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SHORT COMMUNICATION

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cDNA cloning of a new member of the Ras superfamily, *RAB9-like*, on the human chromosome Xq22.1–q22.3 region

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Abstract Members of the RAB protein family regulate vesicular trafficking and reside in specific intercellular compartments. A new member of the RAB family was identified through a public database search, and its full-length cDNA was isolated from a human fetal brain cDNA library. The predicted protein product of the gene consists of 201 amino acid residues, and the protein has 86% similarity to human RAB9 at the amino acid level. We designated the new gene *RAB9-like*. Northern blot analysis showed that the gene was transcribed ubiquitously in various human tissues. A database search revealed that the gene is divided into three exons and spans approximately 7.2 kb of the genome DNA of chromosome Xq22.1–q22.3 region.

Key words RAS superfamily of small GTP-binding proteins · RAB · RAB9 · Chromosome Xq22.1–q22.3

Introduction

The RAS superfamily of small guanosine triphosphate (GTP)-binding proteins, which includes the Ras, Ral, Rho, Rap, and Rab families, is involved in controlling a diverse set of essential cellular functions. The RAB family of small G proteins, consisting of more than 40 members, regulates intercellular vesicle trafficking, including exocytosis, endocytosis, and recycling (Nuoffer and Balch 1994; Novick and Zerial 1997; Chavrier and Goud 1999; Gonzalez and Scheller 1999). The mammalian RAB proteins show striking similarities to *Saccharomyes cerevisiae* YPT1 and SEC4 proteins, and encode Ras-related GTP-binding proteins involved in the regulation of secretion. Initially, several human RAB cDNAs were isolated from a human pheochromocytoma library with a probe derived from the *SEC4* gene (Zahraoui et al. 1989).

Canine Rab9 was first identified in a screen for YPT1 and SEC4 protein-related cDNA clones (Chavrier et al. 1990). The sequence of the full-length protein showed 37% homology to YPT1 protein (Schmitt et al. 1986). Rab9 has been localized to components of the endocytic/exocytic pathway. It has been implicated in the recycling of membrane receptors, such as the mannose 6-phosphate receptor from early endosomes to the trans Golgi network (Chavrier et al. 1990; Lombardi et al. 1993; Shapiro et al. 1993; Riederer et al. 1994). In humans, a cDNA for *RAB9* was isolated from human U937 cells, using a reverse transcriptase-polymerase chain reaction (RT-PCR) method; its amino acid sequence is almost 98% identical to that of canine *Rab9* (Davies et al. 1997). While an antisense inhibition of Rab9 proteins in human fibroblast cells caused severe cell vacuolation resembling that of cells from a patient with Chediak-Higashi syndrome (Davies et al. 1997), the precise functions of Rabs are still poorly understood.

To date, the family is still expanding, with the use of PCR-based cloning approaches employing highly conserved sequence stretches of the RAB proteins. Here we report the sequence features, expression profile, and chromosomal assignment of a novel gene which has high similarity to the human *RAB9* gene.

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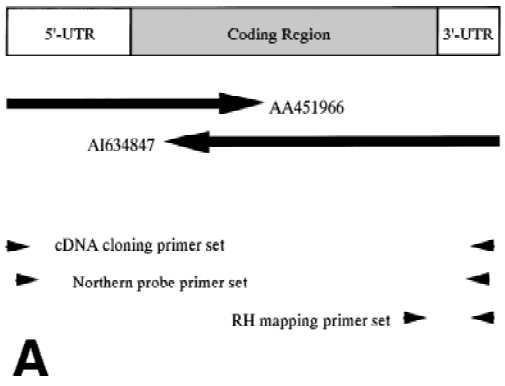
The nucleotide sequence data reported in this paper have been deposited to DDBJ, EMBL, and GenBank database under the accession number AB036693.

