Acta Medica Okayama

Volume 51, *Issue* 4 1997 *Article* 3

AUGUST 1997

cDNA cloning, sequence analysis and expression of a mouse 44-kDa nuclear protein copurified with DNA repair factors for acid-depurinated DNA

Yuko Nakagawa[*] Sekiko Watanabe[†] Kosuke Akiyama[‡]
Altaf H. Sarker[**] Ken Tsutsui[††]
Hajime Inoue[‡‡] Shuji Seki[§]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Okayama University,

cDNA cloning, sequence analysis and expression of a mouse 44-kDa nuclear protein copurified with DNA repair factors for acid-depurinated DNA*

Yuko Nakagawa, Sekiko Watanabe, Kosuke Akiyama, Altaf H. Sarker, Ken Tsutsui, Hajime Inoue, and Shuji Seki

Abstract

We purified a 44-kDa nuclear protein from salt-extract of permeable mouse ascites sarcoma cells in an effort to isolate factors involved in the repair of acid-depurinated DNA. It was copurified with a major AP endonuclease (APEX nuclease) by sequential column chromatography then further purified by sodium dodecyl sulphate-poly-acrylamide gel electrophoresis as a possible DNA repair support factor. Its partial amino acid sequences were determined, and a cDNA clone for the protein was isolated from a mouse T-cell cDNA library using long degenerate oligonucleotide probes deduced from the amino acid sequence. The complete nucleotide sequence of the cDNA (1.7 kilobases) was determined. Northern hybridization using this cDNA detected two transcripts: 1.8kb being the major one and 2.6 kb being the minor one. The complete amino acid sequence for the protein predicted from the nucleotide sequence of the cDNA indicates that the 44-kDa protein consists of 394 amino acids with a calculated molecular weight of 43,698. In tests performed thus far, the recombinant 44-kDa protein expressed in Escherichia coli has not expressed any repair-support activity. It remains to be analyzed whether the protein attains this activity after appropriate posttranslational modifications. Most parts of the 44-kDa protein cDNA and the deduced amino acid sequence were found to be identical to those of the protein p38 -2G4, recently reported as a cell cycle-specifically modulated nuclear protein of 38kDa. The p38-2G4 may be a truncated form of the present 44-kDa protein.

KEYWORDS: 44-kDa protein, nuclear protein, cDNA cloning, cDNA sequencing, recombinant protein

---

*PMID: 9284967 [PubMed - indexed for MEDLINE]

Acta Med Okayama 1997; 51(4): 195-206

cDNA Cloning, Sequence Analysis and Expression of a Mouse 44-kDa Nuclear Protein Copurified with DNA Repair Factors for Acid-Depurinated DNA†

Yuko Nakagawa[a,b], Sekiko Watanabe[a], Kosuke Akiyama[a], Altaf H Sarker[a], Ken Tsutsui[a], Hajime Inoue[b] and Shuji Seki[a,*]

[a]Department of Molecular Biology, Institute of Cellular and Molecular Biology, [b]Department of Orthopaedics Surgery, Okayama University Medical School, Okayama 700, Japan

We purified a 44-kDa nuclear protein from salt-extract of permeable mouse ascites sarcoma cells in an effort to isolate factors involved in the repair of acid-depurinated DNA. It was copurified with a major AP endonuclease (APEX nuclease) by sequential column chromatography then further purified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis as a possible DNA repair support factor. Its partial amino acid sequences were determined, and a cDNA clone for the protein was isolated from a mouse T-cell cDNA library using long degenerate oligonucleotide probes deduced from the amino acid sequence. The complete nucleotide sequence of the cDNA (1.7 kilobases) was determined. Northern hybridization using this cDNA detected two transcripts: 1.8 kb being the major one and 2.6 kb being the minor one. The complete amino acid sequence for the protein predicted from the nucleotide sequence of the cDNA indicates that the 44-kDa protein consists of 394 amino acids with a calculated molecular weight of 43,698. In tests performed thus far, the recombinant 44-kDa protein expressed in *Escherichia coli* has not expressed any repair-support activity. It remains to be analyzed whether the protein attains this activity after appropriate posttranslational modifications. Most parts of the 44-kDa protein cDNA and the deduced amino acid sequence were found to be identical to those of the protein p38-2G4, recently reported as a cell cycle-specifically modulated nuclear protein of 38 kDa. The p38-2G4 may be a truncated form of the present 44-kDa protein.

Key words: 44-kDa protein, nuclear protein, cDNA cloning, cDNA sequencing, recombinant protein

Apurinic/apyrimidinic (AP) sites frequently occur in DNA for several reasons, including spontaneous depurination (1-4), release of the base from a damaged sugar residue, and enzymatic removal of an inappropriate or damaged base by specialized glycosylases (1, 2, 4). Timely repair of the AP sites is necessary, otherwise the lesions, which are devoid of genetic information, have cytotoxic and mutagenic effects. Repair of AP sites is generally initiated by class II AP endonuclease (5' AP endonulease). This class of the enzyme incises the 5' side of the AP site leaving a 3' hydroxyl terminus and a 5' deoxyribose phosphate (dRp) terminus. The 5' terminal base-free sugar phosphate residue should be removed for repair, otherwise it causes a blockage of sealing (5' block). In *E. coli* DNA deoxyribophosphodiesterase (dRpase; RecJ gene product) (5, 6) is thought to be involved in dRp removal. Partial purification of human dRpase having 47 kDa has also been reported (7). However, the actual presence of dRpase in mammalian cells has not yet been proven. We tried to purify enzymes exhibiting such AP site repair-support activity as *E. coli* dRpase in salt extract of mouse ascites sarcoma cells. Our results yielded 44-kDa and 43-kDa proteins as candidates, and we cloned cDNAs for these proteins.

In the present paper we describe purification, cDNA

* To whom correspondence should be addressed.

† The nucleotide sequence data reported in this paper appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases under accession number U43918.

cloning, sequence analysis and expression of the mouse 44-kDa protein, which appears most likely to be involved in AP site repair. The cDNA sequence for the 44 kDa protein showed a strong similarity to that for proliferation-associated nuclear protein, p38-2G4 (8).

Materials and Methods

Materials. The reagents used in this experiment were obtained from the following sources: restriction enzymes from Toyobo Biochem., Osaka, Japan; λgt10 primers, M13 primers and random primers from Takara Shuzo Co., Ltd, Kyoto, Japan; T4 DNA ligase and pGEM-T vector system from Promega Corp., Madison, WI, USA; Taq DNA polymerase from Wako Pure Chemical Industries, Osaka, Japan; mouse spleen cDNA library in phage λgt10 and mouse skeletal muscle poly $(A)^+$ RNA from Clontech Lab., Palo Alto, CA, USA; PRISM Ready Reaction Kit from Applied Biosystems, Mountain View, CA, USA; Megaprime DNA Labeling System, [α-^{32}P] dCTP (3,000 Ci/mmol) and positively charged nylon membrane (Hybond N^+) from Amersham International plc, Buchinghamshire, UK; nylon membranes, positively charged and DIG DNA Labeling and Detection Kit from Boehringer Mannheim, Germany; RNAzol B from Biotecx Lab., Inc., Houston, TX, USA; pET Expression System 16b from Novagen, Inc., Madison, WI, USA; Factor Xa from Danex Biotek, Aarhus, Denmark; BCA Protein Assay Reagent from Pierce, Rockford, IL, USA; 0.24-9.5 Kb RNA Ladder from Life Technologies, Gaithersburg, MD, USA.

A plasmid containing cDNA for the mouse S26 ribosomal protein was kindly provided by Professor J. Fujita of Kyoto University, Kyoto, Japan (9).

The other materials used were obtained as described previously (10, 11). Mouse ascites sarcoma (SR-C3H/He) cells were obtained and maintained as described previously (12).

