

SHORT COMMUNICATION

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cDNA cloning of a human *RAB26-related* gene encoding a Ras-like GTP-binding protein on chromosome 16p13.3 region

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Abstract Members of the RAB protein family are important regulators of vesicular fusion and trafficking. A putative new member of the *RAB* family of genes 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 190 amino acid residues and has 87% identity with rat Rab26. Thus, we designated this gene as the human *RAB26-related* gene. Reverse transcription-coupled polymerase chain reaction (RT-PCR) demonstrated that the *RAB26-related* messenger RNA was predominantly expressed in adult and fetal brain. Furthermore, an RT-PCR experiment for brain subregions showed that the mRNA was highly expressed in the amygdala, cerebellum, caudate nucleus, and hippocampus. By PCR-based analysis with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid panel, the gene was mapped to the chromosome 16p13.3 region between markers WI-7742 and WI-3061. The *RAB26-related* gene consists of eight exons that span about 44 kb of the genome DNA.

Key words Ras superfamily of small GTP-binding proteins · *RAB26-related* · *Rab26* · RT-PCR · RH mapping · Chromosome 16p13.3 · Virtual transcribed sequence (VTS)

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The nucleotide sequence data reported in this paper have been deposited in DDBJ, EMBL and GenBank databases under the accession number AB027137.

Introduction

The RAS superfamily of small 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 the *S.cerevisiae* YPT1 and SEC4 proteins and encode RAS-related GTP-binding proteins involved in the regulation of secretion. Initially, cDNAs for several human *RAB* were isolated from a human pheochromocytoma library with a probe derived from the *SEC4* gene (Zahraoui et al. 1989). To date, the family is still expanding through PCR-based cloning approaches using highly conserved sequence stretches of the RAB proteins.

Pancreatic acinar cells synthesize and secrete digestive enzymes or proenzymes which are stored in zymogen granules located in the apical pole of the cell. Evidence indicates that pancreatic enzyme secretion may also be regulated by low-molecular weight GTP-binding proteins (Kitagawa et al. 1990; Padfield et al. 1991). *Rab26* was identified in a rat pancreas cDNA library, using *Rab 3A* as a probe (Wagner et al. 1995). *Rab3A*, however, which has been suggested to regulate exocytosis of synaptic vesicles, is not present in rat pancreatic acinar cells or in zymogen granules (Wagner et al. 1994).

Recently, we systematically isolated cDNAs for new members of the *RAB* family from oligo-capped cDNA libraries (Maruyama and Sugano 1994; Suzuki et al. 1997). In the present study, by utilization of a predicted gene database, designated the virtual transcribed sequence (VTS) database, (Miyajima et al. 2000), we identified a novel *RAB* family gene and isolated its cDNA for the complete coding sequence of the protein product. The sequence feature of the cDNA, expression profile, chromosomal assignment,

and genomic structure of the human *RAB26-related* gene is described.

Isolation and source of the human *RAB26-related* gene

We searched genes for previously unknown members of the RAB family in the VTS database, using the BLASTP program with Rab9 (accession number, AB027290), RAB9-like (accession number, AB036693), and RAB23 (accession number, AB034244) protein sequences as queries. The database search suggested a novel *RAB* gene located on the genomic sequence (accession number, AC012171) which mapped on human chromosome 16. Then, we performed the polymerase chain reaction (PCR), using specific primers designated from the nucleotide sequence of the predicted exons (5'-GCT CAT GCT CTG CTG CTG CTC-3', corresponding to nucleotides 214 to 234; 5'-AAC TCC TTT GCT ATG GCT GTG-3', corresponding to nucleotides 479 to 459) and isolated a partial cDNA from a human fetal brain cDNA library, showing that the RAB candidate is a real gene. 5' and 3'-RACE (rapid amplification of cDNA ends) were performed to obtain a cDNA with complete coding

sequence (CDS) structure. For 5' and 3'-RACE primers, 5'-GAT GTT GTC AAA GGA GGC CTT GTT GGT GAC-3' (corresponding to nucleotides 270 to 241) and 5'-CGC CAA GAC GGG CCT CAA CGT GGA CTT GGC-3' (corresponding to nucleotides 426 to 455), respectively, were used with a Marathon cDNA amplification kit (Clontech, Palo Alto, CA, USA). Several independent clones were isolated and sequenced by the dideoxy chain-termination method, with an ABI377 DNA sequencer (Perkin Elmer, Norwalk, CT, USA) according to the supplier's instruction.

