DelGEF, an RCC1-related protein encoded by a gene on chromosome 11p14 critical for two forms of hereditary deafness[1]

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Abstract **We have cloned a human cDNA, *DELGEF* (deafness locus associated putative guanine nucleotide exchange factor), derived from a 225 kb genomic sequence of chromosome 11p14, critical for the Usher 1C syndrome and for *DFNB18*, a locus for non-syndromic sensorineural deafness. The amino acid sequence of the protein hDelGEF1 is homologous to the nucleotide exchange factor RCC1 for the small GTPase Ran. hDelGEF2 is derived from the same *DELGEF* gene by alternative splicing. In addition, we have identified a murine homologue, mDelGEF. The ubiquitously expressed soluble protein hDelGEF1 is found both in the cytoplasm and in the nucleus. Overexpressed hDelGEF2 colocalizes with mitochondria.**

Key words: RCC1-like protein; Guanine exchange factor (GEF); Rpgr; Herc2p; Alternative splicing; Mitochondrion

1. Introduction

RCC1, the 'regulator of chromosome condensation' [1], is the first member of a family of structurally related proteins so far identified [2]. It is the guanine nucleotide exchange factor for the small Ras-related GTPase Ran [3], which in the GTP-bound form is required for the dissociation of protein complexes imported into the nucleus and for the formation of similar complexes to be exported into the cytoplasm [4,5]. The three-dimensional structure of RCC1 resembles that of a propeller with seven blades, formed from seven internal repeats in the sequence. They are characterized by invariant glycines in positions where no side chains are sterically permitted [6].

Several genes encoding RCC1-related proteins have been characterized as candidates for genetic disorders. Mutations in the *RPGR* gene are discussed to be involved in the development of the most common form of X-linked retinitis pigmentosa. The predicted 90 kDa protein Rpgr is ubiquitously expressed and displays an RCC1-related repeat structure in its N-terminal region [7,8]. Various mutations in this repeat structure have been found in different patients [9,10]. An unusually large human protein of 532 kDa, p532, which weakly stimulates guanine nucleotide exchange on the small GTPases ARF1, Rab3A and Rab5, contains two regions of seven internal repeats each, distinctly related to RCC1 [11]. *HERC2* is a gene in the region involved in the Prader-Willi/Angelman syndrome. It encodes a large protein thought to be implicated in protein trafficking, which comprises three RCC1-related domains. It is deficient in mice with neuromuscular and spermiogenic abnormalities [12]. The *CHC1L* gene in chromosome region 13q14.3 encodes a ubiquitously expressed mRNA for a protein of 551 amino acid residues, termed Rlg. Its amino-terminal half also shows strong homology to the seven tandem repeat structure of RCC1 [13].

Here we characterize a gene encoding a novel human homologue of RCC1, DelGEF, and show that it is located close to the *DFNB18* and *USHER1C* regions. Usher syndrome is an autosomal recessive, genetically heterogeneous disorder causing severe congenital deafness and retinitis pigmentosa. In Usher 1 patients, vestibular dysfunction is also observed [14]. To date, five candidate loci for this syndrome (*USH1A–E*) have been analyzed, of which *USH1C* maps to 11p13–15 [15]. In addition, a locus for recessive non-syndromic sensorineural deafness *(DFNB18)* has been mapped to this region [16].

