

SHORT COMMUNICATION

Naohiko Seki · Nobuhide Ueki · Kazuhiro Yano
Toshiyuki Saito · Yasuhiko Masuho
Masa-aki Muramatsu

cDNA cloning of a novel human gene *NAKAP95*, neighbor of A-kinase anchoring protein 95 (AKAP95) on chromosome 19p13.11–p13.12 region

Received: July 13, 1999 / Accepted: September 4, 1999

Abstract A-kinase anchoring protein 95 (AKAP95) is a nuclear protein which binds to the regulatory subunit (RII) of cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) and to DNA. A novel nuclear human gene which shares sequence homology with the human *AKAP95* gene was identified by a nuclear transportation trap method. By polymerase chain reaction (PCR)-based analysis with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid panel, the gene was mapped to the chromosome 19p13.11–p13.12 region between markers WI-4669 and CHLC.GATA27C12. Furthermore, alignment with genomic sequences revealed that the gene and human *AKAP95* resided tandemly only approximately 250bp apart from each other. We designated this gene as neighbor of *AKAP95* (*NAKAP95*). The exon-intron structure of *NAKAP95* and *AKAP95* was conserved, indicating that they may have evolved by gene duplication. The predicted protein product of the *NAKAP95* gene consists of 646 amino acid residues, and *NAKAP95* and *AKAP95* had an overall 40% similarity, both having a potential nuclear localizing signal and two C2H2 type zinc finger motifs. The putative RII binding motif in *AKAP95* was not conserved in *NAKAP95*. A reverse transcription coupled (RT)-PCR experiment revealed that the *NAKAP95* gene was transcribed ubiquitously in various human tissues.

Key words Cyclic AMP-dependent protein kinase (PKA) · A-kinase anchoring proteins (AKAPs) · *AKAP95* · Chromosome 19p13.11–p13.12 · RH mapping · Genomic structure · Gene duplication

Introduction

A large number of hormones and neurotransmitters utilize cyclic adenosine monophosphate (cAMP) as an intracellular second messenger. Cyclic AMP regulates a number of key cellular processes such as metabolism, gene regulation, cell growth, cell differentiation, ion channel conductivity, and release of synaptic vesicles (Krebs and Beavo, 1979; Boynton and Whitfield, 1983; Edelman et al. 1987; Roesler et al. 1988; Taylor et al. 1990; McKnight 1991). The main intracellular target for cAMP in mammalian cells is cAMP-dependent protein kinase (PKA or A-kinase). PKA type II is directed to different subcellular loci through interaction of the RII subunits with A-kinase anchoring proteins (AKAPs) (Scott and Macartney, 1994; Rubin 1994; Hausken et al. 1996; Hausken and Scotte, 1996; Faux and Scott 1996). A number of different AKAPs which direct different compartmentalizations have been found: *AKAP79/75* direct the RII to postsynaptic densities and cortical actin (Carr et al. 1992; Li et al. 1996), *AKAP250/Gravin* to filopodia (Nauert et al. 1997), *AKAP350* to centrosomes (Schmidt et al. 1999), *AKAP100* to sarcoplasmic reticulum (McCartney et al. 1995), *AKAP220* to peroxisome (Lester et al. 1996), *AKAP85* to Golgi apparatus (Keryer et al. 1993), and *AKAP84/149* to mitochondria (Chen et al. 1997).

AKAP95 was originally isolated by an interaction cloning strategy with RII α as a probe from a rat pituitary (GH₄C₁) cDNA library (Coghlan et al. 1994). The rat *AKAP95* contained both RII and DNA binding domains. The *AKAP95* was detected in a nuclear matrix fraction, and immunofluorescence, using purified anti-*AKAP95* antibodies, revealed distinct nuclear staining in a variety of cell types (Coghlan et al. 1994). It is proposed that *AKAP95*

N. Seki · T. Saito
Genome Research Group, National Institute Radiological Sciences,
Chiba, Japan

N. Seki
Laboratory of Gene Function II, Kazusa DNA Research Institute,
Chiba, Japan

N. Ueki · K. Yano · Y. Masuho · M. Muramatsu (✉)
Helix Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan
Tel. +81-438-52-3966; Fax +81-438-52-3952
e-mail: mmasaaki@hri.co.jp

M. Muramatsu
Department of Biological Cybernetics, Medical Research Institute,
Tokyo Medical Dental University, Tokyo, Japan

could play a role in targeting type II PKA for cAMP-responsive nuclear events. Recently, human AKAP95 was identified and sequenced, and was shown to be 89% homologous to rat AKAP95 (Eide et al. 1998).