The resultant consensus sequence of the cDNA was 1320bp in length and contained an open reading frame for a predicted protein of 190 amino acid residues with a calculated molecular weight of approximately 21.4 kiloDalton. A canonical polyadenylation signal, aataaa, was located 35 bp upstream of the poly (A) (Fig. 1). Sequence analysis clearly showed the protein is a low molecular weight GTP-binding protein of the RAB family, containing the conserved GTP-binding regions known to be present in this group of proteins (Fig. 1). Homology search for relevant amino acid sequences in the protein database (NIBI Protein Database and Swiss Protein Database) revealed that the protein is most homologous to rat Rab26 (accession number, U18771) (Wagner et al. 1995), having 87% identity at the

Fig. 1. Nucleotide sequence and deduced amino acid sequence of human *RAB26-related* gene. The conserved regions of the amino acid sequence involved in GTP-binding are *underlined*. The polyadenylation signal, aataaa, is *double underlined*. The nucleotide sequence of the human *RAB26-related* gene is deposited in GenBank/EMBL/DDBJ databases under the accession number of AB027137

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gtcATGCTGGTGGGGACTCGGGTGTGGGGAAGACCTGTCTGCTGGTGCGATTCAAGGAT      60
  M L V G D S G V G K T C L L V R F K D      19
GGTGCTTTCCTGGCGGGGACCTTCATCTCCACCGTAGGCATFGACTTCCGGAACAAAGTT      120
  G A F L A G T F I S T V G I D F R N K V      39
CTGGACGTGGATGGTGTGAAGGTGAAGCTGCAGATGTGGGACACAGCTGGTCAGGAGCGG      180
  L D V D G V K V K L Q M W D T A G Q E R      59
TTCCGCAGTGTACCCATGCCCTACTACCGGGATGCTCATGCTCTGCTGTCTCTACGAT      240
  F R S V T H A Y Y R D A H A L L L L Y D      79
GTCACCAACAAGGCCTCCTTTGACAACATCCAGGCCTGGCTGACCAGATCCACGAGTAC      300
  V T N K A S F D N I Q A W L T E I H E Y      99
GCCCAGCACGACGTGGCGCTCATGCTGCTGGGGAACAAGGTGGACTCTGCCCATGAGCGT      360
  A Q H D V A L M L L G N K V D S A H E R      119
GTGGTGAAGAGGGAGGACGGGGAGAAGCTGGCCAAGGAGTATGGACTGCCCTTCATGGAG      420
  V V K R E D G E K L A K E Y G L P F M E      139
ACCAGCGCAAGACGGGCCTCAACGTGGACTTGGCCCTTCACAGCCATAGCAAAGGAGTTG      480
T S A K T G L N V D L A F T A I A K E L      159
AAGCAGCGCTCCATGAAGGCTCCAGCGAGCCGCGCTTCCGGCTGCATGATTACGTTAAG      540
  K Q R S M K A P S E P R F R L H D Y V K      179
AGGGAGGGTTCGAGGGGCCTCCTGCTGCCGCCCTTGAacctggctgagctcagtcctctgg      600
  R E G R G A S C C R P *      190
aggaagccgcccagtccttagaaggctggacagagggctctccaggcccttctgactttgt      660
  tggccagtgccaacgcccagtgctgtgttttcaggagccccaggtaagccttctgacct      720
  tctcctcccagcaacagtcaccaacaagcaggcttctgagagcccgtggccgcacactgg      780
  ccgccacggaaaagcagtccttctgcacgggacggggagcggcaagtggacagactttgcc      840
  acggtgctctgctgccccctcctgggcacgtccaggtgaggagggctggggctggcacc      900
  acgcacagtgccctaaccctagaaaagccatgtcttcagccgcacatgctcaggcagctaa      960
  gggagacgctgcccacgctgggacagaaggcttactgctaatacacaatcgtgcatct      1020
  gtgtgctcctgggagctgctgctcccggcccaccctctaggagctctggctcaaacagc      1080
  aatagggcttctcactgaccttgaggatgctgtggccttgtgataaaatgtgggaa      1140
  atcacagaaaacaccagaacaacaactgccagcccggcctggccacaggtgaggtctgt      1200
  gatcccgagcagctccacctgcaacttggccttttgattgcacaagcctttgt      1260
  tttcagtcctagtgaataaagtgtgttttctggagcgtctgtctcatctgttgaaaaa      1320