2. Materials and methods

2.1. Isolation of a human cDNA clone from a fetal brain library, sequencing, and Northern blot analysis

Databases were searched with the RCC1 and RPGR sequences to identify homologous genes, using tblastx and blastn from the HUSAR program package of the German Cancer Research Center. Sequencing reactions were carried out with the Applied Biosystems automated sequencer 373 A and dye terminator chemistry (Perkin Elmer). Using the clone as template for PCR reaction with the forward primer (5′-GGCTGTCCCATCCAACAG) and the reverse primer (5′-GCTTCAGTGCCATCTCCG), a PCR fragment of 645 bp was obtained. 30 ng of this fragment was labeled using the NonaPrimer Kit II (Appligene/Oncor, Heidelberg, Germany). High density filters with cDNA from a human fetal brain library (DKFZp564, German Cancer Research Center, Heidelberg, Germany) and from a human testis library (DKFZp565, German Cancer Research Center, Heidelberg, Germany) were pre-hybridized in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1 mM EDTA and 0.1 mg/ml salmon sperm DNA [17] at 65°C for 1 h. Hybridization was performed for 14 h at 65°C in the same buffer containing the labeled probe at a concentration of 1.5×10^6 cpm/ml. The filters were washed twice at 65°C in a buffer containing 0.2×SSC and 0.2% SDS and exposed for 20 h to a Kodak X-OMAT AR X-ray film. Human multiple tissue Northern blots (Clontech) were hybridized under the same conditions as described above. To confirm comparable loading of mRNA in each lane the blots were hybridized with labeled human β-actin cDNA.

2.2. Chromosomal mapping of the terminal exon of DELGEF

Chromosomal DNA from mapping panel #2 (Coriell Institute for Medical Research, Camden, NJ, USA) was used as template for PCR reactions under the following conditions: 40 ng of genomic DNA, 1×PCR buffer, 200 μM dNTPs, 1.6 U TaKaRa Ex Taq (Boehringer Ingelheim, Germany) in a total volume of 50 μl. The forward primer was 5′-CATGGCCCAGGAACCCTTTGTTTTGCT-3′, the reverse

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[1] The nucleotide and amino acid sequence data reported in this paper have been deposited in the EMBL Nucleotide Sequence Database under the accession numbers AJ243950 (hDELGEF1), AJ243951 (hDELGEF2), and AJ243952 (mDELGEF).

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primer 5′-GTGACAGTAGGGAAGGGCCGAGGAGGAA-3′. After an initial denaturation (94°C for 1.5 min), 35 cycles of amplification (94°C for 30 s, 2.5 min 72°C) were carried out, followed by a final extension for 7 min at 72°C (PTC-200 thermocycler, MJ Research).

2.3. Antibodies

The antiserum to the C-terminal sequence of DelGEF1 was raised against the synthetic peptide CTSTQSQSDWSRNGGL (Fig. 2) and the antiserum to the N-terminal region was raised against the N-terminal peptide MEREPSASEAAPAAAC present in both proteins. The cysteine residues were introduced for coupling each of these peptides to keyhole limpet hemocyanin (Imject Maleimide Activated Carrier Protein #77105, Pierce, Rockford, IL, USA). Immunization of rabbits was performed according to standard procedures (Eurogentec, Seraing, Belgium). For antibody purification, the synthetic peptides were coupled to UltraLink Iodoacetyl (#53155, Pierce, Rockford, IL, USA). For immunoblotting 30 μg of cell lysate or 15 ng of recombinant hDelGEF protein was run on SDS gel electrophoresis and transferred to nitrocellulose membranes. The hDelGEF protein was detected using affinity-purified rabbit antibody to the C-terminus of hDelGEF1.

2.4. Preparation of subcellular protein fractions

Packed HeLa cells (25 ml) were thawed in 100 ml of lysis buffer (20 mM Tris pH 7.5, 1 mM $MgCl_2$ and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany)), and swollen on ice for 30 min. They were lysed by 10 strokes with an S-type Dounce homogenizer and centrifuged at $100\,000 \times g$ for 90 min. Supernatant and pellet were separated for further analysis.

