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SHORT COMMUNICATION

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

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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 250 bp 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.

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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 RIIα as a probe from a rat pituitary (GH_4C_1) 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

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 2057 bp 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 14 bp 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 139 bp 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 3.89 cR 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 258 bp 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, 668 bp). 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/DDBJ 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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gagcagcagaagccggcgtcgtcggatgttgtgttgcccgccaccATGAGCTACACAGGC      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
ACCTGTGATTATGGATATGGAACTTGGAACTCTGGGACAAATAGAGGCTACGAGGGCTAT     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
GAGACAGACATGATGCAAGGAGGCGTGTACGGCTCAGGTGGAGAAAGGTATGACTCTTAT     420
E  T  D  M  M  Q  G  G  V  Y  G  S  G  G  E  R  Y  D  S  Y      125
GAGTCCTGCGACTCGAGGGCCGTCCTGAGTGAGCGCGACCTGTACCGGTCAGGCTATGAC     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
GACCAGTTCCGCATGCGTGGCAACGACACCTTCGGTCCCAGGGCACAGGGCTGGGCCCGG     600
D  Q  F  R  M  R  G  N  D  T  F  G  P  R  A  Q  G  W  A  R      185
GATGCCCGGAGCGGCCGGCCAATGGCCTCAGGCTATGGGCGCATGTGGGAAGACCCCATG     660
D  A  R  S  G  R  P  M  A  S  G  Y  G  R  M  W  E  D  P  M      205
GGGGCCCGGGGCCAGTGCATGTCTGGTGCCTCTCGGCTGCCCTCCCTCTTCTCCCAGAAC     720
G  A  R  G  Q  C  M  S  G  A  S  R  L  P  S  L  F  S  Q  N      225
ATCATCCCCGAGTACGGCATGTTCCAGGGCATGCGAGGTGGGGGCGCCTTCCCGGGCGGC     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
TGGACCACAGCCGACTTCCGAACCAAGAAGAAGAAGAGAAAGCAGGGCGGCAGTCCTGAT     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
GAGGGCACCGAGGGGGAAGCCACAGAGGGCCTTGAAGGCACCGAGGCTGTGGAGAAGGGC    1020
E  G  T  E  G  E  A  T  E  G  L  E  G  T  E  A  V  E  K  G      325
TCCAGAGTGGACGGAGAGGATGAGGAGGGAAAAGAGGATGGGAGAGAAGAAGGCAAAGAG    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
AGGATCCAGTTTGTGTGTTCTCTGTGCAAATACCGGACCTTCTATGAGGACGAGATGGCC    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  K  L  P      425
AAGCAGACGGCTGACTTTCTGCAGGAGTACGTCACTAACAAGACCAAGAAGACAGAGGAG    1380
K  Q  T  A  D  F  L  Q  E  Y  V  T  N  K  T  K  K  T  E  E      445
CTCCGAAAAACCGTGGAGGACCTTGATGGCCTCATCCACCAAATCTACAGAGACCAGGAT    1440
L  R  K  T  V  E  D  L  D  G  L  I  H  Q  I  Y  R  D  Q  D      465
CTGACCCAGGAAATTGCCATGGAGCATTTTGTGAAGAAGGTGGAGGCAGCCCATTGTGCA    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  Q  F  G  I  I  Q  K  H  L  K  T  M      505
GATCACAACCGGAACCGCAGGCTCATGATGGAGCAGTCCAAGAAGTCCTCCCTCATGGTG    1620
D  H  N  R  N  R  R  L  M  M  E  Q  S  K  K  S  S  L  M  V      525
GCCCGCAGTATTCTCAACAACAAGCTCATCAGCAAGAAGCTGGAGCGCTACCTGAAGGGC    1680
A  R  S  I  L  N  N  K  L  I  S  K  K  L  E  R  Y  L  K  G      545
GAGAACCCTTTCACCGACAGCCCCGAGGAGGAGAAGGAGCAGGAGGAGGCTGAGGGCGGT    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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                CCGGCGCAGCCTCCCGTGCCCCCAGAGCCAGCCCCCGGGGCCGTGTCGCCGCCACCGCCG   1860
                P  A  Q  P  P  V  P  P  E  P  A  P  G  A  V  S  P  P  P  P     605
                CCGCCCCCAGAGGAGGAGGAGGAGGGCGCCGTGCCCTTGCTGGGTGGGGCGCTGCAACGC  1920
                P  P  P  E  E  E  E  E  G  A  V  P  L  L  G  G  A  L  Q  R     625
                CAGATCCGCGGCATCCCGGGCCTCGACGTGGAGGACGACGAGGAGGGCGGCGGGGGCGCC  1980
                Q  I  R  G  I  P  G  L  D  V  E  D  D  E  E  G  G  G  G  A     645
                CCGTGAcccgagctcggggcgggcggagcccgcgtggccgaacgtggaaaccaaacctaa  2040
                P  *                                                           646
          a     taaagttttcccatccc                                              2057