Preparation of acid-depurinated DNA. The superhelical pUC18 DNA and calf thymus DNA were depurinated by incubation in 3 volumes of 50 mM sodium citrate (pH 3.5) at 60°C for 15 min essentially in the same manner as described by Niwa and Moses (13). After incubation, the mixture was chilled to 0°C and dialysed against 50 mM Tris-HCl (pH 7.5) for 3 h and then against distilled water overnight. The dialyzed DNA solution was used to measure AP site repair-support activity.

In vitro repair system for abasic sites and assay of AP site repair-support activity. A repair system for acid-depurinated DNA was constructed using recombinant APEX nuclease (10, 11), Klenow polymerase, T4 DNA ligase and a repair-supporting factor in mouse ascites cell-extract. We monitored circle-closing activity as an indicator of repair-support activity of AP sites. This was done by measuring conformational changes occurred in acid-depurinated pUC18 DNA by the repair incubation essentially as described previously (14, 15). The assay mixture (15 μl in final volume) contained 0.25 μg (0.14 pmol) acid-depurinated pUC18 DNA, Triton-Buffer B (0.0175% Triton X-100, 0.25 M sucrose, 10 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM EDTA, 6 mM 2-mercaptoethanol, pH 8.0 adjusted at 25°C), 25 ng human recombinant APEX nuclease, 0.015 u Klenow polymerase, 40 u T4 DNA ligase, 2.5 mM ATP, 50 mM each of dNTPs and the renatured repair-support protein in an appropriate amount. After incubating the assay mixture at 37°C for 20 min, the reaction was stopped by chilling it to 0°C and then adding 3 μl of 6x concentrated gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol in H_2O). Each sample was loaded into a slot of submerged 0.8% agarose gel. Conformation of pUC18 DNA was analyzed by agarose gel electrophoresis as described previously (14, 15).

Cleavage of the aspartyl-prolyl bond of the 44-kDa protein and partial amino acid sequencing. To generate peptide fragments, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-purified 44-kDa protein was treated at 37°C for 48 h with 70% formic acid, which preferentially hydrolyzes peptide bonds between aspartate and proline. It was then cleaved into two major peptides electrophoresed at the 29 and 18 kDa positions by SDS-PAGE. Each peptide band on the SDS-polyacrylamide gel was cut out, and the peptide was eluted from the gel slice, as described previously (16). Partial amino acid sequences of the peptide were determined by an Applied Biosystem Model 477A automated protein sequencer (in the central research laboratory of the Okayama University Medical School). The amino terminal amino acid sequence of the 18 kDa peptide was PSKQYGLKMKTSRAFFSEVER-RFDAMPFTLRAF. The N-terminals of the original 44-kDa protein (electrophoresed at 47 kDa position on a SDS-polyacrylamide gel) and the 29 kDa peptide were both blocked. The results suggested that the 18 kDa

peptide was a carboxy terminal fragment and the 29 kDa peptide was an amino terminal fragment of the 44-kDa protein.

Preparation of cDNA for mouse muscle mRNA by reverse transcription. One microgram of mouse skeletal muscle poly $(A)^+$ RNA in 4.42 μl of water in an Eppendorf tube was heated to 60 °C for 3 min and then quenched on ice. To this tube we added the following: 4 μl of 5 × RT buffer (1 × RT buffer is 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 1 μl of dithiothreitol (0.1 M), 3 μl of dNTPs (10 mM each), 6.33 μl of random primers (500 pmoles), 0.25 μl (10 units) of RNasin and 1 μl (200 units) of Moloney murine leukemia virus (M-MLV) reverse transcriptase. The reaction was conducted at 42 °C for 2 h, and excess random primers and substrates were removed using Bio-Spin 30 chromatography columns. The synthesized cDNA was collected by ethanol precipitation.

Preparation of a probe for screening a cDNA library. We performed polymerase chain reaction (PCR) with degenerative primers according to the amino acid sequence, and the specific amplified DNA was sequenced and used to screen a cDNA library. The PCR primers were synthesized according to the amino acid sequence information of the 18 kDa peptide as follows: a sense-strand degenerate primer, 5'-TAYGGNYTNAA-RATGAARAC-3' (20-mer); an antisense-strand degenerate primer, 5'-GTRAANGGCATNGCRTCRAA-3' (20-mer). In these sequences, the following degenerate nucleotide symbols are used: Y (C or T), R (A or G), N (A or G or C or T). Degeneracies of the sense and antisense primers were 256-fold and 128-fold, respectively.

A solution containing an aliquot of the cDNA pool (equivalent to 0.4 ng mRNA) and the sense and antisense primers (10 mM each) in 10 μl of PCR cocktail (50 mM KCl, 10 mM Tri-HCl, pH 8.8, 3.5 mM $MgCl_2$, 0.1 % Triton X-100, 0.1 mM dNTPs, 1 unit of Thermus aquaticus DNA polymerase) was incubated in a thermocycler for 5 min at 95 °C and then for 40 cycles of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C. The amplified product (74 bp) was subcloned into T-vector, and sequenced by the dideoxy chain termination method (17) using M13 primers on the Applied Biosystems Model 373A DNA Sequencing System (in the central research laboratory of the Okayama University Medical School).

To prepare a DNA probe for cDNA library screening, PCR was performed using a mouse spleen cDNA library as a template and an oligonucleotides primer synthesized according to the sequence determined as described above and λgt10 reverse primer. The amplified fragments (119 bp) were labeled with [α-^{32}P] dCTP by using PCR (18).

cDNA cloning. The recombinant phages in the mouse spleen cDNA library were plated with *E. coli* C600, and two replica nylon membranes (Hybond N^+) were prepared. Hybridization was carried out at 68 °C for 30 h in 6 × SSC (1 × SSC: 150 mM NaCl and 15 mM sodium citrate, pH 7.0), 5 × Denhardt's solution, 0.5 % SDS and 100 μg/ml denatured salmon testis DNA. The filters were then washed at 68 °C in 1 × SSC, and 0.1 % SDS, air dried, and autoradiographed using Kodak x-ray film (19). Insert DNAs from the isolated bacteriophage clones were subcloned into plasmid vector pUC18. Although 6 positive clones were isolated and their DNA sequences were determined as described above, we could not obtain full-length cDNA copies. Hence, we investigated another cDNA library. The recombinant phages in the mouse T-cell cDNA library were plated with *E. coli* XL-1Blue MRF', and two replica nylon membranes (nylon membrane, positively charged) were prepared. A ^{32}P-labelled probe (684 bp) was prepared from the 3' region of the cDNA insert in a positive clone obtained from the mouse spleen cDNA library by the random primer DNA labelling system (20). Hybridization and autoradiography were performed under the same conditions described above with the exception of substitution of a shorter hybridization time (16 h). Fragments cloned into the Lambda ZAP-II vector were automatically excised with helper phage and recircularized to generate subclones in pBluescript SK (−) phagemid vector (21). The nucleotide sequence was determined as described above.

