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Molecular cloning of a cDNA encoding a novel small GTP-binding protein*

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A cDNA encoding a small GTP-binding protein, S10, was cloned from Jurkat cells. The deduced amino acid sequence of S10 had the structural features characteristic to this family of proteins with highest homology to rab subfamily. Northern blot analysis revealed that this gene is expressed only in lymphoid cell lines and a histiocytic leukemia, U937. Hence, it should have a specialized function in cells derived from the hematopoietic stem cell.

GTP-binding protein; Lymphocyte, Macrophage; Vesicular transport

1. INTRODUCTION

Small GTP-binding proteins are involved in a wide variety of cellular functions [1–3], including signal transduction (ras) [4], vesicular transport (rab) [5], control of actin cytoskeleton (rho and rac) [6,7], and regulation of chromatin structure (TC4/ran) [8]. More than 50 members of small GTP-binding proteins have been reported so far. Many of them are expressed ubiquitously, while some are expressed in tissue-specific manners. Tissue-specific expression of a protein may represent the specificity of the funcitonal role it plays, as suggested for rab3A [9] or rac2 [10,11] protein. In an attempt to isolate small GTP-binding proteins expressed mainly in T lymphocytes, we employed polymerase chain reaction (PCR) method, which was successfully used to isolate new members of this family from marine ray [12]. Here we report the cloning of a cDNA which encodes a new member of the small GTP-binding proteins from a human T lymphocyte cell line, Jurkat. Expression of this gene was restricted to cells of lymphoid and monocytic lineage.

2. MATERIALS AND METHODS

2.1 *Cell culture*

Cell lines of human origin were cultured in either RPMI1640 (for cells of hematopoietic origin) or Dulbecco's modified Eagle's medium (for others) supplemented with 10% fetal bovine serum.

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*The accession number for the nucleotide sequence reported in this paper is D14889.

2.2. *RNA extraction and PCR*

Total cellular RNA from cultured cells were extracted as described [13] Poly(A)$^+$ RNA was prepared by two passages through an oligo(dT)-cellulose column. One μg of poly(A)$^+$ RNA from Jurkat cells was used as a template for oligo(dT)-primed cDNA synthesis by Superscript reverse transcriptase (BRL) One-hundredth of this reaction was subjected to PCR to amplify a region of small GTP-binding proteins. Two degenerate primers were used in this reaction: 5′CCCGAATTCTGGGA(TC)ACNGCNGGNCA(GA)GA3′ and 5′GCCGGATCCNA(GA)(GA)TC(GA)CA(TC)TT(GA)TTNCC3′, each corresponding to the conserved amino acid sequences of G-3 and G-4 [14,15], respectively. The conditions for the PCR were 1 min at 94°C, 1.5 min at 50°C, and 2 min at 72°C.

2.3 *Cloning in plasmid and nucleotide sequencing*

PCR products were separated by agarose gel electrophoresis and a major band of expected size was recovered. After digestion with *Eco*RI and *Bam*HI, fragments were subcloned in pBluescript vector (Stratagene). Double stranded plasmid DNA was sequenced by the dideoxy chain termination method with Sequenase (United States Biochemicals) and [α-^{32}P]dCTP (New England Nuclear).

2.4. *Screening of Jurkat cDNA library*

Jurkat cDNA library in λgt10 vector was purchased from Clonetech. About 1×10^6 plaques were screened with nick translated insert DNA from a PCR clone, S10. Positive phage clones (S10-32 and S10-4) were purified and the cDNA inserts subcloned in pBluescript were sequenced.

2.5. *Northern blot hybridization*

Twenty μg each of total RNA was separated by 1.0% agarose gel electrophoresis in 6% formaldehyde and transferred to a nitrocellulose membrane (Schleicher & Schuell). S10-4 cDNA was labeled by Megaprime DNA labeling system (Amersham) and used as a probe for S10. Final washing condition was 0.1 × SSC, 0.1% SDS at 50°C. After hybridization with S10 probe, membranes were washed twice in 2.5 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 0 025% sodium pyrophosphate and 0.05 × Denhardt's solution, at 70°C for 1 h, and then rehybridized with a β-actin probe.

3. RESULTS AND DISCUSSION

3.1. *PCR amplification of a region between G-3 and G-4 of small GTP-binding proteins*

Small GTP-binding proteins have regions of conserved amino acid sequences (G-1 through G-5) which are involved in GTP/GDP-binding and GTPase activity [14]. A region between G-3 and G-4 was amplified from single-stranded cDNA synthesized from Jurkat poly(A)$^+$ RNA using two degenerate oligonucleotides: a sense oligonucleotide corresponding to the amino acid sequence WDTAGQE of G-3 and an anti-sense oligonucleotide corresponding to GNKCDL of G-4. Nucleotide sequence were determined for inserts of more than 30 PCR clones. Most of them contained sequences of previously reported small GTP-binding proteins, including rab1, rab9, TC4/ran, and rab13. Two clones shared the same sequence which did not correspond to any known small GTP-binding proteins. This sequence was referred to as S10.