2.5. Expression cloning, protein production and purification

For bacterial expression, h*DELGEF1* cDNA (DKFZp564O2248) was used for PCR amplification with forward primer 5′-TTTCATATGGAGCGCGAGCCCAGCGCCT-3′ and 5′-TTTGTCGACTCACAGTCCCCCATTTCTGGAC-3′ as the reverse primer. The PCR product was ligated between the *Nde*I and *Xho*I sites in a pET-14b vector (Novagen). For the expression of the recombinant protein in *Escherichia coli* BL21(DE3), a 1.2 l culture was grown at 16°C. Expression of the recombinant protein was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3.5 h. Cells were lysed in 20 mM HEPES (pH 7.6), 2 mM DTT, 1 mg lysozyme/ml and EDTA-free protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The lysate was sonicated five times, adjusted with 1 M NaCl to a final concentration of 100 mM NaCl and centrifuged for 1 h at $100\,000 \times g$. The supernatant was incubated for 2 h with 0.5 ml of a Ni-NTA agarose suspension. It was washed four times with 20 mM HEPES (pH 7.6), 100 mM NaCl and 20 mM imidazole. Elution was performed with 20 mM HEPES (pH 7.6), 100 mM NaCl and 1 M imidazole.

For expression in mammalian cells, *DELGEF1* and *DELGEF2* (Fig. 4) were cloned into a modified pCDNA3 vector, which contained an in-frame flag coding sequence downstream of the *Eco*RV site in the multiple cloning region. For *DELGEF1* and *DELGEF2* cloning, the forward primer 5′-GGAAGACTAAGCTATGGAGCGCGAGCCCAGCGCCT-3′ was used, while the reverse primer 5′-CAGTCCCCCATTTCTGGACCA-3′ was generated for *DELGEF1* and primer 5′-TGCTCTGAGCCACAAGAGACCTGTCTGA-3′ for *DELGEF2*. Both PCR products were digested with the restriction enzyme *Bpi*I and ligated to the modified pCDNA3 vector between the *Hin*dIII and *Eco*RV restriction sites.

2.6. Immunofluorescence studies

COS-7 cells on coverslips were transfected with plasmid DNA of *DELGEF1* and *DELGEF2* by SuperFect transfection reagent (Qiagen, Hilden, Germany). Sixty hours after transfection cells were fixed in 3% formaldehyde in phosphate buffered saline (PBS) for 10 min and washed twice with PBS. Cells were permeabilized for 5 min in 1% Triton X-100 in PBS and washed twice with PBS. The antibodies to the N- and C-terminal regions of hDelGEF1 were diluted 1:100 in 1% bovine serum albumin in PBS. Cells were incubated at 25°C for 1 h with the antibodies and washed twice with PBS. FITC-coupled goat anti-rabbit antibody (Dianova, Hamburg, Germany) diluted 1:200 was used as secondary antibody. For mitochondrial staining, 'Mitotracker Red CMXRos' (Molecular Probes, Leiden, The Netherlands) was used according to the manufacturer.

3. Results

3.1. Northern blot analysis and elucidation of cDNA and amino acid sequences of human and murine DelGEF

A human expressed sequence tag (EST) Image I.M.A.G.E. Consortium clone ID 291525 (GB N67793) [18] distinctly homologous to human RCC1 and RPGR was identified by database searching. This clone was obtained from Research Genetics and sequenced. The sequence of 1243 bp did not fully match the mRNA size of 1400 bp as determined by Northern blot analysis (Fig. 1), which resulted in one major band of some 1400 bp in all human adult tissues tested. Only in testis a smaller variant of 1 kb was evident. The expression level was high in brain, skeletal muscle, prostate and ovary, while it was low in placenta, lung, and liver. In addition, *DELGEF* was expressed in retina, since a partial clone, GB W25773, was derived from a human retina library.

To obtain the full-length cDNA, high density filters of a human fetal brain library and a testis library were screened. On the fetal brain library filter one positive clone (DKFZp564O2248) was identified. The sequence of this clone showed a potential start ATG, but no in-frame stop codon in the 5′ untranslated region (Fig. 2A). The cDNA clone has a total length of 1431 bp and encodes a protein of 458 amino acid residues (48 987 Da). The 3′ untranslated region contains a polyadenylation signal (AATAAA), 15 nucleotides upstream of the poly(A) tail.