Construction of expression vector pEMD44. A 44-kDa protein cDNA fragment was amplified by PCR with oligonucleotide 5'-CCGCATATGTCGGGCGAA-GACGAG-3' and M13 primer RV as 5' and 3' PCR primers, respectively. After digesting with *Nde* I and *Bgl* II, the 185 bp DNA fragment was ligated together with a 1.1 kb *Bgl* II-*Bam*H I fragment from 44-kDa protein cDNA clone into the *Bgl* II-*Bam*H I-cut pET16b. The construct was designated as expression vector pEMD44 (Fig. 1), which contained the entire 44-kDa protein-coding sequence with additive twenty-codons, including ten histidine codons and Factor Xa cleavage site, at the 5' end. The PCR-amplified portion was sequenced to ensure its correctness. The ten histidine motif serves as an affinity tag to facilitate the one-step purification of

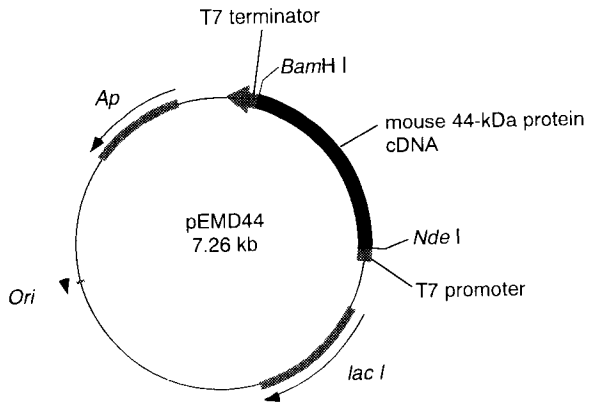

Fig. 1 Schematic representation of pEMD44. The open reading frame of mouse 44-kDa protein cDNA was cloned into the *Nde*I-*Bam*H I site of pET16b under control of strong bacteriophage T7 transcription signals to construct a plasmid designated pEMD44.

the recombinant protein (22, 23).

Expression and purification of recombinant 44-kDa protein. Expression and purification of recombinant 44-kDa protein was performed using the pET vector system according to the manufacturer's instructions. Briefly, overnight, saturated cultures of *E. coli* BL21 (DE3) pLysS cells carrying pEMD44 plasmids were diluted with 50 volumes of LB medium, and then culture was continued at 37°C while being shaking until the absorbance at 600nm reached 0.6. After addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1mM, the incubation was continued for 3h. The cells were harvested by centrifugation at 5,000 × g for 5min. The pellet was resuspended in ice-cold Binding Buffer (5mM imidazole, 500mM NaCl, 20mM Tris-HCl, pH 7.9) and sonicated on ice. All subsequent purification steps were carried out at 0-4°C. Cell debris was removed by centrifugation at 39,000 × g for 20min. The supernatant was loaded on the prepared His · Bind resin column and finally eluted with 6 volumes (1 volume was equivalent to the settled bed volume) of Elution Buffer (1M imidazole, 500mM NaCl, 20mM Tris-HCl, pH 7.9). The eluate was loaded onto a Centricut U-10 (molecular weight limit of filtration, 10,000; Kurabo) to reduce salt and imidazole concentrations by ultra-filtration. To remove the oligohistidine domain, the purified-recombinant protein was treated at 25°C for 15h with Factor Xa (the ratio between Factor Xa and recombinant protein was 1:80) under 50mM Tris-HCl; 100 mM NaCl, 1mM $CaCl_2$, pH 8.0. The concentration of recombinant protein was determined by BCA Protein Assay Reagent.

Northern blot analysis. Total RNA were prepared from SR-C3H/He cells, and male BALB/c mouse organs (kidney, testis, spleen, intestine, brain and liver) by RNAzol B and chloroform according to Chomczynski and Sacchi's method (24). Mice were sacrificed under ether anesthesia at 8 weeks of age. At least 3 mice were used for each experiment. An aliquot of the RNA fraction was denatured with 6% formaldehyde and subjected to electrophoresis on a 1.2% agarose gel in the presence of 6% formaldehyde. RNA was transferred overnight onto a positively charged nylon membrane (Boehringer) using the capillary blotting technique. The 44-kDa protein cDNA was labeled with [α-^{32}P] dCTP or digoxigenin-11-dUTP using the random-primed method (20). The labeling reaction was carried out according to the manufacturer's instructions. When using ^{32}P-labeled probe, the hybridization was conducted at 42°C for 17h in the presence of 50% formamide, essentially as described previously (19). In contrast, when using the digoxigenin-labeled probe, the membranes were prehybridized at 68°C in a prehybridization buffer consisting of 0.25M Na_2HPO_4, pH 7.2, 1mM EDTA, 20% SDS, 0.5% Blocking Reagent (Boehringer) according to the method of Gabi Engler-Blum *et al.* (25). Hybridization was performed by incubating the membranes at 68°C for 42h in the prehybridization buffer supplemented with a denatured digoxigenin-labeled probe. Washing and detection were performed according to the method of Gabi Engler-Blum *et al.* (25). Each membrane was rehybridized with a cDNA probe for the mouse S26 ribosomal protein (9, 26), as a reference for the amount of RNA loaded in each lane. Quantitative analysis of the autoradiograms was performed using a densitometer (Scanning Imager 300SX, Molecular Dynamics, Sunnyvale, CA, USA) in the central research laboratory of the Okayama University Medical School.

Results

Extraction and purification of mouse 44-kDa protein. The mouse 44-kDa protein was extracted from permeable mouse ascites sarcoma cells (SR-C3H/He) with 0.2M potassium phosphate (KPi) buffer (pH 7.5) (27). After adjustment of the KPi concentration to 0.1M, the extract (fraction N1) was mixed with

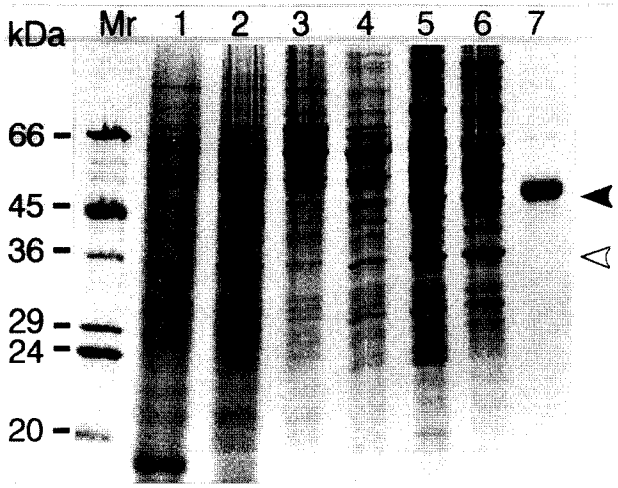

Fig. 2 SDS-polyacrylamide gel electrophoresis of fractions obtained in purification steps of mouse 44-kDa protein. Lane 1, crude extract from permeabilized SR-C3H/He cells; lane 2, 1st phosphocellulose-; lane 3, DEAE-cellulose-; lane 4, 2nd phosphocellulose-; lane 5, single-stranded DNA cellulose-; lane 6, hydroxyl apatite-fractions; lane 7, SDS-PAGE-purified 44-kDa protein. A solid arrow indicates mouse 44-kDa protein which is electrophoresed at the 47kDa position on SDS-PAGE, and an open arrow indicates APEX nuclease coeluted with the 44-kDa protein.

