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Genomic Structure of the Rat Major AP Endonuclease Gene (*Apex*) with an Adjacent Putative O-Sialoglycoprotease Gene (*Prsmg1/Gcpl1*) and a Processed Apex Pseudogene (*Apexp1*)

Ming Yao[a], Kosuke Akiyama[a], Yunshan Tan[a], Altaf Hossain Sarker[a], Shogo Ikeda[b], Shahjalal Shafiul Alam[a], Ken Tsutsui[a], Michihiro C Yoshida[c] and Shuji Seki[ad]*

[a]Department of Molecular Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, Okayama 700-8558, Japan, [b]Department of Biological Chemistry, Okayama University of Science, Okayama 700-0005, Japan, [c]Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060-0180, Japan, [d]Department of Human Nutrition, Chugoku Junior College, Okayama 701-0197, Japan

Genomic sequencing and chromosomal assignment of the gene encoding rat APEX nuclease, a multifunctional DNA repair enzyme, were performed. An active *Apex* gene and a processed pseudogene were isolated from a rat genomic library. The active *Apex* gene consists of 5 exons and 4 introns spanning 2.1 kb. The putative promoter region of the *Apex* gene lacks the typical TATA box, but contains CAAT boxes and a CpG island having putative binding sites for several transcription factors, such as Sp1, AP-2, GATA-1 and ATF. A putative O-sialoglycoprotease (a homologue of *Pasteurella haemolytica* glycoprotease, gcp; abbreviated as Prsmg1/Gcpl1) gene consisting of 11 exons and 10 introns spanning 7.3 kb lies immediately adjacent to the *Apex* gene in a 5'-to-5' orientation. The *Apex* gene locus was mapped to rat chromosome 15p12 using *in situ* hybridization. The processed pseudogene (designated as rat *Apexp1*) has a nucleotide sequence 87.1% identical to that of the rat *Apex* cDNA, although several stop codons interrupting the coding sequences and multiple nucleotide deletions were observed. The *Apexp1* is located in an inactive *LINE* sequence. Calculation of nucleotide substitution rates suggests that the immediate, active progenitor of *Apexp1* arose 23 million years ago and that the non-functionalization occurred 15 million years ago.

Key words: apurinic/apyrimidinic endonuclease, glycoprotease, Apex pseudogene, genomic sequencing, chromosomal mapping

APEX nuclease (also called BAP1, HAP1, APE, Ref-1 and APE1) is a multifunctional DNA repair enzyme having apurinic/apyrimidinic (AP) endonuclease, 3'-5' exonuclease, DNA 3' repair diesterase, DNA 3'-phosphatase and RNase H activities (1-3). It is also known to be a redox factor (Ref-1), stimulating DNA binding activity of AP-1 binding proteins such as Fos and Jun (4). cDNAs for APEX nuclease have been cloned from bovine, human, mouse and rat cDNA libraries (reviewed in Refs. 2 and 3). Genomic cloning and sequencing have also been reported for human *APEX* and mouse *Apex* genes (5-8). The *Apex/Ref-1* disruption in germ cells leads to lethality during embryogenesis (9), and the establishment of Apex/Ref-1 (-/-) cell lines has not yet been reported (10). These results suggest that APEX nuclease performs multiple functions in living cells and is essential for cell survival. To analyze the biological function of APEX nuclease, we studied developmental changes and tissue variations of *Apex* gene expression in mice and rats by *in situ* and northern blot hybridizations (11, 12). In order to further clarify the genetic regulation and biological function of APEX nuclease, we cloned the *Apex* gene of the rat, an important model organism in biomedical research. During this study, we noticed the presence of genomic DNA restriction fragments that were hybridizable with labeled rat *Apex* cDNA by Southern blot analysis but were not derived from the active *Apex*

* To whom correspondence should be addressed.

gene. To investigate the origin of the unexpected restriction fragments, we screened intensively *Apex*-related genes in the rat genomic library, and cloned an *Apex* pseudogene in addition to an active *Apex* gene.