hAKAP95       1 MDQGYGGYGAWSAGPANTQGAYGTGVASWQGYENYNYYGAQNTSVTTGATYSYGPASWEA
rAKAP95       1 MEQSYGGYGAWSAGPANTQGTYGSGVASWQGYENYSYYNAQNTSVPTGTPYSYGPASWEA
NAKAP95       1 ~~~~MSYTGFVQGSETTLQSTYSDTSAQPTCDYGYGTWNSGTNRGYEGYGYGYGYGQDNT

hAKAP95      61 AKANDGGLAAGAPAMHMASYGPEPCTDNS...DSLIAKINQRLDMMSKEGGRGGSGGGGE
rAKAP95      61 TKASDGGLAAGSSAMHVASFAPEPCTDNS...DSLIAKINQRLDMLSKEGGRGGISSGGE
NAKAP95      57 TNYGYGMATSHSWEMPSSDTNANTSASGSASADSVLSRINQRLDMV.PHLETDMMQGGVY

hAKAP95     118 GIQDRESSFRFQPFESYDSRPCLPEHNPYRPSYSYDYEFDLGSDRNGSFGGQYSECRDPA
rAKAP95     118 GMQDRDSSFRFQPYESYDSRPCMPEHTPYRPSYSYDYDFDLGTDRNGSFGGTFNDCRDPT
NAKAP95     116 G....SGGERYDSYESCDSRAVLSERDLYRSGYDYS...ELDPEMEMAYEGQYDAYRDQF

hAKAP95     178 RERGSLDGFMRGRGQGRFQDRSNPGTFMRSDPFVPPAASSEPLSTPWNELNYVGGRGLGG
rAKAP95     178 PERGALDGFLRGRGQGRFQDRSNSSTFIRSDPFMPPSASSEPLSTTWSELNYMGGRGLGG
NAKAP95     169 RMRG..NDTFGPRAQGWARDARSGR..........PMASG..YGRMWEDPMGARGQCMSG

hAKAP95     238 PSPSRPPPSLFSQSMAPDYGV.MGMQGAGGYDSTMPYGCGRSQPRMRDRDRPKRRGFDRF
rAKAP95     238 PSTNRPPPSLFSQSMAPDYSM.MGMQGVGGFGGTMPYGCGRSQTRI..RDWPRRRGFERF
NAKAP95     215 ASRL...PSLFSQNIIPEYGMFQGMRGGGAFPGGSRFGFGFGNGMKQMRRTWKTWTTADF

hAKAP95     297 GPDGTGRKRKQFQLYEEPDTKLARVD.SEGDFSENDDAA.GDFRSG..............
rAKAP95     295 GPDNMGRKRKPFPLYEEPDAKLARAD.SEGDLSENDDGA.GDLRSG..............
NAKAP95     272 RTKK..KKRKQGGSPDEPDSKATRTDCSDNSDSDNDEGTEGEATEGLEGTEAVEKGSRVD

hAKAP95     341 DEEFKGEDELCDSGRQRGEK.....EDEDEDVKK.....RREKQRRRDRTRDRAADRIQF
rAKAP95     339 DEEFRGEDDLCDSRKQRGEK.....EDEDEDVKK.....RREKQRRRDRMRDRAADRIQF
NAKAP95     330 GEDEEGKEDGREEGKEDPEKGALTTQDENGQTKRKLQAGKKSQDKQKKRQRDRMVERIQF

hAKAP95     391 ACSVCKFRSFDDEEIQKHLQSKFHKETLRFISTKLPDKTVEFLQEYIVNRNKKIEKRRQE
rAKAP95     389 ACSVCKFRSFEDEEIQKHLQSKFHKETLRFISTKLPDKTVEFLQEYIINRNKKIEKRRQE
NAKAP95     390 VCSLCKYRTFYEDEMASHLDSKFHKEHFKYVGTKLPKQTADFLQEYVTNKTKKTEELRKT

hAKAP95     451 LMEKETAKPK....PDPFKGIGQEHFFKKIEAAHCLACDMLIPAQPQLLQRHLHSVDHNH
rAKAP95     449 LLEKESPKPK....PDPFKGIGQEHFFKRIEAAHCLACDMLIPAQHQLLQRHLHSVDHNH
NAKAP95     450 VEDLDGLIHQIYRDQDLTQEIAMEHFVKKVEAAHCAACDLFIPMQFGIIQKHLKTMDHNR

