Human rab11a: transcription, chromosome mapping and effect on the expression levels of host GTP-binding proteins

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Abstract Rab11a is a member of the *rab*-branch of the *ras*-like small GTP-binding protein superfamily that is associated with both constitutive and regulated secretory pathways. Using a direct procedure for cDNA cloning of small *ras*-related GTPases, that is based on the screening of eukaryotic cDNA expression libraries using [α-^{32}P]GTP as a probe, we have isolated two cDNA clones encoding rab11a. Both clones share identical coding sequences, but differ in the length and sequence of their 3′ untranslated regions (3′-UTR). Northern blot hybridisation analysis of various human tissues revealed indeed two mRNA species with lengths of 1.0 and 2.3 kb, respectively. Sequence analysis of the cDNAs identified two different putative polyadenylation signals (AATAAA) at positions 927 and 2302 of the larger transcript. In addition, the 3′-UTR of the larger transcript exhibited several AU-rich elements (ARE) that are believed to control gene expression by regulating the rate of mRNA degradation. Southern blots of human DNA digested with several rare restriction enzymes, and separated by pulse-field gel electrophoresis, yielded the same macro-restriction fragment pattern when hybridised with probes that discriminate between the two transcripts. Taken together, these findings imply that the two mRNA species originate from a single gene, which we have mapped to 15q21.3-q22.31, by the use of diferent polyadenylation sites. As expected, both rab11a-cDNAs yielded the same protein product when transiently expressed in COS-1 cells, and surprisingly, upregulated the proteome expression profile (de novo synthesis or posttranslational modification of preexisting proteins) of a few other, yet unknown GTP-binding proteins.

Key words: 2D PAGE; GTP-binding protein; *ras* family

1. Introduction

The small *ras*-related GTP-binding proteins constitute a large superfamily, that has been grouped into several main branches according to their sequence homologies and functional features: *ras*-, *rho*/*rac*-, *rab*/*ypt*-, *ran*/*tc4*-, and *arf*-subfamilies [1–3]. Recently, a novel branch of the *ras*-superfamily has been identified, two homologues of which, termed rheb, have been cloned in rat [4] and in human [5]. The *Rab*/*Ypt* subgroup is perhaps the largest branch of the superfamily as it comprises more than thirty proteins [6], some of which may be key signal components of the pathways that regulate vesicular transport in eukaryotic cells [7,8]. Members of the *rab*-subfamily show cell type dependent variations in cell expression, as well as differential distribution to distinct intracellular compartments and organelles that perform multiple sorting functions [9].

The YPT3-related rab11 protein is probably one of the less studied members of the *rab*-subgroup. Four mammalian cDNA encoding rab11 from human [10], dog [11], rat [12] and rabbit [13] have been cloned and found to be identical to each other. A novel variant, that shared 89% identity to rab11, was recently described in mice and named rab11b [14]. Moreover, a human homolog of rab11b has also been identified strengthening the contention that there are at least two types of rab11 genes in mammalian cells [15]. To distinguish between them, the mammalian YPT3-related rab11 protein has been renamed rab11a [14]. The rab11b genes have been mapped to mouse chromosomes 1 and 17 [14], but the chromosome location of rab11a remains unknown.

The functional role of the rab11 genes in protein traffic is still poorly understood, although there is evidence that it is associated with both constitutive and regulated secretory pathways [16]. Immunohistochemical studies have shown that rab11a is enriched in rabbit parietal cells, and have revealed an association with intracellular tubulovesicles [17]. Interestingly, transcription of the mammalian rab11a gene yields two mRNAs (1 and 2.3 kb) as determined by Northern blotting [11,18], while transcription of the rab11b gene yields only a single species of 1.8 kb [14]. Studies of various mammalian cell lines have shown that rab1, 5, 10 and 11 exhibit two transcripts of different size when hybridised with the corresponding cDNA probes, while rab2, 4 and 7 yield three [11,19]. To date, several mechanisms have been shown to lead to the generation of different transcripts from the same gene: these include (i) the selection of different polyadenylation sites, (ii) the use of alternate transcription start codons and (iii) differential splicing. So far, however, the mechanism underlying the generation of multiple transcripts observed in many of the *rab* genes remains unsolved.

Here we report the isolation of two cDNAs encoding human rab11a, and show that the two observed transcripts originate from a single gene mapping to 15q21.3-q22.31, by the alternative use of two polyadenylation signals. In addition, we present data showing that the overexpression of rab11a in

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Abbreviations: GTP, guanosine 5′-triphosphate; DTT, dithiothreitol; IEF, isoelectric focusing; 2D PAGE, two-dimensional polyacrylamide gel electrophoresis

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COS-1 cells affects the levels of expression of other small GTP-binding proteins.

2. Materials and methods

2.1. Cultured cells and labeling with [^{35}S]methionine

COS-1 monkey kidney cells [20] were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum and antibiotics (penicillin at 100 U/ml and streptomycin at 50 μg/ml). COS-1 cells were labeled for 14 h in DMEM medium lacking methionine and containing 10% dialyzed fetal calf serum and 1 mCi of [^{35}S]methionine per ml.

