

Identification and characterization of mitochondrial abasic (AP)-endonuclease in mammalian cells

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ABSTRACT

Abasic (AP)-endonuclease (APE) is responsible for repair of AP sites, and single-strand DNA breaks with 3' blocking groups that are generated either spontaneously or during repair of damaged or abnormal bases via the DNA base excision repair (BER) pathway in both nucleus and mitochondria. Mammalian cells express only one nuclear APE, 36 kDa APE1, which is essential for survival. Mammalian mitochondrial (mt) BER enzymes other than mtAPE have been characterized. In order to identify and characterize mtAPE, we purified the APE activity from beef liver mitochondria to near homogeneity, and showed that the mtAPE which has 3-fold higher specific activity relative to APE1 is derived from the latter with deletion of 33 N-terminal residues which contain the nuclear localization signal. The mtAPE-sized product could be generated by incubating ³⁵S-labeled APE1 with crude mitochondrial extract, but not with cytosolic or nuclear extract, suggesting that cleavage of APE1 by a specific mitochondria-associated N-terminal peptidase is a prerequisite for mitochondrial import. The low abundance of mtAPE, particularly in cultured cells might be the reason for its earlier lack of detection by western analysis.

INTRODUCTION

ROS-induced damage in DNA includes a plethora of oxidized bases, abasic (AP) sites and DNA strand breaks all of which are repaired via the base excision repair (BER) pathway.

Repair of damaged bases is initiated with excision of a damaged or abnormal base by a DNA glycosylase thereby leaving a non-coding AP site. Oxidized base-specific mammalian DNA glycosylases, such as NTH1 and OGG1 further cleave the AP site via lyase reaction to generate 3' α , β unsaturated deoxyribose (1). ROS also directly attacks deoxyribose and cleaves the DNA strand to produce 3'-glycolate termini (2,3). Both AP sites and 3' blocking groups are processed by abasic (AP)-endonuclease (APE) to generate a 3' OH terminus which serves as the primer for gap-filling DNA synthesis by a DNA polymerase. All APEs have both AP site-specific endonuclease and 3' phosphodiesterase activities (4). Unlike *Escherichia coli* or yeast, mammalian cells express only one APE, 36 kDa APE1, whose sequence is highly conserved among various mammalian species. The human and bovine APE1s have 93% sequence identity. APE1 belongs to the *E.coli* Xth family and has significant homology with APN2 in yeast (4–6).

The mitochondrial genome is much more susceptible to endogenous, oxidative damage than the nuclear genome presumably because of both proximity to the site of ROS generation (in mitochondrial respiratory complexes), and the lack of associated histones (7,8). Oxidative damage to the mitochondrial genome has been implicated in various human degenerative diseases, and in aging (9,10). DNA repair in mitochondria should thus be extremely important, particularly for non-dividing cells (11). Although the mitochondria lack the nucleotide excision repair system (12), repair of oxidative damage via the BER pathway in the mitochondria has been demonstrated for a number of cell types (13–19). Uracil-DNA glycosylase (UDG) is the first DNA glycosylase to be identified in mitochondria (20,21). Nuclear and mitochondria-specific DNA glycosylases are encoded by the same nuclear genes. These mitochondrial enzymes lack the nuclear localization signal (NLS), and contain N-terminal mitochondrial targeting sequence (MTS) (22–24).

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While most BER enzymes in mitochondria have been characterized, the nature of mtAPE remains unclear. APE activity, which was believed to be due to APE1, was demonstrated in *Xenopus* oocyte mitochondria (13). Tell *et al.* (25) showed APE1's localization in rat cell mitochondria. Because APE1 does not appear to possess N-terminal MTS, the targeting of APE1 to the mitochondria was difficult to explain, although APE1 was suggested to diffuse through the mitochondrial permeability transition pore in oxidatively stressed cells (26,27).

The presence of APE activity in mouse cell mitochondria was first demonstrated by Tomkinson *et al.* (28) who partially purified the enzyme, and observed APE1 antibody cross-reacting 66–68 kDa bands. However, these larger proteins were not further characterized. Subsequently a homolog of APE1, named APE2, was identified in the human genome database which encodes a 62 kDa protein (29). Although APE2 with putative MTS was shown to be present in both the nucleus and mitochondria, it has no detectable APE activity (29,30).

One problem in studying mitochondrial proteins, not appreciated earlier, is the difficulty of removing endoplasmic reticulum (ER) and cytosolic contaminants from purified mitochondria (31). Some proteins, originally shown to be present in the mitochondrial matrix, could in fact be adventitiously associated with the mitochondrial outer membrane and ER (32). However, such extraneous proteins are susceptible to trypsin which does not degrade the matrix proteins (33).

Because of the uncertainty about the nature of mtAPE, we decided to identify the mammalian mtAPE by purifying the enzyme, based on activity. We have shown here that mtAPE is derived from APE1 by proteolytic cleavage of 33 N-terminal residues.