3.2. *Isolation of cDNA clones from a library and sequence analysis*

A Jurkat cell cDNA library was screened with the

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   1 CCCTCCCCTGCCTGCATTCCCGGGACGGACCCGAGGGAAGAAGCCTCAGGAGGAGGGTGT   60
  61 GGGCCGAGGCGCGGCGGCGGCTGGAGCAGCGCGGTAGGGTCCTTCGCCAGAGCATCCGGT  120
 121 CCGAGGGCGCACACAGGCAGAAGGCTCGGGGCTCGTCCACTCTCCTCCCTCTCTCCTCCT  180
 181 CTCCCTGGCTTTGTGTTGGTGCCTCCGAGCTGCAAGGAGGGTGCGCTGGAGGAGGAGGAG  240
 241 GGGGGCCCGGAGTGAGAGGCACCCCCTTCACGCGCGCGCGCGCACACGGTGCCGGCGCAC  300
 301 GCACACACGGGCGGACACACACACGCGCGCACACACACGCACAGAGCTCGCTCGCC       360
 361 TCGAGCGCACGAACGTGGACGTTCTCTTTGTGTGGAGCCCTCAAGGGGGGTTGGGGCCCC  420
 421 GGTTCGGTCCGGGGGAGATGGCGCAGCCCATCCTGGGCCATGGGAGCCTGCAGCCCGCCT  480
                     M  A  Q  P  I  L  G  H  G  S  L  Q  P  A  S
 481 CGGCCGCTGGCCTGGCGTCCCTGGAGCTCGACTCGTCGCTGGACCAGTACGTGCAGATTC  540
       A  A  G  L  A  S  L  E  L  D  S  S  L  D  Q  Y  V  Q  I  R
 541 GCATCTTCAAAATAATCGTGATTGGGGACTCCAACGTGGGCAAGACCTGCCCGACCTTCC  600
       I  F  K  I  I  V  I  G  D  S  N  V  G  K  T  C  P  T  F  R
 601 GCTTCTGCGGGGGTACCTTCCCAGACAAGACTGAAGCCACCATCGGCGTGGACTTCAGGG  660
       F  C  G  G  T  F  P  D  K  T  E  A  T  I  G  V  D  F  R  E
 661 AGAAGACCGTGGAAATCGAGGGCGAGAAGATCAAGGTTCAGGTGTGGGACACAGCAGGTC  720
       K  T  V  E  I  E  G  E  K  I  K  V  Q  V  W  D  T  A  G  Q
 721 AGGAACGTTTCCGCAAAAGCATGGTCGAGCATTACTACCGCAACGTACATGCCGTGGTCT  780
       E  R  F  R  K  S  M  V  E  H  Y  Y  R  N  V  H  A  V  V  F
 781 TCGTCTATGACGTCACCAAGATGACATCTTTCACCAACCTCAAAATGTGGATCCAAGAAT  840
       V  Y  D  V  T  K  M  T  S  F  T  N  L  K  M  W  I  Q  E  C
 841 GCAATGGGCATGCTGTGCCCCCACTAGTCCCCAAAGTGCTTGTGGGCAACAAGTGTGACT  900
       N  G  H  A  V  P  P  L  V  P  K  V  L  V  G  N  K  C  D  L
 901 TGAGGGAACAGATCCAGGTGCCCTCCAACTTAGCCCTGAAATTTGCTGATGCCCACAACA  960
       R  E  Q  I  Q  V  P  S  N  L  A  L  K  F  A  D  A  H  N  M
 961 TGCTCTTGTTTGAGACATCGGCCAAGGACCCCAAAGAGAGCCAGAACGTGGAGTCGATTT 1020
       L  L  F  E  T  S  A  K  D  P  K  E  S  Q  N  V  E  S  I  F
1021 TCATGTGCTTGGCTTGCCGATTGAAGGCCCAGAAATCCCTGCTGTATCGTGATGCTGAGA 1080
       M  C  L  A  C  R  L  K  A  Q  K  S  L  L  Y  R  D  A  E  R
1081 GGCAGCAGGGGAAGGTGCAGAAACTGGAGTTCCCACAGGAAGCTAACAGTAAAACTTCCT 1140
       Q  Q  G  K  V  Q  K  L  E  F  P  Q  E  A  N  S  K  T  S  C
1141 GTCCTTGTTGAAACCAAACGATATAAATACAAGATAAATTATCACTGGAGTTTTTTCTTT 1200
       P  C  *
1201 CCCTTTTTTCTGTGCCTGCATAATGCTGACACCTGCTTGTTTCCATACAAATTGATATCA 1260
1261 AAATAAAATTTGTATAGATTATCAAAAAAAAAA                             1293
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of S10 cDNA. The cDNA sequence was established by the combination of two overlapping cDNA clones, S10-32 (nucleotides 1 to 1271) and S10-4 (nucleotides 533 to 1293). S10-4 had C residue at position 742, which results in amino acid substitution of T in place of M. This variation can be an artifact in cDNA cloning or may represent two alleles present in Jurkat cells. G-1 through G-5 regions conserved in small GTP-binding proteins are underlined.

