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SHORT COMMUNICATION

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cDNA cloning, expression profile, and genomic structure of human and mouse *RNF10/Rnf 10* genes, encoding a novel RING finger protein

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Abstract RING finger (C3HC4-type zinc finger) is a variant zinc finger motif present in a new family of proteins including transcription regulators. A new member of the RING finger protein family was identified through a mouse expressed sequence tag (EST) database search, and its full-length cDNA was isolated from a mouse brain full length-enriched cDNA library. The gene was designated as Rnf10, for RING finger protein 10. The cDNA clone consists of 3110 nucleotides and encodes an open reading frame (ORF) of an 804-amino acid protein. A database search revealed that human KIAA0262 protein (accession number, D87451) has strong homology to mouse Rnf10. To confirm that mouse Rnf10 is the homolog or an isolog of human KIAA0262, a human RNF10 cDNA was cloned in our hands from a fetal brain cDNA pool. The newly isolated cDNA contained an ORF for 811 amino acids which had almost identical structure to mouse Rnf10 protein, indicating that the human ORF codes for RNF10 protein. This finding was also supported by comparative chromosome mapping in which both genes were localized in a conserved linkage homology region between mouse and human. Comparison of the RNF10 and KIAA0262 proteins revealed that both were transcribed from the same gene and that the longer RNF10 ORF would be the authentic form. The complete genomic organization of *RNF10* was determined to consist of 17 exons spanning at least 40 kb in the genome.

Key words RING finger (C3HC4-type zinc finger) motif · RNF10 · Rnf10 · KIAA0262 · RH mapping · 12q23–q24.1 · D5Mit318

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The nucleotide sequence data of human RNF10 and mouse Rnf10 genes reported in this paper have been submitted to DDBJ, EMBL, and GenBank database and appear under the accession number AB027196 and AB026621, respectively.

Introduction

Zinc finger motifs are composed of several subfamilies based on their different types of fingers categorized by the nature and spacing of their zinc-chelating residues (Schwabe and Klug 1994; Mackay and Crossley 1998). An increasing number of genes encoding zinc finger motifs is being identified in the course of genome sequencing projects, and to date zinc finger proteins constitute the largest gene superfamily (Tatusov et al. 1997; Neil et al. 1998). The RING finger, named after the Really Interesting New Gene 1 (Hanson et al. 1991), was originally identified as a cysteine-rich, putative zinc-chelating motif in otherwise unrelated proteins (Freemont et al. 1991). RING finger (C3HC4-type zinc finger) motif is a variant type of zinc finger motif and members of RING finger proteins can be divided into several groups according to their sequence homology, domain structure, and assumed biological functions (Freemont 1993; Saurin et al. 1996). Members of the RING finger family are implicated in a variety of functions, such as transcriptional regulation, DNA repair, site-specific recombination, and signal transduction (Freemont et al. 1991; Barlow et al. 1994; Borden et al. 1995; Saurin et al. 1996).

Source and isolation of human and mouse RING finger protein genes *RNF10/Rnf10*

Recently, we systematically isolated cDNA clones encoding a novel RING finger protein (Seki et al. 1998; Ueki et al. 1999). Using a RING finger motif (C-x_2-C-x_{11-27}-C-x-H-x_2-C-x_2-C-x_{6-12}-C-x_2-C) as a query sequence, we searched for the