MATERIALS AND METHODS

Purification of mitochondria

Mitochondria from beef liver (4 lbs) were purified by fractionating cell lysate via differential centrifugation with slight modification of a published procedure (20). The mitochondrial pellet was resuspended in 50 ml buffer A (10 mM HEPES–KOH, pH 7.4, 250 mM sucrose, 10 mM DTT, 0.5 mM EGTA and 2 mM EDTA). The final purification step involved centrifugation in sucrose step gradient containing 1 and 1.5 M sucrose in 10 mM HEPES–KOH (pH 7.4) and 1 mM EDTA. The mitochondria were harvested from the sucrose band interphase, washed twice with buffer A, and then lysed with a buffer containing 20 mM HEPES–KOH (pH 7.4), 1 mM EDTA, 1 mM DTT, 300 mM KCl, 5% glycerol, protease inhibitor cocktail (Roche) and 0.5% Triton X-100. The lysate was centrifuged in a microfuge for 15 min, and the supernatant was stored at -80°C .

Trypsin treatment of mitochondria

Intact mitochondria from beef liver or mouse NIH3T3 cells (1 mg/ml) resuspended in buffer A were treated with trypsin (10 $\mu\text{g}/\text{ml}$) for 20 min at room temperature, followed by the addition of an equivalent amount of bovine trypsin inhibitor (Invitrogen) to inactivate the trypsin (33,34). Protease and

mock-treated mitochondrial suspensions were washed twice in the same buffer before lysis.

Purification of mitochondrial APE from beef liver

The mitochondrial lysates were dialyzed against buffer B containing 25 mM Tris–HCl (pH 7.5), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT and 10% glycerol at 4°C for 3 h to remove Triton X-100, and then loaded on 50 ml HiTrap Q and SP–Sephacrose columns (Amersham) connected in tandem. After washing with buffer B, the HiTrap-SP column was disconnected, from which the proteins were eluted with buffer B containing a linear gradient of KCl (0.05–1.0 M). Fractions with high-APE activity were pooled, dialyzed and then loaded onto a 5 ml HiTrap SP column. After washing, a 40 ml linear gradient of KCl (0.1–0.6 M) in buffer B was used, and the APE activity was eluted at 250–270 mM KCl. The pooled active fractions were similarly processed twice more using a 1 ml HiTrap-SP column. The peak activity present in two fractions from the last column was further purified by chromatography on a 1 ml HiTrap-Heparin column, using a 0.1–0.6 M KCl gradient. APE-containing fractions were pooled, snap-frozen and stored at -80°C .

Mass spectrometry and peptide sequence analysis

Protein bands in SDS–PAGE stained with Coomassie Blue were excised from the gel, digested with trypsin and then analyzed by MALDI-TOF (Applied Biosystems) for identification. The proteins were also independently identified by automated N-terminal sequencing after transferring to PVDF membrane.

Expression and purification of full-length and $\Delta 33$ recombinant human APE1

The coding sequence for full-length human APE1 (hAPE1) was inserted in the pET15b vector (Novagen) at NdeI/XhoI sites for expression of APE1 in *E.coli*. The $\Delta 33$ hAPE1 mutant, lacking 33 N-terminal residues, was similarly expressed using the same vector. Both plasmids encoded 20 additional amino acid residues including His₆ sequence tag at the N-terminus. *E.coli* BL21(DE3) cells transformed with the APE1 plasmid were induced with 0.5 mM isopropyl- β -D-thiogalactopyranoside at 0.6 A_{600} and then grown at 16°C overnight. After harvesting and then resuspending the bacteria in buffer C (20 mM Tris–HCl, pH 8.0 and 0.5 M NaCl), the cells were sonicated, filtered and then applied to a 3 ml Ni-NTA Superflow (Qiagen) column. After washing successively with 30 ml of buffer C and then 30 ml buffer C containing 40 mM imidazole, APE1 was eluted in 1 ml fractions with 10 ml buffer C containing 100 mM imidazole. After pooling active fractions and dialysis in a buffer containing 20 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.1 mM DTT and 10% glycerol, the solution was treated with thrombin (10 U) before SP–Sephacrose chromatography. APE1 was eluted with a linear gradient of NaCl (0.1–0.6 M) in the previous buffer. Thrombin removed all but two additional N-terminal residues in APE1 or $\Delta 33$ APE1. The active fractions of both enzymes were stored at -20°C in a buffer containing 20 mM Tris–HCl (pH 8.0), 300 mM NaCl, 1 mM EDTA, 1 mM DTT and 50% glycerol.

AP-endonuclease assay

A 43mer oligo duplex containing tetrahydrofuran (THF; Midland) at position 31 in the sequence 5'-GATCTGAT-TCCCCATCTCCTCAGTTTCACTXCTGCACCGCATG-3' (X: THF) was 5'-terminally labeled using [γ - 32 P]ATP and T4 polynucleotide kinase (PNK). After annealing to the complementary strand with A opposite THF and subsequent purification by PAGE, the duplex oligo (500 nM) was incubated with mitochondrial fractions or recombinant APE1 at 37°C for 10 min in a 10 μ l reaction mixture containing 50 mM KCl, 2 mM MgCl₂, 0.5 mM DTT, 0.1 mM EDTA and 100 μ g/ml BSA at pH 9.2 (50 mM AMPPO) or at pH 7.5 (50 mM Tris-HCl). After stopping the reaction with 80% formamide/10 mM NaOH containing 0.05% xylene cyanol, the oligos were separated by denaturing gel electrophoresis in 20% polyacrylamide containing 8 M urea. Radioactivity in the separated DNA bands was quantified in a PhosphorImager using Imagequant software (Molecular Dynamics). Preliminary enzyme activity assays were carried out to ensure linearity of product formation with respect to both time of incubation and the amount of extract.