2.2. Screening of cDNA libraries with [α-^{32}P]GTP

An oligo-dT primed expression cDNA library was constructed in phage λ ZAPII (Stratagene) using poly(A)$^+$ RNA from whole human epidermis. Screening of plated libraries with [α-^{32}P]GTP was carried out as previously described [5]. Briefly, amplified phages (600–2000 bp) were plated on 500-cm^2 dishes at a density of approximately 125 000 pfu per plate, using the bacterial strain LE 392 (Stratagene). As soon as the plaques were visible, they were overlaid with nitrocellulose membranes (Amersham) for 12 h. Prior to overlay, the membranes were soaked in 10 mM IPTG and dried. Thereafter, the nitrocellulose replica was washed two times in a solution containing 50 mM Tris-HCl, 0.3% Tween 20 or NP-40, pH 7.5 to remove the attached bacterial lawn and to reduce the high viscosity resulting from bacterial lysis. The filters were then incubated in 2% SDS/100 mM DTT for 15 min at 50°C. The replica was then washed three times with 25 mM Tris/190 mM glycine buffer, pH 8.3 containing 20% methanol (25 min per wash) to remove SDS, and subjected for [α-^{32}P]GTP blot overlay assay as described [21]. Following removal of the nitrocellulose filters from the plates, replicas were taken using nylon membranes which were then used for hybridization with previously identified cDNAs. Several random labeled cDNAs were hybridized at the same time. The corresponding DNA hybridization autoradiographs were then superimposed with the ^{32}P X-ray films containing all the clones revealed by the [α-^{32}P]GTP-binding assay. After picking the first round of clones, the plates were placed at −70°C for subsequent isolation of additional clones.

2.3. DNA sequencing

Cloned cDNAs were rescued as pBluescript plasmid using the automatic excision process of λ ZAP. The fragments were sequenced by automatic sequencing using the ALF system (Pharmacia).

2.4. Pulse-field gel electrophoresis, Southern transfer and hybridisation

Total genomic DNA was isolated from human lymphocytes and digested with several rare restriction enzymes. Pulse-field gel electrophoresis (PFGE) was carried out in 1% agarose gel using the Bio-Rad Mapper system at 13°C for 28 h. We used a voltage gradient of 6 V/cm with the switch time 'ramped' linearly from 20–90 s in the two-state mode. Southern transfer and hybridization were performed as described in the Bio-Rad manual for PFGE. Radioactive probes were prepared by using the Megaprime labeling kit (Amersham).

2.5. Fluorescence in situ hybridization (FISH)

FISH using the biotin-labeled 2.3-kb rab11a cDNA clone (rab11a-2.3), with corresponding DAPI-banding and measurement of the relative distance from the short arm telomere to the signals (FLpter value), was performed as described previously [22].

2.6. Radiation hybrid mapping

The 86 GeneBridge4 clones for radiation hybrid (RH) mapping [23] were obtained from the Human Genome Mapping Project Resource Center (UK MRC HGMP-RC) (http://www.hgmp.mrc.ac.uk/homepage.html). Primers used for RH-mapping were: a1: 5′-TCTCCCCTAGAAGGCTGTGT-3′ and a2: 5′-TCATTCGGGACAAGTGGAT-3′, amplifying a 164-bp fragment in the 3′-UTR region of the rab11a gene (position 755–919 of the rab11-1 clone). A second set of primers, b1: 5′-CCACTTGTCCCGAATGACT-3′ and b2: 5′-CTCTGTGGCAAAATGGTAGC-3′, amplifying a 224-bp fragment originating in the 3′-region shared between the two clones, but extending into the unique region of clone rab11-2.3 (position 868–1092 of the rab11-2.3 clone) were synthesized (Pharmacia). Both primer sets were designed using OLIGO software (National Biosciences) [24]. PCR was performed on a PTC-225 DNA Engine Tetrad thermocycler (MJ Research) for 40 cycles at 95, 55 and 72°C. Each step in the thermocycling program was 20 s. Thermocycling was preceded by a pre-denaturation step at 95°C, 5 min. Succeeding thermocycling, reactions were incubated at 72°C, 5 min. PCR were done in standard PCR buffer (Perkin Elmer) with 10 pmol of each primer, 10 nmol dNTP (Pharmacia) and 0.5 U AmpliTaq (Perkin Elmer) in a total volume of 15 μl. Gel-loading buffer at final concentrations of 0.2 mM cresol red and 12% sucrose, were included in the reaction buffer [25]. Fragments were electrophoresed on a 2% agarose gel (FMC) and photographed. By visual inspection of the gel, PCR products of correct length were scored as positives while PCR products of incorrect length or absent were scored as ambiguities or negatives. Mapping was performed using the UK MRC HGMP-RC interface to the Whitehead Institute/MIT Center for Genome Research's RH mapping facility (http://www.hgmp.mrc.ac.uk/homepage.html; http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl). Following mapping, markers neighboring or coinciding with the RH data vector were looked up using the ENTREZ genome database browser at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/Entrez/Genome/org.html), and the chromosomal location documented.

2.7. Northern blot hybridization

^{32}P-labeled cDNA was generated by random priming (Megaprime labeling kit, Amersham), and used for hybridization. mRNA tissue distribution was analysed using a multiple tissue Northern blot obtained from Clontech.

2.8. Transient expression of rab11a cDNAs in COS-1 cells

The full length rab11a cDNAs containing the entire coding region were subcloned into the pMT21 vector [26], and transiently transfected into COS-1 cells using the LipofectAmine reagent (Gibco-BRL, Life Technology) according to the manufacturer's description.

2.9. 2D gel electrophoresis and [α-^{32}P]GTP blot overlay procedure

2D gel electrophoresis [27], blotting [28], and the GTP blot overlay procedure [21] were performed according to published procedures.