We have recently developed a screening method, designated nuclear transportation trap (NTT), to systematically isolate nuclear proteins (Ueki et al. 1998). Using this method, several novel nuclear genes were isolated, such as *CLIM1/CLIM2*, *PIAS3*, and *HFB30* (Ueki et al. 1999a,b,c). We have also isolated a partial cDNA clone which had a sequence homologous to the human *AKAP95* gene. We describe here the complete cDNA sequence, expression profile, chromosomal assignment, and genomic structure of the gene, *NAKAP95*.

Cloning of human *NAKAP95* gene

A partial cDNA clone (initially called HFB2018) was isolated from a human fetal library, using the NTT method (Ueki et al. 1998). BLAST search revealed that HFB2018 was most homologous to human *AKAP95* (Eide et al. 1998). Specific primers were designed, according to the HFB2018 sequence, to obtain a full-length cDNA from a human fetal brain library, using GeneTrapper (GIBCO BRL, Gaithersburg, MD, USA). The resultant cDNA was 2057bp in length and contained an open reading frame of 646 amino acids (Fig. 1a). The nucleotide sequence of the cDNA will appear in GenBank/EMBL/DDBJ databases under the accession number, AB025905. Since the gene was found to reside next to the human *AKAP95* gene (see below and Fig. 2a) we designated this clone as neighbor of *AKAP95* (*NAKAP95*). The predicted NAKAP protein initiated from the first putative initiation ATG codon was in accordance with Kozak's rule (Kozak 1987). A canonical polyadenylation signal, AATAAA, was located 14bp upstream of a poly(A) (Fig. 1a). The alignment of predicted amino acid sequences of human *AKAP95*, rat *AKAP95*, and human *NAKAP95* proteins is shown in Fig. 1b. Human *NAKAP95* and *AKAP95* are 30% identical (40% similar) at the amino acid level. *NAKAP95* and *AKAP95* possess two C2H2 type zinc finger motifs at a similar position. The regions that included the zinc finger motifs showed the highest identity between the two proteins. A putative nuclear localization signal KKKKRK was found at residues 274–279 (Fig. 1a). From the alignment, the putative RII-binding region of *AKAP95* (Coghlan et al. 1994; Hausken and Scott 1996) was not conserved in *NAKAP95*. Therefore, whether *NAKAP95* can bind to RII remains elusive.

Expression profile of human *NAKAP95* gene

We examined the distribution of the *NAKAP95* transcript in various human tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR) as described previously (Seki et al. 1998; 1999). Primers used for the RT-PCR

corresponded to the coding region of the gene (5'-TGG TGC CGC ATT TGG AGA CAG-3') and (5'-TGC CAA ACC CGA AAC CAA AGC-3'). The primer set gave a longer PCR product from genomic DNA, which was easily distinguished from the 459-bp product from the mRNA. The 459-bp PCR product was generated in all tissues examined, indicating that the transcript is ubiquitously expressed in a wide variety of human tissues (Fig. 3). Therefore, *NAKAP95* described in the present study seems to be involved in a basic house-keeping function of cells.