Further database searches revealed a murine homologue, I.M.A.G.E. Consortium clone ID 698116 (GB AA239362), which was obtained from Research Genetics and sequenced. This clone has a total length of 1430 bp and represents the full-length cDNA (Fig. 2B). The ORF starts at position 19 and ends with the termination signal TAA at nucleotide 1411, encoding a protein of 464 amino acid residues (49 194 Da). The deduced human and murine amino acid sequences have 80% identity and are distinctly homologous (some 25%, Fig.

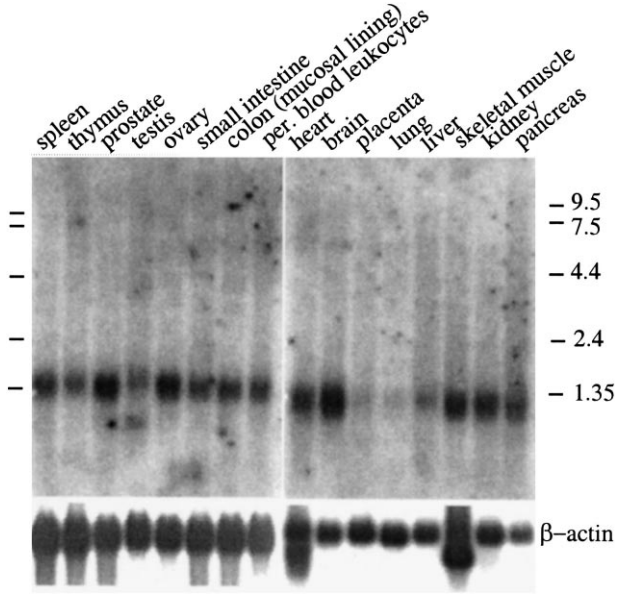

Fig. 1. Ubiquitous expression of h*DELGEF* mRNA. Top: Northern blot analysis of the expression. The bars on both sides indicate RNA size markers. Bottom: Reprobing of the same blot with β-actin to ensure loading of comparable amounts of mRNA.