This paper describes the genomic structure, sequence and chromosomal localization of the rat *Apex* gene along with those of an adjacent *Prsmg1/Gcpl1* gene [a gene for a putative metalloglycoprotease or for gcp (*Pasteurella haemolytica* glycoprotease)-like 1 (EC3.4.24.57)]. This paper also demonstrates the presence of an inactive, processed *Apex* pseudogene in the rat genomic DNA.

Materials and Methods

Materials. The reagents used in these experiments were obtained from the following sources: restriction enzymes from Toyobo Biochem. (Osaka, Japan); T4 DNA ligase from Promega Corp. (WI, USA); rat genomic library constructed using Sprague-Dawley/C adult rat DNA and cloning vector EMBL-3 SP6/T7 from Clontech Laboratories, Inc. (CA, USA); Megaprime™ DNA Labeling System, [α-^{32}P] dCTP (3,000 Ci/mmol), and positively charged nylon membrane (Hybond-N$^+$) from Amersham Japan (Tokyo, Japan); PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit from PE Applied Biosystems (CA, USA); Blood and Cell Culture DNA Maxi Kit, QIAGEN Lambda Mini Kit, QIAprep Spin Miniprep Kit and QIAquick Gel Extraction Kit from QIAGEN K.K. (Tokyo, Japan). The other materials used were obtained as described previously (13).

Cloning of the rat Apex gene and its pseudogenes. Recombinant bacteriophage clones in a rat genomic library in bacteriophage vector EMBL-3 SP6/T7 (Clontech) were plated with *Escherichia coli* K802. Approximately 1×10^6 bacteriophage clones were screened using the ^{32}P-labeled rat *Apex* cDNA probe by the plaque hybridization technique (14). A ^{32}P-labeled probe was prepared using the *Eco*RI-*Sma*I fragment from the rat *Apex* cDNA clone pUC18-RAPEX80 (12) as a template and [α-^{32}P]dCTP (3,000 Ci/mmol) by the random priming labeling method. Prehybridization was carried out at 68°C in 6 × SSC (1 × SSC: 150 mM NaCl and 15 mM sodium citrate, pH 7.0), 5 × Denhardt's reagent (1 × Denhardt's reagent: 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.02% bovine serum albumin), 0.5% sodium dodecylsulfate (SDS) and 100 μg/ml denatured salmon testes DNA for 4 h. Hybridization was carried out at 68°C in the same solution containing the ^{32}P-labeled cDNA probe for 18 h. The membrane was washed at room temperature twice in 2 × SSC and 0.1% SDS for 15 min, and then twice at 68°C in 1 × SSC and 0.1% SDS for 30 min. After the washing, the membrane was air dried and autoradiographed at − 80°C using Fuji X-ray film with an intensifying screen. Screening was repeated 3 times to isolate positive isolated clones. The recombinant phage DNA was isolated from the positive clones by the plate lysate method. The insert DNAs of the positive clones were subcloned into plasmid vector pUC18 and subjected to DNA sequencing.

DNA sequencing and analysis of the rat Apex gene. DNA sequences were determined on both strands by the dideoxy chain termination method using double-stranded templates and modified AmpliTaq polymerase (ABI PRISM Cycle Sequencing Kit from PE Applied Biosystems) and by an ABI 373S DNA Sequencer (Perkin-Elmer Japan Co., Ltd., Chiba, Japan). Sequencing primers used were the M13 universal primer and specific oligonucleotide primers synthesized according to the sequences determined. The CpG islands (CpG-rich regions) were defined according to the criteria reported by Gardiner-Garden and Frommer (15).