Chromosome mapping and genomic structure of human *NAKAP95* gene

Chromosomal assignment of the human *NAKAP95* gene was done by PCR analysis of a human/rodent somatic cell hybrid panel (National Institute of General Medicine Service, Coriell Cell Repositories, Camden, NJ, USA) and a radiation hybrid panel (Genebridge 4; Research Genetics, Huntsville, AL, USA), as described previously (Saito et al. 1997; Seki et al. 1997). The human *NAKAP95* specific PCR primers (5'-TCG GCT GCC CTC CCT CTT CTC-3', 5'-GGT CCG CCT CAT CTG CTT CAT-3') gave rise to an amplified product with a size of 139bp by genomic PCR. First, the specific amplified product for human was detected only from the hybrid containing human chromosome 19 (data not shown). Further mapping analysis, using a radiation hybrid panel with the same primer set, was done. Statistical analysis of the radiation hybrid data was performed using the RHMAPPER software package (<http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl>). The data vector for the human *NAKAP95* gene was 0000000101 0001000010 1000111110 0001010000 1100010101 1011010001 0001000110 1101011001 0001011100 001 and the consequent report indicated that the gene was placed to 3.89cR distal from the marker CHLC.GATA27C12 (lod > 3.0). The region including the marker was cytogenetically mapped to the 19p13.11–p13.12 region (Fig. 2a). Since human *NAKAP95* and *AKAP95* genes have been mapped to the same region, we then searched for genomic sequences in the public database, and found a cosmid clone which contains both genes (accession number, AC005785). The human *NAKAP95* and *AKAP95* resided tandemly and the first exon of human *AKAP95* began 258bp after the end of the *NAKAP95* gene. We have no data about the orientation of *NAKAP95* and *AKAP95* in chromosome 19. To confirm that human *AKAP95* and *NAKAP95* are juxtaposed, primers were designed between the last exon of human *NAKAP95* and the first exon of *AKAP95* (5'-GAC AGC CCC GAG GAG GAG AAG-3' and 5'-CCC ACC AGC AGC CCC GTT TAC-3'; product size, 668bp). Genomic PCR using the primers generated a PCR product of the expected size (data not shown), proving that human *AKAP95* and *NAKAP95* are next to each other. Although the sequence between the genes would provide the promoter for the *AKAP95* gene, it lacked a typical TATA box or a GC-rich region.

Fig. 1. a Nucleotide sequence and deduced amino acid sequence of human *NAKAP95* gene. Asterisk denotes the stop codon. Two C2H2 zinc finger motifs are *underlined* and a putative nuclear localizing signal is shown in *italics*. The polyadenylation signal, aataaa, is *double underlined*. The nucleotide sequence of the *NAKAP95* gene is deposited in GenBank/EMBL/DBJ databases under the accession number, AB025905. The nucleotide sequences of both strands were determined by a primer walking method, using an ABI377 sequencer (Perkin Elmer, Norwalk, CT, USA). **b** Alignment of human AKAP95 (accession number, Y11997), rat AKAP95 (accession number, U01914), and human NAKAP95 (accession number, AB025905) proteins. Identities are indicated by *black background* and similar residues are *shadowed*. Asterisks denote the termination codon