insert DNA of S10 PCR clone. Two positive clones were isolated, subcloned in a plasmid vector, and sequenced. These two clones overlapped with each other comprising the sequence of 1,293 nucleotides. As shown in Fig. 1, the sequence had an open reading frame of 711 bp which can encode a protein of 237 amino acids. The translation start site was compatible with the consensus sequence of Kozak [16], but the first methionine codon at nucleotide 438 was not preceded by any stop codon, leaving the possibility that the open reading frame starts from more 5'-region beyond the end of the isolated cDNA. However, this possibility is unlikely for the following reasons. First, the size of the cDNA was almost the same as the size of transcript (1.3 kb) detected by Northern hybridization. And second, the 5'-region of cloned cDNA contained CA repeat sequences unlikely to encode a functional peptide.

The deduced amino acid sequence of S10 had all characteristics of the small GTP-binding proteins. The regions G-1 to G-5 were conserved and C-terminal cysteine residues susceptible to isoprenoid modification were present. S10 had highest homology to yeast YPT1 protein with 40% identity and 78% similarity. Human rab1 had 39% identity and 72% similarity. Table I lists the percent identity of the guanine nucleotide binding domain [17] between small GTP-binding proteins. Percent identity of S10 in this region were 34–47% with members of rab subfamily, and 24–32% with other small GTP-binding proteins (Table I and data not shown). From these data, we conclude that S10 belongs to rab subfamily. As rab family proteins function in vesicular transport, S10 protein may also play a role in the transport system.

3.3. *Expression of S10 in human cell lines*

In order to determine the cell type specific expression of S10, Northern blot hybridization were performed on RNA extracted from various human tumor cell lines (Fig. 2). Expression of S10 was detected in cell lines of

Table I

S10 Homology to small GTP-binding proteins

	S10	rab1	rab2	rab3A	rab4	rab5	rab6	H-ras	rhoA	TC4
S10	–	47	38	37	36	40	37	32	25	31
rab1		–	50	52	46	47	41	37	29	32
rab2			–	42	60	42	40	35	31	30
rab3A				–	38	37	42	31	29	29
rab4					–	42	39	33	29	33
rab5						–	45	33	28	33
rab6							–	31	32	28
H-ras								–	31	26
rhoA									–	27
TC4										–

The values represent the percentage of amino acid identity within the guanine nucleotide binding domain, corresponding to amino acids 5 to 164 of H-ras protein [17]. Human sequences were used for calculation.

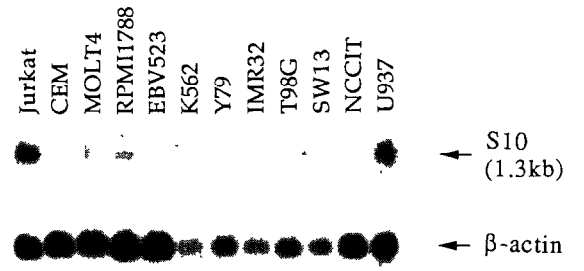

Fig. 2. Northern hybridization with S10 cDNA probe. Twenty μg each of total RNA from human cell lines was electrophoresed, transferred, and hybridized with S10 cDNA probe as described in section 2. After removal of the probe, the same membrane was rehybridized with a β-actin probe.

T lymphocyte (Jurkat, CEM, and MOLT4) and B lymphocyte (RPMI1788 and EBV523). A histiocytic leukemia cell line (U937) also expressed S10 mRNA, although we could not detect transcription in other monocytic cell lines (THP-1 and J111), in a myelo-monocytic cell line (HL60) (data not shown), or in a myelocytic leukemia cell line (K562). S10 was not expressed in cell lines on non-hematopoietic origin: retinoblastoma (Y79), neuroblastoma (IMR32), glioblastoma (T98G), adrenal cortex adenoma (SW13), teratocarcinoma (NCC-IT), hepatoma (HepG2), stomach cancer (AZ521), epidermoid carcinoma (A431), fibrosarcoma (HT1080), and cervical carcinoma (HeLa) (Fig. 2 and data not shown).

Restriction of expression to lymphoid and monocytic lineage may suggest the function of S10 specific to these cells. rab3A [18,19] and rab15 [20] are expressed only in neural tissues and rab3A was proposed to play a role in synaptic vehicle release being consistent with the expression pattern [9]. rac2 is predominantly expressed in lymphoid and myelo-monocytic cells [21–23] and was implicated to control superoxide production [10,11]. Expression pattern of S10 was similar to that of rac2. However, S10 was expressed only in U937 cells among the cell lines of myelo-monocytic lineage tested, and the expression diminished after differentiation of U937 with dibutyryl cyclic AMP (data not shown) in contrast to the augmentation of rac2 expression [21]. Moreover, rac2 is also expressed in HeLa cells [21] and MDCK kidney cells [24]. Thus expression of S10 appears to be more restricted than rac2. Further investigation is required to elucidate the function of S10 in lymphoid and monocytic cells.

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