Southern blot analysis of rat genomic DNA. High molecular weight DNA was isolated from rat liver by using a Blood and Cell Culture DNA Maxi Kit (QIAGEN). Ten micrograms of DNA digested with a restriction enzyme were electrophoresed on a 0.7% agarose gel in the standard electrophoresis buffer containing 89 mM Tris-HCl, 89 mM boric acid and 2 mM EDTA (pH 8.0), and transferred to a nylon membrane (Hybond-N$^+$) by the capillary transfer technique. The blotted membrane was hybridized with ^{32}P-labeled rat *Apex* cDNA probe prepared as described above. Prehybridization was carried out at 68°C in 6 × SSC, 5 × Denhardt's reagent, 0.5% SDS and 100 μg/ml denatured salmon testes DNA for 4 h, and then hybridization was carried out at 68°C in the same solution containing ^{32}P-labeled cDNA probes (2 ng/ml) for 20 h. The membrane was washed twice at room temperature in 2 × SSC and 0.1% SDS for 15 min, twice at 68°C in 1 × SSC and 0.1% SDS for 30 min, and then twice at 68°C in 0.1 × SSC and 0.1% SDS for 5 min. After the washing, the membrane was air dried and autoradiographed at − 80°C using Fuji X-ray film with an intensifying screen for 4 weeks.

Fluorescence in situ hybridization (FISH).

Air-dried chromosome preparations were made from fibroblasts derived from primary skin cultures of a WKAH strain rat by standard methods. Chromosomal DNA was denatured by treatment in 70% formamide, 2 × SSC for 3 min at 75 °C. *In situ* hybridization with a biotinylated rat probe was performed according to a modification of the method of Kondoh *et al.* (16). Briefly, the DNA probe (clone A; 13.4 kb) labeled with biotin-16-dUTP (Boehringer Mannheim, Australia) by nick translation was coprecipitated with sonicated salmon sperm DNA and *E. coli* tRNA, dissolved in 100% formamide, denatured for 10 min at 75 °C, and then resuspended in a hybridization mixture containing 4 × SSC, 0.2% bovine albumin, 10% dextran sulfate, and 50% formamide.

After hybridization to metaphase spreads and incubation with anti-biotin Fab'-alkaline phosphatase (ALP) conjugate (1:50) for 45 min at 37 °C, biotinylated probes were detected with the HNPP-azo dye solution by the method previously described (16). Chromosomes were counterstained with Hoechst-quinacrine (17) and mounted in MacIlavaine's buffer (pH 4.5) for simultaneous observations of fluorescent signals and Q-bands with a Nikon fluorescence microscope equipped with a BV-2A filter set (excitation wavelength, 400–440 nm).

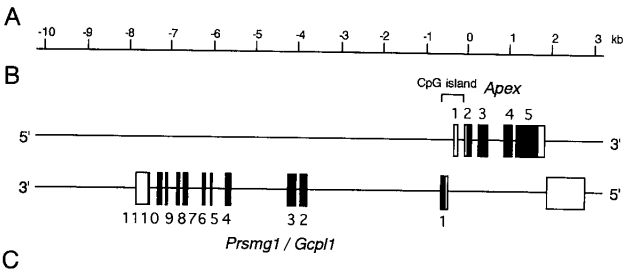
We have used ISCN terminology (18) in describing the chromosomes of the RNO (*Rattus norvegicus*) for rat chromosomes and numbered the rat G-bands according to the system of Satoh *et al.* (19).

Results and Discussion

Isolation of Apex-related genomic clones. A rat genomic library was screened to isolate *Apex* genomic clones as described in Materials and Methods. Fourteen recombinant clones hybridizable to the rat *Apex* cDNA probe were isolated. We selected a clone containing the whole region of the *Apex* gene with a long 5' flanking sequence for further analysis of the active gene. The insert DNA was subcloned into plasmid vector pUC18, and the clone was designated as clone A. Three independent clones having an identical processed pseudogene (*Apexp1*) were also subcloned into plasmid vector pUC18 (clones 2, 21 and 25). Among them, the clone 21 was selected for further analysis, because it contained an insert (8.2 kb) long enough to analyze the pseudogene with the 5'- and 3'-flanking regions.

