MOLECULAR AND CELLULAR BIOLOGY, Sept. 1994, p. 6187–6197
0270-7306/94/$04.00+0

Vol. 14, No. 9

Proliferating Cell Nuclear Antigen-Dependent Abasic Site Repair in *Xenopus laevis* Oocytes: an Alternative Pathway of Base Excision DNA Repair

YOSHIHIRO MATSUMOTO,[1]* KYUNG KIM,[1] AND DANIEL F. BOGENHAGEN[2]

Department of Radiation Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111,[1] and Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794[2]

Received 12 April 1994/Returned for modification 10 May 1994/Accepted 7 June 1994

DNA damage frequently leads to the production of apurinic/apyrimidinic (AP) sites, which are presumed to be repaired through the base excision pathway. For detailed analyses of this repair mechanism, a synthetic analog of an AP site, 3-hydroxy-2-hydroxymethyltetrahydrofuran (tetrahydrofuran), has been employed in a model system. Tetrahydrofuran residues are efficiently repaired in a *Xenopus laevis* oocyte extract in which most repair events involve ATP-dependent incorporation of no more than four nucleotides (Y. Matsumoto and D. F. Bogenhagen, Mol. Cell. Biol. 9:3750–3757, 1989; Y. Matsumoto and D. F. Bogenhagen, Mol. Cell. Biol. 11:4441–4447, 1991). Using a series of column chromatography procedures to fractionate *X. laevis* ovarian extracts, we developed a reconstituted system of tetrahydrofuran repair with five fractions, three of which were purified to near homogeneity: proliferating cell nuclear antigen (PCNA), AP endonuclease, and DNA polymerase δ. This PCNA-dependent system repaired natural AP sites as well as tetrahydrofuran residues. DNA polymerase β was able to replace DNA polymerase δ only for repair of natural AP sites in a reaction that did not require PCNA. DNA polymerase α did not support repair of either type of AP site. This result indicates that AP sites can be repaired by two distinct pathways, the PCNA-dependent pathway and the DNA polymerase β-dependent pathway.

Base excision repair is a major pathway for repair of damaged bases in DNA (8). In this pathway, a DNA-*N*-glycosylase initiates repair by removing a specifically modified base, leaving an apurinic/apyrimidinic (AP) site. AP sites are also generated by spontaneous or induced base loss. Since AP sites arise so frequently, it is reasonable to expect that cells should have evolved very efficient mechanisms to repair this sort of damage.

To study the mechanism of repair of AP sites, we have taken advantage of the ability to insert a single copy of a synthetic analog of an AP site, 3-hydroxy-2-hydroxymethyltetrahydrofuran (tetrahydrofuran), at a specific position in a covalently closed circular DNA (cccDNA) (17, 23, 29). This tetrahydrofuran residue was efficiently repaired in a *Xenopus laevis* oocyte extract. Analyses of this repair reaction revealed several features of the repair mechanism (15, 16). Repair is initiated by cleavage on the 5′ side of the lesion by a class II AP endonuclease. Synthesis of a short patch of no more than four nucleotides displaces the damaged residue. Following DNA synthesis, the AP site and one or two 3′-flanking nucleotides are excised. Repair DNA synthesis requires ATP, suggesting that the repair-related DNA synthesis may not be a simple DNA polymerase reaction but may involve interaction with other protein factors. Thus, the final step in repair, sealing of the DNA strand by DNA ligase, is not the only ATP-requiring step in this repair pathway.

The enzymes and factors involved in this repair reaction have not been identified. The most controversial question is the identity of the DNA polymerase responsible for base excision repair. Many studies using inhibitors to distinguish different DNA polymerases suggested that DNA polymerase β catalyzes repair synthesis in the base excision pathway. However, several lines of evidence suggested that DNA polymerase β might not be the only polymerase involved in repair of AP sites. First, our early experiments showed that repair of the tetrahydrofuran residue was inhibited by aphidicolin, as expected if repair involves a high-molecular-weight DNA polymerase (unpublished data). Second, biochemical analyses of yeast mutants indicated that DNA polymerase ε is responsible for this reaction (33). While a discrepancy concerning the identity of the repair polymerase may be the result of intrinsic differences in the experimental systems, some uncertainties could be eliminated if a reconstituted system which catalyzes the repair reaction with purified factors were available. In this paper, we present results obtained by fractionating the *X. laevis* ovarian extract, leading to development of a reconstituted system. This system consists of five fractions, including proliferating cell nuclear antigen (PCNA) and DNA polymerase δ. DNA polymerases α and β were also tested for their ability to support AP site repair in the place of DNA polymerase δ and were found to be incapable of participating in the PCNA-dependent repair reaction.

MATERIALS AND METHODS

Materials. All the chromatography resins and prepacked columns were purchased from Pharmacia except for phosphocellulose (Whatman P11), single-stranded DNA (ssDNA)-cellulose, double-stranded DNA (dsDNA)-cellulose (Sigma), and hydroxyapatite cartridge (Bio-Rad). Aphidicolin and ddTTP were obtained from Boehringer Mannheim and Pharmacia, respectively. Butylphenyl dGTP (BuPdGTP) was a generous gift from G. Wright, University of Massachusetts. ^{32}P-labeled nucleotides were purchased from DuPont and ICN Biochemicals. Poly(dA), poly(rA), and oligo(dT) were from U.S. Biochemicals. Uracil-DNA glycosylase and *Taq* DNA polymerase were supplied by Perkin Elmer. Polyvinylidene

* Corresponding author. Phone: (215) 728-5272. Fax: (215) 728-4333.

difluoride membrane (Immobilon P) was from Millipore. Goat anti-mouse immunoglobulin G (IgG) antibody conjugated with alkaline phosphatase and reagents for colorimetric development were purchased from Bio-Rad. Recombinant human PCNA purified from bacteria and a monoclonal antibody against human PCNA were generous gifts from K. Fien and B. Stillman, Cold Spring Harbor Laboratory. Recombinant human AP endonuclease, Ref-1, purified from bacteria, and a polyclonal anti-Ref-1 antibody were generous gifts from S. Xanthoudakis and T. Curran, Roche Institute.

Preparation of repair substrates. DNA templates for repair reactions consisted of pBS^- plasmid (Stratagene) produced by in vitro ligation of oligonucleotides containing either a tetrahydrofuran or a uracil residue into the polylinker region of a gapped heteroduplex. Preparation of cccDNA containing a unique tetrahydrofuran was described previously (16). cccDNA containing a natural AP site was prepared in two steps. First, uracil-containing cccDNA was prepared by the same method as for tetrahydrofuran-containing DNA, in which a unique uracil residue was substituted for the tetrahydrofuran residue. The uracil-containing DNA was stored in TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) at 4°C until use. In the second step, this cccDNA was treated with uracil-DNA glycosylase (10 U of the enzyme per μg of DNA, incubated at 37°C for 1 h) immediately before the repair reaction to leave a natural AP site with an intact phosphodiester backbone. The uracil-DNA glycosylase was free from AP endonuclease activity. ^{32}P-labeled DNA containing an AP site was prepared by using a 5′-end ^{32}P-labeled oligonucleotide or 5′-end ^{32}P-labeled vector to incorporate a single labeled residue several nucleotides away on the 5′ or 3′ side of the AP site, as described before (16).

Repair reactions. The standard repair reaction was performed at 25°C for 30 min with 10 ng of AP site-containing cccDNA (200 to 1,000 cpm of prelabeled DNA adjusted to the needed amount with nonradioactive DNA) and proteins as indicated in a 20-μl reaction mixture containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 20 μM each of the four deoxynucleoside triphosphates, 2 mM ATP, and 0.1 mg of bovine serum albumin (BSA) per ml. NAD (0.5 mM) was added to the reaction mixes for F3, S, or Q fractions. The repair reaction was stopped by the addition of sodium dodecyl sulfate (SDS) to a final concentration of 0.4% and 2 μg of proteinase K. After a 20-min incubation at 37°C, the DNA was recovered from the reaction mixture and subjected to digestion with either *Pvu*II or *Hin*fI as described before (16). DNA products were analyzed by electrophoresis on gels containing either 6% (for *Pvu*II digests) or 20% (for *Hin*fI digests) polyacrylamide with 8 M urea and were detected by autoradiography. The radioactivities of repair products on gels were quantified with a Fuji Bio Imaging Analyzer. In experiments to detect intermediate products of the natural AP site-containing DNA, termination of the repair reaction by addition of SDS and proteinase K was followed by treatment with 0.3 M $NaBH_4$ at room temperature for 10 min. After residual reducing reagent was quenched with NH_4Cl, DNA was recovered and analyzed as described above.