The K_m and k_{cat} values of the Δ 33 APE1 and the full-length APE1 were determined after incubating 0.01 nM enzyme with various concentration of THF-containing oligo substrate at pH 9.2, or with 0.05 nM enzyme at pH 8.0, at 37°C for 4 min in the same reaction buffer as stated before.

DNA 3' phosphodiesterase assay

To measure the DNA 3' end-cleaning activity, a 26mer oligodeoxynucleotide with U at the 5'-terminus was labeled with [γ - 32 P]ATP by T4 PNK. This oligo was annealed with a 51mer oligo containing a non-complementary 25 nt extension at the 3' end (30). Subsequent annealing of a complementary 25mer oligonucleotide and treatment with T4 DNA ligase generated a 51mer duplex with U at position 26 in the labeled strand. The 51mer duplex oligonucleotide was treated with *E. coli* Udg (35) and Fpg (36) to generate 5' 32 P-labeled 3' phosphate after excision of U and DNA strand cleavage owing to the $\beta\delta$ lyase activity of Fpg. In a parallel reaction, the duplex oligonucleotide was treated with Udg (35) and Nth (36) to generate 3' 32 P-phospho α,β -unsaturated aldehyde. The oligonucleotides were purified and treated with APE1 or PNK as described earlier (30) and the radioactivity in the free phosphate or phosphoaldehyde was quantified by PhosphorImager analysis.

Western analysis

The proteins after SDS-PAGE were transferred to a nitrocellulose membrane (BioRad) and blocked with TBST (20 mM Tris-HCl), pH 7.5, 500 mM NaCl with 0.1% Tween 20 containing 5% non-fat dry milk (37). The membranes were subsequently probed with rabbit anti-hAPE1 IgG (38) or with anti-lamin b antibody (Santa Cruz Biotechnology), also in TBST containing 5% non-fat dry milk. The bands were visualized using ECL (Amersham Biosciences) and analyzed with Imagequant software (Molecular Dynamics).

Synthesis of the 35 S-labeled APE1

[35 S]hAPE1 was synthesized *in vitro* via coupled transcription-translation using TnT-coupled Reticulocyte

Lysate System (Promega). APE1 cDNA cloned in pRSET B vector (1 μ g) was incubated in 50 μ l reaction mixture containing [35 S]methionine (20 μ Ci) according to the manufacturer's protocol. Aliquots of 1 μ l were individually incubated with 25 μ g extracts of nucleus, cytoplasm or mitochondria purified from beef liver in buffer A (20 μ l) for 30 min at 37°C. After stopping the reaction with the loading buffer, the proteins were separated by SDS-PAGE and visualized with PhosphorImager.

Intracellular localization of full-length and truncated hAPE1

293 cells grown on cover slips in 35 mm dishes were transfected for 6 h with 0.5 μ g plasmid DNA (full-length, Δ 20 and Δ 41 APE1-EGFP), using Lipofectamine 2000 and OptiMEM (Invitrogen), and 18 h later, the live cells were treated with MitoTracker Red (20 nM). In selected experiments, cells were fixed in methanol: acetone (1:1) and stained with DAPI. Fluorescent images were captured using a Photometric Cool-SPAP Fx digital camera mounted on a NIKON Eclipse TE 200 UV microscope.

RESULTS

Identification of Δ 33 APE1 as the major mitochondrial AP-endonuclease

We purified APE activity from beef liver mitochondria as described in Materials and Methods. Final fractions 12–14 from Heparin-Sepharose chromatography contained the most APE activity (Figure 1). SDS-PAGE Analysis of fraction 12 indicated a 33 kDa major protein band (Figure 2A) which showed strong cross-reaction with hAPE1 antibody suggesting that the 33 kDa species is closely related to APE1 (Figure 2B). MALDI-TOF and electrospray mass spectrometry after trypsin digestion of the excised band (\sim 1 μ g) identified the peptides as being derived from APE1 (Figure 3A). Subsequent automated Edman degradation showed the sequences to be EKEAV at the N-terminus of the band, corresponded to residues 34–38 in APE1. Thus mtAPE is derived from APE1 after deletion of 33 N-terminal residues (Figure 3B).

Comparative properties of recombinant human APE1 (hAPE1) and bovine mtAPE

We compared the enzymatic parameters of purified, recombinant hAPE1 and bovine mtAPE. The pH dependence of APE activity showed the pH optimum to be 9.2, and the highest activity was observed at 50 mM KCl and 2 mM MgCl₂, for both enzymes (Figure 4). We then compared the specific activity of full-length hAPE1 and bovine mtAPE by using equimolar amounts of hAPE1 and purified bovine mtAPE (Heparin-Sepharose fraction 12). The amount of the bovine enzyme was estimated by comparison with hAPE1 by quantitative western analysis (Figure 5A). Figure 5B shows that comparable cleavage of THF-oligo (500 nM) required 100 pM hAPE1 and about a third as much bovine enzyme. Thus the specific activity of bovine mtAPE appeared to be about 3-fold higher than of full-length hAPE1.