Sequencing and analysis of the rat Apex gene. The sequence of the clone A insert (13.4 kb) covered the entire *Apex* gene with 9.9 kb, 5' flanking and 1.4 kb, 3' flanking regions. By comparing this sequence with the published *Apex* cDNA sequence (12), we defined the exon/intron structure of the rat *Apex* gene (Fig. 1; accession no. AB023065). The gene consists of 5 exons and 4 introns, with a total length of 2.1 kb. The nucleotide sequence of the exons corresponds exactly to the rat cDNA sequence (12). All exon-intron junctions follow the GT/AG rule (20). The translation initiation and termination codons (ATG and TGA) are located in exon II and exon V, respectively. In order to tentatively assign a base position within the gene and in the flanking regions, the adenine residue in the translation initiation codon of the *Apex* gene was designated as position + 1 and the 5' neighboring residue guanine as position − 1. With this as a starting point, positive numbers were given to positions downstream from the starting point and negative numbers to those upstream. The splicing sites in the amino acid codons are completely conserved as in the mouse and human genes (7, 8). The putative promoter region of the *Apex* gene lacks the typical TATA box, but contains CAAT boxes and a CpG island having putative binding sites for several transcription factors, such as Sp1, AP-2, GATA-1 and ATF.

Genes adjacent to the Apex gene. The sequence analysis of the 9.9 kb, 5' flanking region of the *Apex* gene revealed the presence of a gene coding for a putative glycoprotease homologous to *Pasteurella haemolytica* glycoprotease (gcp) (accession nos.: U15958, M62364) (21). The known substrates for the *Pasteurella haemolytica* glycoprotease are glycoproteins with extensive clusters of negatively charged sugar residues such as O-sialoglycan or sulfoglycan conjugates, including human cell surface glycoproteins (glycophorin A, CD24, CD34, CD43, CD44 and CD45), ligands for P- and L-selectins, the tumor antigen epitectin, the vascular adhesion protein VAP-1, platelet glycoprotein Ib, and cranin, a brain O-sialoglycoprotein (22). Based on the information reported for the prokaryotic gcp enzyme and the information found in EST Data Bases, the gene encoding the putative mammalian homologue of the gcp enzyme is tentatively designated as a metalloprotease (EC3.4.24.57) for glycoprotein (Prsmg1) or gcp (*Pasteurella haemolytica* glycoprotease)-like 1 (Gcpl1). By comparing the 5' flanking sequence of the rat *Apex* gene with mammalian EST-sequences (accession nos.: AA051631, AA105521, AA401938, AA572107, AA822991, AF052137, H31957) homologous to the *Pasteurella haemolytica*



C

No.	Exon size (bp)	5'Splice site sequence	Intron size (bp)	3'Splice site sequence
1	≧50	GGAG gtcagg...	181	...ctgcag GCAGC
2	112	TCCG gtaagg... SerG	204	...ttatag AGCCA luPro
3	188	GGAT gtgagt... Asp	413	...attcag TGGGT Trp
4	193	ATTG gtgaga... IleG	90	...atatag GTGAG lyGlu
5	670			

Fig. 1 Organization of the rat *Apex* gene and adjacent genes. A base position is tentatively assigned as described in the section of Results and Discussion. (**A**) Scale in kilobases (kb). (**B**) A diagram of the rat *Apex* gene and the adjacent genes (the *Prsmg1/Gcpl1* gene and the last exon of an unknown protein) with exons indicated by boxes and introns indicated by solid lines. The exons are numbered from the 5' end of the genes. Filled and open boxes represent translated and untranslated regions, respectively. The CpG island associated with the *Apex* and *Prsmg1/Gcpl1* genes is also indicated. (**C**) Nucleotide sequences of the exon-intron boundaries and the sizes of each exon and intron of the *Apex* gene. Sequences of 5' and 3' splice junctions are given in uppercase letters for exons and in lowercase letters for introns.

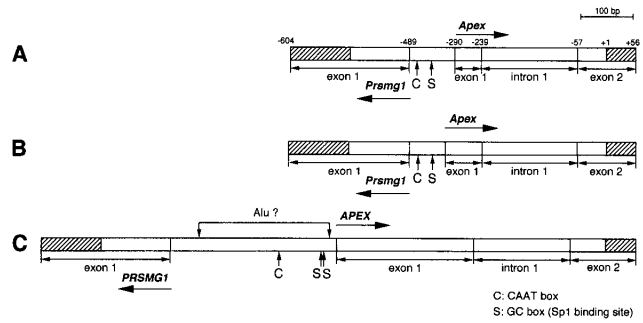


Fig. 2 Comparisons of the genomic structure of 5' terminal and flanking regions of the mammalian major AP endonuclease (*APEX* or *Apex*) gene in rat, mouse and human. The size marker at the top is 100 base pairs. Arrows under the *Apex* (or *APEX*) and *Prsmg1* (or *PRSMG1*) genes indicate the direction of transcription of the respective gene. Coding regions are shaded. Vertical arrows with capital letter C and S indicate the positions of CAAT and GC boxes, respectively. 'Alu?' indicates the Alu-like sequence. (**A**) rat; (**B**) mouse; (**C**) human.