Immunoblotting of PCNA. Samples were subjected to electrophoresis on a 10% polyacrylamide gel with the Tris-glycine-SDS buffer system (12). Proteins were electrophoretically transferred to an Immobilon P membrane. The blot was incubated with a 1:4,000 dilution of anti-human PCNA antibody and then washed and incubated with alkaline phosphatase-conjugated anti-mouse IgG antibody. Immunoreactive protein was visualized by colorimetric development. Intensities of stained bands were quantified with an UltroScan laser densitometer (Pharmacia).

AP endonuclease assay. Prelabeled tetrahydrofuran residue-containing DNA (200 to 1,000 cpm) which had been digested with *Pvu*II prior to the assay and 200 ng of depurinated DNA (approximately 1 pmol of AP sites; prepared as described in reference 16) were incubated at 25°C for 30 min in a 7-μl reaction mixture containing 10 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, and a protein fraction (2 μl of 1:200 to 1:4,000 dilution). After incubation, the DNA was subjected directly to electrophoresis on a 6% polyacrylamide gel containing 8 M urea and analyzed in the same manner as in the repair assay.

DNA polymerase assays. DNA polymerase α activity was measured on a poly(dA)/oligo(dT) template/primer (molar ratio, 5:1; total, 0.5 μg) with 50 mM bis-Tris-HCl (pH 6.5)–10 mM KCl–6 mM $MgCl_2$–0.4 mg of BSA per ml–1 mM DTT–50 μM [α-^{32}P]TTP (1,000 to 2,000 cpm/pmol) in 25 μl at 37°C. DNA polymerase δ activity was measured under the same conditions as for polymerase α but in the presence of 0.5 μg of PCNA (the human recombinant protein for earlier experiments or the *Xenopus* purified protein for later experiments) and 30 μM BuPdGTP. DNA polymerase β activity was measured on a poly(rA)/oligo(dT) template/primer (molar ratio, 1:1; total, 2 μg) with 50 mM Tris-HCl (pH 8.8)–100 mM KCl–0.5 mM $MnCl_2$–0.4 mg of BSA per ml–1 mM DTT–50 μM [α-^{32}P]TTP (1,000 to 2,000 cpm/pmol) in 25 μl at 25°C. One unit of activity corresponds to the incorporation of 1 pmol of TMP into acid-precipitable material in 30 min.

Photolabeling of DNA polymerases. DNA polymerases were preincubated at 25°C for 10 min with 6 pmol of the oligonucleotide described by Insdorf and Bogenhagen (10), 10 μCi of [α-^{32}P]dATP (3,000 Ci/mmol), and approximately 40 μM 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUTP (prepared as described by Bartholomew et al. [2]) in 20-μl reaction mixes containing 50 mM bis-Tris-HCl (pH 6.5), 1 to 40 mM KCl (carried over from DNA polymerase fractions), 6 mM $MgCl_2$, 0.4 mg of gelatin per ml, and 1 mM DTT. The reaction mixtures were then irradiated for 1 min at a distance of 2.5 cm with a small hand-held UV light (UVGL-25; UVP, Inc.; 254 nm) and digested with 0.1 μg of micrococcal nuclease in the presence of 10 mM $CaCl_2$ for 15 min at 37°C. After boiling and precipitation with trichloroacetic acid, the proteins were electrophoresed in an SDS-containing 7% polyacrylamide gel (12) and visualized by silver staining. The gel was then dried and subjected to autoradiography.

Fractionation of repair factors. All the following procedures were performed at below 4°C. Protein concentration was determined by the method of Bradford (3) with bovine gamma globulin as a standard.

An extract derived from whole *X. laevis* ovaries (F3) was prepared as described by Cozzarelli et al. (5) with modifications. The ovarian homogenate was separated from cell debris and organelles by centrifugation at 2,000 rpm for 10 min in a Beckman JA-14 rotor followed by centrifugation at 16,000 rpm for 15 min in a Beckman JA-17 rotor. The S100 extract was prepared by centrifugation at 39,000 rpm for 90 min in a Beckman 45Ti rotor. Proteins precipitated from the S100 extract with 65% saturated ammonium sulfate were resuspended to provide the F3 fraction.

The F3 fraction (5 g of protein) was diluted with buffer A (40 mM Tris-HCl [pH 7.8], 0.5 mM EDTA, 0.1 mM ethylene glycol tetraacetic acid [EGTA], 1 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride [PMSF], 2 mM benzamidine-HCl, 20% glycerol) to give a conductivity equivalent to 100 mM KCl and loaded onto a 100-ml column of DEAE-Sepharose FF which

had been preequilibrated with buffer A plus 100 mM KCl. After the column was washed with the same buffer, the bound proteins were eluted either with a 600-ml linear gradient of 100 to 400 mM KCl in buffer A (as in Fig. 2) or, in later experiments, by step elution with 200 and 400 mM KCl in buffer A. The flowthrough fractions from the DEAE-Sepharose column were loaded directly onto a 100-ml column of SP Sepharose FF which had been preequilibrated with buffer A plus 100 mM KCl. Bound proteins were eluted with buffer A plus 600 mM KCl and dialyzed against buffer A until the KCl concentration became lower than 100 mM. Dialyzed fractions were clarified by centrifugation to provide the S fraction. In later experiments, the 200 mM KCl step eluate from the DEAE-Sepharose column was diluted with buffer A and combined with the DEAE flowthrough fractions to be loaded together onto the S Sepharose column (S′ fraction). The S fraction was diluted with buffer A to 50 mM KCl and loaded onto a 15-ml column of Q Sepharose FF. Bound proteins were eluted either with a 150-ml linear gradient of 50 to 400 mM KCl or with a step gradient of 600 mM KCl in buffer A. The fractions which contained repair activity in the presence of PCNA and AP endonuclease were pooled to provide the Q fraction.

PCNA was purified from the DEAE fractions that supported tetrahydrofuran residue repair in the presence of the S fraction and eluted at approximately 400 mM KCl. These DEAE fractions were adjusted to contain 1 M ammonium sulfate and loaded onto a 10-ml column of phenyl-Sepharose FF which had been preequilibrated with buffer B (20 mM potassium phosphate [pH 7.0], 0.5 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.2 mM PMSF, 2 mM benzamidine-HCl, 10% glycerol) plus 1 M ammonium sulfate. After the column was washed with 0.5 M ammonium sulfate in buffer B, the bound proteins were eluted with 10% ethylene glycol and 20% glycerol in buffer B. The protein peak fractions were loaded onto a Superdex200 16/60 gel filtration column (bed volume, 125 ml) with buffer C (40 mM Tris-HCl [pH 7.8], 0.5 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 μg of aprotinin per ml, 0.5 μg of leupeptin per ml, 1 μg of pepstatin per ml, 20% glycerol, 0.01% Triton X-100) plus 200 mM KCl and 2 mM benzamidine-HCl. The PCNA-containing fractions were diluted to 150 mM KCl with buffer C and loaded onto a MonoQ 5/5 column. After the column was washed with buffer C plus 150 mM KCl, bound proteins were eluted with a linear gradient of 150 to 600 mM KCl. The PCNA-containing fraction was stored at −80°C.

AP endonuclease was purified from the flowthrough fraction of the Q Sepharose column described above. Saturated ammonium sulfate solution was slowly added to the Q flowthrough fraction to 65% saturation. After the precipitate was removed by centrifugation, additional solid ammonium sulfate was added to the supernatant to a final concentration of 80% saturation, and the mixture was stirred overnight. The precipitate was recovered and suspended in 4 ml of buffer C plus 200 mM KCl. The solution was then loaded onto a Superdex200 16/60 column. The fractions containing AP endonuclease activity were adjusted to 100 mM KCl and loaded onto a 0.8-ml dsDNA-cellulose column. Bound proteins were eluted with a 10-ml linear gradient of 100 to 600 mM KCl in buffer C. The AP endonuclease peak fractions were then diluted to 100 mM KCl with buffer C and loaded onto a MonoS 5/5 column. AP endonuclease was eluted with a 10-ml linear gradient of 100 to 600 mM KCl in buffer C. Active fractions were eluted at approximately 240 mM KCl and stored at −80°C.