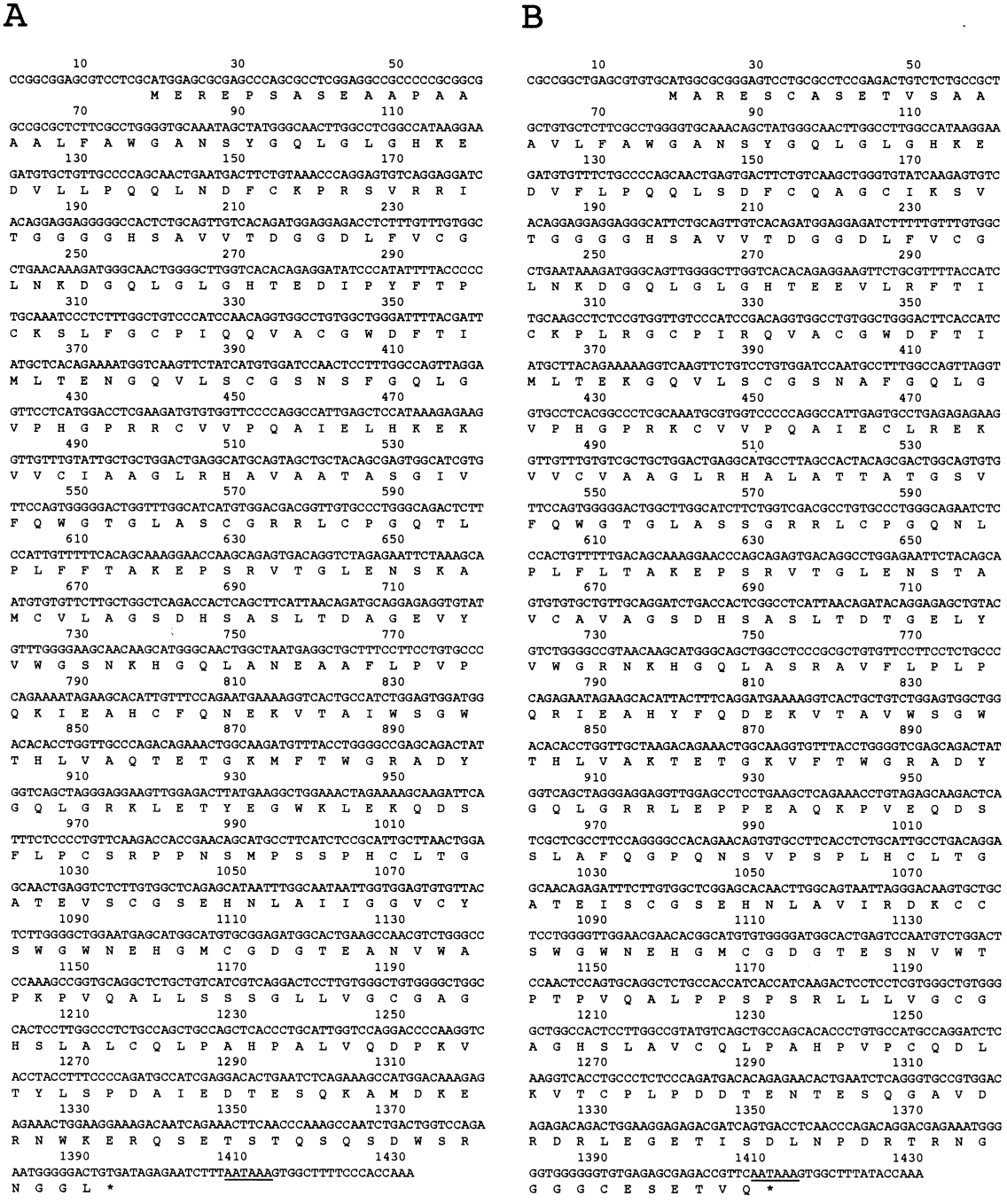

Fig. 2. cDNA and predicted amino acid sequences of (A) human DelGEF1 (accession number AJ243950) and (B) the murine homologue mDelGEF (accession number AJ243952). The polyadenylation signal is underlined.

3) to RCC1, and to regions in Rpgr, HERC2, p532, and Rlg. All these proteins share the repeat structure found in RCC1. The individual putative propeller blades in DelGEF vary from 49 to 67 residues, blade six having an insert of 16 residues (303–318) compared to the other proteins of this family. Secondary structure predictions indicate a flexible conformation for this sequence.

3.2. *Structure of the human DELGEF gene and localization on chromosome 11p14*

A database search revealed the h*DELGEF* gene to be part of the sequenced PAC clones 6-130a9 (GB AC005728), pDJ59m18 (GB AC004582) and 1082L12 (GB AC004736) [19], which have been localized to chromosome 11p14 [20]. A comparison of the sequences of these clones with the h*DELGEF* cDNA showed that the *DELGEF* gene comprises 11 exons. Exons 1–9 are present in PAC clone 6-130a9, exons 10 and 11 are found in clone pDJ59m18 (GB AC004582) (Fig. 4). The Whitehead chromosome marker D11S1310 corresponds to a sequence in intron 9, and D11S1888 is only 32 kb downstream of the 3′-terminal exon 11, which is also part of a third PAC clone 1082L12 (GB AC004736). 5′ and 3′