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gagcagcagaagccggcgtcgtcggatggtgtgttgcgccaccATGAGCTACACAGGC      60
                                     M S Y T G                          5
TTTGTCCAGGGATCTGAAACCACTTTGCAGTCGACATACTCGGATACCAGCGCTCAGCCC    120
F V Q G S E T T L Q S T Y S D T S A Q P                          25
ACCTGTGATTATGGATATGGAACCTGGAACCTGCGGACAAATAGAGGCTACGAGGGCTAT    180
T C D Y G Y G T W N S G T N R G Y E G Y                          45
GGCTATGGCTATGGCTATGGCCAGGATAACACCACCAACTATGGGTATGGTATGGCCACT    240
G Y G Y G Y G Q D N T T N Y G Y G M A T                          65
TCACACTCTTGGGAAATGCCTAGCTCTGACACAAATGCAAACACTAGTGCCTCGGGTAGC    300
S H S W E M P S S D T N A N T S A S G S                          85
GCCAGTGCCGATTCCGTTTTATCCAGAATTAACCAGCGCTTAGATATGGTGCCGCATTTG    360
A S A D S V L S R I N Q R L D M V P H L                          105
GAGACAGACATGATGCAAGGAGGCGTGACGGCTCAGGTGGAGAAAGGTATGACTCTTAT    420
E T D M M Q G G V Y G S G G E R Y D S Y                          125
GAGTCCGCGACTCGAGGGCCGCTCTGAGTGAGCGCGACCTGTACCGGTGAGGCTATGAC    480
E S C D S R A V L S E R D L Y R S G Y D                          145
TACAGCGAGCTTGACCCTGAGATGGAAATGGCCTATGAGGGCCAATACGATGCCTACCGC    540
Y S E L D P E M E M A Y E G Q Y D A Y R                          165
GACCAGTTCGCGATGCGTGGCAACGACACCTTCGGTCCCAGGGCACAGGGCTGGGCCCGG    600
D Q F R M R G N D T F G P R A Q G W A R                          185
GATGCCCGGAGCGGCCGCAATGGCCTCAGGCTATGGGCGCATGTGGGAAGACCCCATG    660
D A R S G R P M A S G Y G R M W E D P M                          205
GGGGCCCGGGCCAGTGCATGTCTGGTGCTCTCGGCTGCCCTCCCTCTTCTCCAGAAC    720
G A R G Q C M S G A S R L P S L F S Q N                          225
ATCATCCCCGAGTACGGCATGTTCAGGGCATGCGAGGTGGGGGCGCCTTCCCGGGCGGC    780
I I P E Y G M F Q G M R G G G A F P G G                          245
TCCCGCTTTGGTTTCGGGTTTGGCAATGGCATGAAGCAGATGAGGCGGACCTGGAAGACC    840
S R F G F G F G N G M K Q M R R T W K T                          265
TGGACCACAGCCGACTTCCGAACCAAGAAGAAGAGAAAGCAGGGCGGCAGTCTTGAT    900
W T T A D F R T K K K K R K Q G G S P D                          285
GAGCCAGATAGCAAAGCCACCCGCACGGACTGCTCGGACAACAGCGACTCAGACAATGAT    960
E P D S K A T R T D C S D N S D S D N D                          305
GAGGGCACCGAGGGGAAGCCACAGAGGGCCTTGAAGGCACCGAGGCTGTGGAGAAGGGC    1020
E G T E G E A T E G L E G T E A V E K G                          325
TCCAGAGTGGACGGAGAGGATGAGGAGGAAAAGAGGATGGGAGAGAAGAAGGCAAAGAG    1080
S R V D G E D E E G K E D G R E E G K E                          345
GATCCAGAGAAGGGGGCCCTAACCACCCAGGATGAAAATGGCCAGACCAAGCGCAAGTTG    1140
D P E K G A L T T Q D E N G Q T K R K L                          365
CAGGCAGGCAAGAAGAGTCAGGACAAGCAGAAAAAGCGGCAGCGAGACCGCATGGTGGAA    1200
Q A G K K S Q D K Q K K R Q R D R M V E                          385
AGGATCCAGTTTGTGTGTTCTCTGTGCAAAATACCGGACCTTCTATGAGGACGAGATGGCC    1260
R I Q F V C S L C K Y R T F Y E D E M A                          405
AGCCATCTTGACAGCAAGTTCCACAAGGAACACTTTAAGTACGTAGGCACCAAGCTCCCT    1320
S H L D S K F H K E H F K Y V G T A K L P                          425
AAGCAGACGGCTGACTTTCTGCAGGAGTACGTCACTAACAAGACCAAGAAGCAGAGGAG    1380
K Q T A D F L Q E Y V T N K T K K T E E                          445
CTCCGAAAAACCGTGAGGACCTTGATGGCCTCATCCACCAATCTACAGAGACCAGGAT    1440
L R K T V E D L D G L I H Q I Y R D Q D                          465
CTGACCCAGGAAATGCCATGGAGCATTTTGTGAAGAAGGTGGAGGCAGCCATTGTGCA    1500
L T Q E I A M E H F V K K V E A A H C A                          485
GCCTGCGACCTCTTCATTCCCATGCAGTTTGGGATCATCCAGAAGCATCTGAAGACCATG    1560
A C D L F I P M O F G I I O K H L K T M                          505
GATCACAACCGGAACCGCAGGCTCATGATGGAGCAGTCCAAGAAGTCTCCCTCATGGTG    1620
D H N R N R R L M M E Q S K K S S L M V                          525
GCCCCGAGTATTCTCAACAACAAGCTCATCAGCAAGAAGCTGGAGCGCTACCTGAAGGGC    1680
A R S I L N N K L I S K K L E R Y L K G                          545
GAGAACCCTTTACCCGACAGCCCCGAGGAGGAGAAGGAGCAGGAGGAGGCTGAGGGCGGT    1740
E N P F T D S P E E E K E Q E E A E G G                          565
GCCCTGGACGAGGGGGCGCAGGGCGAAGCGGCAGGGATCTCGGAGGGCGCAGAGGGCGTG    1800
A L D E G A Q G E A A G I S E G A E G V                          585