The Q fraction was loaded onto a Superdex200 16/60 column preequilibrated with buffer C plus 200 mM KCl and 2 mM benzamidine-HCl, and two separate fractions with base excision repair-complementing activity, BE-1 and BE-2, were recovered. The preparation of more highly purified repair factors from these fractions will be described in greater detail elsewhere. The BE-1 fraction was used as starting material for further purification of DNA polymerases α and δ and for preparation of a separate repair factor designated BE-1B. The BE-1 fraction from the Superdex200 chromatography was adjusted to 40 mM KCl with buffer C and loaded onto a 6-ml ssDNA-cellulose column. During elution with a 50-ml linear gradient of 40 to 500 mM KCl, the BE-1 activity was separated into two fractions: BE-1A, which coeluted with DNA polymerases α and δ, and BE-1B. The fractions containing DNA polymerases α and δ were loaded onto a 5-ml HiTrap heparin column. Elution with a 30-ml linear gradient of 150 to 550 mM KCl in buffer C partially separated DNA polymerase α from δ, which coeluted with the BE-1A activity. The two DNA polymerases were then separately purified through P11, MonoQ 5/5, and MonoS 5/5 columns. The BE-1B active fractions from the ssDNA-cellulose chromatography step were further purified through P11 and MonoS 5/5 columns.

The BE-2 fraction from the gel filtration column was adjusted to 70 mM KCl with buffer C and loaded onto a 4-ml ssDNA-cellulose column. Elution with a 20-ml gradient of 70 to 300 mM KCl followed by another 20-ml gradient of 300 mM to 1 M KCl in buffer C separated the BE-2 activity from DNA polymerase β. The BE-2 active fraction was further purified through MonoS 5/5, MonoQ 5/5, and a 1-ml hydroxyapatite column. DNA polymerase β was purified through MonoQ 5/5 and MonoS 5/5 columns.

RESULTS

Fractionation of *X. laevis* ovarian extracts. Tetrahydrofuran residues can be quantitatively repaired during a 20-min incubation in an S150 extract prepared by crushing size-selected large oocytes by centrifugation (15). To expedite biochemical characterization of the enzymes and factors involved in repair, we optimized the repair reaction for an ammonium sulfate-precipitated fraction referred to as F3, derived from a homogenate of whole ovaries. We observed that different batches of extract showed variable repair efficiencies. Addition of NAD to the repair reaction mix decreased this variability and stimulated the activity of suboptimal preparations, possibly by alleviating blockage of DNA damage by poly(ADP-ribose) polymerase (25, 26). The ability of the F3 extract to repair tetrahydrofuran residues was comparable to that previously reported for the S150 extract (data not shown). We routinely assay DNA repair in complementation reactions with cccDNAs prelabeled at a site several residues away from a precisely positioned abasic site. Although this assay is less sensitive than monitoring the incorporation of labeled precursors, it has two advantages. First, this assay directly shows the fraction of input DNA molecules repaired. Second, it allows us to monitor partial reactions in the repair process.

To identify protein factors responsible for AP site repair, we subjected the F3 fraction to a series of column chromatography procedures (Fig. 1). As a first step, F3 was fractionated on DEAE-Sepharose. The column eluate was assayed for DNA polymerase activity and tetrahydrofuran residue repair activity as well as for PCNA by immunoblotting with a monoclonal antibody directed against human PCNA (Fig. 2). Most, but not all, of the DNA polymerase α- and δ-like activities bound to DEAE-Sepharose at 100 mM KCl and were eluted with increasing KCl concentration, while most of the DNA polymerase β-like activity was detected in the flowthrough fraction. The DNA polymerase ε-like activity (activity resistant to

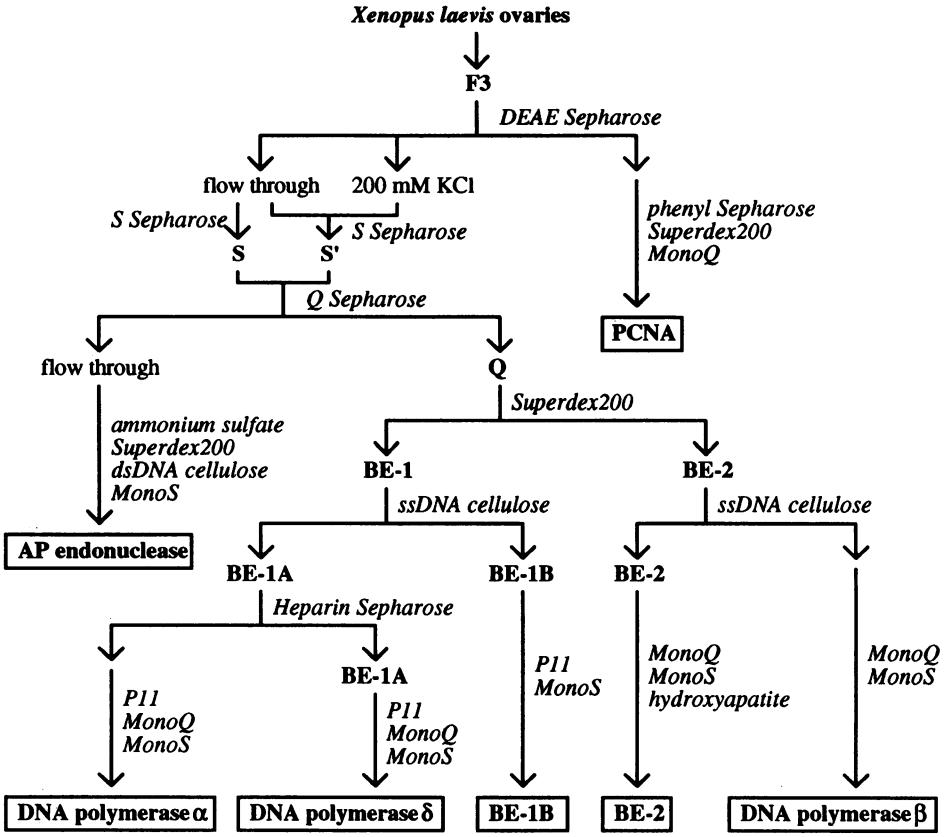

FIG. 1. Fractionation scheme for the purification of *X. laevis* repair factors.

BuPdGTP and ddTTP but not dependent on exogenously added PCNA) formed a peak positioned between the peak of DNA polymerase α- and δ-like activities and the protein peak which was recognized by anti-human PCNA antibody. We attempted to separate this activity from other DNA polymerase activities. The DNA polymerase ε-like activity, however, disappeared after several chromatography steps (data not shown). We interpret this activity as DNA polymerase δ activity stimulated by endogenous PCNA. We have not succeeded in clearly identifying DNA polymerase ε from *X. laevis* ovarian extracts.

The proteins which failed to bind to DEAE-Sepharose were loaded onto S Sepharose, and the bound proteins were recovered by stepwise elution with 0.6 M KCl (S fraction). The S fraction contained most of the DNA polymerase β-like activity and minor portions of DNA polymerase α- and δ-like activities. Neither the S fraction nor the individual DEAE-Sepharose-bound fractions were capable of repairing tetrahydrofuran residues. However, repair was reconstituted by combining the S fraction with DEAE-Sepharose-bound fractions which eluted after the main peak of DNA polymerase activities (Fig. 2B). This repair-complementing activity coeluted with PCNA from the DEAE-Sepharose column. Other experiments showed that the broad peak of repair-complementing activity in Fig. 2B results from the fact that repair is stimulated by very low concentrations of this DEAE-bound factor (Y. Matsumoto, data not shown). Additional chromatography of the main peak of complementing activity showed that it did not bind to P11 at 0.1 M KCl (data not shown). The activity bound to phenyl-Sepharose in a buffer containing 1 M ammonium sulfate and eluted with 10% ethylene glycol and 20% glycerol in the absence of ammonium sulfate. This chromatographic behavior resembled that of PCNA (22). To determine whether this DEAE-bound repair-complementing activity is identical to PCNA, we loaded the phenyl-Sepharose eluate onto a Superdex200 gel filtration column and assayed the eluted fractions for both repair-complementing activity and PCNA immunoblotting. The PCNA-like protein and the repair-complementing activity again coeluted from this column (Fig. 3). In addition, cloned human PCNA protein purified from bacteria could substitute for the Superdex200 fraction in tetrahydrofuran residue repair (Fig. 3A, lane H). Thus, PCNA was identified as an essential factor for repair of tetrahydrofuran residues. The *X. laevis* protein was further purified and concentrated by MonoQ chromatography (Fig. 4B, lane 1). We concluded that this protein was *Xenopus* PCNA, based on the observations that it was a 36-kDa protein that reacted with anti-human PCNA antibody and that it stimulated DNA polymerase δ (see below and Table 1).