glycoprotease, we defined the exon/intron structure of the rat *Prsmg1/Gcpl1* gene (Fig. 1; accession no. AB023065). The gene consists of 11 exons and 10 introns spanning 7.3 kb. All the exon-intron junctions follow the GT/AG rule (20). The *Prsmg1/Gcpl1* gene lies immediately adjacent to the *Apex* gene in a 5'-to-5' orientation. The closest exons for the *Apex* and *Prsmg1/Gcpl1* genes, the exon 1 of the *Apex* gene and the exon 1 of the *Prsmg1/Gcpl1* gene, lie within a 366 bp sequence. The CpG island is located between the introns 1 of the *Apex* gene and of the putative *Prsmg1/Gcpl1* gene (Fig. 1). The rat Prsmg1/Gcpl1-coding sequence (accession no. AB023065) deduced from the exons appears to code an enzyme consisting of 335 amino acids with a calculated molecular weight of 36,356. The predicted amino acid sequence of the rat Prsmg1/Gcpl1 protein conserves a putative zinc-binding site (His-109 and His-113).

We also found putative human *PRSMG1/GCPL1* and mouse *Prsmg1/Gcpl1* genes in homologous, 5' flanking regions of the human *APEX* and mouse *Apex* genes on chromosomes 14q11.2-q12 and 14C2-D1, respectively. The putative rat Prsmg1/Gcpl1 protein shows 31.8% identity in 321 amino acid residues to the *Pasteurella haemolytica* glycoprotease (accession nos.: U15958, M62364), 94.9% identity in 335 residues to the putative human PRSMG1/GCPL1 protein, and 97.6 % identity in 335 residues to the putative mouse Prsmg1/Gcpl1 protein (unpublished results).

To investigate consensus sequences present commonly in the probable promoter regions of the rat, mouse and human major AP endonuclease genes, the genomic sequences of these *APEX* (or *Apex*) genes were compared in 5' terminal and 5' flanking regions (Fig. 2). A CAAT box and a GC box (Sp1 binding site) are equally located in the intervening sequences between these *APEX* (or *Apex*) and *PRSMG1/GCPL1* (or *Prsmg1/Gcpl1*) genes. The intervening sequences are short and quite similar between the rat and mouse. The intervening human sequence is relatively long, fairly different from those of the rat and mouse, and contains Alu-like sequences as described previously (8). The promoters of the *Apex* and *Prsmg1/Gcpl1* genes are probably overlapped, based on the fact that the intervening sequence is short and that CAAT and GC boxes having characteristics that allow them to function in either orientation are equally present.

The sequence analysis using EST data bases (accession no.: H31707) of the 3' flanking region (1.4 kb) of the *Apex* gene in the clone A indicated the presence of the last exon of an unidentified gene laid in a 3'-to-3' orientation (Fig. 1). The polyadenylation signal (AATAAA) of the 3'-adjacent gene is located at position 1908/1903 (accession no.: AB023065).

Chromosome mapping of the Apex gene. A total of 10 metaphases were examined. All the metaphases had twin spots on one homologue at region p12 of chromosome 15, indicating that the rat *Apex* gene is located on RNO15p12. A representative example of the FISH mapping is shown in Figure 3. Although no obvious morphological similarity is detectable between RNO15 and MMU14, on which the mouse *Apex* gene is located, the present mapping demonstrates that the *Apex* gene is a member of a conserved syntenic group between rat 15 and mouse 14, such as nucleoside phosphorylase on RNO15 and MMU14 (19.50 map units).