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CCGGCGCAGCCTCCCGTGCCCCAGAGCCAGCCCCGGGGCCGTGTCGCCGCCACCGCCG 1860
P A Q P P V P P E P A P G A V S P P P P 605
CCGCCCCAGAGGAGGAGGAGGAGGGCGCGCTGCCCTTGCTGGGTGGGGCGCTGCAACGC 1920
P P P E E E E E G A V P L L G G A L Q R 625
CAGATCCGCGGCATCCCGGGCCTCGACGTGAGGACGACGAGGAGGGCGGGGGGGCGCC 1980
Q I R G I P G L D V E D D E E G G G G A 645
CCGTGAcceggagctcgggggggggagcccgcgtggccgaactggaaaccaaactaa 2040
P * 646
a taaagttttcccatccc 2057

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hAKAP95 1 MDQGYGGYGAW SAGPANTQGA YGTVASWQGYENYNY YGAQNTSVTTGATYSYGPASWEA
rAKAP95 1 MEQSYGGYGAW SAGPANTQGT YSGVASWQGYENYS YNAQNTSVPTGTPYSYGPASWEA
NAKAP95 1 ~~~MSYTG FVQGS ETTLQST YSDTSAQPT CDYGYGTWNSG TNRGYEGYGYGYGQDNT

hAKAP95 61 AKANDGGLAAGAPAMHMAS YGPEPCTDNS...DSLIAKINQRLDMM SKEGGRGGS SGGGGE
rAKAP95 61 TKASDGLAAGSSAMHVASF APEPCTDNS...DSLIAKINQRLDMM SKEGGRGGS ISSGGE
NAKAP95 57 TNYGYGMATSHS WEMPSSD TNANTSASGSASA DSVLSRINQRLDMV.PHLETDMMQGGVY

hAKAP95 118 GIQDRBSSFRFQPFESYDSR PCLPEHN PYRPSYSYDYDFDLGSDRNGSFGGQYSECRDPA
rAKAP95 118 GMQDRDSSFRFQPYESYDSR PCLPEHTPYRPSYSYDYDFDLGIDRNGSFGGTFND CRDPT
NAKAP95 116 G...SGGERYDSYES CDSRAVLSE RDLYRSGVDYS...ELDP EMEMAYEGQYDAYRDQF

hAKAP95 178 RERGS LDGFM RGRGQGRFQDRSNPGTFMRSDPFVPPAASSEPLSTPWNEELNYVGGRLGG
rAKAP95 178 PERGALDGF LRGRGQGRFQDRSNSS TFRSDPFMPPSASSEPLSTWSELNYMGGRLGG
NAKAP95 169 RMRG..NDTFGPRAO GWARDARSGR.....PMASG..YGRM WEDPMGARGQCMMSG

hAKAP95 238 PSPSRPPPSLFSOSMAPDYGV.MGMQGAGGYDS TMPYGCGRSQPRMRDRDRPKRRGFDRF
rAKAP95 238 PSTNRPPPSLFSOSMAPDYSM.MGMQGVGGFGG TMPYGCGRSQTRL..RDWPRRRGFDRF
NAKAP95 215 ASRL...PSLFSQNIIP EYGMFOGMRGCGGAFPGGSRFGFGFGNGMKQMRRTWK TWTTADF

hAKAP95 297 GPDGTGRKRKQFQLYE EPDTKLARVD.SEGDFSEND DAA.GDFRSG.....
rAKAP95 295 GPDNMGRKRKPFPLYE E PDAKLARAD.SEGDLSEND DGA.GDLRSG.....
NAKAP95 272 RTKK..KRRKQGGSPDE PDSKATR TDCSDNSDSDNDEGTEGEATEGLEGTEAVEKGS RVD