To identify other repair factors, we subjected the S fraction (or the S′ fraction in later experiments) to further chromatography and assayed repair activity in the presence of PCNA. Chromatography on a Q Sepharose column resulted in loss of activity for the bound fractions. Repair activity was markedly stimulated by readdition of the flowthrough fraction (Fig. 4A, lane 2). Experiments with prelabeled DNA substrates showed that virtually all of the AP endonuclease activity was contained in the Q flowthrough fraction. We purified this AP endonuclease as described in Materials and Methods. Both purified *Xenopus* AP endonuclease and human recombinant Ref-1

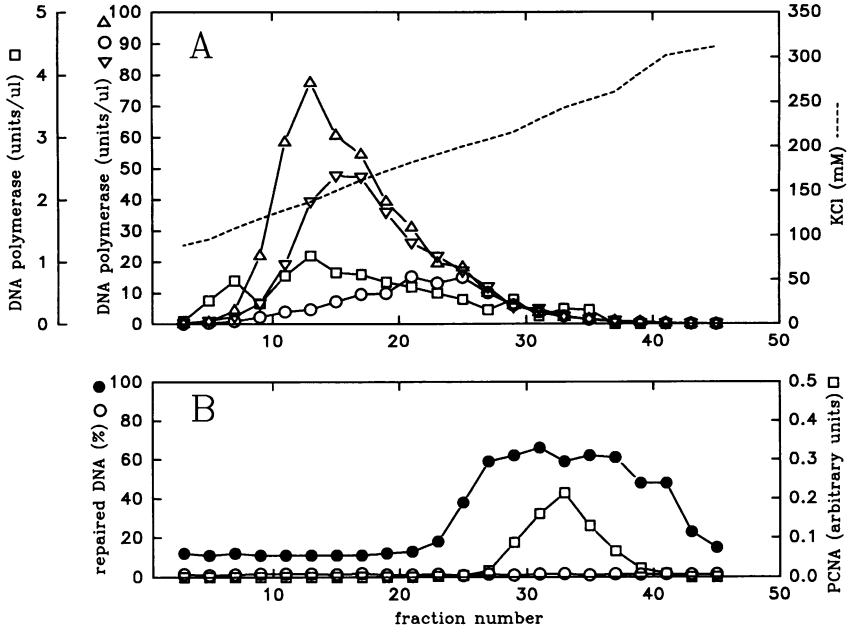

FIG. 2. DEAE-Sepharose chromatography of F3. (A) DNA polymerase activities in the DEAE-bound fractions. For DNA polymerases α, δ, and ε, the activity was measured on poly(dA)/oligo(dT) as described in Materials and Methods in the absence (▽) or the presence of BuPdGTP (○) or BuPdGTP plus PCNA (△). For DNA polymerase β, the activity was measured on poly(rA)/oligo(dT) (□). (B) DNA repair activity and PCNA in the DEAE-bound fractions. The repair activity was measured on the tetrahydrofuran residue-containing DNA by adding 1 μl of DEAE fractions only (○) or 0.1 μl of DEAE fractions plus 2 μl of the S fraction (●) to the repair reaction mix. The PCNA-like protein in DEAE fractions (10 μl) (□) was detected by immunoblotting with monoclonal anti-human PCNA antibody, and the blot intensity was quantified by densitometry as described in Materials and Methods.

stimulated the repair activity of the Q fraction (Fig. 4A, lanes 3 and 4). The *Xenopus* AP endonuclease eluted from MonoS consisted of a single polypeptide of approximately 40 kDa (Fig. 4B, lane 2) and was recognized by polyclonal antibody directed against human class II AP endonuclease, Ref-1 (data not shown) (24, 35). The *Xenopus* enzyme cleaved tetrahydrofuran residues as well as natural AP sites at their 5′ side, as observed for the initial incision during repair in the crude extract (15).

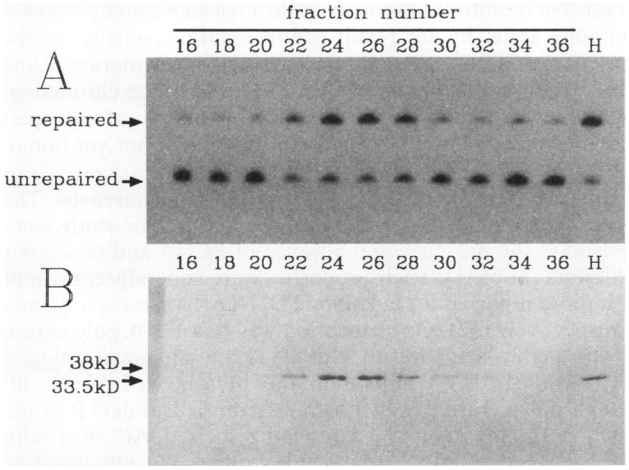

FIG. 3. Copurification of repair-complementing activity and PCNA. (A) DNA repair assays of Superdex200 fractions. The indicated Superdex200 fractions (1 μl of a 1:50 dilution) were assayed for tetrahydrofuran residue repair in the presence of 2 μl of the S fraction. Lane H, 5 ng of recombinant human PCNA purified from bacteria was used in the repair complementation assay. (B) Immunoblot of Superdex200 fractions for PCNA. The indicated Superdex200 fractions (5 μl) or 200 ng of human PCNA was used for blotting as described in Materials and Methods. Arrows indicate the positions of molecular weight marker proteins.

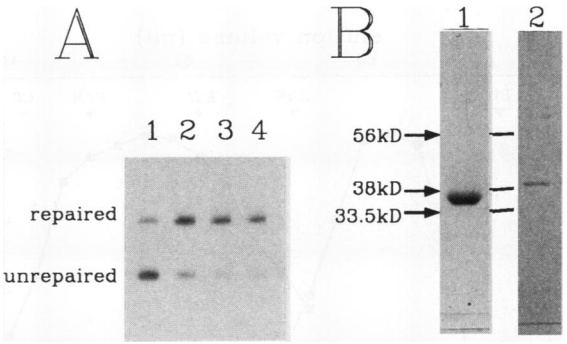

FIG. 4. AP endonuclease as a repair factor. (A) Complementation of the Q fraction with purified AP endonuclease. The repair of tetrahydrofuran residues was assayed with *X. laevis* PCNA and 2 μl of the Q-bound fraction alone (lane 1) or with 2 μl of the Q flowthrough fraction (lane 2), 0.1 μl of *X. laevis* AP endonuclease from MonoS (lane 3), or 5 ng of recombinant human AP endonuclease, Ref-1 (lane 4). The DNA recovered from the reaction mixture was treated with AP endonuclease so that the unrepaired DNA which was not incised at its AP site during the repair reaction was distinguished from the repaired DNA. (B) Gel analysis of purified AP endonuclease and PCNA. PCNA (5 μl) from the MonoQ column step (lane 1) and 2 μl of AP endonuclease from the MonoS column step (lane 2) were loaded on SDS-containing 10% polyacrylamide gels and visualized by staining with Coomassie brilliant blue.

TABLE 1. Effects of PCNA and inhibitors on *X. laevis* DNA polymerases

DNA polymerase	Relative residual activity with PCNA and inhibitor[a]:				
	None	PCNA[b]	Aphidicolin	ddTTP	BuPdGTP
α	1.0	1.19 ± 0.08 (A)	0.18 ± 0.004	0.94 ± 0.07	0.04 ± 0.02
β	1.0	0.9 ± 0.02 (A)	0.97 ± 0.002	<0.01	0.04 ± 0.01
δ + PCNA	1.0	0.1 ± 0.02 (O)	0.05 ± 0.008	1.02 ± 0.05	1.02 ± 0.02

[a] Values represent residual activities relative to the control value with no inhibitor, calculated from averages (± standard deviation) for three assays. Note that the assay condition for DNA polymerase β was distinct from those for DNA polymerases α and δ plus PCNA (see Materials and Methods). Activity in the control assay was 23.5 U for α, 47.8 U for β, and 12.4 U for δ plus PCNA. The concentrations of inhibitors were 30 μg of aphidicolin per ml, 100 μM ddTTP (ddTTP-dTTP, 2:1), and 30 μM BuPdGTP.

[b] PCNA was added (A) (0.5 μg/25-μl reaction mix) for the DNA polymerase α and β assays. For DNA polymerase δ, PCNA was always present in the reaction mix but was omitted (O) for this experiment.

These properties are characteristic of a class II AP endonuclease.

The requirement of PCNA for tetrahydrofuran residue repair suggested that DNA polymerase δ or ε could be involved in this reaction. To directly identify the DNA polymerase responsible for the repair, we further fractionated the Q fraction, which still included DNA polymerase α, β, and δ activities. After size exclusion chromatography of this fraction on Superdex200, we found that no single fraction retained good repair-complementing activity. However, two separate peaks of activity were identified when we assayed late-eluting fractions in the presence of the pooled high-molecular-weight proteins (fractions 8 to 16) and when we assayed early-eluting fractions in the presence of the pooled lower-molecular-weight proteins (fractions 26 to 34), as shown in Fig. 5. The two gel filtration fractions were referred to as BE-1 (high molecular weight) and BE-2 (low molecular weight), respectively. The purification of repair activities in these fractions is briefly described below and will be reported in greater detail elsewhere.

