading of the proteins on the substrate, thus passing the substrate from one enzyme to another as has been proposed in the "passing the baton" model (*12*). If DNA polymerase β is not able to remove the 5′-dRP and has to dissociate from the repair APE1−Pol β−DNA intermediate complex (*38*), then, at the switching point from short-patch to long-patch repair, recruitment of FEN1 through APE1 may play an important role.

PCNA may play multiple roles in BER. It forms a toroidal homotrimeric clamp around the DNA molecule and is believed to serve as a scaffold that brings together proteins that are not physically interacting (*40*). As has been shown previously, PCNA interacts with FEN1 (*25*, *26*) and the role of PCNA may be simply to stimulate FEN1-dependent long-patch BER repair (*6*, *35*, *41*). However, PCNA may also be involved in coordination of the BER reaction. In our experiments, we detected an APE1−PCNA complex. Although the PCNA−APE1 interaction is not as robust as the FEN1−PCNA or FEN1−APE1 interactions, it may be very important because PCNA has multiple interaction sites and potentially may coordinate multiprotein reactions. PCNA also interacts with DNA ligase I (*28*, *29*). These observations support the idea that the interaction of APE1, FEN1, and DNA ligase I with PCNA may provide a molecular mechanism for the transition from the excision step to the DNA strand-sealing step during long-patch BER. This function may be similar to the role of the XRCC1 protein, which acts as a scaffold protein bringing together Pol β and DNA ligase III during single-nucleotide patch repair (*18*, *42*). Other polymerases (Pol δ/ϵ) may also support long-patch BER (*43*). We were unable to detect physical interaction between FEN1 and Pol δ or APE1 and Pol δ (data not shown); however, Pol δ directly interacts with PCNA (*44*) and may also participate in long-patch BER (*32*).

It appears that the BER is coordinated and directed by multiple interactions between proteins participating in BER. Reports from several laboratories have indicated that after removal of the altered base, DNA glycosylase remains bound to the AP site and is later displaced by APE1 (*14*, *15*). Moreover, there has been a recent accumulation of evidence suggesting that such displacement stimulates the 8-oxoguanine-DNA glycosylase activity by increasing the rate of glycosylase turnover (*45*−*47*). In turn, APE1 incises the AP site and also remains tightly bound to the incision product (*48*) and may serve as a mediator of the next step in BER. APE1, when bound to DNA, communicates with Pol β (*16*) and, as we demonstrate here, also physically interacts with FEN1 and PCNA. thus providing multiple protein-protein communications during DNA repair.

Two mechanisms for the BER process have been discussed. Prasad et al. (*17*) purified a BER complex that was able to conduct complete uracil-initiated BER, and they suggested the existence of a pre-assembled BER complex. Other models consider the assembly of the short lifetime intermediate complexes at the site of damage (*12*, *49*, *50*). Our finding of APE1−FEN1 and APE1−PCNA interactions supports the idea of coordination of BER, but the question of whether this coordination is provided through preexisting BER subcomplexes or by sequential assembly of the specific repair complex at the site of DNA damage requires further studies.

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