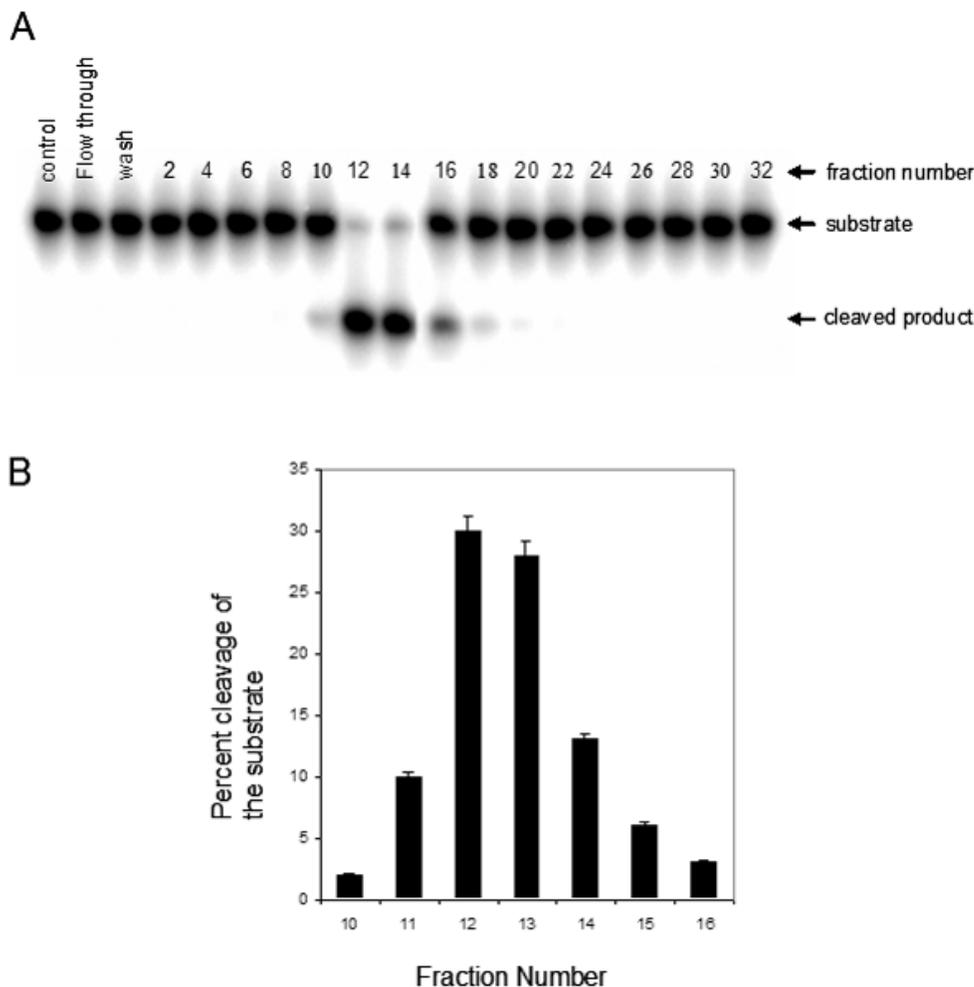


Figure 1. APE activity of Heparin-Sepharose fractions. (A) APE activity of fractions was measured at 37°C for 10 min with 1:200 diluted fractions and 500 nM substrate. (B) Quantitative representation of APE activity in active fractions (10–16) after 1:500 dilution.

Enhanced endonuclease Activity of NΔ33 APE1 is due to increased enzyme turnover

In order to eliminate the possibility that the higher turnover of bovine mtAPE relative to hAPE1 is due to intrinsic difference of human and bovine APEs, we purified recombinant NΔ33 hAPE1 in the same way as the full-length hAPE1. We confirmed that the NΔ33 hAPE1 was indeed three times more active than the full-length hAPE1 (Figure 6A and B). We then compared the 3' phosphoesterase activities of full-length and truncated enzymes with DNA containing 3' phospho α,β unsaturated aldehyde. This 3' phosphodiesterase activity was ~100-fold lower compared with the endonuclease activity of APE1, in confirmation of earlier observations (4). Nevertheless, NΔ33 hAPE1 had 3-fold higher specific activity than the full-length APE1 (Figure 6C and D).

On the other hand, full-length and NΔ33 hAPE1 showed comparable K_m (~18 nM) with the THF-oligo substrate similar to that observed earlier (39). This indicates N-33 deletion of APE1 does not affect its substrate affinity. However, the k_{cat} of NΔ33 hAPE1 was 3-fold higher than that of the full length enzyme (Table 1). Although APE1's specific activity was

5-fold higher at pH 9.2 than at pH 7.4, a similar difference in specific activities of full-length and truncated APE1 was maintained at pH 7.4.

NΔ33 APE1 is the predominant APE in beef liver mitochondria

That the APE species with N-33 deletion is indeed present in the mitochondrial matrix, and was not generated artifactually from full-length APE1 during its purification, was ensured by immunoblotting extracts of crude, purified and trypsin-treated mitochondria. Controlled trypsin treatment cleaves most proteins bound to the mitochondrial outer membrane but not the matrix proteins. Western analysis of extracts of mitochondria purified through two cycles of sucrose gradient centrifugation and subsequent trypsin treatment, showed the presence of only the NΔ33 APE1 species, and not the full-length protein (Figure 7A). However, considerable loss of the total APE in trypsin-treated mitochondria was observed, probably because of damage to mitochondrial membrane during purification. Nuclear contamination of mitochondrial preparations as tested by the presence of lamin B was negligible (data not shown).