The BE-1 fraction contained DNA polymerase α and δ activities, while the BE-2 fraction contained DNA polymerase β activity. Subsequent fractionation of BE-2 on ssDNA-cellulose separated the repair-complementing activity for tetrahydrofuran residues from DNA polymerase β activity. DNA polymerase β activity was further purified as described in Materials and Methods. The final fraction from MonoS was nearly homogeneous, as determined by SDS-polyacrylamide gel electrophoresis, with a single major polypeptide of 42 kDa (Fig. 6C). This observation and the characteristic sensitivities to various inhibitors (see Table 1) identified this enzyme as DNA polymerase β. BE-2 was separated from DNA polymerase β and purified through several chromatography steps as described in Materials and Methods. The BE-2 active fraction from a hydroxyapatite column still contained several polypeptides detected by silver staining (data not shown).

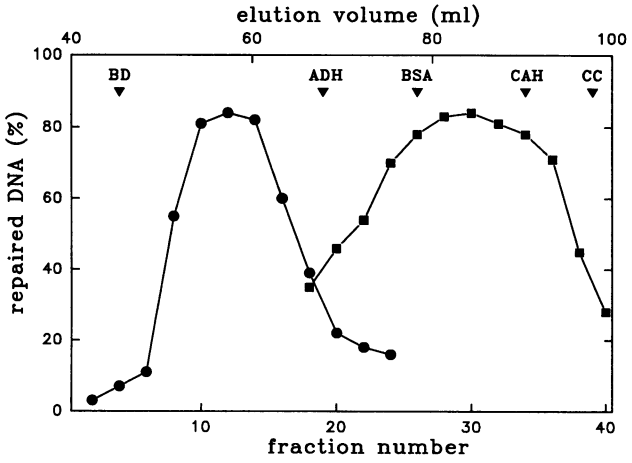

FIG. 5. Superdex200 gel filtration chromatography of the Q fraction. The repair activity for tetrahydrofuran residues was assayed with 1 μl of the Superdex200 fractions in the presence of *X. laevis* PCNA, *X. laevis* AP endonuclease, and 1 μl of the pooled Superdex200 fractions, either 8 to 16 (■) or 26 to 34 (●). Arrows indicate the peak positions of the gel filtration standards blue dextran (BD; 2,000 kDa), alcohol dehydrogenase (ADH; 150 kDa), BSA (66 kDa), carbonic anhydrase (CAH; 29 kDa), and cytochrome *c* (CC; 12.4 kDa).

Further chromatography of gel filtration fraction BE-1 on an ssDNA-cellulose column divided it into two activities, designated BE-1A and BE-1B, both of which were required for tetrahydrofuran residue repair in addition to PCNA, AP endonuclease, and BE-2. Fraction BE-1A contained DNA polymerase α and δ activities. These two polymerase activities were purified further as described in Materials and Methods. The BE-1A activity coeluted with DNA polymerase δ activity through three chromatography steps. BE-1B eluted after DNA polymerase, and its function in repair is not completely understood. We monitored this activity through subsequent chromatography steps by its repair-complementing activity in the presence of PCNA, AP endonuclease, DNA polymerase δ, and BE-2. The fraction obtained after P11 and MonoS chromatography showed no cross-contamination by the other four repair activities (see below), although this fraction is not yet homogeneous.

Characterization of three *X. laevis* DNA polymerases. The three *Xenopus* DNA polymerases purified in this study were assayed in the presence and absence of PCNA and of various inhibitors (Table 1). Their properties were generally consistent with those reported for eukaryotic DNA polymerases α, β, and δ, respectively (32). One exception was that DNA polymerase β was highly sensitive to BuPdGTP in an assay using a poly(rA)/oligo(dT) template in the presence of Mn^{2+}, although polymerase β was more resistant to BuPdGTP in the reaction on the template containing incised AP sites with Mg^{2+} instead of Mn^{2+} (data not shown). We also tried to identify a catalytic subunit of each enzyme. For this purpose, we used the DNA polymerase trap method developed by Insdorf and Bogenhagen (10). In this experiment, we used 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUTP as a photoreactive analog of TTP instead of the bromodeoxyuridine triphosphate used in the original study. This modification significantly improved the efficiency of photolabeling in control experiments with bacterial DNA polymerase (data not shown). However, DNA polymerase β was not labeled, probably be-

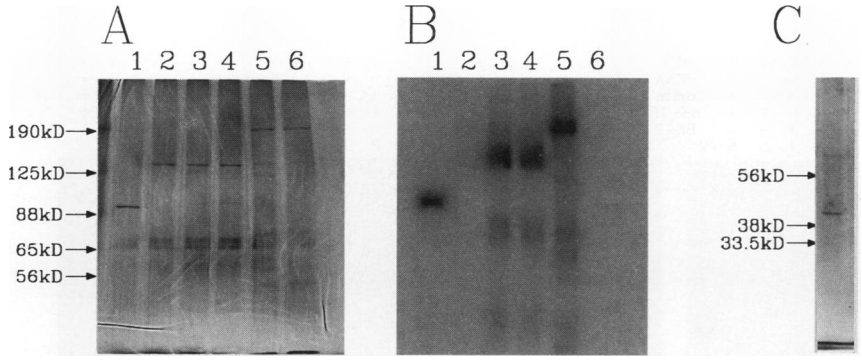

FIG. 6. Purified *X. laevis* DNA polymerases. (A) Silver staining of photolabeled DNA polymerases α and δ in an SDS-containing 7% polyacrylamide gel. (B) Autoradiogram of the gel shown in panel A. The photolabeling experiment was performed as described in Materials and Methods with the following DNA polymerases and additions: 1 U of *Taq* DNA polymerase (lane 1), 60 U of *X. laevis* DNA polymerase δ (lane 2), 60 U of *X. laevis* DNA polymerase δ plus 0.5 μg of PCNA (lane 3), 60 U of *X. laevis* DNA polymerase δ plus 0.5 μg of PCNA and 30 μM BuPdGTP (lane 4), 94 U of *X. laevis* DNA polymerase α (lane 5), and 94 U of *X. laevis* DNA polymerase α plus 30 μM BuPdGTP (lane 6). (C) Silver staining of *X. laevis* DNA polymerase β (950 U) in an SDS-containing 10% polyacrylamide gel.

cause this enzyme did not incorporate 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUTP efficiently. Therefore, we used this method only for DNA polymerases α and δ.

Photolabeling of these enzymes clearly identified the largest polypeptides in DNA polymerases α and δ, approximately 180 and 130 kDa, respectively, as catalytic subunits (Fig. 6). The size of this large subunit in our preparation of DNA polymerase α enzyme is close to that of the large subunit of *X. laevis* DNA polymerase α reported by Kaiserman and Benbow (11), although we do not know which of their two species corresponds to our enzyme. In the case of DNA polymerases δ and ε, there have been no reports of these enzymes purified from *X. laevis*. DNA polymerase δ from HeLa cells and calf thymus has catalytic subunits of 125 kDa and requires PCNA for activity. DNA polymerase ε from HeLa cells is a 220-kDa polypeptide (21), and that purified from calf thymus contains a 210-kDa polypeptide and a 145-kDa polypeptide that is likely to be a proteolytic product of the 210-kDa polypeptide (28). Lee et al. reported that DNA polymerase ε was stimulated by PCNA as well as single-stranded binding protein and activator 1 protein (A1; also called replication factor C [RF-C]) in the presence of salt (14). The DNA polymerase δ purified here required PCNA for polymerase activity at both low and high salt concentrations, implying that it is genuine DNA polymerase δ.

Reconstitution of AP site repair with purified factors. To elucidate the functional roles of each purified factor in AP site repair, we performed repair reactions for either a tetrahydrofuran residue or a natural AP site with various combinations of five fractions. The use of the AP site-containing DNAs which were labeled at a distance of several nucleotides from either the 5′ or 3′ side of the AP site allowed us to analyze DNA products accumulated during incomplete repair reactions. In experiments with the natural AP site, the DNA was treated with $NaBH_4$ immediately after the repair reaction. This treatment protects the remaining AP site from β-elimination during subsequent processing (9).