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amino acid level (Fig. 2). Thus, we designated this gene as the human *RAB26-related* gene. Sequence comparison revealed close homology to human MEL, RAB3A, and yeast SEC4; 52%, 40%, and 45%, respectively, identical at the amino acid level. The *MEL* gene was identified following transfection of NIH3T3 mouse fibroblasts with DNA from a human melanoma cell line (Nimmo et al. 1991). Rab3A is abundant in brain synaptic vesicle (Fischer et al. 1991; Matteoli et al. 1991). The yeast protein SEC4 is well characterized, and it has been genetically determined that the gene is essential for life and that its product regulates a post-Golgi event in secretion (Salminen and Novick 1987). The alignment of the deduced amino acid sequences of human RAB26-related and the other proteins is shown in Fig. 2. The nucleotide sequence of the human *RAB26-related* cDNA will appear in GenBank/EMBL/DBJ databases under the accession number AB027137.

Expression profile of *RAB26-related* gene

We examined the distribution of human *RAB26-related* transcripts in various human adult and feta tissues by reverse transcription-coupled polymerase chain reaction (RT-PCR), as described previously (Seki et al. 1999). Primers used for the RT-PCR corresponded to the coding region

of the gene (5'-TGT CTG CTG GTG CGA TTC AAG-3', corresponding to nucleotides 37 to 57, and 5'-GTC GTG CTG GGC GTA CTC GTG-3', corresponding to nucleotides 312 to 292). The primer set gave the longer PCR product from genomic DNA, which was easily distinguished from the 276-bp product from the mRNA. The 276-bp PCR product was predominantly detected in fetal and adult brain (Fig. 3A,B). An RT-PCR experiment for brain subregions indicated that *RAB26-related* mRNA was highly expressed in amygdala, cerebellum, caudate nucleus, and hippocampus (Fig. 3C). Therefore, the *RAB26-related* gene described in the present study seems to be involved in a specific function of the nerve system. The conditions for non-saturated RT-PCR of the *RAB26-related* gene were determined by changing the number of PCR cycles from 22, 25, 30 to 35. Amplification of 30 cycles gave minimum signals and was considered to detect a non-saturated PCR process. To facilitate comparison of transcript accumulation in different tissues, an RT-PCR using a GAPDH primer set was performed as an internal control experiment.

Rat *Rab26* is expressed in brain, kidney, and lung, and submandibular gland, but not in liver (Wagner et al. 1995). Although the *Rab26* was cloned first from pancreas, it was not specific for pancreas, but was expressed in other tissues as well (Wagner et al. 1995). Such a discrepancy between the expression profiles of rat *Rab26* and the present human small G protein gene, *RAB26-related*, suggested that the two genes may be different ones.