3. Results and discussion

3.1. Isolation and sequencing of two cDNAs encoding rab11a

Using a direct approach for the cloning of small GTP binding proteins [5], we identified and sequenced two cDNAs coding for rab11a having sizes of 1 kb (rab11a-1) and 2.3 kb (rab11a-2.3), respectively. Sequence alignment showed that the region of overlap between the two cDNAs (951 bp) was identical, and corresponded to a published sequence of human rab11a [10]. The rab11a-2.3 cDNA on the other hand exhibited 1372 bp of additional sequence distal to the 3′ end of rab11a-1, and represented a novel cDNA encoding for this protein (Fig. 1). Two distinct canonical polyadenylation sites are present in rab11a-2.3 at positions 926 and 2303, the first being common for both cDNAs. Taken together these

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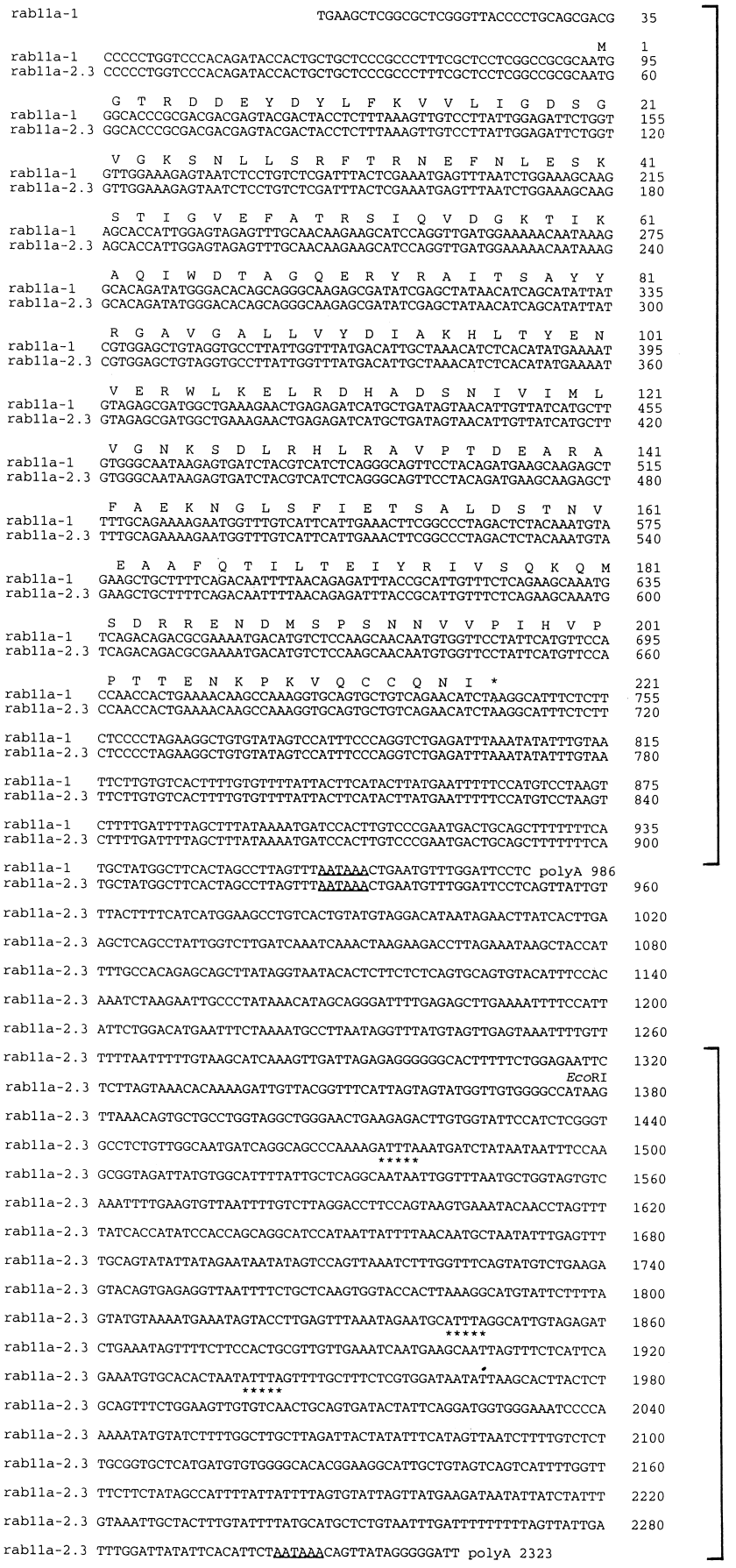
Fig. 1. Sequence alignment of the coding and 3′ untranslated regions of two types of cDNAs encoding rab11a. The sequences from nucleotide 1–951 (rab11a-2.3), including the coding regions, are identical in both cDNAs. The Rab11a-2.3 kb cDNA has an additional 3′-UTR sequence of 1372 bp in length. Two putative polyadenylation signals (AATAAA) are underlined. Several motifs corresponding to the AU-rich elements (ATTTA) are indicated with asterisks. The *Eco*RI site at position 1313 was used to cut the rab11a-2.3 kb 3′-UTR fragment. The regions of the rab11a cDNAs used as a probe for Northern and Southern hybridisation (see Figs. 2 and 3) are indicated on the right.


