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Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: Definition of a family of DNA repair enzymes

(*Homo sapiens*/DNA alkylation damage/interspecies complementation/exonuclease III)

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ABSTRACT **Abasic (AP) sites are common, potentially mutagenic DNA damages that are attacked by AP endonucleases. The biological roles of these enzymes in metazoans have not been tested. We have cloned the human cDNA (APE) that encodes the main nuclear AP endonuclease. The predicted Ape protein, which contains likely nuclear transport signals, is a member of a family of DNA repair enzymes that includes two bacterial AP endonucleases (ExoA protein of *Streptococcus pneumoniae* and exonuclease III of *Escherichia coli*) and Rrp1 protein of *Drosophila melanogaster*. Purified Ape protein lacks the 3′-exonuclease activity against undamaged DNA that is found in the bacterial and *Drosophila* enzymes, but the lack of obvious amino acid changes to account for this difference suggests that the various enzyme functions evolved by fine tuning a conserved active site. Expression of the active human enzyme in AP endonuclease-deficient *E. coli* conferred significant resistance to killing by the DNA-alkylating agent methyl methanesulfonate. The APE cDNA provides a molecular tool for analyzing the role of this central enzyme in maintaining genetic stability in humans.**

A prominent insult to cellular DNA is the continuous loss of bases, either through spontaneous reactions such as hydrolytic depurination (1) and free-radical attack (2) or by the action of DNA glycosylases that remove various altered bases (3). The resulting abasic (AP) sites can block the progress of the DNA replication apparatus and cause mutations in *Escherichia coli* (4). Evidently, AP sites must be corrected to restore genetic integrity. The major enzymes initiating this repair process, AP endonucleases, have been identified, and their *in vivo* roles in correcting alkylation-induced AP sites and other DNA damages have been confirmed for bacteria and yeast (4–7). Such molecular information has been lacking for metazoans and particularly for human cells.

The major human AP endonucleases have been purified from various sources (8, 9). These enzymes are modest in size ($M_r \approx 37{,}000$), act efficiently in the absence of other proteins, and lack activity against undamaged DNA (8, 9). Like the microbial AP endonucleases, the human enzymes are multifunctional activities that not only attack AP sites but also remove fragments of deoxyribose from the 3′ termini of DNA strand breaks produced by free-radical attack. For the main AP endonuclease of HeLa cells, this 3′-repair activity is relatively weak [≈1% of the AP-cleaving activity (10)], in contrast to exonuclease III and endonuclease IV of *E. coli* and Apn1 of *Saccharomyces cerevisiae*, in which the 3′-repair and AP-cleaving activities are about equal (11, 12). However, all of these enzymes cleave DNA AP sites in the same way, as hydrolytic (so-called class II) AP endonucleases that incise the phosphodiester just 5′ to the site to generate a normal 3′-terminal 3′-hydroxyl nucleotide and a 5′-terminal deoxyribose-5-phosphate (13). A second class II AP endonuclease is also present in HeLa cells in lesser amounts, and this enzyme has about equal levels of 3′-repair and AP-cleaving activity *in vitro* (41). Neither enzyme appears to be affected by various human genetic diseases (14), including xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome, and Fanconi anemia.

Clearly, molecular tools will be necessary to define the biological role of the human AP endonucleases. We report here the cloning and analysis of the cDNA that codes for the major human AP endonuclease,† which we have named APE.

MATERIALS AND METHODS

The HeLa S3 and MG63 osteosarcoma (15) cell lines were obtained from T. Maniatis (Harvard University). The strains used for cloning and propagation of recombinant DNA were *E. coli* XL1-Blue (Stratagene) and Y1090 (16). Strains BW9109 (Δ*xth*) and BW528 (Δ*xth nfo1::kan*) were stocks of this laboratory, obtained originally from B. Weiss (University of Michigan, Ann Arbor; see ref. 17). Bacteria were cultured at 37°C in Luria–Bertani (LB) broth (16) supplemented with the appropriate antibiotics. The expression and sequencing vector pBluescript KS was obtained from Stratagene. The pPROK1 expression vector and the human placental cDNA library in λgt11 (no. HL1008b) were obtained from Clontech.

Immunological Techniques. The rabbit antisera directed against the major AP endonuclease of HeLa cells are comparable to those described by Kane and Linn (8) but are of higher titer (41). Immunopurification was carried out in either of two ways: by affinity chromatography on a column of purified HeLa AP endonuclease crosslinked to Sepharose CL-4B (LKB-Pharmacia, used according to the manufacturer's specifications) or by adsorption to nitrocellulose blots containing cell-free extracts of selected λgt11 lysogens (18). For immunoblotting, protein samples were transferred from SDS/polyacrylamide gels to nitrocellulose membranes, which were probed by standard procedures (18) with the affinity-purified antisera. Bound antibody was detected with ^{125}I-labeled protein A (New England Nuclear) and autoradiography.