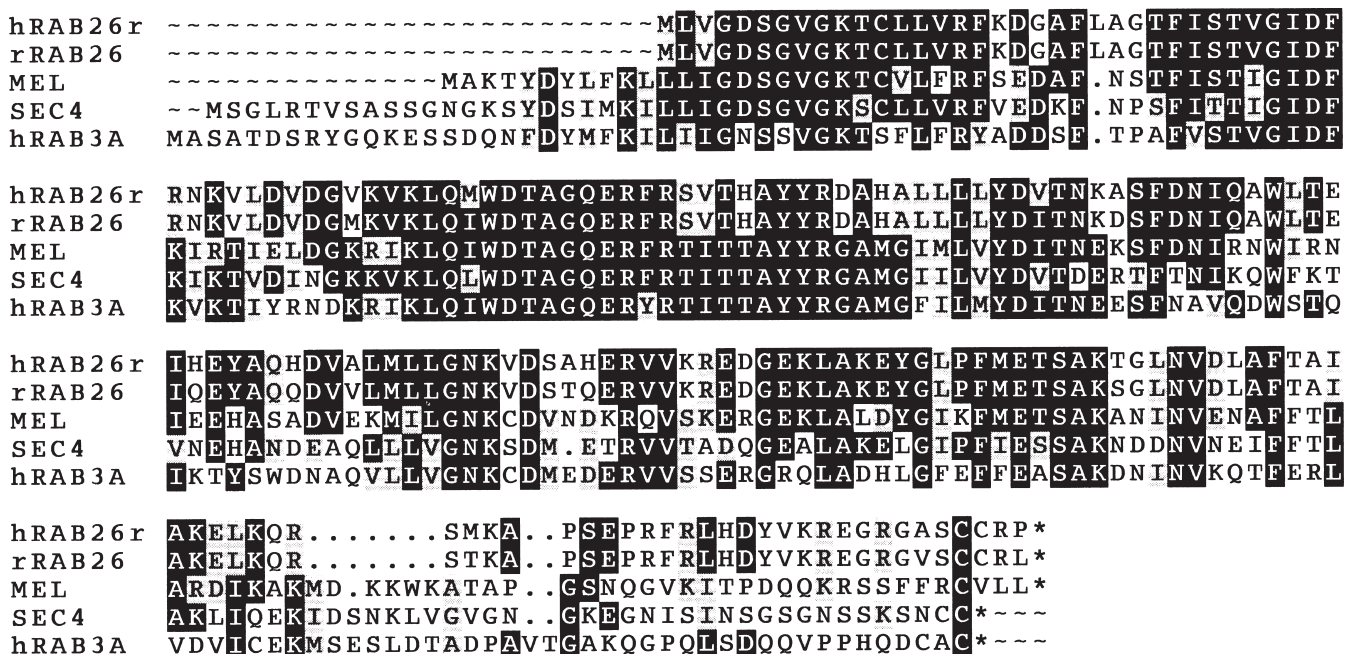


Fig. 2. Alignment of human RAB26-related (hRAB26r, accession number AB027137), rat Rab26 (rRAB26, accession number, U18771), human MEL (MEL, accession number, NM_005370), yeast SEC (SEC, accession number, M16507) and human RAB3A (hRAB3A, accession number, NM_002866). Identities are indicated by black background and similar

residues are shadowed. Asterisks denote the termination codons. A search of the public databases and alignment of these genes was performed with the FASTA program of the UWGCG package