rab11a-1 TGAAGCTCGGCGCTCGGGTTACCCCTGCAGCGACG 35
M 1
rab11a-1 CCCCCTGGTCCCACAGATACCACTGCTGCTCCCGCCCTTTCGCTCCTCGGCCGCGCAATG 95
rab11a-2.3 CCCCCTGGTCCCACAGATACCACTGCTGCTCCCGCCCTTTCGCTCCTCGGCCGCGCAATG 60
G T R D D E Y D Y L F K V V L I G D S G 21
rab11a-1 GGCACCCGCGACGACGAGTACGACTACCTCTTTAAAGTTGTCCTTATTGGAGATTCTGGT 155
rab11a-2.3 GGCACCCGCGACGACGAGTACGACTACCTCTTTAAAGTTGTCCTTATTGGAGATTCTGGT 120
V G K S N L L S R F T R N E F N L E S K 41
rab11a-1 GTTGGAAAGAGTAATCTCCTGTCTCGATTTACTCGAAATGAGTTTAATCTGGAAAGCAAG 215
rab11a-2.3 GTTGGAAAGAGTAATCTCCTGTCTCGATTTACTCGAAATGAGTTTAATCTGGAAAGCAAG 180
S T I G V E F A T R S I Q V D G K T I K 61
rab11a-1 AGCACCATTGGAGTAGAGTTTGCAACAAGAAGCATCCAGGTTGATGGAAAAACAATAAAG 275
rab11a-2.3 AGCACCATTGGAGTAGAGTTTGCAACAAGAAGCATCCAGGTTGATGGAAAAACAATAAAG 240
A Q I W D T A G Q E R Y R A I T S A Y Y 81
rab11a-1 GCACAGATATGGGACACAGCAGGGCAAGAGCGATATCGAGCTATAACATCAGCATATTAT 335
rab11a-2.3 GCACAGATATGGGACACAGCAGGGCAAGAGCGATATCGAGCTATAACATCAGCATATTAT 300
R G A V G A L L V Y D I A K H L T Y E N 101
rab11a-1 CGTGGAGCTGTAGGTGCCTTATTGGTTTATGACATTGCTAAACATCTCACATATGAAAAT 395
rab11a-2.3 CGTGGAGCTGTAGGTGCCTTATTGGTTTATGACATTGCTAAACATCTCACATATGAAAAT 360
V E R W L K E L R D H A D S N I V I M L 121
rab11a-1 GTAGAGCGATGGCTGAAAGAACTGAGAGATCATGCTGATAGTAACATTGTTATCATGCTT 455
rab11a-2.3 GTAGAGCGATGGCTGAAAGAACTGAGAGATCATGCTGATAGTAACATTGTTATCATGCTT 420
V G N K S D L R H L R A V P T D E A R A 141
rab11a-1 GTGGGCAATAAGAGTGATCTACGTCATCTCAGGGCAGTTCCTACAGATGAAGCAAGAGCT 515
rab11a-2.3 GTGGGCAATAAGAGTGATCTACGTCATCTCAGGGCAGTTCCTACAGATGAAGCAAGAGCT 480
F A E K N G L S F I E T S A L D S T N V 161
rab11a-1 TTTGCAGAAAAGAATGGTTTGTCATTCATTGAAACTTCGGCCCTAGACTCTACAAATGTA 575
rab11a-2.3 TTTGCAGAAAAGAATGGTTTGTCATTCATTGAAACTTCGGCCCTAGACTCTACAAATGTA 540
E A A F Q T I L T E I Y R I V S Q K Q M 181
rab11a-1 GAAGCTGCTTTTCAGACAATTTTAACAGAGATTTACCGCATTGTTTCTCAGAAGCAAATG 635
rab11a-2.3 GAAGCTGCTTTTCAGACAATTTTAACAGAGATTTACCGCATTGTTTCTCAGAAGCAAATG 600
S D R R E N D M S P S N N V V P I H V P 201
rab11a-1 TCAGACAGACGCGAAAATGACATGTCTCCAAGCAACAATGTGGTTCCTATTCATGTTCCA 695
rab11a-2.3 TCAGACAGACGCGAAAATGACATGTCTCCAAGCAACAATGTGGTTCCTATTCATGTTCCA 660
P T T E N K P K V Q C C Q N I * 221
rab11a-1 CCAACCACTGAAAACAAGCCAAAGGTGCAGTGCTGTCAGAACATCTAAGGCATTTCTCTT 755
rab11a-2.3 CCAACCACTGAAAACAAGCCAAAGGTGCAGTGCTGTCAGAACATCTAAGGCATTTCTCTT 720
rab11a-1 CTCCCCTAGAAGGCTGTGTATAGTCCATTTCCCAGGTCTGAGATTTAAATATATTTGTAA 815
rab11a-2.3 CTCCCCTAGAAGGCTGTGTATAGTCCATTTCCCAGGTCTGAGATTTAAATATATTTGTAA 780
rab11a-1 TTCTTGTGTCACTTTTGTGTTTTATTACTTCATACTTATGAATTTTTCCATGTCCTAAGT 875
rab11a-2.3 TTCTTGTGTCACTTTTGTGTTTTATTACTTCATACTTATGAATTTTTCCATGTCCTAAGT 840
rab11a-1 CTTTTGATTTTAGCTTTATAAAATGATCCACTTGTCCCGAATGACTGCAGCTTTTTTTCA 935
rab11a-2.3 CTTTTGATTTTAGCTTTATAAAATGATCCACTTGTCCCGAATGACTGCAGCTTTTTTTCA 900
rab11a-1 TGCTATGGCTTCACTAGCCTTAGTTTAATAAACTGAATGTTTGGATTCCTC polyA 986
rab11a-2.3 TGCTATGGCTTCACTAGCCTTAGTTTAATAAACTGAATGTTTGGATTCCTCAGTTATTGT 960
rab11a-2.3 TTACTTTTCATCATGGAAGCCTGTCACTGTATGTAGGACATAATAGAACTTATCACTTGA 1020
rab11a-2.3 AGCTCAGCCTATTGGTCTTGATCAAATCAAACTAAGAAGACCTTAGAAATAAGCTACCAT 1080
rab11a-2.3 TTTGCCACAGAGCAGCTTATAGGTAATACACTCTTCTCTCAGTGCAGTGTACATTTCCAC 1140
rab11a-2.3 AAATCTAAGAATTGCCCTATAAACATAGCAGGGATTTTGAGAGCTTGAAAATTTTCCATT 1200
rab11a-2.3 ATTCTGGACATGAATTTCTAAAATGCCTTAATAGGTTTATGTAGTTGAGTAAATTTTGTT 1260
rab11a-2.3 TTTTAATTTTTGTAAGCATCAAAGTTGATTAGAGAGGGGGGCACTTTTTCTGGAGAATTC 1320
EcoRI
rab11a-2.3 TCTTAGTAAACACAAAAGATTGTTACGGTTTCATTAGTAGTATGGTTGTGGGGCCATAAG 1380
rab11a-2.3 TTAAACAGTGCTGCCTGGTAGGCTGGGAACTGAAGAGACTTGTGGTATTCCATCTCGGGT 1440
rab11a-2.3 GCCTCTGTTGGCAATGATCAGGCAGCCCAAAAGATTTAAATGATCTATAATAATTTCCAA 1500
*****
rab11a-2.3 GCGGTAGATTATGTGGCATTTTATTGCTCAGGCAATAATTGGTTTAATGCTGGTAGTGTC 1560
rab11a-2.3 AAATTTTGAAGTGTTAATTTTGTCTTAGGACCTTCCAGTAAGTGAAATACAACCTAGTTT 1620
rab11a-2.3 TATCACCATATCCACCAGCAGGCATCCATAATTATTTTAACAATGCTAATATTTGAGTTT 1680
rab11a-2.3 TGCAGTATATTATAGAATAATATAGTCCAGTTAAATCTTTGGTTTCAGTATGTCTGAAGA 1740
rab11a-2.3 GTACAGTGAGAGGTTAATTTTCTGCTCAAGTGGTACCACTTAAAGGCATGTATTCTTTTA 1800
rab11a-2.3 GTATGTAAAATGAAATAGTACCTTGAGTTTAAATAGAATGCATTTAGGCATTGTAGAGAT 1860
*****
rab11a-2.3 CTGAAATAGTTTTCTTCCACTGCGTTGTTGAAATCAATGAAGCAATTAGTTTCTCATTCA 1920
rab11a-2.3 GAAATGTGCACACTAATATTTAGTTTTGCTTTCTCGTGGATAATATTAAGCACTTACTCT 1980
*****
rab11a-2.3 GCAGTTTCTGGAAGTTGTGTCAACTGCAGTGATACTATTCAGGATGGTGGGAAATCCCCA 2040
rab11a-2.3 AAAATATGTATCTTTTGGCTTGCTTAGATTACTATATTTCATAGTTAATCTTTTGTCTCT 2100
rab11a-2.3 TGCGGTGCTCATGATGTGTGGGGCACACGGAAGGCATTGCTGTAGTCAGTCATTTTGGTT 2160
rab11a-2.3 TTCTTCTATAGCCATTTTATTATTTTAGTGTATTAGTTATGAAGATAATATTATCTATTT 2220
rab11a-2.3 GTAAATTGCTACTTTGTATTTTATGCATGCTCTGTAATTTGATTTTTTTTTAGTTATTGA 2280
rab11a-2.3 TTTGGATTATATTCACATTCTAATAAACAGTTATAGGGGGATT polyA 2323
PROBE 1
PROBE 2