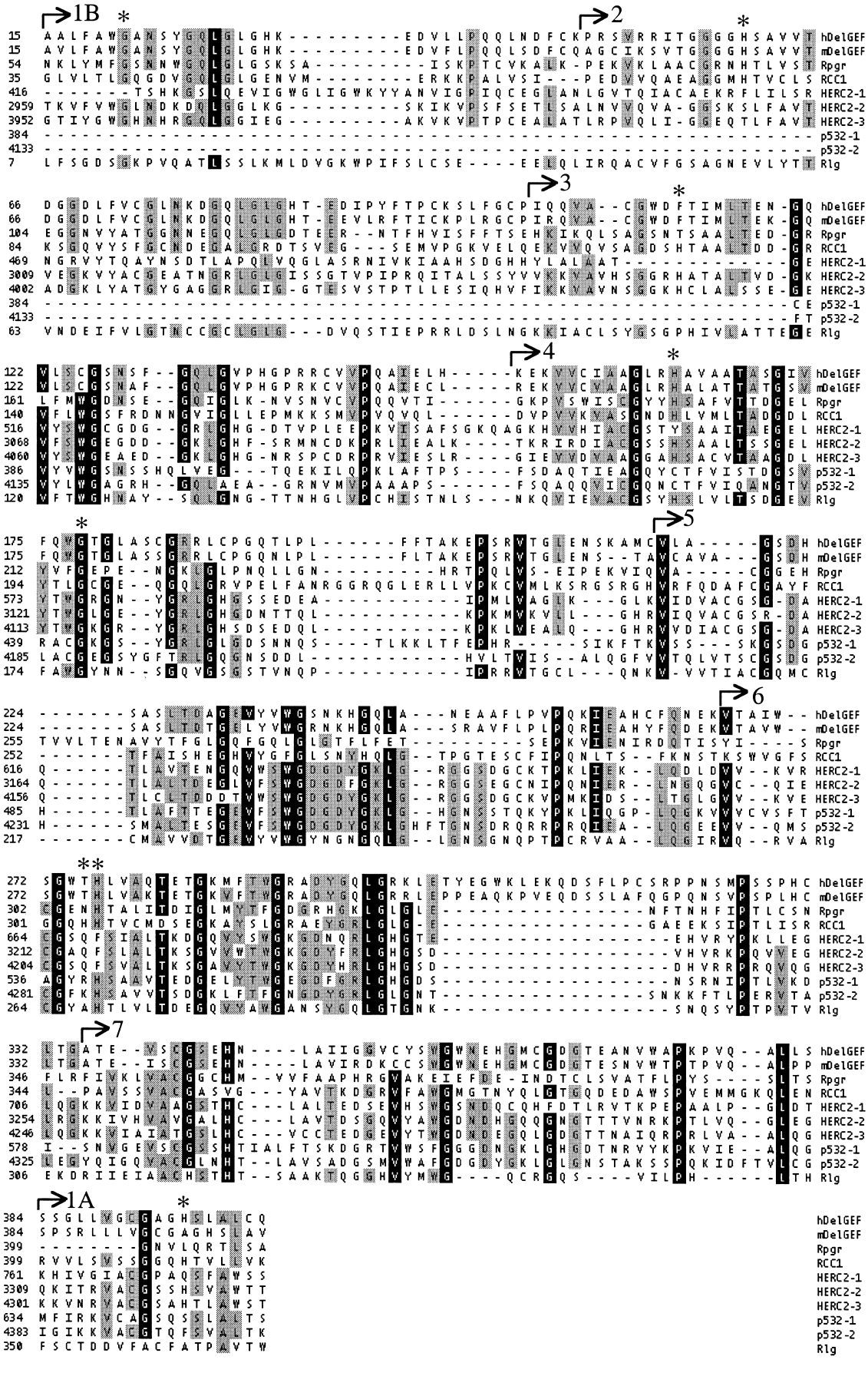
1B
2
3
4
5
6
7
1A
hDelGEF
mDelGEF
Rpgr
RCC1
HERC2-1
HERC2-2
HERC2-3
p532-1
p532-2
Rlg