packed phosphocellulose equilibrated with 0.1M KPi. Proteins bound to the phosphocellulose were eluted with 0.3M KPi. The eluant (fraction N2) was passed through DEAE-cellulose mainly to remove nucleic acid as described previouly (27). Materials (fraction N3) passing through the DEAE-cellulose column were loaded onto the second phosphocellulose column equilibrated with 0.075M KPi buffer (pH 7.5) containing 6mM 2-mercaptoethanol. The protein was eluted with a linear gradient of KPi from 0.075 to 0.35M and the 44-kDa protein eluted in almost the same fractions which the APEX nuclease and the 43-kDa protein eluted. The fractions containing the 44-kDa protein were collected and dialyzed against TEMG (0.02M Tris-Cl, pH 8.0, 1mM EDTA, 6mM 2-mercaptoethanol and 10% glycerol) supplemented with 0.01M NaCl. The dialyzed sample (fraction N4) was loaded onto a single-stranded DNA cellulose column equilibrated with the NaCl-TEMG. Proteins were eluted with a linear gradient of NaCl from 0.01 to 0.7M. The 44-kDa protein was coeluted with APEX nuclease. The fractions containing the 44-kDa protein were collected and dialyzed against KPi-MG (6mM 2-mercaptoethanol and 10% glycerol) supplemented with 0.1M KPi. The dialyzed sample (fraction N5) was loaded onto a hydroxyl apatite column equilibrated with KPi-MG. Proteins were eluted with a linear gradient of KPi-MG from 0.1 to 0.45 M. The 44kDa protein was maximally eluted at 0.17M KPi, and APEX nuclease was maximally eluted at 0.2M KPi (11). However, a lot of APEX nuclease was still coeluted with the 44-kDa protein (Fig. 2). The eluted sample was electrophoresed on a large SDS-polyacrylamide gel ($17 \times 15 \times 0.1$ cm) according to the method of Laemmli (19, 28). The 44-kDa protein band was cut out from the gel in which the band was well resolved from its surroundings. The 44-kDa protein was eluted from the gel band, denatured and renatured as described previously (16). Fig. 2 shows the result of SDS-PAGE of fractions obtained in purification steps of the protein. In lane 7, SDS-PAGE-purified 44-kDa protein was electrophoresed. Besides the major 44-kDa protein band, a faint band is observable at the position of about 60kDa. This protein was considered not to be a primary contaminant of the SDS-PAGE-purified 44-kDa protein but to be an aggregates or contaminant incurred during the loading process for SDS-PAGE from the surrounding lanes.

AP site repair-support (circle-closing) activity of fractions containing 44-kDa protein. When acid-depurinated pUC18 DNA was treated at 37°C for 30min with APEX nuclease, nicks were introduced and the plasmid DNA in superhelical form was converted into open circular form. By incubating the acid-depurinated DNA with APEX nuclease, Klenow polymerase (or DNA polymerase β), dNTPs, T4 DNA ligase and the 44-kDa protein, circle-closing (repair) occurred and the conformation of the pUC18 DNA having AP sites was changed into closed circular DNA through open circular DNA (Fig. 3). No circle-closing (repair) of the acid-depurinated DNA occurred in absence of the 44-kDa protein.

Cloning and sequencing of cDNA for the 44-kDa protein. Using the ^{32}P-labelled probes as described in the Materials and Methods section, 1×10^6 independent clones of a mouse spleen cDNA library (cloned into the *Eco*R I site of λgt10) and a mouse T-cell cDNA library (cloned into the *Eco*R I and *Xho* I sites of the Uni-ZAP XR vector) were screened. Positive clones were isolated and DNA sequences were determined. We were able to obtain two candidate clones, p4703 from the mouse spleen cDNA library and pBlue4710 from the mouse T-cell cDNA library, respectively. Although the inserts of the two clones contained an overlapping, identi-

cal region, the sequence of each clone was thought to be incomplete. The insert of p4703 was thought to lack the 3' region of the cDNA, and the insert of pBlue4710 was thought to lack the 5' region. The *Eco* RI-*Pma*C I fragment from clone p4730 and the *Pma*C I-*Sal* I fragment from clone pBlue4710 were ligated, and the resulting fragment was subcloned into pUC18 plasmid to generate the mouse cDNA construct p4739 for the 44-kDa protein (Fig. 4). DNA sequencing identified an open reading frame of 1185 nucleotides (nt) with 266 nt of 5'-flanking and 263 nt of 3'-flanking (untranslated) regions. The cDNA terminated with a poly (A) tail, 10 nt downstream of an authentic polyadenylation signal, AATAAA. The cDNA for mouse 44-kDa protein contained an open reading frame encoding 394 amino acids with a calculated molecular weight of 43.7 kDa, which is a little shorter than 47 kDa, as measured for the purified protein on SDS-PAGE. The C-terminal amino acid sequence of the 44-kDa protein contains a putative nuclear localization signal, KKKKKK (Fig. 5).

Northern hybridization analysis. Northern hybridization analysis with the cDNA for the mouse

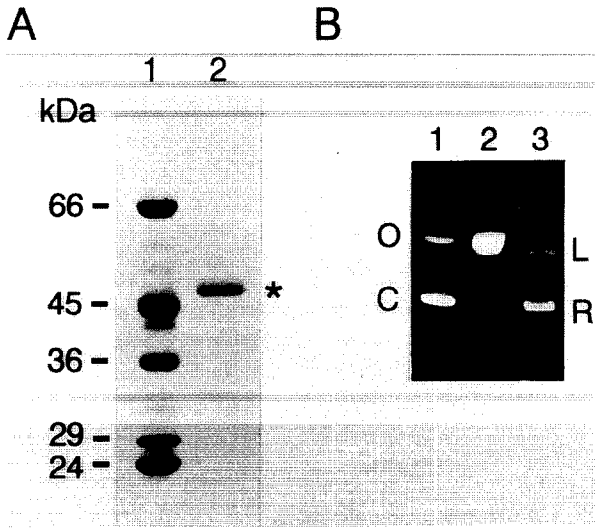

Fig. 3 Assay for the AP site repair-support (circle-closing) activity of purified mouse 44-kDa protein. **A**: lane 1, molecular weight markers; lane 2, SDS-PAGE-purified 44-kDa protein which is electrophoresed at the 47 kDa position on SDS-PAGE (asterisk). **B**: The AP site repair-support (circle-closing) activity of mouse 44-kDa protein was measured as described in the Materials and Methods section. The complete assay mixture contained acid-depurinated pUC18 DNA, APEX nuclease, klenow polymerase, dNTPs, T4 DNA ligase, ATP and renatured 44-kDa protein. The amount of DNA loaded into each slot was 0.25 μg. Lane 1, acid-depurinated pUC18 DNA; lane 2, acid-depurinated pUC18 DNA incubated with APEX nuclease; lane 3, acid-depurinated pUC18 DNA incubated with the complete assay mixture.

Abbreviations: O, nicked, open circular DNA; C, closed circular, supercoiled DNA; L, linear DNA; R, repaired, closed circular DNA.

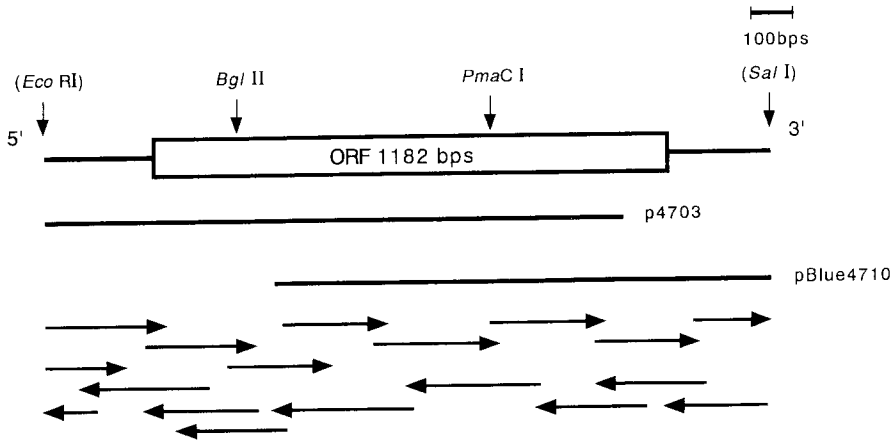

Fig. 4 cDNA for mouse 44-kDa protein, designated p4739, constructed with cloned p4703 and pBlue4710 cDNAs and sequence strategy of cDNA for 44-kDa protein. An open bar indicates the protein coding region and solid lines indicate untranslated regions, determined using the clone p4739. Each arrow indicates the length and direction of the sequence determined.

44-kDa protein identified a major transcript of about 1.8 kb and a faint transcript of about 2.6 kb (Fig. 6). The size of the major transcript was close to the size of cDNA sequenced.