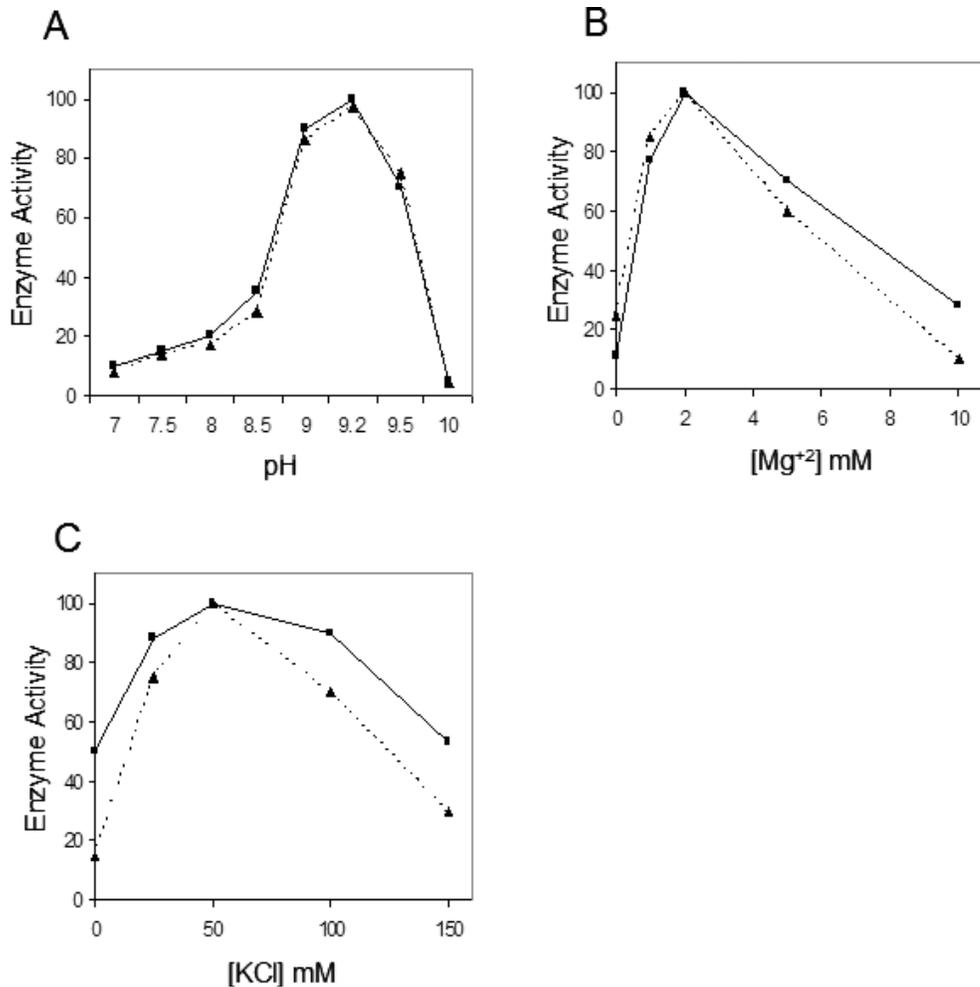


Figure 4. Kinetic parameters for recombinant hAPE1 (closed square) and bovine mtAPE (closed triangle). Dependence on (A) pH, (B) Mg²⁺ and (C) KCl.

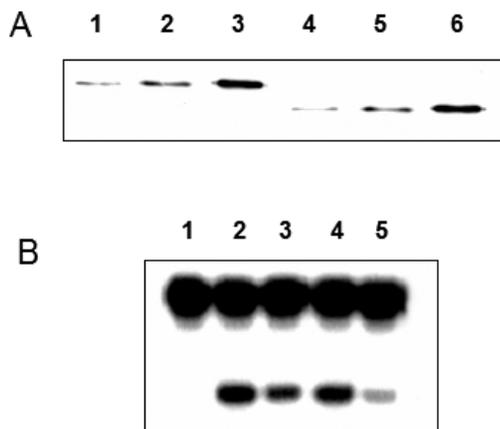


Figure 5. Comparative endonuclease activity of recombinant hAPE1 and bovine mtAPE (fraction 12). (A) Quantification of hAPE1 and bovine mtAPE from western analysis. Lanes 1–3, 4, 8 and 16 ng of APE1, respectively; lanes 4–6, 0.5, 1 and 2 μl of fraction 12, respectively. (B) THF-oligo substrate was incubated with hAPE1 or fraction 12 as described in Materials and Methods. Lane 1, no enzyme; lane 2, 1 fmol hAPE1; lane 3, 0.5 fmol hAPE1; lanes 4–5, fraction 12, at 1000- and 2500-fold dilution, respectively.

outside the matrix. The NΔ33 APE1 lacking the NLS is imported into the mitochondrial matrix possibly via an internal MTS as with cytochrome C. We tested this possibility by examining subcellular distribution of ectopic full-length, NΔ20 and NΔ41 hAPE1 C-terminally fused to EGFP. The full-length APE1 was mostly localized to the nucleus in live cells as observed earlier (40) and confirmed by colocalization with DAPI in fixed cells (Figure 9A and B). The truncated proteins were predominantly distributed in the cytosol, as well as mitochondria as confirmed by colocalization with Mitotracker Red (Figure 9C and D). These results are consistent with our conclusion that the absence of NLS is a prerequisite for mitochondrial import of APE1.