hAKAP95 341 DEEFKGEDELCD SGRORGEK.....EDED EDVKK.....RREKQRRRDRTRDRAADRIQF
rAKAP95 339 DEEFRGEDDLCD SRKORGEK.....EDED EDVKK.....RREKQRRRDRMRDRAADRIQF
NAKAP95 330 GEDEEGKEDGRE EGKEDPEK GALTTQDENGQTKRKLQAGKKSODKQKQRDRMVERIQF

hAKAP95 391 ACSVCKFRSFDDEEIQKHLQSKFHKETLRFISTKLPDKTVEFLQEYIVN RNKKIEKRROE
rAKAP95 389 ACSVCKFRSFEDEEIQKHLQSKFHKETLRFISTKLPDKTVEFLQEYIIN RNKKIEKRROE
NAKAP95 390 VCSLCKYRTFYEDEMASHLDSKFHKEHF KYVGTKL PKQTADFLQEYV TNKTKKTEELRKT

hAKAP95 451 LMEKETAKPK...PDPFKGIGOE HFFKKIEAAHCLACDMLIPAQPOLLORHLHSVDHNN
rAKAP95 449 LLEKESPKPK...PDPFKGIGOE HFFKRIEAAHCLACDMLIPAQHOLLORHLHSVDHNN
NAKAP95 450 VEDLDGLIHQIYRDQDLTQEIAMEHFVKKV EAAHCAACDLFIPM OFGTIQKHLKTM DHNR

hAKAP95 507 NRRLAAEQFKK TSLHVAKSVLNNRHIVKMLEKYLKGEDPFTSETVDPEMEGDDNLGGEDK
rAKAP95 505 NRRLAAEQFKK TSLHVAKSVLNNKHIVKMLEKYLKGEDP FVNETA DLETEG DENLG..EE
NAKAP95 510 NRRLMMEQSKK S SLMVAR S I LNNKLI SKKLE RYLKGENPFTDSPEE KEQEFAEGGALDE

hAKAP95 567 KETPEEVAADVLAEVITA AAVRAVDGEGAPAP ESSGEP ADEGPTDTAEAGSDPQAEOLLE
rAKAP95 563 KETPEEVAAEVLAEVITA AVKAVEGDGEPAAEHS DVLAEVEGPVDTAEAGSDSHTGK LLE
NAKAP95 570 GAQGEAAGISEGAEGVPAQ.PPV PPEPAPGAVSP PPPP...PEEEEECAVPLLG GALQ

hAKAP95 627 EQVPCGTAHE..KGVPKARSEAAEAGNGAETMAAEAES AQTRVAPAPAAADA EVEQ TDAE
rAKAP95 623 EQT.CETASETRNME DMARGEAAEARN EAAVPA AAGSPVPVIA.IPGILEDELEQ TDAE
NAKAP95 625 RQIRGIPGLDVEDDEEGGGGAP*-----