Fig. 3. Sequence identity between human and murine DelGEF and members of the RCC1-related protein family. The aligned sequences are hDelGEF1 (this work), mDelGEF (this work), Rpgr [7,8], RCC1 [2]. HERC2 [12], p532 [11], and Rlg [13]. There are three RCC1 homology regions in HERC2 and two in p532. Gaps are inserted to achieve maximum similarity. Positions in which 80% of the sequences have an identical residue are highlighted in black, positions with at least 50% identical residues are shaded in gray. The conserved glycines are characteristic of the structural repeats in this protein family. The three-dimensional structure of RCC1 resembles a propeller with seven blades [6]. Each of these blades is indicated by an arrow. The C-terminal region 1A and the N-terminal region 1B jointly form one blade. Positions marked by an asterisk are referred to in the discussion section.
←

splice sites of the exon-intron boundaries match the consensus sequence patterns (Table 1). The exon sizes vary from 37 bp (exon 10) to 366 bp (exon 11), and the intron sizes from 1.2 kb (intron 2) to 89.8 kb (intron 10). To confirm the published mapping result we constructed a forward and reverse primer surrounding the terminal exon of the h*DELGEF* gene and used chromosomal DNA from a hybrid panel as template for PCR. It resulted in the expected PCR fragment of 998 bp (according to GB ACC004582) only for chromosome 11 and for total human DNA, not for murine and hamster DNA (not shown).

3.3. Alternatively spliced transcripts

Comparison of the sequences in Fig. 2A (*DELGEF1*) and the Image I.M.A.G.E. Consortium clone ID 291525 (GB N67793, *DELGEF2*) indicated alternative splicing. *DELGEF2* lacks exon 9 (863–1029) (Fig. 4). This alters the ORF in exon 10 to a total of only 288 amino acid residues and 30 546 Da for the encoded protein. The two proteins are identical up to residue 281 (Thr), which in DelGEF2 is followed by the C-terminal heptapeptide sequence GLLWLRA. This results in the deletion of two out of the seven repeats. Both variants are represented by EST clones in the database: I.M.A.G.E. Consortium clones ID 173819 (GB H23780) corresponds to *DELGEF1*, whereas clones 665437 (GB AA195166) and 174457 (GB H21559) represent *DELGEF2*. The alternatively spliced transcripts are not detectable as separate bands on Northern blots, because they do not differ significantly in size (Fig. 1).

3.4. Expression of human DELGEF and subcellular localization of the protein

We raised antisera in rabbits to synthetic peptides derived from the N- and C-terminal sequences of hDelGEF1. On immunoblots of cell lysates, the affinity-purified antibodies recognized DelGEF1 as a soluble protein of 50 kDa (HeLa supernatant, Fig. 5). Recombinant DelGEF1 migrates slightly more slowly because of its His tag. The smaller DelGEF2 is not detected by the antiserum to the C-terminal region of DelGEF1, since it lacks the corresponding epitope. The antiserum to the N-terminal region, which is identical in both proteins, did not detect either form in whole cell lysates, but only DelGEF1 enriched by ion exchange chromatography (not shown). An unidentified additional band of approximately 200 kDa was stained in HeLa cell lysates. Preincubation of the antiserum with the corresponding synthetic peptide abolished this staining as well as that of DelGEF1 (not shown). This might indicate cross-reaction with a similar structure. However, on Northern blots with multiple tissues (Fig. 1) no mRNA hybridized with our probe that corresponded in size to such a large protein. We therefore believe