public mouse expressed sequence tag (EST) database with the tBLASTN program (www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform = 1) and found a cluster of several ESTs with a RING finger motif (accession numbers, AA413547, AA117803, AA471771, AI115110). To obtain the full-length cDNA structure of the new RING finger protein gene, we performed polymerase chain reaction (PCR), using specific primers designed from the consensus sequences of these ESTs and vector primers. Several independent cDNA clones were isolated from a mouse brain full-length enriched cDNA library and sequenced by the dideoxy chain-termination method with an ABI377 DNA sequencer (Perkin Elmer, Norwalk, CT, USA) according to the supplier's instructions. The resultant consensus sequence was 3110 bp in length and the presumed initiation methionine was preceded in the sequence by an in-frame stop codon. The open reading frame encoded a protein of 804 amino acids residues (Fig. 1) having a molecular mass of 100.5 kDa. The putative protein contained a canonical RING finger motif (CPICLYPPTAAKITRCGHIFCWACILHYLSLSEKTWSKCPIC) at the amino-terminal region (between residues 225 aa and 266 aa) (Fig. 1). A polyadenylation signal, AATAAA, was located 18 bp upstream of the poly(A). Three putative nuclear localization signals, KRKRQRQKK (592 aa–600 aa), RRRERR (605 aa–610 aa), and KKRKKQKQK (785 aa–793 aa) existed (Fig. 1). We designated the gene as RING finger protein 10 (*Rnf10* as the gene symbol approved by the Human Gene Nomenclature Committee). The nucleotide sequence data of mouse *Rnf10* cDNA will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number, AB026621.

Rnf10 protein showed striking homology to human KIAA0262 protein (accession number, D87451). Then we determined the human RNF10 cDNA sequence by our hands to confirm whether mouse Rnf10 is the homolog or an isolog of human KIAA0262. The human EST database was searched with the Rnf10 amino acid sequence as a query with the tBLASTN program, and many ESTs were obtained as transcripts from the probable *RNF10* gene. With primers designed from a consensus nucleotide sequence of the hit ESTs', 5′ and 3′-RACE (rapid amplification of cDNA ends) were performed with a human fetal brain-derived RACE cDNA library (Clontech, Palo Alto, CA, USA). The obtained cDNA contained an 811-amino acid open reading frame (ORF) which is entirely homologous to Rnf10 protein (91% identity and 93% similarity), indicating that the cDNA represents human RNF10, the counterpart of mouse Rnf10. Multiple alignment of RNF10, Rnf10, and KIAA0262 protein amino acid sequences is shown in Fig. 1. The KIAA0262 protein lacks 52 amino acid residues of amino terminal present in the RNF10/Rnf10 protein. Conservation of the N-terminal structure between human and mouse suggests that the cDNA sequence of RNF10 is authentic, although how the cDNA sequence of KIAA0262 is created (alternative form or any artifacts) is unknown. The approved symbol RNF10 for this human counterpart (accession number, AB027196) has been offered by Human Nomenclature Committee.

We examined the distribution of the mouse *Rnf10* transcript in various mouse fetal and adult tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR). A clear common signal of the expected size was detected in all the tissues examined (data not shown), indicating that the mouse *Rnf10* gene is ubiquitously transcribed in various tissues and would be involved in the basic housekeeping function of cells. Such a ubiquitous expression profile has good accordance with the northern blot analysis of KIAA0262 (Nagase et al. 1996).

Chromosome mapping of *RNF10* and *Rnf10* genes

We determined the chromosomal location of the mouse *Rnf10* gene using a radiation hybrid panel (T31 Mouse Radiation Hybrid Panel; Research Genetics, Huntsville, AL, USA) in the same manner as in previous reports (Saito et al. 1997; Seki et al. 1999). Primers used for PCR amplification correspond to the 3′ untranslated region of the gene (5′-AGG GGA AGC TGG AAA ATA CAC-3′) and (5′-ACA GAT TGA TTA GCT TGG GGC-3′) (95-bp PCR product). The radiation hybrid mapping data were processed using the RHMAPPER software package (http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl). The data vector for the gene was 0000121100 0001000000 0100001001 0001000000 1000000010 0100000010 0000001001 0000001111 1000000200 1001100110 (lod > 3.0) and the consequent statistical report indicated the gene was mapped to chromosome 5, with the nearest public locus the D5Mit318 region. This region contains homologous organization to the human chromosome 12q22–q24 region (www.ncbi.nlm.nih.gov/Homology). A search in the Unigene database (www.ncbi.nlm.nih.gov/UniGene/Hs.Home.html) showed that human KIAA0262 (Hs.5094) was mapped to the chromosome 12q23–q24.1 region, with markers between D12S366 and D12S340. Thus, it was confirmed that the mouse *Rnf10* and human *RNF10* are localized in a region with conserved linkage homology among these species. These mapping data support the idea that the two genes are homologs of each other.