Isolation and source of human *RAB9-like* gene

Recently, we have systematically isolated cDNAs for novel RAB family genes, human *RAB26-related* (Seki et al. 2000) (accession number, AB027137), human *RAB23* (accession number, AB034244), and mouse *Rab9* (accession number,

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gaaagaataaaacaacaacaggaaaaaaaggaaaatatttaaattgtgacaaaaacccac   240
tgggttctcttggttacaaactccttcccttctggtgctgcaaaaATGAGTGGGAAATCC   300
                                          M  S  G  G  K  S      5
CTGCTCTTAAAGGTCATTCTCTTGGGTGATGGTGGAGTTGGGAAAAGTTCGCTTATGAAC   360
L  L  L  K  V  I  L  L  G  D  G  G  V  G  K  S  S  L  M  N     25
CGTTACGTAACCAACAAATTTGACTCCCAGGCTTTTCACACCATAGGGGTAGAGTTCTTA   420
R  Y  V  T  N  K  F  D  S  Q  A  F  H  T  I  G  V  E  F  L     45
AATCGAGATCTGGAGGTAGATGGACGCTTTGTAACCCTCCAGATCTGGGACACTGCAGGG   480
N  R  D  L  E  V  D  G  R  F  V  T  L  Q  I  W  D  T  A  G     65
CAGGAACGTTTCAAGAGCCTTAGGACACCCTTCTACAGGGGAGCAGACTGCTGCCTCTTG   540
Q  E  R  F  K  S  L  R  T  P  F  Y  R  G  A  D  C  C  L  L     85
ACCTTCAGCGTGGATGATCGGCAGAGCTTCGAGAATCTTGGTAACTGGCAGAAAGAATTT   600
T  F  S  V  D  D  R  Q  S  F  E  N  L  G  N  W  Q  K  E  F    105
ATTTACTATGCGGATGTGAAGGACCCTGAGCATTTCCCCTTTGTAGTTCTGGGTAACAAG   660
I  Y  Y  A  D  V  K  D  P  E  H  F  P  F  V  V  L  G  N  K    125
GTAGACAAAGAGGATAGGCAAGTGACTACTGAGGAGGCACAAACCTGGTGCATGGAGAAT   720
V  D  K  E  D  R  Q  V  T  T  E  E  A  Q  T  W  C  M  E  N    145
GGGGATTACCCTTATTTAGAAACTAGTGCCAAAGATGATACTAATGTGACAGTGGCCTTT   780
G  D  Y  P  Y  L  E  T  S  A  K  D  D  T  N  V  T  V  A  F    165
GAAGAAGCTGTCAGGCAGGTGCTGGCTGTAGAGGAACAGCTGGAGCATTGCATGTTGGGT   840
E  E  A  V  R  Q  V  L  A  V  E  E  Q  L  E  H  C  M  L  G    185
CACACCATTGACTTGAACAGTGGCTCCAAAGCAGGGTCTTCGTGCTGTTAAagataggga   900
H  T  I  D  L  N  S  G  S  K  A  G  S  S  C  C  *             201
gccttttaaaaatgtgccccaaattgatcagtcagtagtgtaagaataactgtgcccctc   960
taagagtgcacacacacgcacacaagagggtaagagacaaggttctgattgtgaaacaga  1020
gcctttcaaattgaagtgtagattgattt                                 1049
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B

Fig. 1. A Schematic representation of the relationship between the *RAB9-like* gene and the two expressed sequence tag (EST) sequences. The polymerase chain reaction (PCR) primer sets are shown below the representation. *UTR*, Untranslated region; *RH*, Radiation hybrid. **B** Nucleotide sequence and deduced amino acid sequence of the *RAB9-like* gene. *Asterisk* denotes the termination codon. The nucleotide sequence data reported here will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB036693. The conserved regions of the amino acid sequence involved in guanosine triphosphate (GTP) binding are *underlined*

AB027290), using a predicted gene database, designated as Virtual Transcribed Sequence (VTS) (Miyajima et al. 2000). Using a cDNA sequence of mouse *Rab9* gene (accession number, AB027290) as a query sequence, we searched for the novel human RAB-related sequence in the public human expressed sequence tag (EST) database, using the tBLASTN program (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform=1), and found two ESTs (accession numbers, AI634847 and AA451966) with a high similarity sequence with the human *RAB9* gene (accession numbers, U44103), but not identical. Then, we performed PCR, using specific primers designed from the two EST sequences, to obtain a cDNA containing the putative complete coding sequence. Primers used for the cDNA cloning were 5′-CGA GCG GCA CGT GTC AGG CCA CCG AGG-3′, corresponding to nucleotides 1 to 27, and 5′-AAA TCA ATC TAC ACT TCA ATT TGA AAG-3′, corresponding to nucleotides 1026 to 1049 (Fig. 1A). A single-sized RT-PCR product was obtained from human brain RNA, showing that the sequence is really transcribed. The RT-PCR band was cloned into TA cloning vector (Invitrogen, Carlsbad, CA, USA), and several cDNA clones were subjected to sequencing to eliminate possible artificial mutations by PCR. The structural relationship between the determined cDNA and the two EST sequences, AI634847 and AA451966, is shown in Fig. 1A.