data suggest that the differential processing of the rab11a primary transcript could generate two mature mRNAs due to the use of the alternative polyadenylation sites in the pre-mRNA.

Sequence analysis also revealed that the 3′ untranslated region (3′-UTR) of the larger transcript contains a high level of adenine and thymidine, as well as several adenylate/uridylate-rich elements (ARE) which are believed to represent the most common determinant of RNA stability in mammalian cells [29,30]. It is well established that the rate of mRNA degradation can be determined in part by specific AREs that are found in the 3′-UTR of many messenger RNAs coding for proto-oncogenes, transcription factors and cytokines [30]. There are two common structural features among the different AREs: (i) a high content of adenylate and uridylate residues, and (ii) the presence of various copies of an AUUUA pentanucleotide [31]. The corresponding sequence motif ATTTA is repeated three times within the 3′-UTR of rab11a-2.3 cDNA (Fig. 1). However, as the presence of AUUUA motif(s) in mRNA does not guarantee a destabilizing function of ARE [30], it is not clear whether these sequences play a role in the stability of the large rab11a-2.3 transcript.

3.2. Northern blot analysis of rab11a

To determine the relation between the two rab11a cDNAs and the transcripts revealed by Northern hybridisation with rab11a, we examined a number of human tissues using two probes specific for rab11a-1 and rab11a-2.3 cDNAs, respectively (Fig. 1). As shown in Fig. 2, hybridisation with the ^{32}P-labeled rab11a-1 cDNA (probe 1, see Fig. 1) revealed two major mRNA transcripts with lengths of 1 kb and 2.3 kb (Fig. 2A) in line with earlier observations [11,18]. To assess the identity of the larger rab11a mRNA (2.3 kb), we cut the 3′-UTR portion of the rab11a-2.3 cDNA using the *Eco*RI site at position 1312 (probe 2, see Fig. 1), and used it to probe the same blot. Rehybridisation of the filter with probe 2 detected only the larger transcript (2.3 kb) in all tissues (Fig. 2). It may be concluded, therefore, that the two rab11a mRNA species

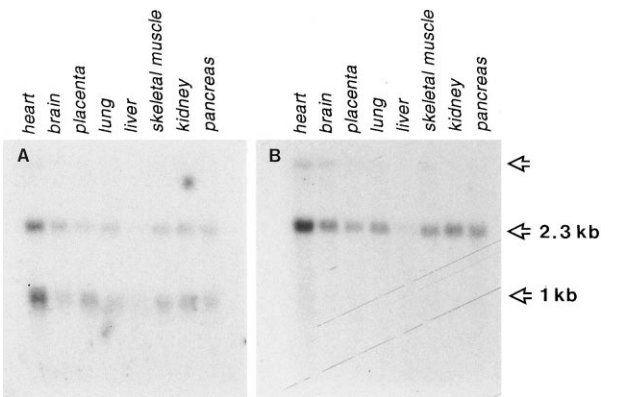


Fig. 2. Rab11a gene transcripts in adult human tissues. A multiple tissue Northern blot (Clontech) containing 2 mg of pure poly(A)$^+$ RNA per line was first hybridized with ^{32}P-labeled rab11a-1 kb cDNA (probe 1), washed under high stringency, and exposed to X-ray films at −80°C using an intensifying screen (A, left panel). The filter was then washed in 0.1% SDS at 100°C for 3 min to remove the probe, and rehybridised with the ^{32}P-labeled fragment designed from the 3′-UTR of rab11a-2.3 kb cut using the *Eco*RI site at position 1313 (probe 2) (B, right panel). The samples were: heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.