After finishing the present experiments, we noticed a report of Johansson *et al.* (23) on cytogenetic localization of rat cancer-related genes including two Apex-related genes which were designated *Apex1* and *Apex2*. These *Apex*-related genes have not yet been cloned or sequenced. However, Johansson *et al.* reported that there appear to be two loci for the Apex gene in the rat, one located on RNO4q12 and the other on RNO15p14. Based on previous reports that the chromosomal localizations of mouse *Apex* and human *APEX* genes are MMU14C3 and HSA14q11.2-q12, respectively, and that MMU14 shows homology to part of RNO15, they described that the gene located on RNO15 appears to be the *Apex1* gene, homologous to the active human and mouse genes. Further, they suggested without any sequence information that the *Apex2* gene on RNO4q12 might represent a pseudogene. As described above, we assigned the rat *Apex* gene to RNO15p12 after cloning and sequencing it. We could not detect any significant hybridization signal on RNO4. The difference between our results and those of Johansson *et al.* is likely to be due to the difference in the probes used for *in situ* hybridization. Johansson *et al.* (23) used the rat *Apex* cDNA cloned by us (12), whereas we used the rat *Apex* genomic clone A as the probe. Our present results agree with theirs in that the active *Apex* gene is located on rat chromosome 15. It is not clear, however, why their location (RNO15p14) for *Apex1*, the active *Apex* gene described in their study, was somewhat different from the location (RNO15p12) of the active *Apex* gene in the present paper. We suggest that their *Apex2* gene on RNO4q12 is not an active *Apex* gene, as will be discussed later.

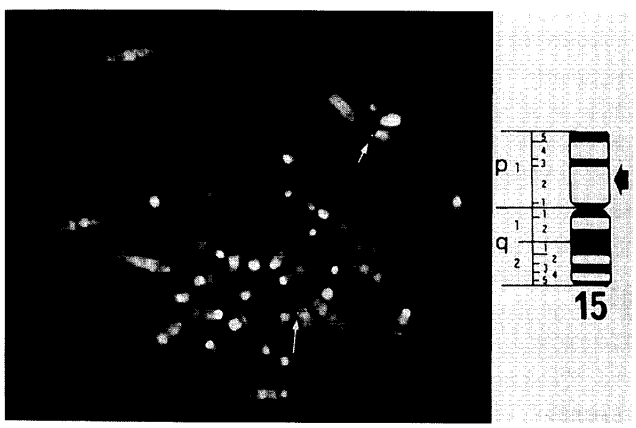


Fig. 3 Chromosome localization of rat *Apex* gene. Arrows indicate the position of the hybridization signals.

Characterization of the rat Apex pseudogene 1 (Apexp1). The clone 21 having an 8.2 kb long fragment encompassing the *Apex* pseudogene (*Apexp1*) was sequenced and analyzed precisely (the sequence is deposited in Genome Data Bases with the accession no. AB023066). In order to assign tentatively a base position within the insert of the clone 21, the guanine residue at the 5' end of the insert sequence on which the *Apexp1* lies was designated as position + 1. With this as a starting point, positive numbers were given to positions downstream.

Apexp1 is a processed pseudogene comprising almost the entire sequence of rat *Apex* cDNA, from its exon 1 to the polyA signal (AATAAA), with the exception of the polyA tail. Although the *Apexp1* sequence is 87.1 % identical to the *Apex* cDNA, in-frame termination codons and deletions or insertions that change the reading frame preclude the possibility that this pseudogene encodes a functional APEX nuclease. The nucleotide sequence encompassing *Apexp1*, which is located at base positions from 1837 to 3026 in the insert of the clone 21, is related to long interspersed repetitive elements (LINEs) (about 74 % identical nucleotides/4350 bp to a LINE3 element-accession no. M13100), with the exception of the sequences from 1 to 780 nt and from 6231 to 8236 nt in the insert, which have no significant sequence homologies with LINES (Fig. 4; accession no. AB023066). *Apexp1* is located in a LINE element in the opposite direction and