DISCUSSION

Since the discovery of robust BER activity in mammalian mitochondria several years ago (13,19,41), most mitochondrial BER enzymes were identified and characterized. However, the identity of APE, a key BER enzyme was not established. Several recent studies indicated the presence of

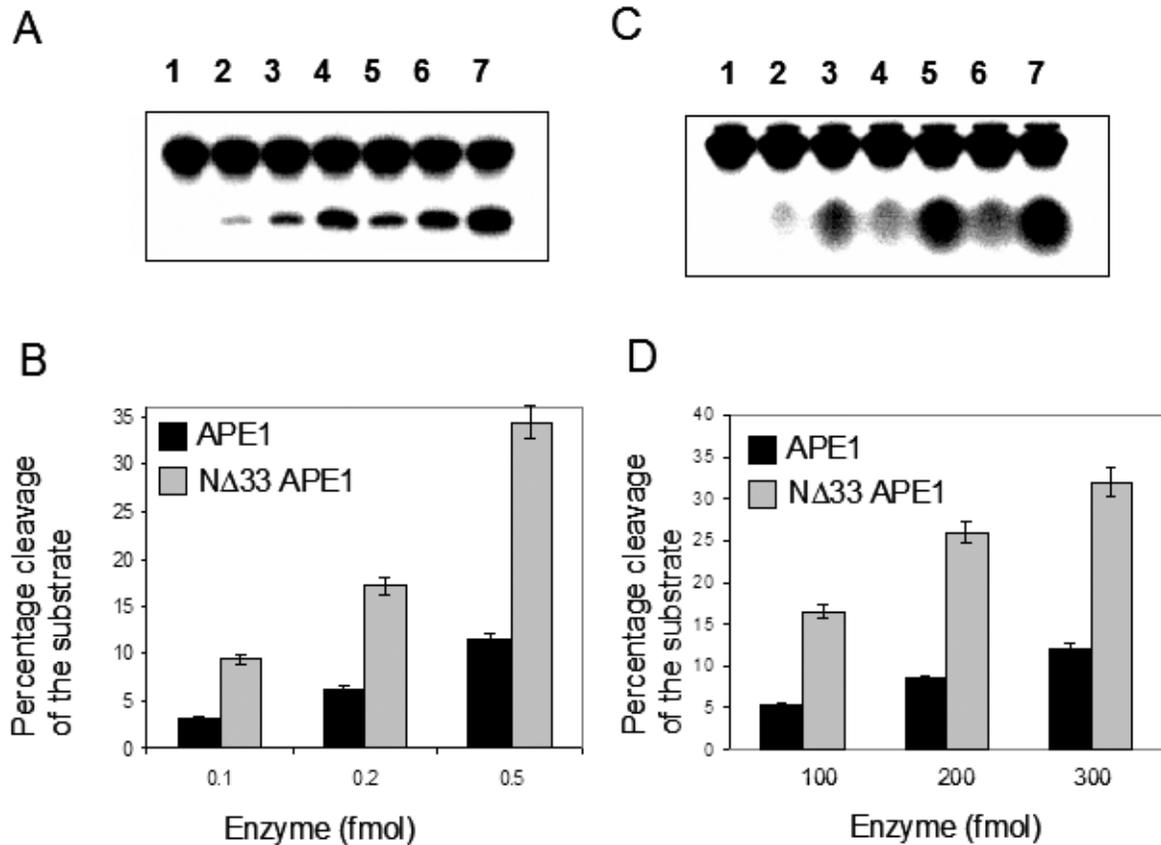


Figure 6. Relative activity of NΔ33 and full-length hAPE1. (A) Endonuclease activity, lane 1, control; lanes 2–4, 0.1, 0.2 and 0.5 fmol full-length APE1; lanes 5–7, 0.1, 0.2 and 0.5 fmol NΔ33 APE1. (C) 3' Phosphodiesterase activity, lane 1, control; lanes 2, 4 and 6; 100, 200 and 300 fmol APE1; lanes 3, 5 and 7; 100, 200 and 300 fmol NΔ33 APE1. (B and D) Graphical representation of the results in (A) and (C), respectively.

Table 1. Kinetic parameters of full-length and truncated hAPE1

	APE1	NΔ33 APE1
K_m (nM)	17	19
K_{cat} (min^{-1})	68	200
K_{cat}/K_m ($\text{min}^{-1} \text{nM}^{-1}$)	4	10.5

nuclear APE1 in the mitochondria, suggesting that APE1 is not altered after its mitochondrial import. However, this was unexpected because of the apparent absence of N-terminal MTS in APE1. We decided to adopt the *ab initio* approach for establishing the identity of mtAPE, based on activity. We have shown here that the mtAPE lacks the N-terminal 33 amino acid residues including the NLS required for nuclear import. We have further shown that the extract of mitochondria but not of nuclei or cytosol, cleaves recombinant hAPE1 to generate a mtAPE-sized product. Our recent studies suggest that a serine protease associated with mitochondria/ER is responsible for this activity (B. Szczesny, unpublished data). These results imply that the loss of NLS is a prerequisite for mitochondrial import of APE. Most mitochondrial proteins contain N-terminal MTS which is cleaved off by the mitochondrial processing peptidase localized within the matrix (42). In the absence of a candidate MTS at the N-terminus, it appears that mtAPE, like a few other mitochondrial proteins, has an alternative, internal MTS which is not cleaved for mitochondrial