hAKAP95     507 NRRLAAEQFKKTSLHVAKSVLNNRHIVKMLEKYLKGEDPFTSETVDPEMEGDDNLGGEDK
rAKAP95     505 NRRLAAEQFKKTSLHVAKSVLNNKHIVKMLEKYLKGEDPFVNETADLETEGDENLG..EE
NAKAP95     510 NRRLMMEQSKKSSLMVARSILNNKLISKKLERYLKGENPFTDSPEEEKEQEEAEGGALDE

hAKAP95     567 KETPEEVAADVLAEVITAAVRAVDGEGAPAPESSGEPAEDEGPTDTAEAGSDPQAEQLLE
rAKAP95     563 KETPEEVAAEVLAEVITAAVKAVEGDGEPAAEHSDVLAEVEGPVDTAEAGSDSHTGKLLE
NAKAP95     570 GAQGEAAGISEGAEGVPAQ.PPVPPEPAPGAVSPPPPP....PPEEEEEGAVPLLGGALQ

hAKAP95     627 EQVPCGTAHE..KGVPKARSEAAEAGNGAETMAAEAESAQTRVAPAPAAADAEVEQTDAE
rAKAP95     623 EQT.CETASETRNMEDMARGEAAEARNEAAVPAAAAGSPVPVIA.IPGILEDELEQTDAE
NAKAP95     625 RQIRGIPGLDVEDDEEGGGGAP*~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

hAKAP95     685 SKDAVPTE*
rAKAP95     681 AKD.TPTE*
b NAKAP95   647 ~~~~~~~~~~
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Fig. 1. *Continued*