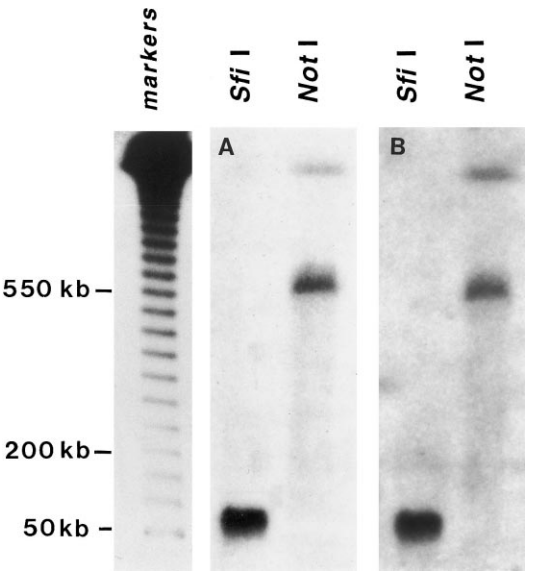


Fig. 3. Southern blot analysis of DNA fragments resolved by pulse field electrophoresis. Intact total DNA was isolated from normal human lymphocytes, digested with *Sfi*I and *Not*I, and separated by pulse-field gel electrophoresis. DNA fragments were then transferred to Hybond N$^+$ membrane and hybridized with (A) ^{32}P-labeled rab11a-1 kb cDNA and (B) the rab11a-2.3 kb 3′-UTR cDNA fragment The filters were then washed under high stringency conditions, i.e. 0.1% SSPE/1% SDS at 65°C for 1 h. Size markers are shown on the left.

most likely result from the use of the two different polyadenylation signals at positions 927 and 2302, respectively. As seen in Fig. 2, an additional weak band is also visible on the blot reacted with both probes suggesting the existence of a larger size rab11a transcript that may be differentially spliced from the same gene. However, the possibility that the largest mRNA piece may be a product of a highly related gene cannot be ruled out.

Presently, we have no information as to the functional implications of having two messengers encoding rab11a, although the presence of putative ARE elements in the 3′-UTR of the larger transcript as well as the variation of their relative abundance in different tissues point towards mechanism(s) by which cellular localisation and/or decay of the two rab11a mRNAs may be differentially regulated. The importance of the 3′ untranslated region in cytoplasmic regulation of mRNA function has been well documented ([31], and references therein), in particular, 3′-UTRs have been shown to signal many events in mRNA metabolism, i.e. intracellular localisation [32], control of polyadenylation [33], and regulation of degradation [29,30]. Other functions associated with the 3′-UTR have been reported that include growth control and differentiation of myoblasts [34].

As shown in Fig. 2, both rab11a mRNAs are ubiquitously expressed in human tissues, but their relative abundance varied markedly. The highest level of both transcripts was observed in heart and the lowest in liver. The level of the larger 2.3-kb mRNA is higher in brain and lung as compared to placenta and heart, where the smaller 1-kb transcript is relatively more abundant. The level of both transcripts was more or less the same in skeletal muscle, kidney and pancreas. Whether the differences in levels are due to preferential poly(A) site selection, and/or tissue specific effects on the

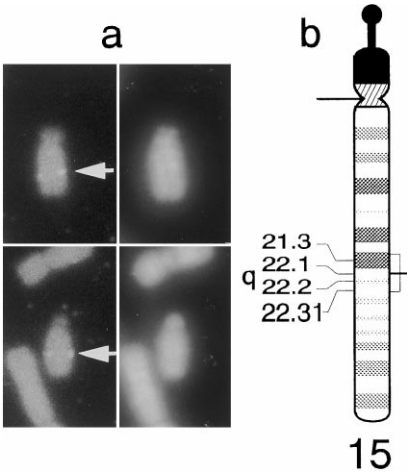


Fig. 4. FISH mapping of RAB11A. a: Two chromosomes 15 showing specific FISH signals (arrows) on both chromatids following hybridization with rab11a-2.3 cDNA, and with the simultaneously DAPI-stained chromosomes. b: A chromosome ideogram [38] with the localization of RAB11A based on the FLpter value and DAPI-band pattern. The horizontal box indicates the variation in FLpter values on individual chromosomes.

stability, translatability, and cellular localisation of each mRNA species remains to be elucidated.

3.3. The two mRNA species encoding rab11a originate from a single gene that maps to 15q21.3-q22.31

From the data presented above it may be concluded that the two rab11a transcripts arise from the differential use of polyadenylation sites. However, the data does not rule out the possibility of having several copies of rab11a gene in the human genome. For example, two copies of the rab11b gene, the close homologue of rab11a (89% of identity) have been recently mapped to mouse chromosomes 1 and 17 [14]. To address this possibility we analysed the rab11a genomic locus by Southern hybridization of large DNA fragments resolved by pulse-field gel electrophoresis. Intact genomic DNA was isolated from human leukocytes and digested using the rare cutting restriction enzymes *Sfi*I and *Not*I. The resulting large DNA fragments were resolved by pulse-field electrophoresis and transblotted to Nylon N-plus membranes as described in Section 2. Southern blot analysis of the DNA fragments was carried out using the same probes that were used for Northern hybridisation (see above). The DNA blot was first hybridized with rab11a-1 cDNA (probe 1) and exposed to an X-ray film. Following autoradiography, the filter was washed, checked by autoradiography and rehybridized with the 3′-UTR portion of the large rab11a cDNA 2.3 kb (probe 2). As shown in Fig. 3, identical macro-restriction fragment patterns were obtained with the two probes, as both markers hybridized to common 500-kb *Not*I and 50-kb *Sfi*I fragments. From these data, we concluded that the genomic regions encoding for the two different transcripts of rab11a are physically located within a DNA fragment of less than 50 kb, and therefore most likely originate from the single gene.