Expression of the 44-kDa protein mRNA in various mouse organs (kidney, testis, spleen, intestine, brain and liver) was examined by Northern blot hybridization using the digoxigenin-labeled cDNA as a probe. The 1.8 kb transcript was detected in all organs examined. After normalization of sampling by densitometric quantitations, the expression level of this transcript in various organs did not vary significantly. This is based on comparison with

```
CTCATGTAATATTGTCTTTTCCAGCTCTGTCCATTTACATGCAAATGTTATGATCTCATTTTTCTTTATGGCTGAATAAAATTCTAGTGT      90
ATATGTAGCAGCTTTTCGGTCCATCCGTCTATGCAGACATCCAGGCTGACACTGTTCCCTTGCTTGCTCGCGCTTTCCTCGCGGATCGAA     180
GAGACTCTAGCTACAGCCTGTGGCTGGGAAGGGAGACGGAGGCCGCAGCTCAGGGAAAGTGAAGCTGCAGTAGTAGCAGTAGGAAGATGT     270
                                                                                M  S       2
CGGGCGAAGACGAGCAGCAGGAGCAAACTATCGCCGAGGACCTGGTCGTGACCAAGTATAAGATGGGGGGCGACATCGCCAACCGGGTGC     360
  G  E  D  E  Q  Q  E  Q  T  I  A  E  D  L  V  V  T  K  Y  K  M  G  G  D  I  A  N  R  V  L      32
TTCGATCTTTGGTGGAGGCTTCCAGCTCAGGTGTGTCTGTGCTGAGCTTGTGTGAGAAAGGTGATGCCATGATTATGGAAGAGACAGGGA     450
  R  S  L  V  E  A  S  S  S  G  V  S  V  L  S  L  C  E  K  G  D  A  M  I  M  E  E  T  G  K      62
AGATCTTCAAGAAGGAAAAGGAGATGAAGAAAGGTATTGCCTTTCCTACCAGCATTTCCGTAAATAACTGTGTGTGTCACTTCTCCCCTT     540
  I  F  K  K  E  K  E  M  K  K  G  I  A  F  P  T  S  I  S  V  N  N  C  V  C  H  F  S  P  L      92
TGAAGAGTGACCAGGACTATATACTCAAGGAAGGTGACTTGGTAAAAATTGACCTTGGGGTTCATGTGGATGGCTTCATTGCCAATGTGG     630
  K  S  D  Q  D  Y  I  L  K  E  G  D  L  V  K  I  D  L  G  V  H  V  D  G  F  I  A  N  V  A     122
CTCACACTTTTGTAATTGGTGTAGCTCAGGGGACCCAGGTAACAGGGCGGAAAGCAGATGTCATTAAGGCCGCTCACCTATGTGCTGAAG     720
  H  T  F  V  I  G  V  A  Q  G  T  Q  V  T  G  R  K  A  D  V  I  K  A  A  H  L  C  A  E  A     152
CTGCCTTACGACTGGTCAAACCTGGAAACCAGAACACACAAGTGACTGAAGCATGGAACAAGGTTGCTCACTCATTTAACTGCACACCAA     810
  A  L  R  L  V  K  P  G  N  Q  N  T  Q  V  T  E  A  W  N  K  V  A  H  S  F  N  C  T  P  I     182
TAGAAGGTATGCTGTCACACCAATTGAAGCAGCATGTGATTGATGGAGAGAAGACGATTATCCAGAACCCTACAGACCAGCAGAAGAAGG     900
  E  G  M  L  S  H  Q  L  K  Q  H  V  I  D  G  E  K  T  I  I  Q  N  P  T  D  Q  Q  K  K  D     212
ACCATGAAAAGGCAGAATTTGAGGTGCATGAGGTTTATGCTGTAGATGTCCTCGTCAGCTCAGGAGAAGGCAAGGCCAAAGATGCAGGAC     990
  H  E  K  A  E  F  E  V  H  E  V  Y  A  V  D  V  L  V  S  S  G  E  G  K  A  K  D  A  G  Q     242
AGAGAACCACCATCTACAAGCGAGACCCCTCTAAACAATATGGCCTGAAAATGAAAACTTCACGTGCCTTTTTCAGTGAGGTGGAACGGC    1080
  R  T  T  I  Y  K  R  D  P  S  K  Q  Y  G  L  K  M  K  T  S  R  A  F  F  S  E  V  E  R  R     272
GTTTTGATGCCATGCCGTTTACTTTAAGAGCATTTGAAGATGAGAAGAAGGCTCGAATGGGTGTGGTAGAGTGTGCCAAACATGAGTTAC    1170
  F  D  A  M  P  F  T  L  R  A  F  E  D  E  K  K  A  R  M  G  V  V  E  C  A  K  H  E  L  L     302
TACAGCCATTTAACGTTCTCTATGAGAAGGAGGGTGAATTTGTTGCCCAGTTTAAATTTACAGTTCTACTCATGCCCAACGGCCCCATGC    1260
  Q  P  F  N  V  L  Y  E  K  E  G  E  F  V  A  Q  F  K  F  T  V  L  L  M  P  N  G  P  M  R     332
GGATAACCAGTGGTCCCTTTGAGCCTGACCTGTACAAGTCTGAGATGGAGGTTCAAGATGCAGAGCTGAAGGCTCTTCTCCAGAGTTCTG    1350
  I  T  S  G  P  F  E  P  D  L  Y  K  S  E  M  E  V  Q  D  A  E  L  K  A  L  L  Q  S  S  A     362
CAAGTAGAAAAACCCAGAAAAAGAAGAAAAAGAAGGCCTCCAAGACTGTAGAGAATGCCACCAGTGGAGAAACCTTAGAAGAGAATGGAG    1440
  S  R  K  T  Q  K  K  K  K  K  K  A  S  K  T  V  E  N  A  T  S  G  E  T  L  E  E  N  G  A     392
CTGGGGACTGAGGTGGGTCCCCTCCCCAGCTTGTCATTCCTGCCTCACCCCCTCCCACCGCACCCCAGGCTCTGTCAAGTCGAGTTCGTC    1530
  G  D  ***                                                                                      394
TTCTCCACCCAAGACTACCAGCAGAGCGGGGGGTCTTCAGCCCTCATCCCGGTCCCCAACCCACCCACCCACTCCTTTCAACAAAAAACC    1620
AGCTCCGACTGACTCTGGTGTTGGGAGGCCAGGCTTCCCAGCCACCGAAGACTACTTTTAGTAGAAAAGAAATTGAATAATAAAATCAGG    1710
AGTC                                                                                           1714
```

Fig. 5 Nucleotide sequence of cDNAs encoding mouse 44-kDa protein. The deduced amino acid sequence in the standard one-letter code is shown beneath the nucleotide sequence. Amino acids are numbered, starting with the first in-frame methionine as 1. The translation termination codon and polyadenylation signal are indicated with triple asterisks and double underlines, respectively. The amino acid residues obtained by sequencing of the 18-kDa fragment are underlined.

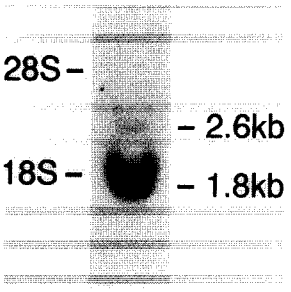

Fig. 6 The mRNA sizes for the mouse 44-kDa protein of SR-C3H/He cells. Denatured total RNAs (10 μg/lane) of SR-C3H/He cells were electrophoretically separated and then transferred to a nylon membrane (HybondN$^+$). The filter was hybridized with the ^{32}P-labeled cDNA. To determine molecular weights of the RNA, ribosomal RNA was used for calibration.