hAKAP95 685 SKDAVPTE*
rAKAP95 681 AKD.TPTE*
b NAKAP95 647 ~~~~~

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Fig. 1. Continued

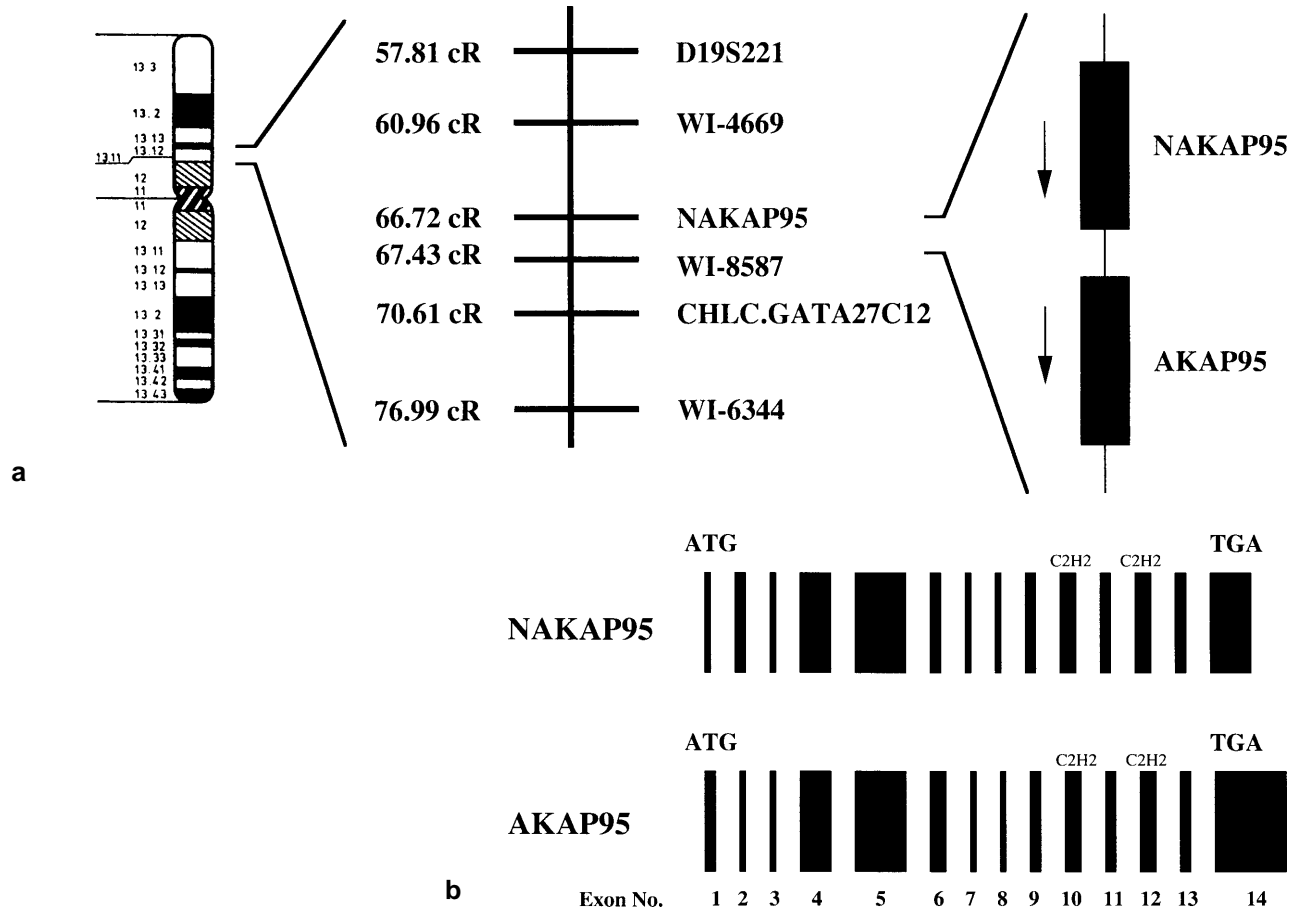


Fig. 2. a Chromosomal placement of the *NAKAP95* gene at a relative distance to framework markers on the WICGR radiation hybrid map of the human genome (http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map). The approximate corresponding cytogenetic location of

the gene on chromosome 19p13.11–p13.12 region is indicated. Distances are in centirays (cR) from the top of the chromosome 19 linkage group. **b** Schematic exon-intron structure of human *NAKAP95* and *AKAP95* genes