Chromosome mapping of the rab11a gene was carried out both by radiation hybrid mapping and FISH as described in Section 2. Both primer sets mapped within −0.00 cR (lod > 3.0) to a published STS, i.e. NIB1778 [35], which is located within chromosomal region 15q22-15q24. Likewise, by FISH using rab11a-2.3 cDNA as a probe, specific signals were observed on the long arm of chromosome 15, with 32 of 41 analysed metaphases displaying at least one specific signal, and with 52 of 164 chromatids being labeled, with 15, 14, 3 and 0 metaphases showing specific signals on 1, 2, 3, and 4 chromatids, respectively. The FLpter value based on the measurement of 14 signal bearing chromosomes was 0.55 ± 0.05, corresponding to a localization at 15q21.3-22.31 (Fig. 4). Other specific labeled regions were not observed. The same localization was obtained with the rab11a-1 probe, although with a lower frequency of signals (data not shown). Thus, the radiation hybrid mapping and FISH mapping confirmed the presence of a single RAB11A-locus on chromosome 15, where the FISH-mapping places the gene in the proximal part of the region (15q21.3-q22.31) delineated by radiation hybrid mapping.

3.4. Overexpression of rab11a in COS-1 cells affects the levels of a few host GTP-binding proteins

Transient transfection of COS-1 cells with the pMT21-rab11a-1 construct yielded two distinct polypeptides as revealed by 2D PAGE (Fig. 5), which we have recently shown to be related via posttranslational geranylgeranylation [36]. The same results have been observed in the case of COS-1 cells transfected with the larger rab11a-2.3 cDNA (data not shown), implying that both rab11a mRNAs are used as a translation template in vivo.

Interestingly, careful analysis of the IEF (isoelectric focusing) 2D gels shown in Fig. 5A,B, revealed that the overexpression of rab11a-1 in COS-1 cells leads to subtle changes in the proteome expression profile of the host, in particular of four polypeptides that are indicated with thin arrows (upregulated polypeptides a, b, c and d). Surprisingly, these polypeptides which are unknown in the Aarhus keratinocyte database (http://biobase.dk/cgi-bin/celis), corresponded to small GTP-binding proteins as determined by the [α-^{32}P]GTP blot overlay assay (Fig. 5C,D). Proteins A and B (indicated with open arrowheads in Fig. 5C,D) correspond to unknown GTP-binding proteins found in most cell types and therefore may serve as a reference for comparing the gels [37]. Presently, we have some evidence indicating that the new polypeptide spots (a–d)

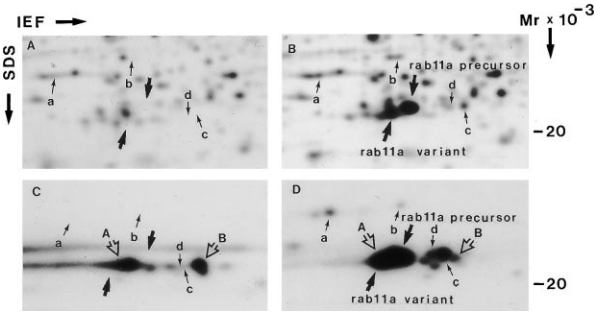


Fig. 5. Transient expression of rab11a in COS-1 cells. 2D PAGE (IEF) gels of [^{35}S]methionine labeled polypeptides from COS-1 cells transfected with (A) pMT21 alone and (B) the pMT21-rab11a-1 construct. C and D: 2D gel [α-^{32}P]GTP blot autoradiographs of proteins from COS-1 cells transfected with (C) pMT21 alone and (D) the pMT21-rab11a-1 construct. Proteins A and B correspond to unknown GTP-binding proteins derived from the COS-1 cells that may serve as reference for comparison. Large arrows indicate the overexpressed Rab11a variants. Small arrows indicate the positions of the additional protein spots (a–d) upregulated in the rab11a transfected COS-1 cells. Only a section of the 2D gels is shown.

are not derived by readthrough, or some unknown transcriptional feature of the pMT21 vector. Sequence analysis of the rab11a-1 cDNA revealed the presence of several termination codons located within the open reading frame, three of them being located less than 80 bp from the first stop codon at position 741. It seems therefore unlikely that proteins a and b, which have an apparent molecular weight of about 30–32 kDa, could be derived via a readthrough effect. Also, the appearance of these proteins cannot be explained by an unknown transcriptional feature of the pMT21 vector, as two other GTP-binding proteins, $p21^{H-ras}$ and the ADP-ribosylation factor, when overexpressed in COS-1 cells do not yield similar changes (data not shown). It is possible, however, that spots c and d (Fig. 5), which exhibit nearly the same M_r but different p*I* as the rab11a primary translation product, may be derived by a yet unknown posttranslational modification(s) of rab11a, but proteins a and b have apparent molecular weights that are far too high to correspond to posttranslation modifications of this protein. Thus, the possibility must be considered that the upregulation (de novo synthesis or posttranslational modification of preexisting proteins) of these polypeptides is caused by the overexpression rab11a, opening the exciting possibility that this protein may modulate the expression levels of some GTP-binding proteins.