The nucleotide sequences of both strands were determined by the primer walking method, using an ABI377 sequencer (Perkin Elmer, Norwalk, CT, USA) according to the supplier's instructions. The isolated cDNA clones were 1049 bp in length and contained an open reading frame for a predicted protein of 201 amino acid residues with a calculated molecular weight of approximately 37.7 kiloDaltons (Fig. 1B). The typical polyadenylation signal was not observed in the 3′ untranslated sequence. However, the present sequence should contain the entire protein-coding sequence, referring the other family proteins as follows and in-frame termination stop codon near its 3′ terminus.

Homology search for relevant amino acid sequences in the protein database (NCBI Protein Database and Swiss Protein Database) revealed that the protein was most homologous to human RAB9 (accession number, U44103), having 76% identity and 86% similarity at the amino acid level. Human RAB7 (accession number, U44104) and the identified protein showed 53% identity and 67% similarity at the amino acid level (Fig. 2). We designated the gene, *RAB9-like*. The nucleotide sequence of the human *RAB9-like* gene will appear in GenBank/EMBL/DDBJ databases under the accession number, AB036693. Sequence analysis and comparison showed that the *RAB9-like* gene is clearly a low-molecular wight GTP-binding protein of the Rab family, containing the conserved GTP-binding regions known to be present in this group (Fig. 1B).

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RAB9-like  ~MSGKSLLLKVILLGDGGVGKSSLMNRYVTNKFDSQAFHTIGVEFLNRDLEVDGRFVTLQ
RAB9       ~MAGKSSLFKVILLGDGGVGKSSLMNRYVTNKFDTQLFHTIGVEFLNKDLEVDGHFVTMQ
RAB7       MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ

RAB9-like  IWDTAGQERFKSLRTPFYRGADCCLLTFSVDDRQSFENLGNWQKEFIYYADVKDPEHFPF
RAB9       IWDTAGQERFRSLRTPFYRGSDCCLLTFSVDDSQSFQNLSNWKKEFIYYADVKEPESFPF
RAB7       IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLVQASPRDPENFPF

RAB9-like  VVLGNKVDKEDRQVTTEEAQTWCMENGDYPYLETSAKDDTNVTVAFEEAVRQVLAVEEQL
RAB9       VILGNKIDISERQVSTEEAQAWCRDNGDYPYFETSAKDATNVAAAFEEAVRRVLATEDRS
RAB7       VVLGNKVDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV

RAB9-like  E...HCMLGHTIDLNSGSKAGSSCC*~~
RAB9       D...HLIQTDTVNLHRKPKPSSSCC*~~
RAB7       ELYNEFPEPIKLDKNDRAKASAESCSC*
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Fig. 2. Alignment of human *RAB9-like* (accession number, AB036693), *human RAB9* (accession number, U44103), and human *RAB7* (accession number, U44104) genes. Identities are indicated by *black background* and similar residues are *shadowed*. *Asterisks* denote termination codons