Indirect immunofluorescence staining experiments were carried out with rabbit anti-AP endonuclease IgG, by using methods previously described (19). The IgG fraction was purified from rabbit anti-AP endonuclease antiserum through

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Abbreviations: AP, abasic; MMS, methyl methanesulfonate; IPTG, isopropyl β-D-thiogalactoside.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M80261).

a DEAE-cellulose DE-52 column (18), and the protein was concentrated to 2 mg/ml. The purified IgG was used as the first antibody, and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cappel no. 55646; Organon Teknika–Cappel) as the second antibody.

Molecular Cloning and Sequence Analysis. The placental cDNA expression library in λgt11 was screened (18) using the crude anti-HeLa AP endonuclease antisera of two rabbits. Three successive screens were employed to isolate two positive phages (λHAP1 and λHAP2), which contained identical inserts of human DNA, as judged by restriction mapping. The insert DNA of λHAP1 was released by digestion with *Eco*RI, isolated, and cloned into the pBluescript KS vector to yield plasmid pCW26.

Double-stranded plasmid DNA was sequenced by the dideoxy chain-termination method (16) with Sequenase (United States Biochemical). In addition to the KS and SK primers (Stratagene), other synthetic oligonucleotide primers (5′-GAAAGGATTAGATTGGG, 5′-CCCCTTGTGCTGTGTGG, 5′-TGTTGTCCCACTCTCTG, 5′-GGCGCCAACCAACATTC, 5′-TACTCCAGTCGTACCAG, and 5′-CTTCTTAATCCAGCTCG) were used to complete the sequence of both strands. Computer analysis of the sequence and comparison of the DNA and predicted protein sequences to data bases was performed by using Genetics Computer Group software (Madison, WI).

Expression in *E. coli*. Two oligonucleotides, 5′-CAGCTGCCATGGGGTTCG (for the sense strand) and 5′-TCTCTGAAGCTTGTTTAAAG (for the antisense strand), were used to amplify, by PCR (16, 18), a 1-kilobase fragment from PCW26 plasmid DNA, simultaneously introducing *Nco* I (5′) and *Hin*dIII (3′) sites (underlined above). After digestion with these enzymes, the PCR fragment was subcloned into the pPROK1 plasmid at its *Nco* I and *Hin*dIII sites, to generate plasmid pCW30.

For the assay of human AP endonuclease expressed in *E. coli*, cells from fresh overnight cultures were diluted 100-fold into 5 ml of LB broth and grown at 37°C to ≈2 × 10^8 cells per ml. Where indicated, isopropyl β-thiogalactoside (IPTG) was then added to a final concentration of 1 mM, and the cultures were incubated another 60 min. The cells were harvested and washed in cold extraction buffer (150 mM KCl/50 mM Hepes·KOH, pH 7.5/10% glycerol/1 mM phenylmethylsulfonyl fluoride) and resuspended in 0.5 ml of extraction buffer. The cells were disrupted by mixing in a mini-Beadbeater (Biospec Products, Bartlesville, OK) with glass beads (0.1 mm). Cell debris and glass beads were removed by centrifugation at 12,000 × *g* for 30 min at 4°C. Protein concentrations were determined by the method of Bradford (20). The crude extracts were assayed for class II AP endonuclease activity as described (13). Resistance to toxic agents was measured by using gradient plates as described (6, 17).

RESULTS

The predominant AP endonuclease/3′-repair diesterase has been purified from HeLa cells (8, 10, 41). Rabbit antisera were prepared against this protein and screened for specificity by immunoprecipitation, immunofluorescence, and immunoblotting experiments. One rabbit produced especially high-titer antiserum, which was immunopurified for some experiments by using an AP endonuclease affinity column (see *Materials and Methods*). When employed in immunoblots, these affinity-purified antibodies recognized a single protein (M_r ≈ 37,000) in crude extracts of HeLa cells (Fig. 1*A*, lane 1), which corresponded to the mobility of the purified protein (Fig. 1*A*, lane 2).