As shown in Fig. 7, all five fractions were essential for repair of both types of AP sites. AP endonuclease is required to initiate the multistep repair reaction. When it was omitted from the reaction mixture, the substrate DNA was not incised adjacent to the lesion (data not shown). Three factors, DNA polymerase δ, PCNA, and BE-1B, were required for DNA synthesis, which involved incorporation of a single nucleotide at the 3′ end produced by AP endonuclease cleavage (lanes 5 in Fig. 7A and B). This result is consistent with the observation made for the mechanism of repair in the crude extract, that DNA synthesis precedes excision of the damage (16). The 5′ terminus resulting from AP endonuclease cleavage remained intact except when DNA polymerase was omitted (lanes 9 in Fig. 7A and B). It is noteworthy that the hypothetical product lacking a deoxyribose phosphate group at its 5′ terminus was not detected at all during repair of the synthetic or natural AP site (this hypothetical intermediate should comigrate with molecular marker III). This observation suggests that DNA deoxyribophosphodiesterase (dRPase), which catalyzes the release of a deoxyribose phosphate residue after incision by class II AP endonuclease at AP sites (7), is not involved in repair in this reconstituted system. A 5′→3′ exonuclease appeared to be involved in the repair reaction in place of dRPase. This exonuclease activity seemed to be suppressed by DNA polymerase δ (see lanes 9 of Fig. 7A and B). Comparison of panels A and B of Fig. 7 shows that in this reconstituted system, synthetic and natural AP sites appear to be repaired in the same manner.

DNA polymerase β-dependent natural AP site repair. The series of chromatographic steps reported here removed DNA polymerases α and β from the set of factors essential for tetrahydrofuran residue repair. To test the possibility that these DNA polymerases may be able to substitute for DNA polymerase δ in the repair reaction, we compared the three DNA polymerases for repair of synthetic and natural AP sites in the presence of the other four fractions. As shown in Fig. 8A, only DNA polymerase δ repaired tetrahydrofuran residues. On the other hand, DNA polymerase β supported the repair of natural AP sites, although the efficiency of repair reached a plateau level lower than that observed for DNA polymerase δ (Fig. 8B). The DNA polymerase β-dependent repair did not require PCNA (data not shown). However, our preparation of DNA polymerase β contained dRPase activity (data not shown). It is likely that the ability of DNA polymerase β to function in PCNA-independent repair depends on this dRPase activity (6). DNA polymerase α did not substitute for DNA polymerase δ in the repair of either type of AP site.

Effects of aphidicolin and ddTTP on AP site repair. Various inhibitors have been used to determine which DNA polymerase is involved in DNA repair. These experiments are based on the assumption that each DNA polymerase should

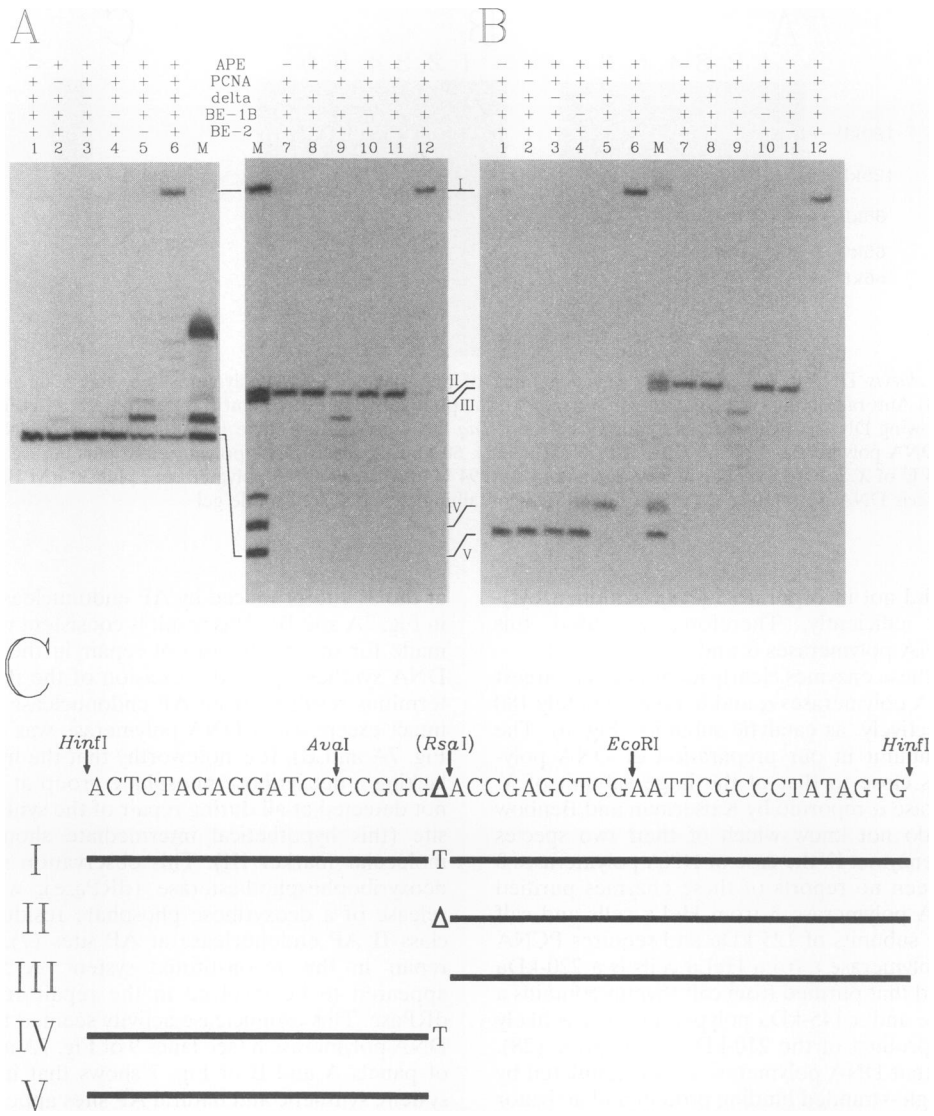

FIG. 7. AP site repair reconstituted with purified factors. (A) Repair of tetrahydrofuran residues. (B) Repair of natural AP sites. The fractions indicated above each lane were added as indicated (+) to the repair reaction mix. The fractions of AP endonuclease (APE), PCNA, DNA polymerase δ (delta), BE-1B, and BE-2 used in this experiment are also shown boxed in Fig. 1. After the repair reaction, the DNA was digested with *Hin*fI and AP endonuclease and analyzed as described in Materials and Methods. In both panels, the DNA prelabeled at the *Ava*I site on the 5′ side of the AP site was used as a repair substrate in lanes 1 to 6, and the DNA prelabeled at the *Eco*RI site on the 3′ side of the AP site was used as a repair substrate in lanes 7 to 12. Lanes M, molecular size markers (identified in panel C). (C) Structure of the AP site-containing *Hin*fI fragment and repair intermediates. The molecular size markers used in panels A and B were prepared by digestion of the tetrahydrofuran residue-containing prelabeled DNA with *Hin*fI and AP endonuclease (markers II and V) or by digestion of the unmodified prelabeled DNA with *Hin*fI and *Rsa*I (markers III and IV). An AP site is designated by △.

reveal the same pattern of sensitivities to inhibitors in the DNA repair reaction as in the DNA polymerase assay, although this assumption has not been extensively tested. We used our reconstituted system of AP site repair to test this. As shown in Fig. 9, tetrahydrofuran residue repair with the early F3 fraction was moderately sensitive to aphidicolin and ddTTP, while natural AP site repair with this fraction was relatively resistant to aphidicolin but sensitive to ddTTP. The repair of both AP sites by the reconstituted system, including DNA polymerase δ as the only DNA polymerase, was strongly inhibited by aphidicolin but not by ddTTP. The natural AP site repair by the DNA polymerase β-dependent pathway was blocked by ddTTP but not by aphidicolin. These results with individual enzymes indicate that the effects of aphidicolin and ddTTP on AP site repair reflect the sensitivities of DNA polymerases to the inhibitors as measured in standard DNA synthesis assays. When both DNA polymerases β and δ were added to the reconstituted system, however, the repair of tetrahydrofuran residues was blocked by either inhibitor. This observation indicates that DNA polymerase β may interfere with the repair by DNA polymerase δ even though DNA polymerase β does not support repair of the tetrahydrofuran residue. The mechanism of this interference has not been established. However, we have observed that DNA polymerase β was able to attach a single nucleotide to the 3′ terminus produced by AP endonuclease at a tetrahydrofuran residue (unpublished result). This incomplete repair product seems to be fully repaired by the DNA polymerase δ-mediated reaction.