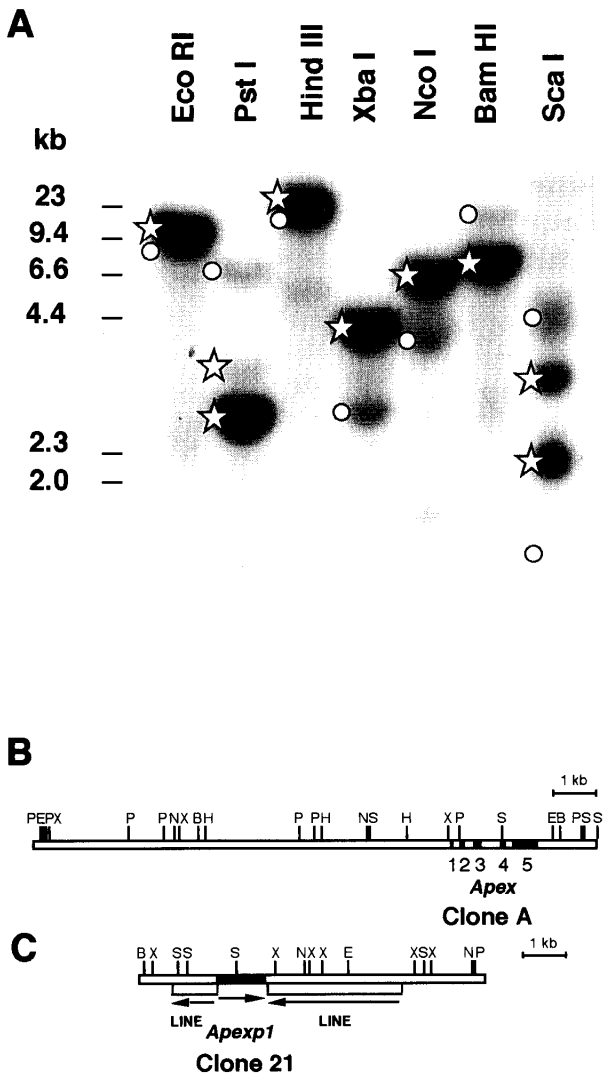


Fig. 4 Southern blot analysis and restriction maps of the rat *Apex* gene and its pseudogene. (**A**) Southern blot analysis of genomic DNA from the rat liver. Rat genomic DNA was digested with the restriction enzyme indicated on the top. Ten micrograms of DNA per lane were subjected to electrophoresis on 0.7% agarose gel and then transferred to a nylon membrane. The blots were hybridized with the ^{32}P-labeled rat *Apex* cDNA. Numbers in the left column indicate size markers in kilobase pairs. Bands corresponding to the *Apex* gene and its pseudogene (*Apexp1*) are indicated by stars and circles, respectively. (**B, C**) Organization and restriction maps of the isolated genomic clone A (**B**) containing the *Apex* gene and clone 21 (**C**) containing the *Apexp1* gene. Recognition sites for restriction enzymes *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Nco*I (N), *Pst*I (P), *Sca*I (S) and *Xba*I (X) are shown over the genes. (**B**) Five exons of the *Apex* gene in clone A are indicated by solid boxes. The Arabic numeral under each exon indicates the exon number. (**C**) The *Apexp1* sequence in clone 21 is indicated by the shaded box. Arrows under the diagram of the clone 21 indicate the coding directions of the progenitor genes (*Apex* and LINE).

flanked by short direct repeats (such as CATTT, TTTTTC and TCTTCAG) which might be involved in the retrotransposition of the gene. The LINE element is thought to be functionally inactive due to the presence of in-frame termination codons and deletions or insertions that change the reading frame.

Processed pseudogenes have been described for numerous housekeeping genes in mammals (24). In terms of *Apex*-related genes, however, the presently described rat *Apexp1* is the first pseudogene reported at the nucleotide level in vertebrates, although Johansson *et al.* (23) recently suggested the presence of a pseudogene in their cytogenetic study. Pseudogenes are formed during the process of evolution. The extent of nucleotide sequence divergence from a functional mRNA can be regarded as a quantifiable interval in an evolutionary clock.

