Cloning of the multifunctional rat apurinic/aprimidinic endonuclease (rAPEN)/redox factor from an immature T cell line

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Received December 13, 1993; Accepted December 21, 1993

GenBank accession no. L27076

We have cloned a cDNA for the rat apurinic/aprimidinic (AP) endonuclease DNA repair enzyme that, in humans, has also been shown to function as a redox protein, altering the reduction/oxidation state of cFos protein (1). The cDNA was initially isolated by polymerase chain reaction (PCR) from the rat immature T cell line Nb2 (2), using oligonucleotides made to the mouse AP endonuclease (4). The oligonucleotides were located at the translation initiation and termination codons. Following the isolation and initial sequencing of this clone to verify that it was the rat AP endonuclease, it was used to screen a rat testis gt11 cDNA library. A cDNA of 1198 bp was isolated

and sequenced in its entirety. The rat clone predicts an open reading frame of 316 amino acids with a predicted molecular weight of 35,371 D. The rat AP endonuclease has 85% DNA identity to the human (3) and 93% to the mouse AP endonucleases (4). Amino acid identity between the rat and mouse is 97% at the amino acid level, while rat and human share 93% identical amino acids. The region surrounding the conserved cysteine at position 65 (hAPE) near the amino terminus is identical in all three proteins (rat, mouse and human). This cysteine has been shown to be crucial for the redox activity of this enzyme and resides in a domain separate from that required for the DNA

hAPE	M P K R G K K G A V A E D G D E L R T E P E A K K S K T A A K K N D K E
rAPEN	R - - - E P K S T G T E
APEX	- A D E P K S T G T E
hAPE	A A G E G P A L Y E D P P D Q K T S P S A K P A T L K I C S W N V D G L
rAPEN	V A G S
APEX	V G S
hAPE	R A W I K K K 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
rAPEN	
APEX	
hAPE	Q 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 L K V S Y
rAPEN	T
APEX	T
hAPE	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 A Y V P N A G R G L V
rAPEN	E E I
APEX	E E
hAPE	R L E Y R Q R W D E A F R K F L K G L A S R K P L V L C G D L N V A H E
rAPEN	D D
APEX	Y
hAPE	E I D L R N P K G N K K N A G F T P Q E R Q G F G E L L Q A V P L A D S
rAPEN	A M
APEX	
hAPE	F R H L Y P N 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
rAPEN	A A
APEX	A
hAPE	H 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 -- 318 aa
rAPEN	Q G -- 316 aa
APEX	G -- 317 aa

Figure 1. Comparison of the amine acid sequence of rat AP endonuclease (rAPEN) with human (hAPE) (3) and mouse (APEX) (4) homologs. Differences in amino acids are noted below the hAPE sequence. The conserved cysteine residue is indicated by an asterisk.

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repair activity (5). The 5' untranslated and promoter regions have also been cloned and results will be presented elsewhere.

Northern blot analysis using the cloned rat AP endonuclease cDNA revealed a mRNA of approximately 1.5 kb with the testis and spleen having the highest levels of AP endonuclease mRNA, although the gene is expressed in all tissues examined (data not shown). Furthermore, examination of testis cells separated into germ, sertoli and leydig fractions indicates that all cell types express AP endonuclease mRNA, with the germ cells showing the highest level. We are currently performing *in situ* hybridization analysis with rAPEN strand specific probes to confirm these results and to further investigate the pattern of rAPEN gene expression in testis. Other preliminary data obtained from the treatment of adult male rats with the reproductive toxicant methoxy acetic acid (MAA) leads us to conclude that the rAPEN gene is predominantly expressed in immature germ cells when compared to those cells in the post-pachytene stages of spermatocyte development.

The cloning of the rat AP endonuclease gene affords us the opportunity to investigate the regulation of a crucial multifunctional enzyme having both DNA repair and redox activity following various paradigms in an organism that has been extensively used in research, particularly in the areas of aging and hormonal control of gene expression.

ACKNOWLEDGEMENTS

This work was supported by NIH grants RR09884 to M.R.K. and predoctoral fellowship MH10571 to J.P.C. and a Council for Tobacco Institute grant 3739 to M.R.K. We would like to thank Amy M.Saunders for her manuscript preparation assistance.

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