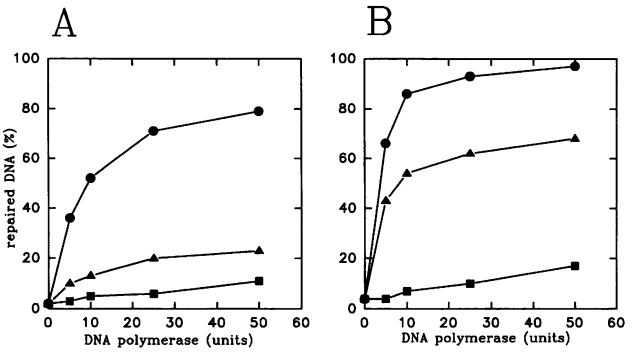

FIG. 8. Comparison of DNA polymerases α, β, and δ in AP site repair. (A) Tetrahydrofuran residue repair. (B) Natural AP site repair. The repair activity was measured with the indicated amounts of *X. laevis* DNA polymerase α (■), β (▲), or δ (●) along with fixed amounts of AP endonuclease, PCNA, BE-1B, and BE-2, as described in Materials and Methods.

It is likely that once the chain terminator analog ddTTP is incorporated by DNA polymerase β, the subsequent action by DNA polymerase δ cannot overcome this block. The result shown in Fig. 9 implies that the effects of these inhibitors on the repair reaction in the presence of more than one DNA polymerase may not predict correctly which enzyme is responsible for the reaction.

DISCUSSION

PCNA is involved in base excision repair. In this paper, we present biochemical fractionation of a *Xenopus* oocyte extract that efficiently repairs natural and synthetic abasic sites in DNA. Three of the repair factors have been purified to near homogeneity. Repair is initiated by cleavage on the 5′ side of the lesion by a class II AP endonuclease that appears to be the *Xenopus* homolog of the major human AP endonuclease, also known as Ref-1 (24, 35). The other two purified repair factors are PCNA and DNA polymerase δ. PCNA has a well-established identity as a processivity factor that stimulates DNA polymerase δ and, under some conditions, ε. It is required for DNA replication from the simian virus 40 origin in an in vitro reconstituted system (22). Recently, two groups have reported that PCNA was an essential factor for nucleotide excision repair in reconstituted systems derived from HeLa cells (20, 27). There is a significant mechanistic difference between these studies of nucleotide excision repair and the present work on base excision repair. Nucleotide excision repair requires synthesis of approximately 30 nucleotides of DNA per damaged residue. Removal of 30 nucleotides surrounding the site of damage may explain the apparent requirements for ssDNA-binding (SSB) protein and helicase (4, 34). The resulting gap might be considered large enough to resemble a replication fork. In contrast, incorporation of no more than four nucleotides is sufficient for repair of tetrahydrofuran residues (15), and there is no reason to suggest the occurrence of a substantial gap in the damaged DNA strand.

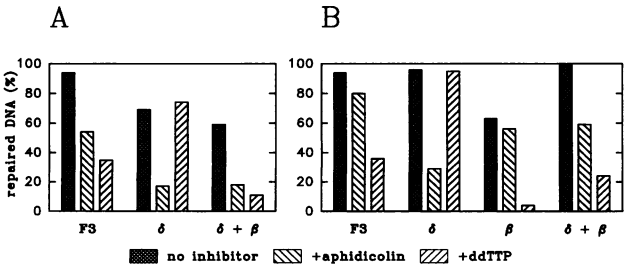

FIG. 9. Effects of inhibitors on AP site repair. (A) Repair of tetrahydrofuran residues. (B) Repair of natural AP sites. The repair efficiency with the F3 fraction (20 μg of protein) or the indicated DNA polymerase (25 U of each) plus four other factors was measured as described in Materials and Methods. Aphidicolin (30 μg/ml) or ddTTP (100 μM) was added to the reaction mix as indicated.

The demonstration that PCNA can function in short-patch DNA repair is paradoxical in light of its role as a processivity factor for DNA polymerase δ and/or ε (14, 30). It is likely that PCNA would serve some function other than to increase the processivity of DNA polymerase in base excision repair. Ng et al. (19) have demonstrated that PCNA can stimulate the incorporation of even a single nucleotide by DNA polymerase δ on oligonucleotide templates. Several reports indicated that PCNA interacts with RF-C on a primed DNA template in an ATP-dependent manner, suggesting a function for the PCNA–RF-C complex in increasing the binding of DNA polymerase to a DNA primer (13, 31). A similar mechanism may function in base excision repair. This model is consistent with our previous observation that repair factors form a complex at an abasic site in an ATP-dependent manner prior to repair-related DNA synthesis (16). It remains to be determined whether RF-C, SSB protein, or helicase is included in the two incompletely purified fractions, designated BE-1B and BE-2, that are essential for PCNA-dependent AP site repair. Fractions BE-1B and BE-2 may be expected to contain other enzymatic activities required for repair, such as a 5′→3′ exonuclease and DNA ligase. However, we note that the BE-1B fraction might include RF-C, since it behaves in an appropriate manner through several chromatography steps. If RF-C can be shown to be involved in repair, its interaction with the damaged DNA substrate would presumably differ from that observed at a synthetic replication fork, where it is thought to make extensive contacts with ssDNA (31).

A replicative DNA polymerase is active in short-patch repair. Over the last several years, a variety of studies using crude complementation assays or drug inhibitors have suggested that DNA polymerase δ or ε plays an important role in DNA repair. The demonstration that PCNA is required for nucleotide excision repair provided additional circumstantial evidence to implicate either DNA polymerase δ or ε in DNA repair. The present study is the first to directly show that highly purified DNA polymerase δ is able to participate in DNA repair synthesis. It is possible that DNA polymerase ε may be able to substitute for DNA polymerase δ in PCNA-dependent base excision repair. We were not able to examine this possibility because the *X. laevis* oocyte extracts that we used in this study did not provide a sufficient yield of DNA polymerase ε for fractionation. Recently, Wang et al. used both genetic and biochemical methods to show that DNA polymerase ε is involved in repair of AP sites in *Saccharomyces cerevisiae* (33). Unlike DNA polymerase α, DNA polymerase ε shares many characteristics with DNA polymerase δ, such as sensitivity to aphidicolin, resistance to ddNTP and BuPdGTP, and stimulation by PCNA and RF-C.

We also observed that natural AP sites but not tetrahydrofuran residues were repaired by the DNA polymerase β-mediated pathway, which was not dependent on PCNA. The inability of DNA polymerase β to repair tetrahydrofuran residues seems to be due to a structural difference between natural AP sites and synthetic AP sites. The tetrahydrofuran

residue has a hydrogen in the place of a hydroxyl group at the 1′ position of the natural AP site and is resistant to cleavage by β-elimination. Several repair-related enzymes, such as AP lyase (formerly called class I AP endonuclease) and dRPase appear to be inactive at a tetrahydrofuran residue (1, 6). It is likely that one of these enzymes is essential for DNA polymerase β-dependent repair.

Base excision repair of uracil residues by mammalian cell extracts was characterized by Dianov et al. (6). They demonstrated that a uracil residue was repaired through a single-nucleotide replacement and that this reaction was more sensitive to ddNTP than to aphidicolin. Thus, the repair mechanism observed by Dianov et al. (6) seems to be equivalent to the DNA polymerase β-dependent pathway in this study. One of the differences between the experimental system in our study and the one employed by Dianov et al. (6) is the origin of extracts: one was prepared from *X. laevis* oocytes, and the other was prepared from mammalian cultured cells. This may not be crucial, however, since we observed efficient repair of tetrahydrofuran residues in HeLa cell extracts, suggesting that the PCNA-dependent pathway is functional in mammalian cells as well (unpublished data). Another difference between the two experimental systems is that we used 3.2-kb cccDNA as a repair substrate, while Dianov et al. (6) used 30-nucleotide duplex oligonucleotides. This substrate may have contributed to the apparent dominance of the DNA polymerase β-dependent pathway in their system if short oligonucleotides do not permit assembly of the PCNA-dependent repair complex. We observed that a tetrahydrofuran residue in a 54-bp restriction fragment was not efficiently repaired (Y. Matsumoto, unpublished observation).

The results described here indicate that natural AP sites can be repaired by two distinct pathways, one of which may serve as the other's back-up in vivo. We can then ask whether PCNA-dependent short-patch repair is essential for cellular resistance to DNA damage. Two reports suggested the importance of the PCNA-dependent pathway in DNA repair processes that are unlikely to employ a nucleotide excision pathway. Miura et al. (18) observed that X irradiation as well as UV irradiation induced tight association of PCNA at sites of unscheduled DNA synthesis. Satoh et al. (26) reported that DNA synthesis relating to repair of single-strand breaks generated by γ irradiation was inhibited by aphidicolin but not by ddTTP, suggesting the involvement of DNA polymerase α, δ, or ε. The DNA damage which is induced by ionizing radiation such as X and γ rays has been considered to be corrected through short-patch repair. These radiation sources generate various types of DNA lesions, including modified bases and strand breaks. Like the tetrahydrofuran residue that we used, some of these lesions may not be good substrates for the DNA polymerase β-dependent pathway. In these cases, it is possible that the PCNA-dependent pathway described here may be utilized for repair.