The cellular compartment of the AP endonuclease was also determined. The IgG fraction of the above antiserum was employed (without immunopurification) to probe the intracellular location of the AP endonuclease in cultured human and mouse cells. The human osteosarcoma line MG63, which grows as a flat monolayer (15), showed cross-reactive material highly localized to the nucleus (Fig. 1*C*). HeLa S3 cells, which are more rotund, and a mouse fibroblast line (L929; provided by T. Maniatis) also revealed immunoreactive material that appeared to be nuclear-localized (data not shown). This localization to the nucleus is consistent with the putative DNA repair function of the AP endonuclease. The detection of cross-reactive material in mouse cell nuclei corroborates biochemical experiments (22) indicating a murine protein with properties similar to the HeLa AP endonuclease.

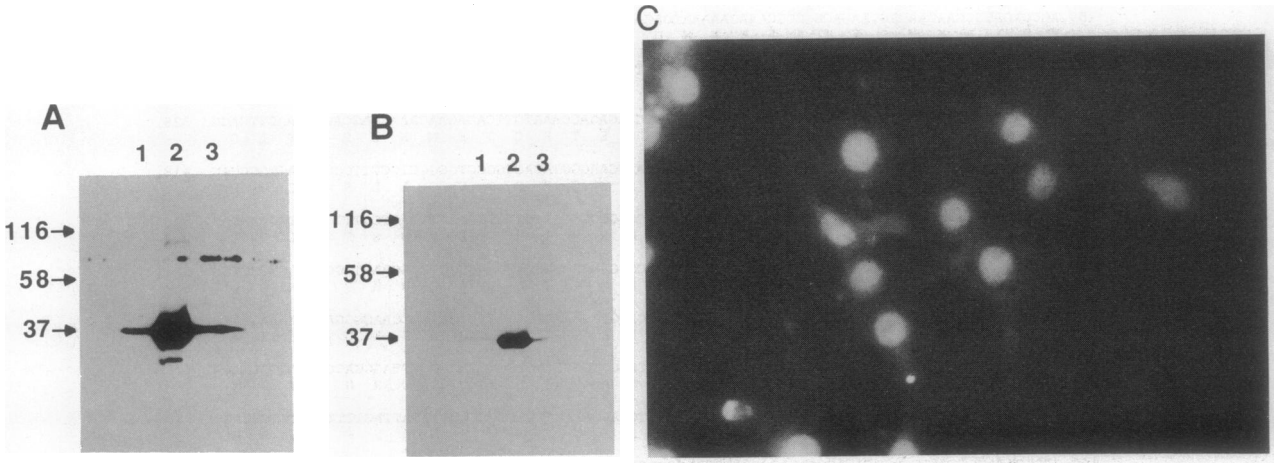

FIG. 1. Specificity of anti-HeLa AP endonuclease antiserum. (*A*) Immunoblot with antiserum immunopurified by using a HeLa AP endonuclease affinity column. Lane 1, HeLa crude extract (100 μg); lane 2, purified enzyme (0.5 μg; fraction Vb; ref. 41); lane 3, extract (100 μg) from a λHAP lysogen induced with IPTG (21). Marker proteins (indicated at left in $M_r \times 10^{-3}$) are β-galactosidase (116,000), pyruvate kinase (58,000), and HeLa AP endonuclease (37,000). The staining between M_r values of 58,000 and 116,000 is an artifact, because it appears in lanes where no sample was loaded (to the left of lane 1 and the right of lane 3) and was not seen in other experiments. (*B*) Immunoblot with antiserum immunopurified using λHAP1-encoded protein. The samples and markers were as in *A*. Lanes 1 and 3 are fainter than in *A* but are clearly visible after longer autoradiography. These bands are not due to spillover from lane 2, because they were also observed in samples loaded in isolation. (*C*) Intracellular location of AP endonuclease. Human osteosarcoma MG63 cells (15) were grown, fixed, and stained with the IgG fraction of rabbit anti-HeLa AP endonuclease antibodies as described (15, 18, 19). A faint staining of the cell bodies can be seen, but the predominant location of the immunoreactive material is clearly nuclear.

Molecular Cloning. A human placental cDNA expression library in λgt11 (21) was screened (16, 18) with the crude antisera of two rabbits. From 1.6×10^6 recombinant phage, two phage plaques were identified that tested positive through three successive screens. Extracts containing the protein(s) expressed by one of these phages, λHAP1, was used to adsorb antibodies from crude antiserum, which were then eluted and used for immunoblotting. These antibodies were specific for the HeLa AP endonuclease (Fig. 1*B*), as expected for recombinant phage expressing the human protein. Both these antibodies and those purified by affinity chromatography on an AP endonuclease column recognized a λHAP1-encoded protein of M_r ≈37,000 in immunoblots (lanes 3 in Fig. 1 *A* and *B*). Thus, although expressed from λgt11 (21), the human protein encoded by λHAP1 is apparently not fused to β-galactosidase ($M_r \approx 116{,}000$).