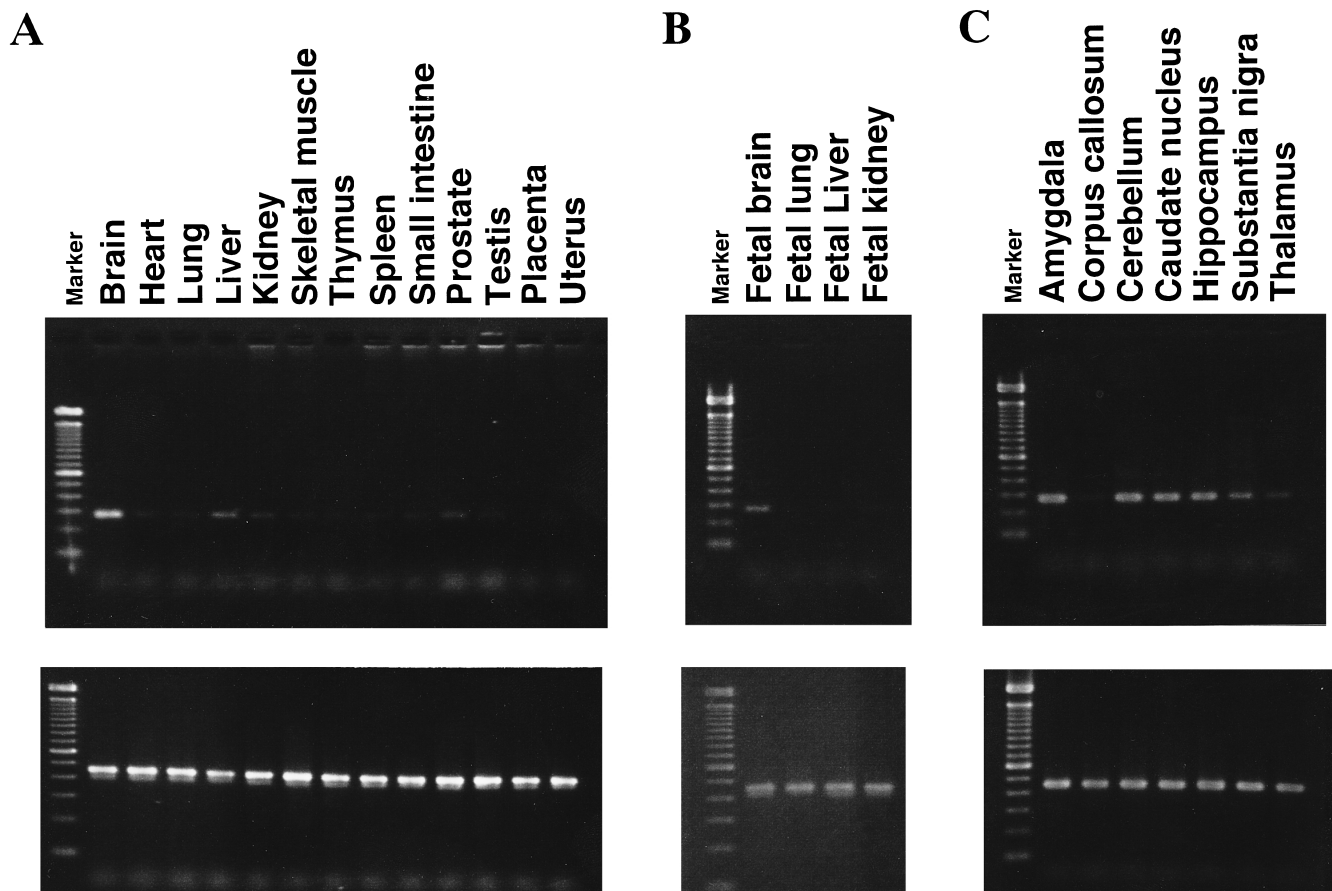


Fig. 3A–C. Tissue distribution analysis using reverse transcription-coupled polymerase chain reaction (RT-PCR). **A** The 13 adult tissues, **B** 4 fetal tissues, and **C** 7 brain subregions are indicated above each lane. The templates of the human tissues of poly(A)⁺ RNAs were purchased from Clontech (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 oligo-dT 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). Temperature and time schedules were: 30 cycles at 95°C for 20s, and 64°C for 1 min. PCR products were separated on 2.0% Nusieve GTG agarose gel (FMC, Rockland, ME, USA) with a 100-bp ladder DNA marker (GIBCO BRL, Gaithersburg, MD, USA)

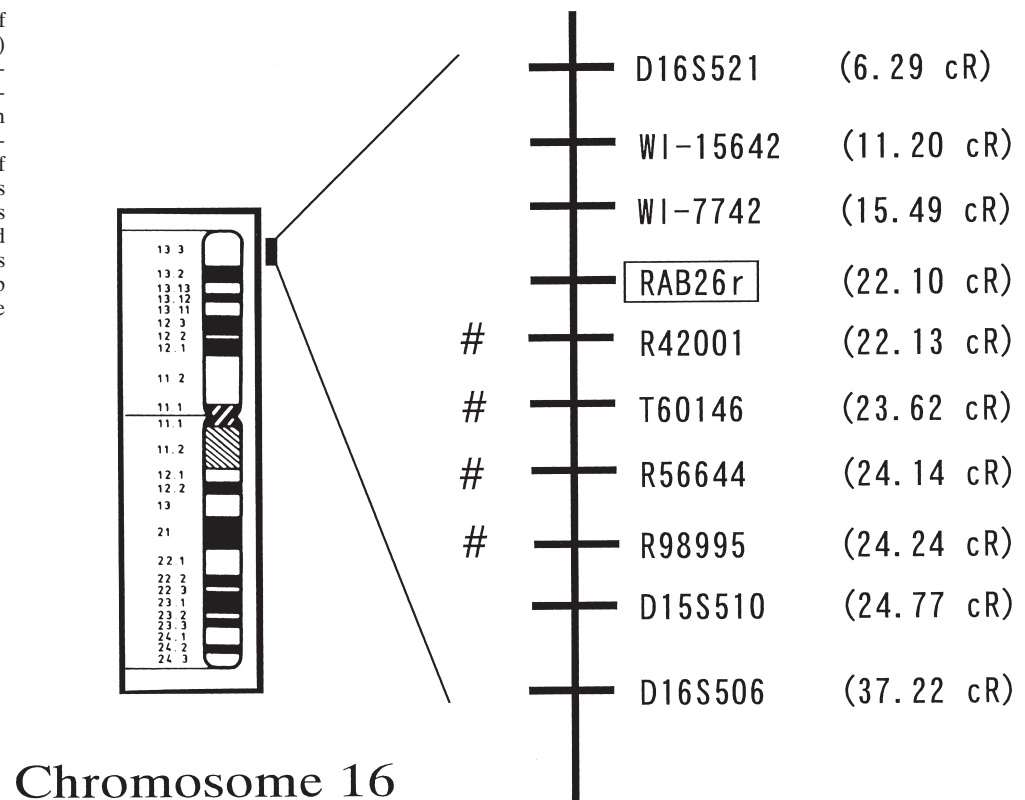
Chromosome mapping and genomic structure of *RAB26-related* gene