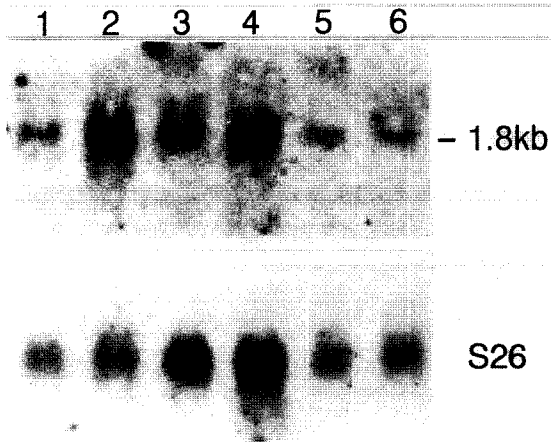

Fig. 7 Expression of 44-kDa protein in various mouse tissues. Each lane contained 20 μg of denatured total RNA extracted from the indicated tissues which were obtained from 8-week old BALB/c male mice. The positively charged nylon membrane (Boehringer) was hybridized with digoxigenin-11-dUTP labeled cDNA. The filter was rehybridized with a cDNA probe for the mouse S26 ribosomal protein, to correct for the amount of RNA loaded. To determine molecular weights of the RNA, 0.24–9.5 kb RNA ladder and ribosomal RNA were used for calibration. Lane 1, kidney; lane 2, testis; lane 3, spleen; lane 4, intestine; lane 5, brain; lane 6, liver.

the signals obtained by rehybridizing the same blots with a cDNA probe for the mouse S26 ribosomal proteins which are expressed at comparable levels in various organs (Fig. 7) (26).

Expression of mouse 44-kDa protein in E. coli. Recombinant 44 kDa protein was clearly expressed in the *E. coli* strain BL21 (DE3) pLysS transformed with the recombinant plasmid and induced with IPTG. The recombinant protein was purified as described in the Materials and Methods section (Fig. 8). Its AP site repair-support activity was tested after purification using the His · Bind resin column and also after removing the histidine-tag by treatment with Factor Xa. The effect of denaturation and renaturation of the expressed protein on the repair support activity was also tested. However, this recombinant protein did not exhibit AP site repair-support (circle-closing) activity.

Database analyses of the cDNA and deduced amino acid sequences of the 44-kDa protein. Database search revealed that the cDNA and deduced amino acid sequences of the 44-kDa protein are markedly similar to those of p38-2G4 recently reported as a novel cell cycle-specifically modified and proliferation-associated nuclear protein in mammals (Fig. 9) (8). The amino acid sequence of the p38-2G4 protein

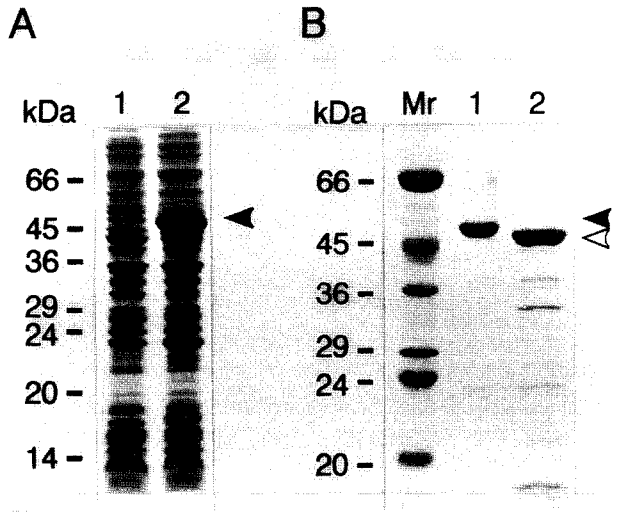

Fig. 8 Coomassie-Brilliant-Blue stained SDS-PAGE demonstrating expression, one step purification and Factor Xa-cleavage of recombinant mouse 44-kDa protein. **A**) Lane 1, bacterial total cell extract equivalent to 120 μl of a culture with an OD600 of 0.65; lane 2, bacterial total cell extract after the induction of 1 mM IPTG and continuous incubation for 3 h, equivalent to 60 μl of a culture with an OD600 of 1. 3. **B**) Lane 1, one step purification of His-tagged mouse 44-kDa protein with an His · Bind resin column, 4.8 μg recombinant protein was loaded; lane 2, Factor Xa-cleaved recombinant mouse 44-kDa protein, 4.8 μg Factor Xa-cleaved protein was loaded. A solid arrow indicates the induced recombinant 44-kDa protein, an open arrow indicates cleaved recombinant protein (on SDS-PAGE, His-tagged 44-kDa protein and Factor Xa-cleaved protein are electrophoresed at the 49 kDa and 47 kDa position, respectively). Mr; Molecular weight markers.

```
M44KP         1' MSGEDEQQEQTIAEDLVVTKYKMGGDIANRVLRSLVEASSSGVSVLSLCEKGDAMIMEET
                                                                      ******
p38-2G4       1"                                                      MIMEET

M44KP        61' GKIFKKEKEMKKGIAFPTSISVNNCVCHFSPLKSDQDYILKEGDLVKIDLGVHVDGFIAN
                 ************************************************************
p38-2G4       7" GKIFKKEKEMKKGIAFPTSISVNNCVCHFSPLKSDQDYILKEGDLVKIDLGVHVDGFIAN

M44KP       121' VAHTFVIGVAQGTQVTGRKADVIKAAHLCAEAALRLVKPGNQNTQVTEAWNKVAHSFNCT
                 ************************************************************
p38-2G4      67" VAHTFVIGVAQGTQVTGRKADVIKAAHLCAEAALRLVKPGNQNTQVTEAWNKVAHSFNCT

M44KP       181' PIEGMLSHQLKQHVIDGEKTIIQNPTDQQKKDHEKAEFEVHEVYAVDVLVSSGEGKAKDA
                 ************************************************************
p38-2G4     127" PIEGMLSHQLKQHVIDGEKTIIQNPTDQQKKDHEKAEFEVHEVYAVDVLVSSGEGKAKDA

M44KP       241' GQRTTIYKRDPSKQYGLKMKTSRAFFSEVERRFDAMPFTLRAFEDEKKARMGVVECAKHE
                 ************************************************************
p38-2G4     187" GQRTTIYKRDPSKQYGLKMKTSRAFFSEVERRFDAMPFTLRAFEDEKKARMGVVECAKHE

M44KP       301' LLQPFNVLYEKEGEFVAQFKFTVLLMPNGPMRITSGPFEPDLYKSEMEVQDAELKALLQS
                 ************************************************************
p38-2G4     247" LLQPFNVLYEKEGEFVAQFKFTVLLMPNGPMRITSGPFEPDLYKSEMEVQDAELKALLQS

M44KP       361' SASRKTQKKKKKKASKTVENATSGETLEENGAGD
                 *********************************
p38-2G4     307" SASRKTQKKKKKKASKTVENATSGETLEENGAGD
```