Subcloning and Sequence Analysis. Both λHAP1 and a second positive phage, λHAP2, contained the same human cDNA insert of ≈1.3 kilobases, as determined from their physical maps (data not shown). The insert from λHAP1 was subcloned into the pBluescript KS vector to yield plasmid pCW26. Determination of the cDNA sequence in this recombinant plasmid revealed a single long open reading frame that would encode a protein of M_r 35,589 (Fig. 2). No other initiation codon is specified by the DNA 5′ to the ATG designated as base pairs 1–3 in Fig. 2, and the DNA sequence 5′ to this putative initiator codon had at least one translational stop codon in each reading frame. Since the DNA sequence at base pairs −18 to −14 encodes a potential bacterial ribosome-binding site (AAGGG), the M_r ≈37,000 polypeptide expressed in *E. coli* (λHAP1) lysogens is probably not synthesized as a fusion protein. Most importantly, residues 2–26 of the predicted protein matched the N-terminal sequence we had already determined for the AP endonuclease purified from HeLa cells (Fig. 2 and legend). This observation simultaneously confirmed the correctness of the suggested open reading frame delineated in Fig. 2 and the authenticity of the cloned DNA. We have named this cDNA APE to signify human *AP E*ndonuclease.

The predicted Ape polypeptide has one additional N-terminal residue compared to the purified enzyme, the initiator methionine (Fig. 2), which is presumably removed by conventional posttranslational processing in HeLa cells. As was already apparent from the limited sequence obtained from the purified protein, this N-terminal region contains a likely nuclear localization signal (KRGK, residues 3–6); two other possible nuclear targeting signals are also present (KKSK, residues 24–27; KKNDK, residues 31–35). These putative signals are closely related to consensus targeting sequences (24, 25) and are consistent with the strong localization of this protein to the nucleus of human cells (Fig. 1*C*).

A Family of AP Endonucleases. A comparison of the predicted Ape protein to polypeptides in available data bases revealed significant homology to only two proteins, ExoA of *Streptococcus pneumoniae* (26) and exonuclease III of *E. coli* (27), with identities of 41% and 28%, respectively (Fig. 3). The predicted *Homo sapiens* Ape protein (M_r 35,600) contained at its N terminus additional peptide sequences not found in the somewhat smaller bacterial enzymes (ExoA, M_r 31,263; exonuclease III, M_r 30,921). Both ExoA and exonuclease III are AP endonucleases that are also active as 3′ → 5′ exonucleases against undamaged duplex DNA (28, 29, 41), an activity not found for purified HeLa or placental AP endonucleases (8, 9).

A *Drosophila melanogaster* protein that produces joint DNA molecules *in vitro* has been partially purified (30), and its structural gene has been cloned (31). The predicted Rrp1 protein has a M_r of 75,000 but contains a 240-residue, C-terminal region that is obviously homologous to Ape (53% identity) and, to a lesser extent, to ExoA and exonuclease III (Fig. 3). Notably, the extensively purified Rrp1 preparation harbors both substantial AP endonuclease and 3′ → 5′ DNA exonuclease activity (31). The Rrp1 protein could be related to the high molecular weight (M_r = 63,000) AP endonuclease reported for *Drosophila* ovaries (32) and to that of mouse mitochondria (M_r = 65,000–82,000), which crossreacts with antiserum against the HeLa AP endonuclease (33).

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-122 GAATTCGGGGGTTGCTCTTTTGCTCATAAGAGGGGCTTCGCTGGCAGTCTGAACGGCAAGCCGGTAAAAATATTGCTTCGGTGGGTGACG  -31
 -32 CGGTACAGCTGCCCAAGGGGTTCGTAACGGGAATGCCGAAGCGTGGGAAAAAGGGAGCGGTGGCGGAAGACGGGGATGAGCTCAGGACAG   58
                                  M  P  K  R  G  K  K  G  A  V  A  E  D  G  D  E  L  R  T

  59 AGCCAGAGGCCAAGAAGAGTAAGACGGCCGCAAAGAAAAATGACAAAGAGGCAGCAGGAGAGGGCCCAGCCCTGTATGAGGACCCCCCAG  148
     E  P  E  A  K  K  S  K  T  A  A  K  K  N  D  K  E  A  A  G  E  G  P  A  L  Y  E  D  P  P

 149 ATCAGAAAACCTCACCCAGTGCGAAACCTGCCACACTCAAGATCTGCTCTTGGAATGTGGATGGGCTTCGAGCCTGGATTAAGAAGAAAG  238
     D  Q  K  T  S  P  S  A  K  P  A  T  L  K  I  C  S  W  N  V  D  G  L  R  A  W  I  K  K  K