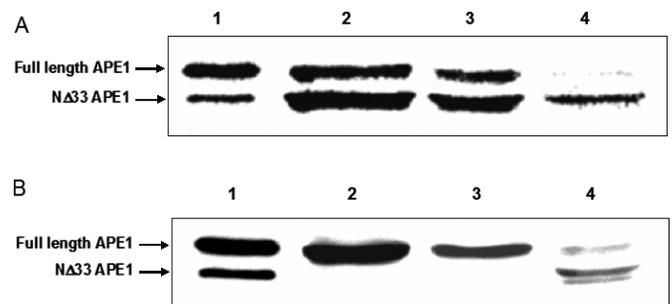


Figure 7. Presence of NΔ33 APE1 in beef liver mitochondria. (A) Lane 1, full-length and NΔ33 APE1 markers; lane 2, mitochondrial extract (50 μg); lane 3, extract (50 μg) of sucrose density gradient-purified mitochondria; lane 4, 25 μg extract after trypsin treatment for 20 min. (B) Lane 1, APE1 and NΔ33 APE1 markers; lanes 2–4, extract (25 μg) of nucleus, cytoplasm and mitochondria, respectively.

import. In any event, the level of mtAPE is generally low, and could not be detected in many cell lines by Western analysis of total mitochondrial extracts (B. Szczesny, unpublished data). The mtAPE could be distinguished from APE1 only by size difference in Western blots, while immunostaining with APE1 antibody will show the presence of the protein in both nucleus and mitochondria (25). This may explain failure to observe mtAPE as a distinct species in some earlier studies. We would like to point out that the truncated NΔ33

APE1 was present at a significant level only in beef liver (Figure 7, lane 2). In contrast, we could detect primarily the full-length APE1 with a trace of mtAPE-sized band in variety of human cell lines. We observed the mtAPE in the

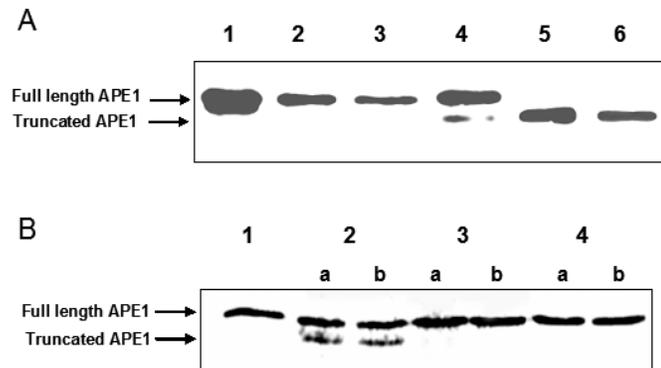


Figure 8. Presence of N Δ 33 APE1 in NIH3T3 cells. (A) Western analysis. Lane 1, recombinant full length hAPE1; lane 2, 20 μ g nuclear extract; lane 3, 20 μ g cytoplasmic fraction; lane 4, 50 μ g mitochondrial extract; lane 5, 50 μ g mitochondrial extract after trypsin treatment for 20 min; lane 6, recombinant N Δ 33 APE1. (B) Specific cleavage of full-length 35 S-labeled hAPE1 by mitochondrial extract. Lane 1, APE1 control; lanes 2–4, treatment with mitochondrial, nuclear and cytoplasmic extracts, respectively as described in Materials and Methods. Full-length and cleaved products in duplicates (a and b) are indicated by arrows.

mitochondrial extract of mouse NIH 3T3 cells only after trypsin treatment (Figure 8A, lane 5). Our results are thus consistent with earlier observation that the full-length APE1 is the major species in cultured cells.

It is interesting that deleting 33 N-terminal amino acid residues increases the specific activity of mtAPE by 3-fold, primarily because of reduced affinity for the product relative to the substrate. Product inhibition of early BER enzymes, i.e. DNA glycosylases and APE has been observed before, leading to the concept of repair-coordination where the consecutive steps in the BER pathway could be coupled (43–45). The disordered N-terminal domain of APE1 is involved in interaction with other BER proteins including DNA polymerase β and XRCC1 (46,47). The absence of some N-terminal residues in mtAPE suggests that such coordination of BER may not be critical in mitochondrial DNA repair. At the same time, we have shown that up to 61 N-terminal amino acid residues in hAPE1 are dispensable for during *in vitro* repair activity (48). It is not known whether residues 34–61 present in mtAPE have a regulatory role during *in vivo* repair of mitochondrial genomes. We should also note that APE1 has two distinct roles in transcriptional regulation. It acts as a reductive activator of many transcription factors, e.g. C-Jun and p53, and was named Ref1 for this activity (49,50). Cys65 in APE1 was shown to be the active residue for Ref1 function which requires 127 N-terminal residues (51). APE1 was subsequently shown to