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References

[1] Valencia, A., Chardin, P., Wittinghofer, A. and Sander, C. (1991) Biochemistry 30, 4637–4648.
[2] Bokoch, G.M. and Der, C.J. (1993) FASEB J. 7, 750–759.
[3] Bos, J.L. (1997) Biochim. Biophys. Acta 1333, 10608–10615.
[4] Yamagata, K., Sanders, L.K., Kaufmann, W.E., Yee, W., Barnes, C.A., Nathans, D. and Worley, P.F. (1994) J. Biol. Chem. 269, 16333–16339.
[5] Gromov, P.S., Madsen, P., Tommerup, N. and Celis, J.E. (1995) FEBS Lett. 377, 221–226.
[6] Zerial, M. and Lukas, H. (Eds.) (1995) Guidebook to the Small GTPases. Sambrook and Tooze Publications, Oxford University Press.
[7] Zerial, M. and Stenmark, H. (1993) Cell Biol. 5, 613–620.
[8] Simons, K. and Zerial, M. (1993) Neuron 11, 1–20.
[9] Lütke, A., Parton, R.G., Murphy, C., Olkkonen, V.M., Dupree, P., Valencia, A., Simons, K. and Zerial, M. (1994) J. Cell Sci. 107, 3437–3448.
[10] Drivas, G.T., Shih, A., Coutavas, E.E., D'Estachio, P. and Rush, M.G. (1991) Oncogene 6, 3–9.
[11] Chavrier, P., Vingron, M., Sander, C., Simons, K. and Zerial, M. (1990) Mol. Cell. Biol. 10, 6578–6585.
[12] Sakurada, K., Uchida, K., Yamaguchi, K., Aisaka, K., Ito, S., Ohmori, T., Takeyama, Y., Ueda, T., Hori, Y., Ohyanagi, H., Saitoh, Y., Kaibuchi, K. and Takai, Y. (1991) Biochem. Biophys. Res. Commun. 177, 1224–1232.
[13] Goldenring, J.R., Soroka, C.J., Shen, R.K., Tang, L.H., Rodriguez, W., Vaughan, H.D. and Modlin, I.M. (1993) EMBL/GeneBank/DDJJ databases, accession number L19260.
[14] Lai, F., Stubbs, L. and Artzt, K. (1994) Genomics 22, 610–616.
[15] Ueno, H. (1994) EMBL/GeneBank/DDJJ databases, accession number D38516.
[16] Urbé, S., Huber, L.A., Zerial, M., Tooze, S.A. and Parton, R.G. (1993) FEBS Lett. 334, 175–182.
[17] Goldenring, J.R., Soroka, C.J., Shen, K.R., Tang, L.H., Rodriguez, W., Vaughan, H.D., Stoch, S.A. and Modlin, I.M. (1994) Am. J. Physiol. 267, 187–194.
[18] Goldenring, J.R., Shen, K.R., Vaughan, H.D. and Modlin, I.M. (1993) J. Biol. Chem. 268, 18419–18422.
[19] Zahraoui, A., Touchot, N., Chardin, P. and Tavitian, A. (1989) J. Biol. Chem. 264, 12394–12401.
[20] Gluzman, Y. (1981) Cell 23, 175–182.
[21] Gromov, P.S. and Celis, J.E. (1997) in: Cell Biology: A Laboratory Handbook, Vol. 4 (Celis, J.E., Carter, N., Hunter, T., Shotton, D., Simons, K. and Small, J.V., Eds.) pp. 454–457, Academic Press, San Diego, CA.
[22] Tommerup, N. and Vissing, H. (1995) Genomics 27, 259–264.
[23] Gyapay, G., Schmitt, K., Fizames, C., Jones, H., Vega-Czarny, N., Spillett, D., Musselet, D., Prud'Homme, J.-F., Dib, C., Auffray, C., Morissette, J., Weissenbach, J. and Goodfellow, P.N. (1996) Hum. Mol. Genet. 5, 339–346.
[24] Rychlik, W. and Rhoads, R.E. (1989) Nucleic Acids Res. 17, 8543–8551.
[25] Hoppe, B.L., Conti-Tronconi, B.M. and Horton, R.M. (1992) BioTechniques 12, 679–680.
[26] Kaufman, R.J., Davies, M.V., Wasley, L.C. and Michnick, D. (1989) Nucleic Acids Res. 19, 4485–4490.
[27] Celis, J.E., Ratz, G., Basse, B., Lauridsen, J.B., Celis, A., Jensen, N.A. and Gromov, P. (1997) in: Cell Biology: A Laboratory Handbook, Vol. 4 (Celis, J.E., Carter, N., Hunter, T., Shotton, D., Simons, K. and Small, J.V., Eds.) pp. 375–385, Academic Press, San Diego, CA.
[28] Celis, J.E., Lauridsen, J.B. and Basse, B. (1997) in: Cell Biology: A Laboratory Handbook, Vol. 2 (Celis, J.E., Carter, N., Hunter, T., Shotton, D., Simons, K. and Small, J.V., Eds.) pp. 429–437, Academic Press, San Diego, CA.
[29] Sachs, A.B. (1993) Cell 74, 413–421.
[30] Chen, C.-Y.A. and Shyu, A.-B. (1995) Trends Biochem. Sci. 20, 465–470.
[31] Jackson, R.J. (1993) Cell 74, 9–14.
[32] Johnston, D.S. (1995) Cell 81, 161–170.
[33] Wahle, E. and Keller, W. (1996) Trends Biochem. Sci. 21, 247–250.
[34] Rastinejad, F. and Blau, H.M. (1993) Cell 72, 903–917.
[35] Berry, R., Stevens, T.J., Walter, N.A.R., Wilcox, A.S., Rubano, T., Hopkins, J.A., Weber, J., Goold, R., Soares, M.B. and Sikela, J.M. (1995) Nat. Genet. 10, 415–423.
[36] Gromov, P.S. and Celis, J.E. (1998) Electrophoresis, in press.
[37] Huber, L.A., Ullrich, O., Takai, Y., Lutke, A., Dupree, P., Olkonenen, V., Virta, H., de Hoop, M.J., Alexandrov, K., Peter, M., Zerial, M. and Simons, K. (1994) Proc. Natl. Acad. Sci. USA 91, 7874–7878.
[38] Francke, U. (1994) Cytogenet. Cell Genet. 65, 206–219.