 239 GATTAGATTGGGTAAAGGAAGAAGCCCCAGATATACTGTGCCTTCAAGAGACCAAATGTTCAGAGAACAAACTACCAGCTGAACTTCAGG  328
     G  L  D  W  V  K  E  E  A  P  D  I  L  C  L  Q  E  T  K  C  S  E  N  K  L  P  A  E  L  Q

 329 AGCTGCCTGGACTCTCTCATCAATACTGGTCAGCTCCTTCGGACAAGGAAGGGTACAGTGGCGTGGGCCTGCTTTCCCGCCAGTGCCCAC  418
     E  L  P  G  L  S  H  Q  Y  W  S  A  P  S  D  K  E  G  Y  S  G  V  G  L  L  S  R  Q  C  P

 419 TCAAAGTTTCTTACGGCATAGGCGATGAGGAGCATGATCAGGAAGGCCGGGTGATTGTGGCTGAATTTGACTCGTTTGTGCTGGTAACAG  508
     L  K  V  S  Y  G  I  G  D  E  E  H  D  Q  E  G  R  V  I  V  A  E  F  D  S  F  V  L  V  T

 509 CATATGTACCTAATGCAGGCCGAGGTCTGGTACGACTGGAGTACCGGCAGCGCTGGGATGAAGCCTTTCGCAAGTTCCTGAAGGGCCTGG  598
     A  Y  V  P  N  A  G  R  G  L  V  R  L  E  Y  R  Q  R  W  D  E  A  F  R  K  F  L  K  G  L

 599 CTTCCCGAAAGCCCCTTGTGCTGTGTGGAGACCTCAATGTGGCACATGAAGAAATTGACCTTCGCAACCCCAAGGGGAACAAAAAGAATG  688
     A  S  R  K  P  L  V  L  C  G  D  L  N  V  A  H  E  E  I  D  L  R  N  P  K  G  N  K  K  N

 689 CTGGCTTCACGCCACAAGAGCGCCAAGGCTTCGGGGAATTACTGCAGGCTGTGCCACTGGTCGACAGCTTTAGGCACCTCTACCCCAACA  778
     A  G  F  T  P  Q  E  R  Q  G  F  G  E  L  L  Q  A  V  P  L  A  D  S  F  R  H  L  Y  P  N

 779 CACCCTATGCCTACACCTTTTGGACTTATATGATGAATGCTCGATCCAAGAATGTTGGTTGGCGCCTTGATTACTTTTTGTTGTCCCACT  868
     T  P  Y  A  Y  T  F  W  T  Y  M  M  N  A  R  S  K  N  V  G  W  R  L  D  Y  F  L  L  S  H

 869 CTCTGTTACCTGCATTGTGTGACAGCAAGATCCGTTCCAAGGCCCTCGCGAGTGATCACTGTCCTATCACCCTATACCTAGCACTGTGAC  958
     S  L  L  P  A  L  C  D  S  K  I  R  S  K  A  L  A  S  D  H  C  P  I  T  L  Y  L  A  L

 959 ACCACCCCTAAATCACTTTGAGCCTGGGAAATAAGCCCCCTCAACTACCATTCCTTCTTTAAACACTCTTCAGAGAAATCTGCATTCTAT 1048
1049 TTCTCATGTATAAAACGAGGAATCCTCCAACCAGGCTCCTGTGATAGAGTTCTTTTAAGCCCAAGATTTTTATTTGAGGGTTTTTTGTT 1138
1139 TTTTAAAAAACCCGAATTC 1157
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FIG. 2. Sequence of the human APE cDNA. The DNA sequence encoding the predicted start (ATG) and stop (TGA) codons is italicized. The predicted protein encoded by the long open reading frame is indicated in the single-letter code below the DNA sequence. The underlined amino acid residues correspond to the N-terminal sequence determined by Edman degradation (23) of the purified AP endonuclease (41) transferred to an Immobilon membrane (Millipore): PKRGKKGAVAEDGDELRTEPPAKKS (reading N to C). The only observed difference (E predicted from the DNA sequence for amino acid residue 22, in contrast to P assigned by direct determination) is likely due to a protein sequencing error.