Fig. 9 Comparison of mouse 44-kDa protein and p38-2G4 sequences. Amino acid residues are numbered from the N terminus of full length polypeptides. The amino acid sequences of mouse 44-kDa protein (M44KP, upper) and p38-2G4 (lower) are aligned to give maximal correspondence. Asterisks indicate exact matches.

```
M44KP         1'     MSGEDEQQEQTIAEDLVVTKYKMGGDIANRVLRSLVEASSSGVSVLSLCEKGDAMI
                      ...... . ....  .*.***..*.....*....** . .*... ..* .**...
SP42KP        1" MSTKEATSETAVDYSLSNPETVNKYKIAGEVSQNVIKKVVELCQPGAKIYDICVRGDELL

M44KP        57' MEETGKIFKKEKEMKKGIAFPTSISVNNCVCHFSPLKSDQD--YILKEGDLVKIDLGVHV
                  *.. *.. ..*.  *******..* *. . *.******..    **.**.*** **.*.
SP42KP       61" NEAIKKVY-RTKDAYKGIAFPTAVSPNDMAAHLSPLKSDPEANLALKSGDVVKILLGAHI

M44KP       115' DGFIANVAHTFVIGVAQGTQVTGRKADVIKAAHLCAEAALRLVKPGNQNTQVTEAWNKVA
                 *** . ** * *.     ...***. **** **    .** * .**** * ***.  .*.*
SP42KP      120" DGFASLVATTTVV---SEEPVTGPAADVIAAASAALKAAQRTIKPGNTNWQVTDIVDKIA

M44KP       175' HSFNCTPIEGMLSHQLKQHVIDGEKTIIQNPTDQQKKDHEKAEFEVHEVYAVDVLVSSG-
                  *..*.*..****** ...****.* .* **.* *... .. .**  ***.**.***..
SP42KP      177" TSYGCKPVAGMLSHQQEREVIDGKKQVILNPSDSQRSEMDTFTFEEGEVYGVDILVSTSP

M44KP       234' EGKAKDAGQRTTIYKRDPSKQYGLKMKTSRAFFSEVERRFDAMPFTLRAFEDEKKARMGV
                 .**.* ..  * ***.. .. * **...**  .**.. .*...**. *... .....**.
SP42KP      237" SGKVKRSDIATRIYKKTDTT-YMLKLQASRKVYSEIQTKFGPFPFSTRNISFDSRTNMGL

M44KP       294' VECAKHELLQPFNVLYEKEGEFVAQFKFTVLLMPNGPMRITSGPFEPDLYKSEMEVQDAE
                  **..*.** *..** .*.*..**.*  *. *  .*.. ....  . *. **...*.*.*
SP42KP      296" NECTSHKLLFPYEVLLDKDGGIVAEFYSTIALTKKGTIILSDSEPKEDFIKSDKKVEDPE

M44KP       354' LKALLQSSASRKTQKKKKKASKTVENATSGETLEENGAGD
                 . ***... . ...***.**.**. *
SP42KP      356" IVALLETPIKVTKNKKKSKKPSKANE
```

Fig. 10 Comparison of mouse 44-kDa protein and *S. pombe* 42-K protein sequences. Amino acid residues are numbered from the N termi of full lenght polypeptides. The amino acid sequences of mouse 44-kDa protein (M44KP, upper) and *S. pombe* 42-K protein (SP42KP, lower) are aligned to give maximal correspondence. Dashes indicate gaps produced by this alignment in one or other sequence. Exact matches and similar amino acids are indicated with asterisks and dots, respectively.

starts from the amino acid position 54 (Met) in the amino acid sequence of the present 44-kDa protein (Fig. 9). The amino acid sequence which follows is completely identical in both proteins. It is possible that the p38-2G4 (38kDa) proteis is a truncated from of the present 44-kDa protein. The amino acid sequence of the 44-kDa protein exhibits a significant homology (41% identity/375 residue; 81% similarity/375 residue) with that of the *S. pombe* 42 K-protein (29) which preferentially associates with curved DNA (Fig. 10).

Discussion

Apurinic/apyrimidinic (AP) sites are known to be one of the most frequent DNA lesions in cells and amount to over 10,000 residues/mammalian cells per day (1). We have been studing enzymes (factors) involved in the repair of AP sites, and cloned cDNAs and genes of a major 5' (class II) AP endonuclease from mammalian cells. It was designated as APEX nuclease (or APEX) (10, 11, 30, 31) for its AP endonuclease and exonuclease activities. It is also referred to as HAP-1 (32, 33), APE (34, 35) and Ref-1 (36). We tried further to purify enzymes exhibiting AP site repair-support activity such as *E. coli* dRpase (37). The present 44-kDa protein was selected as a candidate exhibiting dRpase-like, AP site repair-support activity. It was copurified with APEX nuclease by several column chromatographies, and showed AP site repair-support activity. We purified the protein, determined its partial amino acid sequences, cloned cDNA for the protein and expressed it in *E. coli*. Unexpectedly, the recombinant 44-kDa protein did not show any AP site repair-support activity, in tests thus far. We propose two possible explanations for this lack of repair-support activity. One is that the protein requires posttranslational modification for the activity, which does not occur in *E. coli*. The sequence contains a number of putative phosphorylation sites (consensus motifs) for known protein kinases. Putative myristilation, sulfatation, and glycosylation sites are also found. The second possible explanation is that a minor contamination occurred which had no effect on the amino acid sequence (33 amino acid residues) determined by protein sequencing and exhibited AP site repair-support activity in the SDS-PAGE purified preparation. Further study is necessary to clarify this.

The cDNA and the deduced amino acid sequences of the protein do not show any significant homologies with not only those of RecJ (5) (supposed dRpase in *E. coli*) but also those of supposed AP site repair-support enzymes such as DNA polymerase β and flap endonuclease 1 (FEN-1; DNase IV) (38-40).

Recently, Radomski and Jost (8) reported cloning and characterization of a novel mitogen-inducible gene from murine macrophages that predicts a cell cycle-specifically modulated nuclear protein of 38kDa, designated p38-2G4. They also showed that p38-2G4 expression vanished in G0-arrested cells and restored after release from growth arrest, suggesting that it is proliferation-associated nuclear protein (8). Analyses of cDNA and its deduced amino acid sequence indicated that the 44-kDa protein is virtually identical to the p38-2G4 protein. The amino acid sequence identity between the 44-kDa protein and the p38-2G4 protein occurs from the 54th amino acid (Met) of the former and the first methionine of the latter. These cDNA sequences show a strong similarity (99.2% identity/1579bp), but they are not identical. The open reading frame of the cDNA for the 44-kDa protein encodes 394 amino acids, whereas that of the cDNA for the p38-2G4 protein encodes 340 amino acids. Comparing the 44-kDa protein cDNA with the p38-2G4 protein cDNA, the six guanine tract (GGGGGG) at the position 335-340nt in the 44-kDa protein cDNA (Fig. 4) is replaced five guanine tract in the p38-2G4 protein cDNA, hence, a frame shift occurred and a stop codon (TAA) appeared at the position of 329-331nt. Deletion or insertion of one guanine nucleotide at the position caused the difference between them. Amino acid sequences of both proteins have significant homologies with the *S. pombe* 42K-protein that preferentially binds to a synthetic curved DNA sequence and which is thought to play a role in certain biological processes, such as replication, recombination and transcription (29). The homology of the amino acid sequences in 44-kDa protein and *S. pombe* 42K-protein extends to almost the entire region. The N-terminal 50 amino acid sequence of the *S. pombe* 42K-protein is homologous to the corresponding region of the 44-kDa protein sequence without any gap insertion (Fig. 10), but not with the p38-2G4 protein. This homology data may indicate that the p38-2G4 protein is a truncated form of the 44-kDa protein.

Northern hybridization analysis using the cDNA of the mouse 44-kDa protein revealed two transcripts of about 1.8kb and 2.6kb (Fig. 6). The size of the 1.8 kb-transcript was close to the size of the cDNA sequenced, and the signal of the 1.8-kb product was more than ten times of that of the 2.6-kb product in all tissues

examined. The 1.8-kb transcript is thought to be the mRNA for the 44-kDa protein. The biological significance of the faint 2.6 kb-transcript is unknown. Significant expression of the mRNA for the 44-kDa protein was observed in all tissues studied, even in brain tissue consisting of mainly non-proliferative cells. These results suggest that the 44-kDa protein functions not only in proliferative cells but also in resting cells.

Further studies are necessary to know the actual function of the 44-kDa protein in relation to AP site repair-support, proliferation-related functions including DNA segregation, and/or other functions such as replication, recombination and transcription.