Genomic structure of the human *RNF10* gene

Several members of the RING finger protein family are implicated in human diseases. For example, the RING finger protein, BRCA1, is a tumor suppressor in early onset breast cancer (Miki et al. 1994), and another member, PML, produces a fusion protein with the retinoic acid receptor alpha in acute promyelocytic leukemia (de The et al. 1991; Goddard et al. 1991; Kakizaki et al. 1991; Kastner et al. 1992), and the human 52-kDa SS-A/Ro RING finger protein is the nuclear antigen in Sjogren's autoimmune disease (Chan et al. 1991; Itoh et al. 1991). The chromosomal position, genomic structure, and expression profile of such genes may contribute to ongoing positional candidate ap-

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  1  MPLSSPNAAA TASDMDKNSG SNSSSASSGS SKGQQPPRSA SAGPAGESKP  humanRNF10
                                                            KIAA0262
     ..Q...S... .......... .......... .......... ..........  mouseRnf10
 51  KSDGKNSSGS KRYNRKRELS YPKNESFNNQ SRRSSSQKSK TFNKMPPQRG  humanRNF10
     MM........ .......... .......... .......... ..........  KIAA0262
     .......N.. ........P. .....N..S. ....N..... ..........  mouseRnf10
101  GGSSKLFSSS FNGGRRDEVA EAQRAEFSPA QFSGPKKINL NHLLNFTFEP  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .....P.... S......... .......... .......... ..........  mouseRnf10
151  RGQTGHFEGS GHGSWGKRNK WGHKPFNKEL FLQANCQFVV SEDQDYTAHF  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     ...A...... ...G...... .......... .......... ......A...  mouseRnf10
201  ADPDTLVNWD FVEQVRICSH EVPSCPICLY PPTAAKITRC GHIFCWACIL  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... .......... .......... ..........  mouseRnf10
251  HYLSLSEKTW SKCPICYSSV HKKDLKSVVA TESHQYVVGD TITMQLMKRE  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... .......... ...R..A... ..........  mouseRnf10
301  KGVLVALPKS KWMNVDHPIH LGDEQHSQYS KLLLASKEQV LHRVVLEEKV  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... ..V......N .....L.... .......... .........G  mouseRnf10
351  ALEQQLAEEK HTPESCFIEA AIQELKTREE ALSGLAGSRR EVTGVVAALE  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... ....V.I... ....V..GGG ..........  mouseRnf10
401  QLVLMAPLAK ESVFQPRKGV LEYLSAFDEE TTEVCSLDTP SRPLALPLVE  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     H........T ..A....... ........D. AAQ.....P. G ........  mouseRnf10
451  EEEAVSEPEP EGLPEACDDL ELADDNLKEG TICTESSQQE PITKSGFTRL  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......        ....E.A .V...S.G.. .VGP.M..E. ....P...Q.  mouseRnf10
501  SSSPCYYFYQ AEDGQHMFLH PVNVRCLVRE YGSLERSPEK ISATVVEIAG  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... .......... .....Q.... ..........  mouseRnf10
551  YSMSEDVRQR HRYLSHLPLT CEFSICELAL QPPVVSKETL EMFSDDIEKR  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... .......... .......... ..........  mouseRnf10
601  KRQRQKKARE ERRRERRIEI EENKKQGKYP EVHIPLENLQ QFPAFNSYTC  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     ..PTK..... .........M ....R..R.. .......... ..........  mouseRnf10
651  SSDSALGPTS TEGHGALSIS PLSRSPGSHA DFLLTPLSPT ASQGSPSFCV  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     P......... ......YPL. .......... .......... ..........  mouseRnf10
701  GSLEEDSPFP SFAQMLRVGK AKADVWPKTA PKKDENSLVP PAPVDSDGES  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     ....D....L .......... ....G..... ....D..... ..........  mouseRnf10
751  DNSDRVPVPS FQNSFSQAIE AAFMKLDTPA TSDPLSEEKG GKKRKKQKQK  humanRNF10
     .......... .......... .......... .......... ..........  KIAA0262
     .......... .......... .......... .......DR. .....R....  mouseRnf10
801  LLFSTSVVHT K*                                           humanRNF10
     .......... .*                                           KIAA0262
     .......... .*                                           mouseRnf10
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Fig. 1. Alignment of human RNF10 (accession number, AB027196), mouse Rnf10 (AB026621), and KIAA0262 (D87451) proteins. *Asterisks* denote the terminal codon. The potential RING finger-motif is *double underlined*, and putative nuclear localizing signals are *underlined with a single line*