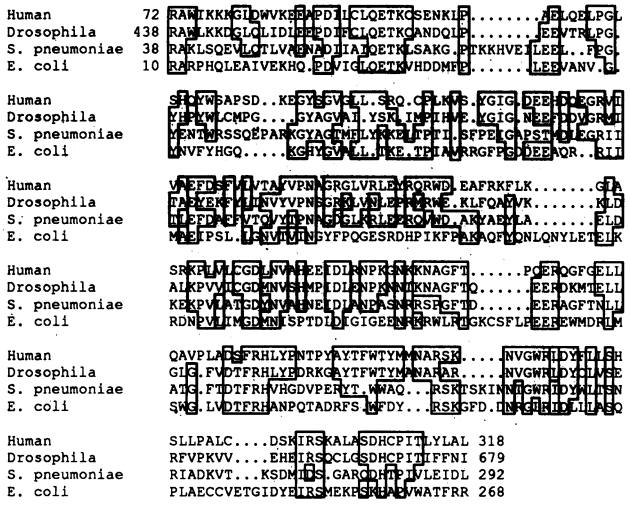

FIG. 3. Homology among AP endonucleases. Blocks of sequence identity or positions where the same amino acid is found in three of the four proteins are indicated (with a few exceptions). The structurally or functionally conservative substitutions are not indicated. The identities relative to *H. sapiens* Ape protein (human) are 52.6% for *D. melanogaster* Rrp1 (Drosophila), 40.5% for *S. pneumoniae* ExoA, and 27.5% for *E. coli* exonuclease III. Other alignments are possible, but the one shown was chosen to maximize the identities among all four sequences.

The cloned cDNA encoding a bovine AP endonuclease (Bap1; ref. 34), reported as this paper was being prepared, predicts a protein 93% identical to the Ape sequence, in keeping with the enzymological similarities of these proteins (8, 9, 35), and adds another member to this family of DNA repair enzymes. However, the intracellular location of the bovine enzyme is unknown.

Expression in *E. coli*. We determined whether the protein expressed from APE cDNA was enzymatically and biologically active. An expression vector, pCW30, introduced into AP endonuclease-deficient *E. coli*, directed the synthesis of the active enzyme, the level of which was increased by induction of the *tac* promoter just 5′ to the cDNA (Table 1). Note that the assay employed here is specific for class II (hydrolytic) AP endonucleases (13), of which the HeLa enzyme is an example (8, 13). The expression of the enzymatic activity was correlated with expression of a $M_r \approx 37{,}000$ polypeptide recognized by the anti-HeLa AP endonuclease antibodies in immunoblots (data not shown).

The human enzyme produced in *E. coli* was biologically effective. Expression from pCW30 conferred resistance to the alkylating agent methyl methanesulfonate (MMS) in AP endonuclease-deficient bacteria (Fig. 4). Only a modest increase in cellular resistance was observed for a strain that lacks the main AP endonuclease, exonuclease III (27, 29), but a more substantial difference due to the expression of Ape was measured for a strain that lacks both exonuclease III and its backup enzyme, endonuclease IV (17). Unexpectedly, the presence of the inducer IPTG did not significantly increase cellular resistance to MMS (Fig. 4*A*).

Table 1. Expression of human AP endonuclease in *E. coli*

Plasmid	IPTG	AP endonuclease, units/mg of protein
pPROK1	−	1.2
	+	1.2
pCW30	−	5.6
	+	72

Strain BW528 (deficient in both exonuclease III and endonuclease IV; ref. 17) containing the indicated plasmid was grown and (where indicated) treated with 1 mM IPTG (isopropylthiogalactoside) before extraction. Cell-free extracts were assayed for hydrolytic (class II) AP endonuclease activity (13). The experiment was repeated twice, with identical results.

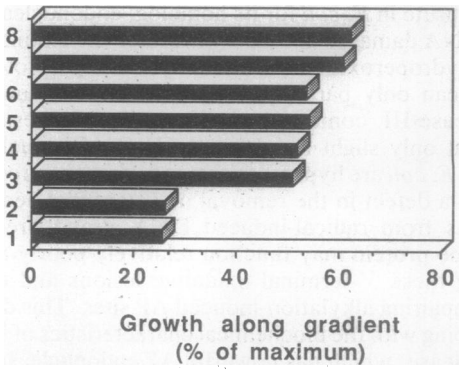

FIG. 4. Functional complementation of AP endonuclease deficiency in *E. coli*. Bars represent growth along gradient plates (ref. 6; 60 μl of 100% MMS in the bottom layer; maximum = 85 mm). Lanes: 1 and 2, strain BW528/plasmid pPROK1; 3 and 4, strain BW528/plasmid pCW30; 5 and 6, strain BW9109/plasmid pPROK1; 7 and 8, strain BW9109/plasmid pCW30. Lanes 2, 4, 6, and 8 were determined in plates with 1 mM IPTG. BW9109 lacks exonuclease III; BW528 lacks both exonuclease III and endonuclease IV (17). The means of three or four repetitions with two independent transformants of each type are shown, with standard deviations that ranged 0.5–5 percentage points and were typically <2.5 percentage points.