Acknowledgments. We thank Prof. J. Fujita of Kyoto University for providing a plasmid containing cDNA of the mouse S26 ribosomal protein, Prof. T. Mizuno of Nagoya University for his valuable information, Mr. T. Nakamura and Ms. K. Tabuchi for their technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture and in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture (Molecular Mechanism for Maintenance of Genetic Information, 0520101).

References

1. Lindahl T and Andersson A: Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. Biochemistry (1972) **11**, 3618-3623.
2. Lindahl T: DNA repair enzymes. Annu Rev Biochem (1982) **51**, 61-87.
3. Friedberg EC: DNA Repair. W. H. Freeman and Company, New York (1985) pp1-77.
4. Sancer A and Sancar GB: DNA repair enzymes. Annu Rev Biochem (1988) **57**, 26-97.
5. Lovett ST and Kolodner RD: Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the recJ gene of *Escherichia coli*. Proc Natl Acad Sci USA (1989) **86**, 2627-2631.
6. Dianov G, Sedgwick B, Daly G, Olsson M, Lovett S and Lindahl T: Release of 5'-terminal deoxyribose-phosphate residues from incised abasic sites in DNA by the *Escherichia coli* RecJ protein. Nucleic Acids Res (1994) **22**, 993-998.
7. Price A and Lindahl T: Enzymatic release of 5'-terminal deoxyribose phosphate residues from damaged DNA in human cells. Biochemistory (1991) **30**, 8631-8637.
8. Radomski N and Jost E: Molecular cloning of a murine cDNA encoding a novel protein, p38-2G4, which varies with the cell cycle. Exp Cell Res (1995) **220**, 434-445.
9. Kaneko Y, Nonoguchi K, Fukuyama H, Takano S, Higashitsuji H, Nishiyama H, Takenawa J, Nakayama H and Fujita J: Presence of alternative 5' untranslated sequences and identification of cells expressing ctk transcripts in the brain and testis. Oncogene (1995) **10**, 945-952.
10. Seki S, Akiyama K, Watanabe S, Hatsushika M, Ikeda S and Tsutsui K: cDNA and deduced amino acid sequence of a mouse DNA repair enzyme (APEX nuclease) with significant homology to *Escherichia coli* exonuclease III. J Biol Chem (1991) **266**, 20797-20820.
11. Seki S, Ikeda S, Hatsushika M, Tsutsui K, Akiyama K and Zang B: A mouse DNA repair enzyme (APEX nuclease) having exonuclease activities: Purification and characterization. Biochim Biophys Acta (1991) **1079**, 57-64.
12. Seki S and Oda T: DNA synthesis in permeable mouse ascites sarcoma cells. Cancer Res (1977) **37**, 137-144.
13. Niwa O and Moses RE: Synthesis by DNA polymerase I on bleomycin-treated deoxyribonucleic acid: A requirement for exonuclease III. Biochemistry (1981) **20**, 238-244.
14. Seki S, Ikeda S, Hatsushika M, Tsutsui K and Teraoka H: Repair of X-ray-induced single-strand breaks by a cell-free system. Carcinogenesis (1990) **11**, 1213-1216.
15. Seki S, Arakaki Y and Oda T: A cell-free system for studying a priming factor involved in repair of bleomycin-damaged DNA. Acta Med Okayama (1989) **43**, 73-80.
16. Seki S, Akiyama K, Watanabe S and Tsutsui K: Activity gel and activitiy blotting methods for detecting DNA-modifying (repair) enzymes. J Chromatogr (1993) **618**, 147-166.
17. Sanger F, Nicklen S and Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA (1977) **74**, 5463-5467.
18. Schowalter DB and Sommer SS: The generation of radiolabeled DNA and RNA probes with polymerase chain reaction. Anal Biochem (1989) **177**, 90-94.
19. Sambrook J, Fritsch EF, and Maniatis T: Molecular Cloning, A Laboratory Manual, 2nd Ed, Clod Spring Harbor Laboratory Press, Cold Spring Harbor (1989).
20. Feinberg AP and Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochim (1984) **137**, 266-267.
21. Short JM, Fernandez JM, Sorge JA and Huse WD: λ ZAP: A bacteriophage λ expression vector with *in vivo* excision properties. Nucleic Acids Res (1988) **16**, 7583-7600.
22. Hochuli E, Döebeli H and Schacher A: New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residue. J Chromatogr (1987) **411**, 177-184.
23. Hoffmann A and Roeder RG: Purification of his-tagged proteins in non denaturing conditions suggests a convenient method for protein interaction studies. Nucleic Acids Res (1991) **19**, 6337-6338.
24. Chomczynski P and Sacchi N: Single-step purification method of RNA isolation by acid guanidinum thiocyanate-phenol-chloroform extraction. Anal Biochem (1987) **162**, 156-159.
25. Engler-Blum Gabi, Meier M, Frank J and Müller GA: Reduction of background problems in nonradioactive northern and southern blot analyses enables higher sensitivity than ^{32}P-based hybridizations. Anal Biochem (1993) **210**, 235-244.
26. Vincent S, Marty L and Philippe F: S26 ribosomal protein RNA: An invariant control for gene regulation experiments in eucaryotic cells and tissues. Nucleic Acids Res (1993) **21**, 1498.
27. Seki S and Oda T: An exonuclease possibly involved in the initiation of repair of bleomycin-damaged DNA in mouse ascites sarcoma cells. Carcinogenesis (1988) **9**, 2239-2244.
28. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (1970) **227**, 680-685.
29. Yamada H, Mori H, Momoi H, Nakagawa Y, Ueguchi C and Mizuno, T: A fission yeast gene encoding a protein that preferentially associates with curved DNA. Yeast (1994) **10**, 883-894.
30. Akiyama K, Seki S, Oshida T and Yoshida MC: Structure, promoter analysis and chromosomal assignment of the human *Apex* gene. Biochim Biophys Acta (1994) **1219**, 15-25.
31. Akiyama K, Nagao K, Seki S, Oshida T, Tsutsui K, Yoshida MC and

Seki S: Cloning, sequence analysis, and chromosomal assignment of the mouse *Apex* gene. Genomics (1995) **26**, 63-69.
32. Robson CN, Milne AM, Pappin DJC and Hickson ID: 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 (1991) **19**, 5519-5523.
33. Robson CN, Hochhauser D, Craig R, Rack K, Buckle VJ and Hickson ID: Structure of the human DNA repair gene HAP I and its localization to chromosome 14q11. 2-12. Nucleic Acid Res (1992) **20**, 4417 4421.
34. Demple B, Herman T and Chen DS: Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: Definition of a family of DNA repair enzymes. Proc Natl Acad Sci USA (1991) **88**, 11450 11454.
35. Harrison L, Ascione G, Menninger JC. Ward DC and Demple B: Human apurinic endonuclease gene (APE): Structure and genomic mapping (chromosome 14q11. 2-12). Hum Mol Genet (1992) **1**, 677 680.
36. Xanthoudakis S, Miao G, Wang F, Pan Y-CE and Curran T: Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. EMBO J (1992) **11**, 3323-3335.
37. Franklin WA and Lindahl T: DNA deoxyribophosphodiesterase. EMBO J (1988) **7**, 3617-3622.
38. Harrington JJ and Lieber MR: The characterization of mammalian DNA structure-specific endonuclease. EMBO J (1994) **13**, 1235-1246.
39. Hiraoka LR, Harrington JJ, Gerhard DS, Lieber MR and Hsieh CL: Sequence of human FEN-1, a structure-specific endonuclease, and chromosomal localization of the gene (FEN-1) in mouse and human. Genomics **25**, 220-225.
40. Robins P, Pappin DJC, Wood RD and Lindahl T: Structural and functional homology between mammalian DNase IV and the 5'-Nuclease Damain of *Escherichia coli* DNA polymerase I. J Biol Chem (1994) **269**, 28535 28538.

Received February 19, 1997; accepted March 21, 1997.