To estimate the time elapsed since the divergence of *Apex* and *Apexp1*, we aligned the rat *Apexp1* sequence with those from rat *Apex* cDNA (accession no.: D44495) (12) and human *APEX* cDNA (accession no.: D90373) (25) and calculated the rate of nucleotide substitutions for each position of the codons, according to the method reported previously (26, 27) (Table 1). According to the computation from Li *et al.* (26), which assumes different substitution rates for inactive pseudogenes and functional genes, it can be estimated that the immediate, active progenitor of the *Apexp1* gene diverged from the *Apex* gene about 23 million years ago and that non-functionalization occurred 15 million years ago. Therefore, it appears that *Apexp1* is a late pseudogene that must be limited to a few species of rodents.

We have attempted to localize the chromosome of the *Apexp1* gene using the 8.2 kb *Apexp1* genomic fragment, but so far have failed to localize it probably due to the presence of the repetitive LINE sequence in the genomic fragment. The Southern blot analysis indicated the presence of a single active *Apex* gene and a single *Apex* pseudogene, as described bellow (Fig. 4). Combining our present results and the results of Johansson *et al.* (23), we suggest that the *Apex2* of Johansson *et al.* on RNO4q12 may be *Apexp1*.

Southern blot analysis. To analyze the exsistence of Apex-related genes in the rat genome, Southern blot analysis of rat genomic DNA was performed using the ^{32}P-labeled, rat *Apex* cDNA probe and several restriction enzymes (*Eco*RI, *Pst*I, *Hin*dIII, *Xba*I, *Nco*I, *Bam*HI and *Sca*I) (Fig. 4). Restriction fragment lengths of genomic DNA containing the true *Apex* gene or a part

Table I Evolutionary rate of rat *Apex* and *Apexp1* genes[a]

	Nucleotide position of codons		
	First	Second	Third
Nucleotide differences[b] (p) between			
rApexp1-rApex	18/306	11/306	23/306
rApexp1-hAPEX	23/305	18/305	100/305
rApex-hAPEX	17/317	8/317	104/317
Nucleotide substitution per site ($-d = (3/4) \ln [1 - (4/3) p]$)			
rApexp1-rApex	0.06126	0.03684	0.07920
rApexp1-hAPEX	0.07948	0.06147	0.43107
rApex-hAPEX	0.05564	0.02567	0.43143
Rate of nucleotide substitution per site per year for functional genes[c]			
	0.28×10^{-9}	0.13×10^{-9}	2.16×10^{-9}

a: Calculated in the manner of Li *et al.* (1981). The sequences are: *rApexp1*, pseudogene from the rat; *rApex* and *hAPEX*, coding sequences from the rat *Apex* and human *APEX* genes, respectively.
b: Number of mismatches per number of aligned codons.
c: Taking into account divergence between the rat and the human 100 million years ago.

of the gene, and those of genomic DNA containing *Apexp1* (a pseudogene) or a part of the gene, were deduced from the 13.4 kb sequence of the clone A and the 8.2 kb sequence of the clone 21, respectively (Fig. 4). The presence of both a single *Apex* gene and a single *Apexp1* gene in the rat genome was confirmed by the Southern blot analysis. Almost all the hybridizing fragments detected by the Southern blot experiment corresponded to the restriction enzyme fragments deduced from the sequences of the *Apex* and *Apexp1* genes, although minor unidentified fragments were also observed (Fig. 4). The origins of the unidentified fragments are not known. They may indicate the presence of a gene(s) distantly related to the *Apex* gene, because the unidentified fragments show only weak hybridization signals.

In summary, we performed genomic cloning, sequencing and chromosomal assignment of Apex-related genes. A single active *Apex* gene is located on RNO15p12. A putative glycoprotease gene is located immediately adjacent to the *Apex* gene in a head-to-head orientation. An Apex pseudogene (*Apexp1*) located in a LINE element is also shown to be present in rat genomic DNA. The present results should provide useful information for the further genetic study of APEX nuclease.

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Foot notes: The nucleotide sequence data reported in this paper have been deposited in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence data bases under the Accession Nos. AB023065 and AB023066.

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