These DNA repair-deficient bacteria are also hypersensitive to oxidative agents such as hydrogen peroxide (6, 17, 36). However, the expression of Ape in a strain lacking both exonuclease III and endonuclease IV gave only a slight increase in resistance to H_2O_2 (≤1 mM; unpublished data).

DISCUSSION

We have isolated the human cDNA, APE, encoding the major cellular enzyme that attacks AP sites in DNA. The predicted Ape protein is a member of a family of AP endonucleases that is represented by enzymes from bacteria, insects, and mammals. Because AP sites in DNA are produced by so many pathways, and because such damages block DNA replication and can target mutagenesis in *E. coli* (4), the biological roles of these enzymes in mammalian, and specifically human, cells need to be defined. The APE cDNA represents a critical tool for this effort.

The Ape family of AP endonucleases includes enzymes with vigorous 3′ → 5′ exonuclease activity against undamaged DNA, typified by *E. coli* exonuclease III (29). Such exonuclease activity against undamaged DNA has not been detected in members of the other family of class II AP endonucleases, represented by *E. coli* endonuclease IV and *S. cerevisiae* Apn1 protein (11, 12, 37). The amino acids responsible for the differences between the exonuclease-active (exonuclease III, ExoA, and Rrp1) and -inactive (Ape and Bap1) enzymes in the Ape family are not obvious from comparisons such as that of Fig. 3. This observation implies that only a fine tuning of the fundamental AP endonuclease mechanism (which is a metal-dependent hydrolytic reaction) of a common ancestor was needed to prevent or allow cleavage of normal nucleotides. This possibility is reminiscent of the suggestion made by Weiss (38) for exonuclease III: that a single active site could account for that enzyme's AP endonuclease, 3′ → 5′ exonuclease, and 3′-phosphatase functions. Protein structural studies of these enzymes would help address this issue.

Functional complementation between species has been useful in the analysis of yeast Apn1 AP endonuclease, which

can substitute in *E. coli* for its homolog, endonuclease IV, to repair DNA damages caused by MMS or the oxidizing agent *t*-butyl hydroperoxide (6). Evidently, the Ape protein of *H. sapiens* can only partially replace its *E. coli* counterpart, exonuclease III, conferring significant cellular resistance to MMS but only slight resistance to H_2O_2. Exonuclease III-deficient *E. coli* are hypersensitive to hydrogen peroxide (36), owing to a defect in the removal of 3′-terminal deoxyribose fragments from radical-induced DNA strand breaks (39). Thus, Ape protein may function relatively poorly *in vivo* in repairing these 3′-terminal oxidative lesions and relatively well in repairing alkylation-induced AP sites. This difference is in keeping with the biochemical characteristics of HeLa AP endonuclease, which has powerful AP endonuclease activity (8, 41) but poor 3′-repair diesterase activity *in vitro* (10).

The presence of IPTG in gradient plates did not substantially increase the resistance of repair-deficient *E. coli* to MMS. The uninduced expression of Ape (≈100 molecules per cell) may be sufficient for repair of all the Ape-sensitive, lethal DNA damages caused by this agent. Alternatively, the induction in agar plates may be insufficiently rapid to cope with the rate of DNA damage caused by MMS. The contribution of the *E. coli* Uvr(A)BC system to AP site repair (40) may also partially mask complementation by the human enzyme.

The work presented here represents a key step in addressing some fundamental questions concerning a major DNA repair activity in human cells. As indicated above, AP endonucleases act on lesions that are produced by many different mutagens. The generation of conditionally Ape-deficient cell lines (e.g., using a regulated antisense RNA or a ribozyme) will allow a test of whether the enzyme operates in multiple repair pathways *in vivo*. Since AP sites are formed by spontaneous mechanisms, the Ape protein may also contribute to genetic stability, as seen for the major AP endonuclease of the yeast *S. cerevisiae* (7).

Various human genetic diseases conferring putative defects in DNA repair or genetic stability have been examined for possible deficiencies in the major AP endonuclease, with negative results (14), but human syndromes may exist in which the cellular AP endonuclease is compromised. The use of APE cDNA to map the precise position of the locus in the human genome, which may then be correlated with possible known genetic defects, may reveal unsuspected etiology of diseases, the clinical symptoms of which are not obviously connected to AP endonuclease deficiency.