Chromosomal assignment of the human *RAB26-related* 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 (Seki et al. 1999, 2000). The human *RAB26-related* specific PCR primers, (5'-GCT TCA CTG CTA ATC ACA TCG-3', corresponding to nucleotides 993 to 1013; 5'TTT TAT CAC AAG GCC ACT GGC-3', corresponding to nucleotides 1132 to 1112), gave rise to an amplified product with a size of 140bp by genomic PCR. First, the specific amplified product for human was detected only from the hybrid containing human chromosome 16 (data not shown). Further mapping analysis was done, using a radiation hybrid panel

with the same primer set. 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 *RAB26-related* gene was 1001000101 0000000001 1000010110 0000001000 0000000001 0000001010 2121100110 0111000000 0011002002 001 and the consequent report indicated that the gene was placed to 6.61 cR proximal from the marker WI-7742 (lod > 3.0). The region including the marker was cytogenetically mapped to the 16p13.3 region (Fig. 4). The exon-intron boundaries of human *RAB26-related* gene were determined by aligning the cDNA sequence with the genomic sequence (accession number, AC012171). As shown in Table 1, all splicing sites conformed to the canonical splicing acceptor and donor rule of AG-GT. The *RAB26-related* gene was divided into eight exons, which ranged in size from 42bp (exon 2) to 847bp (exon 8). Exons 1 and 8 contained the ATG and TAG codons, respectively.

An autosomal recessively inherited disorder, familial Mediterranean fever (FMF; MIM 249100), is mapped in the chromosome 16p13.3 region, and with the aim of cloning the familial Mediterranean fever gene, several groups constructed a high-resolution physical map and transcriptional map around the marker D16S3070 (Bernot et al. 1998, Centola et al. 1998). From the database search (http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map), the *RAB26-related* gene was found to be closely located to four independent gene-derived ESTs (Bernot et al. 1998), such as R42001 (22.13 cR), T60146 (23.62 cR), R56644 (24.14cR), and R98995 (24.24 cR) (Fig. 4). Determination of the chromosomal position and expression profile of the gene may contribute to ongoing positional candidate approaches for potential disease genes linked to this genomic locus.

Fig. 4. Chromosomal placement of human *RAB26-related* (*RAB26r*) gene at a relative distance to framework markers on the WICGR radiation hybrid map of the human genome. The approximate corresponding cytogenetic location of the gene on the 16p13.3 region is indicated. Closely mapped ESTs (Bernot et al. 1998) are marked with hatch symbols (#). Distances are in centirays (cR) from the top of the chromosome 16 linkage group



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Table 1. Intron-exon boundaries of the *RAB26-related* gene

Exon no.	Exon size ^a	Splice acceptor ^b	Splice donor ^b
1	111		TGACTTCCGG gtgagtgaggc
2	42	tgtctgtttc ag AACAAAGTTC	GAAGCTGCAG gtaagtgactg
3	67	ctgtcgtct cgag ATGTGGGACG	GATGCTCATG gtgagccctgg
4	53	ctctgtccc ag CTCTGCTGCT	CAACATCCAG gtcagtgcttt
5	66	tgccctccc ag GCCTGGCTGA	GGGAACAAG gtggaggcccg
6	57	tgccctggg ccag GTGGACTCTG	GCTGGCCAAG gtgagtcaggc
7	77	agctgttt acag GAGTATGGAC	CCATAGCAAA gtaagtcctgcc
8	847	cccactcc gag GGAGTTGAAG	

^aSize, in base pairs

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

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