ACKNOWLEDGMENTS

We thank G. Wright for the gift of BuPdGTP, K. Fien and B. Stillman for the gift of monoclonal anti-human PCNA antibody and purified human PCNA protein, S. Xanthoudakis and T. Curran for the gift of purified Ref-1 protein and anti-Ref-1 antibody, and R. Rieger for synthesis of modified oligonucleotides.

This work was supported by the National Institute of Environmental Health Sciences (grant ES04068), the American Cancer Society (grant IRG-191), the National Institutes of Health (grant CA-06927), the Pew Charitable Trusts, and an appropriation from the Commonwealth of Pennsylvania.

REFERENCES

1. **Bailly, V., and W. G. Verly.** 1988. Possible roles of β-elimination and δ-elimination reactions in the repair of DNA containing AP (apurinic/apyrimidinic) sites in mammalian cells. Biochem. J. **253:**553–559.
2. **Bartholomew, B., G. A. Kassavetis, B. R. Braun, and E. P. Geiduschek.** 1990. The subunit structure of *Saccharomyces cerevisiae* transcription factor IIIC probed with a novel photocrosslinking reagent. EMBO J. **9:**2197–2205.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Coverley, D., M. K. Kenny, M. Munn, W. D. Rupp, D. P. Lane, and R. D. Wood.** 1991. Requirement for the replication protein SSB in human DNA excision repair. Nature (London) **349:**538–541.
5. **Cozzarelli, N. R., S. P. Gerrard, M. Schlissel, D. D. Brown, and D. F. Bogenhagen.** 1983. Purified RNA polymerase III accurately and efficiently terminates transcription of 5S RNA genes. Cell **34:**829–835.
6. **Dianov, G., A. Price, and T. Lindahl.** 1992. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. Mol. Cell. Biol. **12:**1605–1612.
7. **Franklin, W. A., and T. Lindahl.** 1988. DNA deoxyribophosphodiesterase. EMBO J. **7:**3617–3622.
8. **Friedberg, E. C.** 1985. DNA repair. W. H. Freeman Co., New York.
9. **Hadi, S.-M., and D. A. Goldthwait.** 1971. Endonuclease II of *Escherichia coli*. Degradation of partially depurinated deoxyribonucleic acid. Biochemistry **10:**4986–4994.
10. **Insdorf, N. F., and D. F. Bogenhagen.** 1989. DNA polymerase γ from *Xenopus laevis*. I. The identification of a high molecular weight catalytic subunit by a novel DNA polymerase photolabeling procedure. J. Biol. Chem. **264:**21491–21497.
11. **Kaiserman, H. B., and R. M. Benbow.** 1987. Characterization of a stable, major DNA polymerase α species devoid of DNA primase activity. Nucleic Acids Res. **15:**10249–10265.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
13. **Lee, S.-H., and J. Hurwitz.** 1990. Mechanism of elongation of primed DNA by DNA polymerase δ, proliferating cell nuclear antigen, and activator 1. Proc. Natl. Acad. Sci. USA **87:**5672–5676.
14. **Lee, S.-H., Z.-Q. Pan, A. D. Kwong, P. M. J. Burgers, and J. Hurwitz.** 1991. Synthesis of DNA by DNA polymerase ε *in vitro*. J. Biol. Chem. **266:**22707–22717.
15. **Matsumoto, Y., and D. F. Bogenhagen.** 1989. Repair of a synthetic abasic site in DNA in a *Xenopus laevis* oocyte extract. Mol. Cell. Biol. **9:**3750–3757.
16. **Matsumoto, Y., and D. F. Bogenhagen.** 1991. Repair of a synthetic abasic site involves concerted reactions of DNA synthesis followed by excision and ligation. Mol. Cell. Biol. **11:**4441–4447.
17. **Millican, T. A., G. A. Mock, M. A. Chauncey, T. P. Patel, M. A. W. Eaton, J. Gunning, S. D. Cutbush, S. Neidle, and J. Mann.** 1984. Synthesis and biophysical studies of short oligodeoxynucleotides with novel modifications: a possible approach to the problem of mixed base oligodeoxynucleotide synthesis. Nucleic Acids Res. **12:**7435–7453.
18. **Miura, M., M. Domon, T. Sasaki, and Y. Takasaki.** 1992. Induction of proliferating cell nuclear antigen (PCNA) complex formation in quiescent fibroblasts from a *xeroderma pigmentosum* patient. J. Cell. Physiol. **150:**370–376.
19. **Ng, L., C.-K. Tan, K. M. Downey, and P. A. Fisher.** 1991. Enzymologic mechanism of calf thymus DNA polymerase δ. J. Biol. Chem. **266:**11699–11704.
20. **Nichols, A. F., and A. Sancar.** 1992. Purification of PCNA as a nucleotide excision repair protein. Nucleic Acids Res. **20:**2441–2446.
21. **Nishida, C., P. Reinhard, and S. Linn.** 1988. DNA repair synthesis in human fibroblasts requires DNA polymerase δ. J. Biol. Chem. **263:**501–510.
22. **Prelich, G., M. Kostura, D. R. Marshak, M. B. Mathews, and B. Stillman.** 1987. The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication *in vitro*. Nature

(London) **326:**471–475.

23. **Randall, S. K., R. Eritja, B. E. Kaplan, J. Petruska, and M. F. Goodman.** 1987. Nucleotide insertion kinetics opposite abasic lesions in DNA. J. Biol. Chem. **262:**6864–6870.
24. **Robson, C. N., and I. D. Hickson.** 1991. Isolation of cDNA clones encoding a human apurinic/apyrimidinic endonuclease that corrects DNA repair and mutagenesis defects in *E. coli xth* (exonuclease III) mutants. Nucleic Acids Res. **19:**5519–5523.
25. **Satoh, M. S., and T. Lindahl.** 1992. Role of poly(ADP-ribose) formation in DNA repair. Nature (London) **356:**356–358.
26. **Satoh, M. S., G. G. Poirier, and T. Lindahl.** 1993. NAD^+-dependent repair of damaged DNA by human cell extracts. J. Biol. Chem. **268:**5480–5487.
27. **Shivji, M. K. K., M. K. Kenny, and R. D. Wood.** 1992. Proliferating cell nuclear antigen is required for DNA excision repair. Cell **69:**367–374.
28. **Siegal, G., J. J. Turchi, C. B. Jessee, L. M. Mallaber, R. A. Bambara, and T. M. Myers.** 1992. Structural relationships between two forms of DNA polymerase ε from calf thymus. J. Biol. Chem. **267:**3991–3999.
29. **Takeshita, M., C.-N. Chang, F. Johnson, S. Will, and A. P. Grollman.** 1987. Oligodeoxynucleotides containing synthetic abasic sites: model substrates for DNA polymerases and apurinic/apyrimidinic endonucleases. J. Biol. Chem. **262:**10171–10179.
30. **Tan, C.-K., C. Castillo, A. G. So, and K. M. Downey.** 1986. An auxiliary protein for DNA polymerase-δ from fetal calf thymus. J. Biol. Chem. **261:**12310–12316.
31. **Tsurimoto, T., and B. Stillman.** 1991. Replication factors required for SV40 DNA replication *in vitro*. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins. J. Biol. Chem. **266:**1950–1960.
32. **Wang, T. S.-F.** 1991. Eukaryotic DNA polymerases. Annu. Rev. Biochem. **60:**513–552.
33. **Wang, Z., X. Wu, and E. C. Friedberg.** 1993. DNA repair synthesis during base excision repair in vitro is catalyzed by DNA polymerase ε and is influenced by DNA polymerases α and δ in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**1051–1058.
34. **Weeda, G., L. Ma, R. C. A. van Ham, A. J. van der Eb, and J. H. J. Hoeijmakers.** 1991. Structure and expression of the human *XPBC/ERCC-3* gene involved in DNA repair disorders xeroderma pigmentosum and Cockayne's syndrome. Nucleic Acids Res. **19:**6301–6308.
35. **Xanthoudakis, S., G. Miao, F. Wang, Y.-C. E. Pan, and T. Curran.** 1992. Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. EMBO J. **11:**3323–3335.