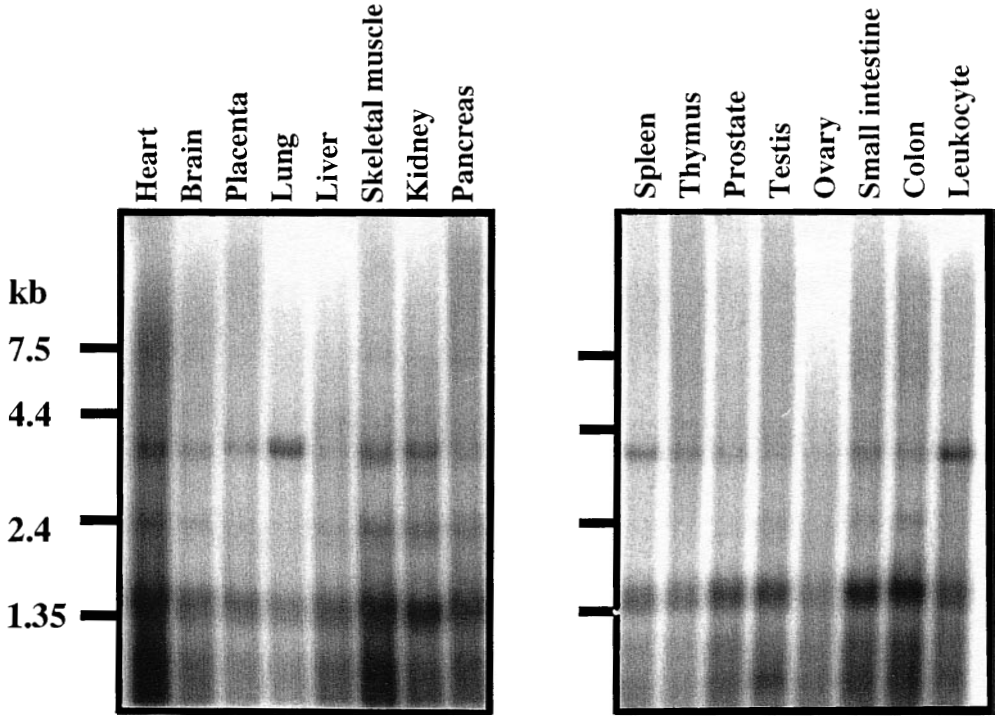

Fig. 3. Northern blot analysis of human *RAB9-like* gene. Northern blot filters containing adult human poly $(A)^+$ RNAs (2 μg/lane) were purchased from Clontech Laboratories (Palo Alto, CA, USA), and hybridization and washing were performed following the manufacturer's instructions. The 961-bp cDNA fragment containing the entire open reading frame was labelled with [α-^{32}P] dCTP and used as a hybridization probe. Size markers (*left*) are in kilobases

Expression profile of *RAB9-like* gene

We examined the distribution of human *RAB9-like* transcript in various human tissues by Northern blot analysis, as described previously (Seki et al. 1998). The 961-bp cDNA fragment containing the entire open reading frame was used as a hybridization probe. Primers used for the probe preparation were 5′-ACT TGC TGC CCC ATT GAG GAC-3′, corresponding to nucleotides 38 to 58, and 5′-GTC TCT TAC CCT CTT GTG TGC-3′, corresponding to nucleotides 978 to 998. A major band of 1.4 kb was observed, and two minor bands, of approximately 2.3 and 4.0 kb, were also detected (Fig. 3). The 1.4-kb size of the major band is consistent with the notion that our clone is full-length. The 1.4-kb signal was detected in all the tissues examined, suggesting that the *RAB9-like* gene described in the present study may be involved in the basic housekeeping function of cells. It is not known whether the longer signals were derived from alternative form(s) of *RAB9-like* mRNA or another related gene transcript(s), and this remains to be elucidated.

Genomic structure and chromosome mapping of the *RAB9-like* gene

The exon-intron boundaries of the human *RAB9-like* gene were determined by aligning the cDNA sequence with the genomic sequence (accession numbers, AL035553;

Table 1. Intron-exon boundaries of the *RAB9-like* gene

Exon no.	Exon size[a]	Splice acceptor[b]	Splice donor[b]
1	169		ATTGTGTTGG **gt**aggtgcca
2	74	gtttttat**ag** CACCCTAAAA	CTAAAAATGG **gt**aggtattt
3	806	ttattttc**ag** GTTCTCTTGG	

Intron-exon junctions were established by comparison of cDNA and genomic sequences
[a] Size in base pairs
[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

clone RP4-540A13). 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 *RAB9-like* gene was divided into three exons, the lengths of which were: 169 bp (exon 1), 74 bp (exon 2), and 806 bp (exon 3), and spanned approximately 7.2 kb of the genome DNA. Exon 3 contained the ATG codon for the initial methionine and TAG termination.