Table 1. Intron-exon boundaries of the human RNF10 gene

Exon No.	Exon size[a]	Splice acceptor[b]	Splice donor[b]
1	604		CCCAAGAGCG **gt**aaggacgggc
2	197	tcttgctttc**ag** ATGGAAAGAA	ACGAGATGAG **gt**atggaatttg
3	200	cattctctct**ag** GTAGCAGAGG	TACAGGCCAA **gt**gagtattgct
4	91	tcctatttct**ag** CTGCCAATTT	GGAACAAGTG **gt**gagtagctca
5	185	attttgtttc**ag** CGCATTTGTA	ATCTCAAGAG **gt**gagattgaga
6	137	tccatgtttc**ag** TGTTGTTGCC	CATCTAGGAG **gt**gagttcttta
7	161	gcattcttat**ag** ATGAACAGCA	GGAGCTCAAG **gt**gagaggatgc
8	126	tggccacgtt**ag** ACTCGGGAAG	ACCCAGGAAG **gt**tagtgtgtcc
9	277	ttgctgcaac**ag** GGTGTGCTGG	TTTTACCAAG **gt**gagggtgccg
10	134	ccttgtcctc**ag** CGGAAGATGG	CATGTCTGAG **gt**gaggccttcc
11	118	tttccaatgt**ag** GATGTTCGAC	ATGTTCTCAG **gt**gagaatgccc
12	102	accaatctgc**ag** ATGACATTGA	CAGGGCAAGT **gt**aagttcagga
13	156	tttcctttgc**ag** ACCCAGAAGT	TCCCATGCAG **gt**aaacaggtga
14	101	gttccctttc**ag** ACTTTCTGCT	CTTTGCCCAG **gt**aaatcctttg
15	58	ccactttggc**ag** ATGCTGAGGG	CCAAAGAAAG **gt**gaggatggtc
16	159	ggtatttttt**ag** ATGAGAACAG	CCCCTCTCTG **gt**aagggcagag
17	298	gtctcccttt**ag** AAGAGAAAGG	

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) are in boldface type

proaches for disease genes linked to the genomic locus.

Sequences of the exon-intron boundaries of the gene were identified by aligning the cDNA sequence with a genomic sequence (Accession numbers, AL022340, Z97199). As summarized in Table 1, all the splicing sites conformed to the AG-GT rule, in that there are always AG and GT dinucleotides at the splice acceptor and donor sites, respectively. The *RNF10* gene is divided into 17 exons, which range in size from 58 bp (exon 15) to 604 bp (exon 1). The first exon is 604 bp and it contains the putative ATG start codon. The last exon of 298 bp contains the TAG translation termination codon followed by a 221-bp 3′-untranslated region. The genome structure of the *RNF10* gene, consisting of 17 exons, spans approximately 40 kb of the genome DNA.

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