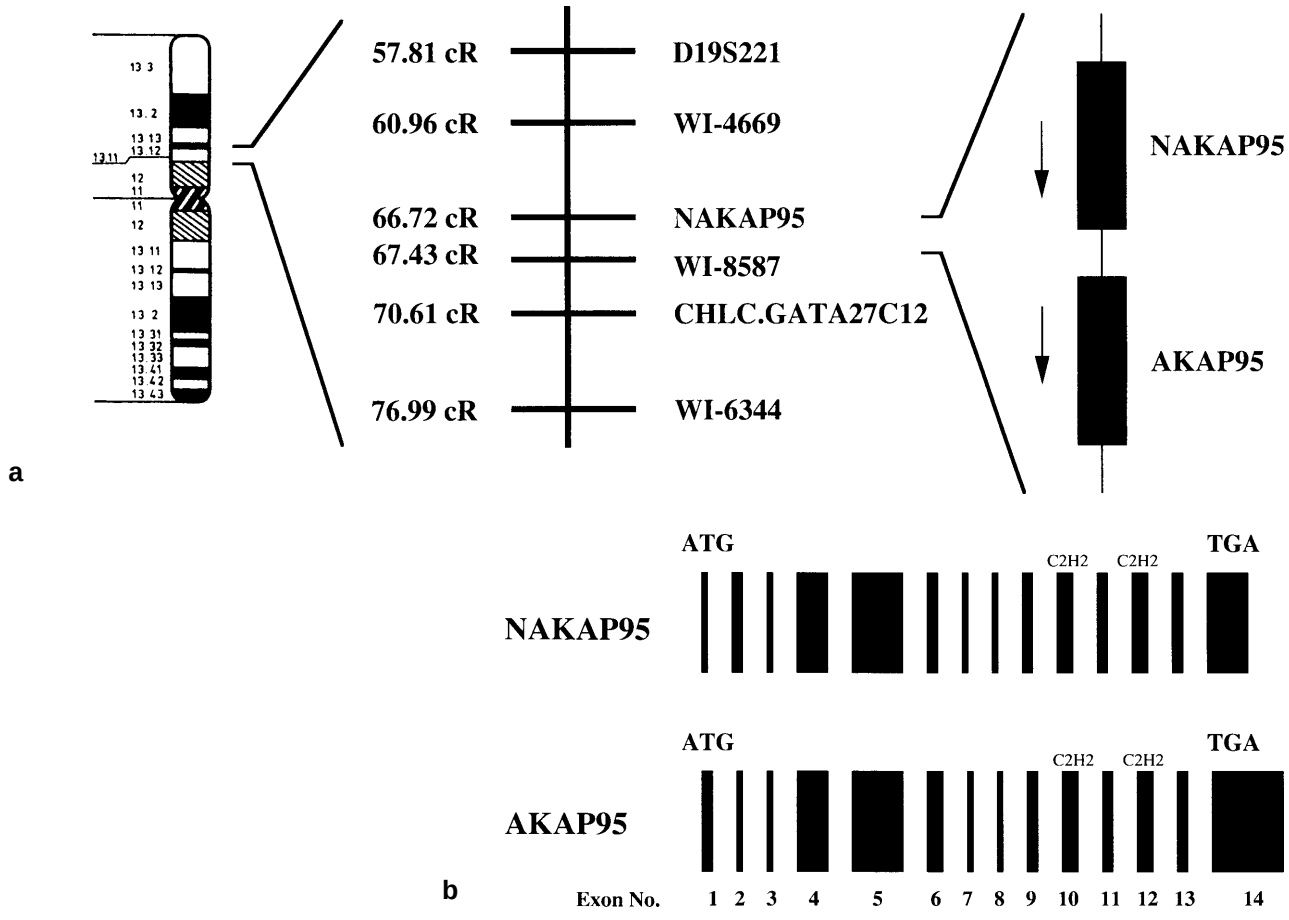

Fig. 2. **a** Chromosomal placement of the *NAKAP95* gene at a relative distance to framework markers on the WICGR radiation hybrid map of the human genome (http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map). The approximate corresponding cytogenetic location of the gene on chromosome 19p13.11–p13.12 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 **gt**gggcctggc
2	75	ctttatgttt**ag** GCTTTGTCCA	TGTGATTATG **gt**aagtgtggac
3	33	tacttttt**gt**ag GATATGGAAC	ACAAATAGAG **gc**aagtgtcatt
4	241	ccctcactgc**ag** GCTACGAGGG	GTGGAGAAAG **gt**gagtggacac
5	454	atgcctctgc**ag** GTATGACTCT	CGACTTCCGA **gt**gagtggaggc
6	97	cttatgaccc**ag** ACCAAGAAGA	TCAGACAATG **gt**gagcccacta
7	71	tttccctttc**ag** ATGAGGGCAC	CTCCAGAGTG **gt**aagaggctct
8	64	tgaaattgtt**ag** GACGGAGAGG	CCAGAGAAGG **gt**gagttttcct
9	109	ctgctcccac**ag** GGGCCCTAAC	TGGTGGAAAG **gt**aaccagcttc
10	142	ttcctgtggc**ag** GATCCAGTTT	CTTTCTGCAG **gt**gagccttgga
11	106	tattccttgc**ag** GAGTACGTCA	CTGACCCAGG **gt**gaggagattt
12	131	tccccactac**ag** AAATTGCCAT	GAACCGCAGG **gt**gagtggccac
13	96	ccctgcccgc**ag** CTCATGATGG	CTACCTGAAG **gt**gaggcactgg
14	380	ccctccccac**ag** CCGCTCTTGG	AAGGGTAGGG

Intron–exon junctions were established by comparison of cDNA and genomic sequences
[a] Size in basepairs
[b] Sequences 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

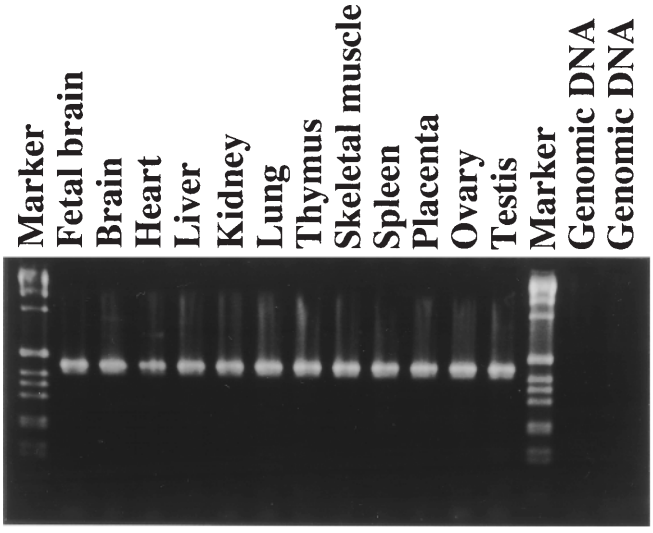

Fig. 3. Tissue distribution analysis, using reverse transcription coupled-polymerase chain reaction (RT-PCR). The 12 tissues and genomic DNA examined are indicated *above each lane*. The templates of the human tissues of poly(A)+ RNAs were purchased from Clontech (Palo Alto, CA, USA). The cDNA templates 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 20 s 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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