Chromosomal assignment of the human *RAB9-like* gene was done by PCR analysis of a radiation hybrid panel (Genebridge4; Research Genetics, Huntsville, AL, USA), as described previously (Seki et al. 1999, 2000). The human *RAB9-like*-specific PCR primers, (5′-CAC ACC ATT GAC TTG AAC AGT-3′, corresponding to nucleotides 841 to 861, and 5′-CTT ACC CTC TTG TGT GCG TGT-3′, corresponding to nucleotides 974 to 994), gave rise to an amplified product with a size of 154 bp by genomic PCR. 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 *RAB9-like* gene was 1000000100 0000001111 0000000000 0110000000 0100000000 0000010000 0100010000 0101000000 0002101100 200 and the consequent report indicated that the gene was placed to 4.08 cR distal from the marker AFMA275ZC9 (lod > 3.0). The region including the marker was cytogenetically mapped to the Xq22 region. On the other hand, the UniGene database (http://www.ncbi.nlm.nih.gov/UniGene/) has noted that the human *RAB9* gene was mapped to the XpTEL-DXS1061 region. The chromosomal positions of the *RAB9-like* and *RAB9* genes are indicated in Fig. 4.

Vesicular transport to and from the lysosome and late endosome is defective in patients with Chediak-Higashi syndrome, and in mutant beige mice (Barbosa et al. 1996, 1997). In Chediak-Higashi syndrome, and in mutant beige mice, cells have giant, perinuclear vesicles with characteristics of late endosomes and lysosomes that arise from dysregulated homotypic fusion (Brandt et al. 1975; Willingham et al. 1981; Burkhardt et al. 1993). Antisense expression of Rab9 produced cells that contained numerous large vacuoles, a phenotype resembling that of fibroblasts from patients with Chediak-Higashi syndrome (Davies et al. 1997). Although the recently identified responsible genes in Chediak-Higashi syndrome and beige mutation do not code any Rab proteins, the above antisense experiment suggests that Rab proteins may be involved in the same biochemical pathway in which the disease genes take part.

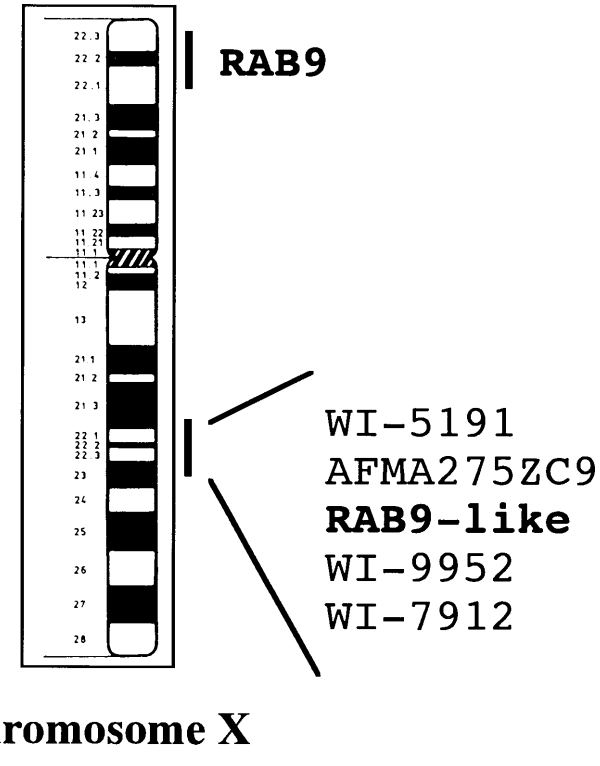

Fig. 4. Chromosomal placement of human *RAB9-like* and *RAB9* genes at a relative distance to framework markers on the WICGR (Whitehead Institute/MIT Center of Genome Research) radiation hybrid map of the human genome. The approximate corresponding cytogenetic locations of the *RAB9-like* and *RAB9* genes are on human chromosome Xq22 and Xp22.3 regions, respectively

The data described in this present report may serve as tools for the investigation of mammalian *RAB* genes and function.

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