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1. Lindahl, T. & Nyberg, B. (1972) *Biochemistry* **11**, 3610–3618.
2. von Sonntag, C. (1977) *The Chemical Basis of Radiation Biology* (Taylor and Francis, New York).
3. Sancar, A. & Sancar, G. B. (1988) *Annu. Rev. Biochem.* **57**, 29–67.
4. Loeb, L. A. & Preston, B. D. (1986) *Annu. Rev. Genet.* **20**, 201–230.
5. Wallace, S. S. (1988) *Envir. Mol. Mutagen.* **12**, 431–477.
6. Ramotar, D., Popoff, S. C. & Demple, B. (1991) *Mol. Microbiol.* **5**, 149–155.
7. Ramotar, D., Popoff, S. C., Gralla, E. B. & Demple, B. (1991) *Mol. Cell. Biol.* **11**, 4537–4544.
8. Kane, C. M. & Linn, S. (1981) *J. Biol. Chem.* **256**, 3405–3414.
9. Shaper, N. L., Grafstrom, R. H. & Grossman, L. (1982) *J. Biol. Chem.* **257**, 13455–13458.
10. Demple, B., Greenberg, J. T., Johnson, A. W. & Levin, J. D. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, UCLA Symposia on Molecular and Cellular Biology, New Series, eds. Friedberg, E. C. & Hanawalt, P. C. (Liss, New York), Vol. 83, pp. 159–166.
11. Levin, J., Johnson, A. W. & Demple, B. (1988) *J. Biol. Chem.* **263**, 8066–8071.
12. Johnson, A. W. & Demple, B. (1988) *J. Biol. Chem.* **263**, 18017–18022.
13. Levin, J. D. & Demple, B. (1990) *Nucleic Acids Res.* **18**, 5069–5075.
14. Friedberg, E. C. (1985) *DNA Repair* (Freeman, San Francisco), pp. 505–542.
15. Fan, C.-M. & Maniatis, T. (1990) *Genes Dev.* **4**, 29–35.
16. Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 2nd Ed.
17. Cunningham, R. P., Saporito, S., Spitzer, S. G. & Weiss, B. (1986) *J. Bacteriol.* **168**, 1120–1127.
18. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
19. Munro, S. & Pelham, H. R. (1987) *Cell* **48**, 899–907.
20. Bradford, M. (1976) *Anal. Biochem.* **76**, 248–254.
21. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
22. Nes, I. F. (1980) *Eur. J. Biochem.* **112**, 161–168.
23. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
24. Roberts, B. (1989) *Biochim. Biophys. Acta* **1008**, 263–280.
25. Silver, P. A. (1991) *Cell* **64**, 489–497.
26. Puyet, A., Greenberg, B. & Lacks, S. (1989) *J. Bacteriol.* **171**, 2278–2286.
27. Saporito, S. M., Smith-White, B. J. & Cunningham, R. P. (1988) *J. Bacteriol.* **170**, 4542–4547.
28. Lacks, S. (1970) *J. Bacteriol.* **101**, 373–383.
29. Rogers, S. G. & Weiss, B. (1980) *Methods Enzymol.* **65**, 201–211.
30. Lowenhaupt, K. Y., Sander, M., Hauser, C. & Rich, A. (1989) *J. Biol. Chem.* **264**, 20568–20575.
31. Sander, M., Lowenhaupt, K. & Rich, A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6780–6784.
32. Venugopal, S., Guzder, S. N. & Deutsch, W. A. (1990) *Mol. Gen. Genet.* **221**, 421–426.
33. Tomkinson, A. E., Bonk, R. T. & Linn, S. (1988) *J. Biol. Chem.* **263**, 12532–12537.
34. Robson, C. N., Milne, A. M., Pappin, D. J. C. & Hickson, I. D. (1991) *Nucleic Acids Res.* **19**, 1087–1092.
35. Henner, W. D., Kiker, N. P., Jorgensen, T. J. & Munck, J.-N. (1987) *Nucleic Acids Res.* **15**, 5529–5544.
36. Demple, B., Halbrook, J. & Linn, S. (1983) *J. Bacteriol.* **153**, 1079–1082.
37. Popoff, S. C., Spira, A. I., Johnson, A. W. & Demple, B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4193–4197.
38. Weiss, B. (1976) *J. Biol. Chem.* **251**, 1896–1901.
39. Demple, B., Johnson, A. W. & Fung, D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7731–7735.
40. Lin, J.-J. & Sancar, A. (1989) *Biochemistry* **28**, 7979–7984.
41. Chen, D. S., Herman, T. & Demple, B. (1991) *Nucleic Acids Res.*, in press.