Table 1. Intron–exon boundaries of the *NAKAP95* gene

Exon No.	Exon size ^a	Splice acceptor ^b	Splice donor ^b
1	58		AGCTACACAG gtgggcctggc
2	75	ctttatgtttag GCTTGTCCA	TGTGATATG gtaagtgtggac
3	33	tactttttgtag GATATGGAAC	ACAAATAGAG gcaagtgtcatt
4	241	ccctcaactgcag GCTACGAGGG	GTGGAGAAAG gtgagtgagcac
5	454	atgcctctgcag GTATGACTCT	CGACTTCCGA gtgagtgaggc
6	97	cttatgaccag ACCAAGAAGA	TCAGACAATG gtgagccacta
7	71	tttccctttcag ATGAGGGCAC	CTCCAGAGTG gtaagagctct
8	64	tgaattgttag GACGGAGAGG	CCAGAGAAGG gtgagtttctc
9	109	ctgctcccacag GGGCCCTAAC	TGGTGGAAAG gtaaccagcttc
10	142	ttcctgtggcag GATCCAGTTT	CTTCTGCAG gtgagccttggg
11	106	tattccttgcaag GAGTACGTCA	CTGACCCAGG gtgaggagattt
12	131	tccccactacag AAATTGCCAT	GAACCGCAGG gtgagtgccac
13	96	ccctgccgcag CTCATGATGG	CTACCTGAAG gtgaggcactgg
14	380	ccctccccacag CCGCTCTTGG	AAGGGTAGGG

Intron–exon junctions were established by comparison of cDNA and genomic sequences

^aSize in basepairs

^bSequences at the splice junction. Exonic sequences are shown in capital letters, with intronic sequences shown in lowercase letters. Invariant nucleotides (ag/gt, gc) are in boldface type

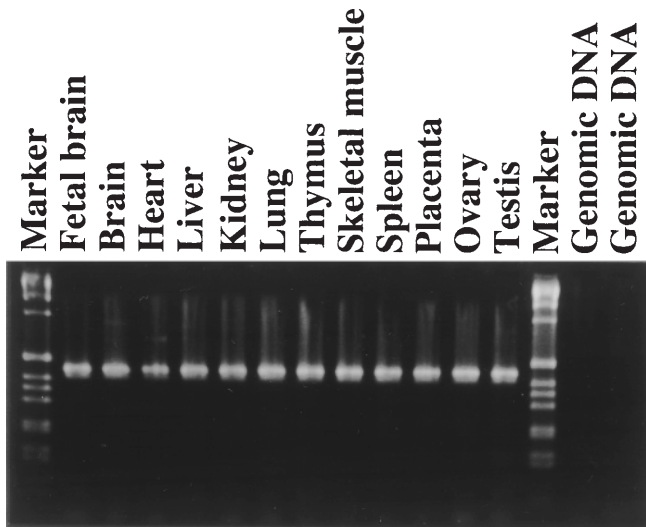


Fig. 3. Tissue distribution analysis, using reverse transcription coupled-polymerase chain reaction (RT-PCR). The 12 tissues and genomic DNA examined are indicated *above each lane*. The templates of the human tissues of poly(A)⁺ RNAs were purchased from Clontech (Palo Alto, CA, USA). The cDNA templates for RT-PCR were synthesized from 2 µg of poly(A)⁺, using excess amounts of Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA) and random hexamer primers. PCR was carried out in a final volume of 10 µl containing 1 × LA-PCR buffer (Takara, Kyoto, Japan), 2 µM each primer, 200 µM each dNTP, 1 µl of template DNA, and 0.01 units of LA-Taq DNA polymerase (Takara). Temperatures and time schedule were: 30 cycles of 95°C for 20s and 62°C for 1 min. PCR products were separated on 2.5% Nusieve GTG agarose gel (FMC, Rockland, ME, USA) with a 1-kb ladder DNA marker (GIBCO BRL)

The exon-intron boundaries of the human *NAKAP95* and *AKAP95* genes were determined by aligning the cDNA sequence with the genomic sequence from the two cosmid clones (accession numbers, AC005785, AC006128) (Fig. 2b). As shown in Table 1, all but one of the splicing sites conformed to the canonical splicing acceptor and donor rule of AG-GT; one was AG-GC. The *NAKAP95* gene was divided into 14 exons, which ranged in size from 33 bp (exon 3) to 454 bp (exon 5). Exons 1 and 14 contained the ATG and TAG codons, respectively. The exon-intron boundary of the human *AKAP95* gene was also determined in the same manner, revealing that human *NAKAP95* and *AKAP95* had a very similar gene structure (Fig. 2b). In both cases, the C2H2 type zinc finger motif resided in exons 10 and 12. These results strongly argue that the *NAKAP95* and *AKAP95* genes could have been established by tandem gene duplication.

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