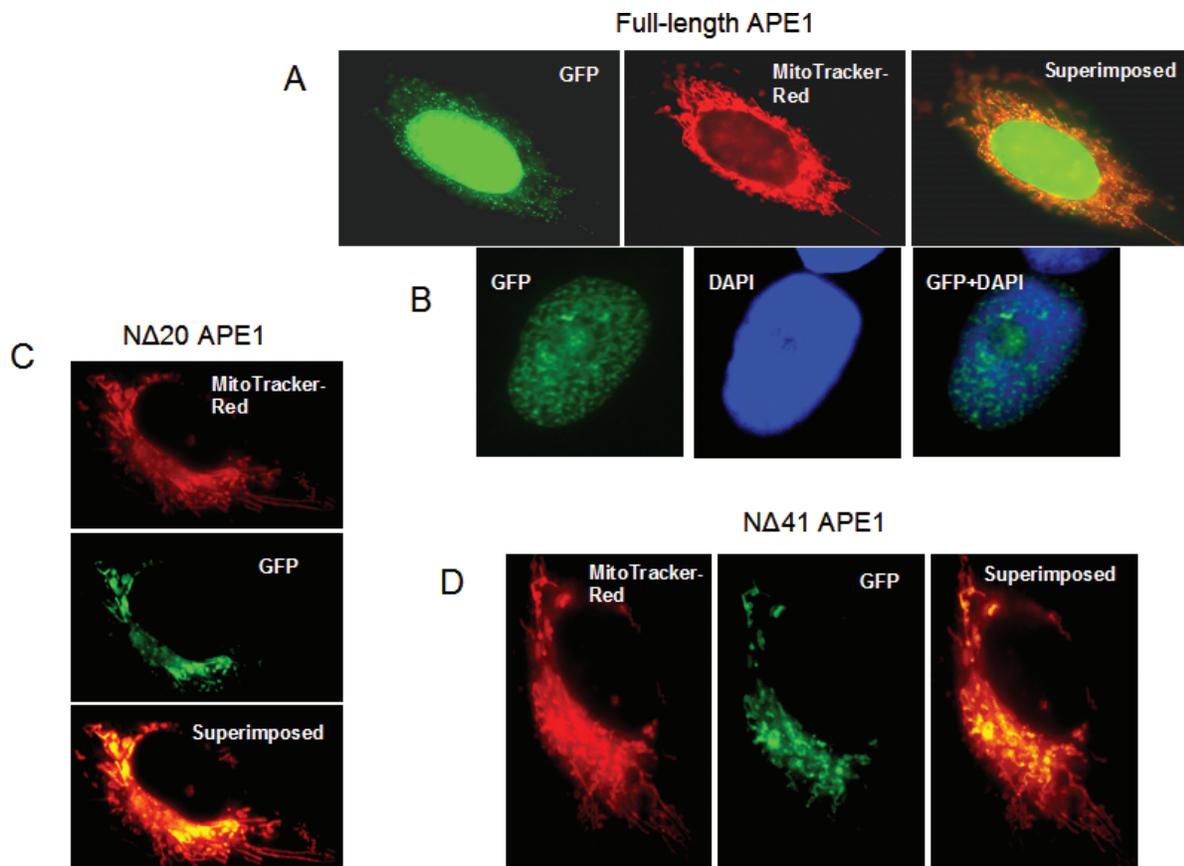


Figure 9. Intracellular localization of full-length and truncated hAPE1 with C-terminal EGFP tag. (A) Nuclear as well as cytoplasmic and mitochondrial localization of full-length APE1-EGFP in live cells. (B) Nuclear localization of full-length APE1 in fixed cells with nuclear DAPI staining. (C and D) Colocalization of N Δ 20 APE1-EGFP and N Δ 41 APE1-EGFP with MitoTracker Red showing their presence in the mitochondria.

act directly as a transcriptional repressor of renin, parathyroid and possibly other genes by participating in protein complexes which bind to the negative Ca²⁺ response elements (nCaRE) (52,53). We have shown the involvement of Lys6/Lys7 acetylation in this process (54). Thus the mtAPE lacks the acetylation-dependant regulatory activity, while retaining the Cys65-dependant redox function.

We have examined age-dependent changes in the intracellular distribution of APE1, whose cytosolic distribution in many cell types is surprising. Analysis of APE activity in nuclear, cytosolic and mitochondrial fractions of hepatocytes from various ages of BALB/C mice showed that the total APE activity in hepatocyte extracts does not vary significantly, but its intracellular redistribution occurs with age (31). The specific activity of APE is ~4-fold higher in the nuclei of old hepatocytes, relatively to the cells obtained from young liver. The mtAPE level increases even more in old mouse livers. We hypothesized that chronic oxidative stress associated with aging is responsible for targeting of APE1 to the nucleus and mitochondria. Oxidative stress in cultured cells also increases the levels of APE in mitochondria and nuclei, in support of our hypothesis (38).

Lieberman and her collaborators have shown that APE1 is a component of the SET complex in cytotoxic T lymphocytes and natural killer cells, and is inactivated by the protease granzyme A which cleaves 31 N-terminal residues from APE1 (55). We could not explain these results because deletion of up to 61 N-terminal residues does not affect APE1's enzymatic activity (48). In fact, deletion of 33 N-terminal residues enhances APE1's repair activity as shown here. It is however possible that, N-terminal truncation leads to APE1's degradation in those lymphocytes.

Finally, we have shown recently that APE1 is essential for survival of APE1 conditional null mutant mouse embryo fibroblasts (56). Others have shown that APE1 downregulation by siRNA causes apoptosis of both untransformed and tumor cells (57,58). APE1 is generally believed to be essential for repairing nuclear DNA damage. However, in view of our earlier studies raising the possibility of APE1-independent BER in the nucleus (59), It is tempting to speculate that APE1's essentiality is due to its role as a precursor of mtAPE, and that the lack of mtDNA repair triggers apoptosis in APE1 deficient cells. It should now be possible to test this hypothesis using our APE1 conditional mutant cells.

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Conflict of interest statement. None declared.

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