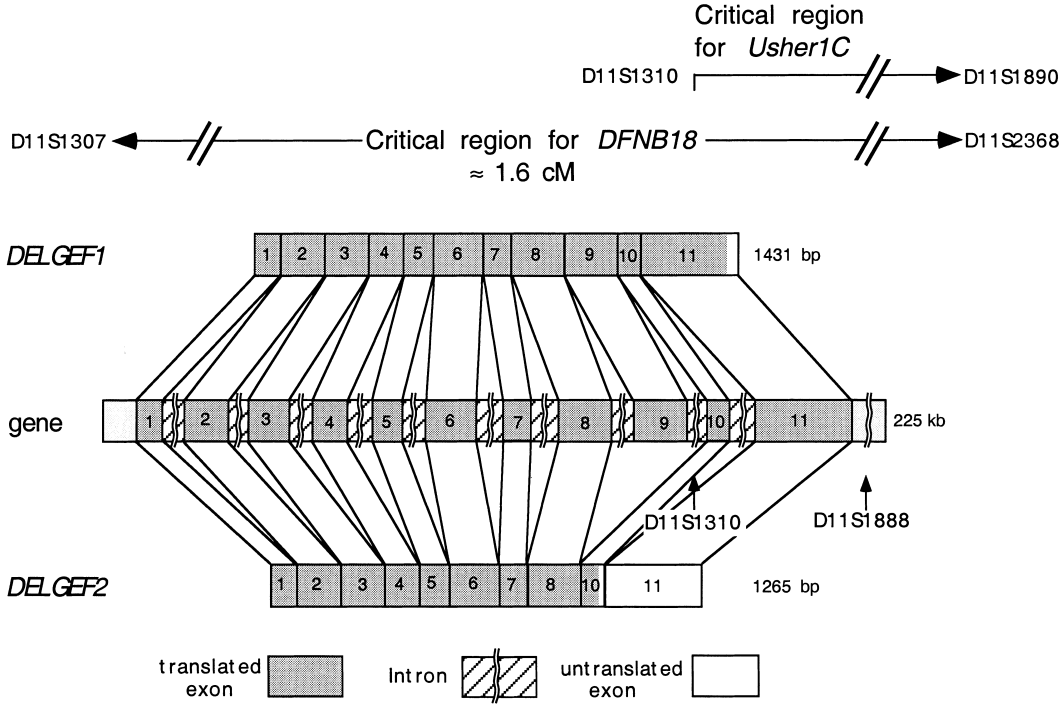

Fig. 4. Structure of the human *DELGEF* gene. The transcript *DELGEF1* is represented by the sequence in Fig. 1 (top) and the I.M.A.G.E. Consortium clone ID 173819 (GB H23780). The structure of the gene is derived from the sequences of the overlapping PAC clones 6-130a9 (GB AC005728), pDJ59m18 (GB AC004582) and 1082L12 (GB AC004736). The transcript *DELGEF2* was found in the I.M.A.G.E. Consortium clones IDs 291525, 665437 and 174457 (partial sequences GB N67793, AA195166 and H21559).

Table 1
Exon-intron junctions of human *DELGEF*

Exon	Size (bp)	cDNA position	Donor sites	Intron	Size (kb)	Acceptor sites
1	78	1–78	CTGG**gt**acgt	1	4.9	ccct**ag**GGTG
2	136	79–214	ACAG**gt**aatt	2	1.2	ttgc**ag**ATGG
3	156	215–370	ACAG**gt**gagt	3	2.1	ttgc**ag**AAAA
4	95	371–465	TGAG**gt**gagg	4	3.9	tgac**ag**CTCC
5	61	466–526	ACAG**gt**gatc	5	4.6	ggga**ag**CGAG
6	114	527–640	ACAG**gt**aaga	6	2.8	ttgt**ag**GTCT
7	63	641–703	ACAG**gt**aggt	7	4.3	ttta**ag**ATGC
8	159	704–862	ACAG**gt**gaga	8	29.0	tttt**ag**AAAC
9	167	863–1029	TGAG**gt**agga	9	80.8	ttac**ag**GTCT
10	37	1030–1066	ATTG**gt**aagt	10	89.8	tcac**ag**GTGG
11	366	1067–1432				

that the 200 kDa protein on protein blots is not closely related to DelGEF.

In immunofluorescence studies, none of the sera stained untransfected cells. However, when COS cells were transfected with vectors carrying a variety of DelGEF constructs, the overexpressed proteins were readily detected. Overexpression of *DELGEF1* results in nuclear plus cytoplasmic staining (Fig. 6B), whereas DelGEF2 appears to be associated with mitochondria (Fig. 6D). The same two patterns were seen when flag-tagged DelGEF was stained with anti-flag antibodies (not shown).

4. Discussion

We have identified a human cDNA, h*DELGEF1*, and its murine homologue, m*DELGEF*, encoding proteins of 458 and 464 amino acid residues. In contrast to its small transcript, the human *DELGEF* gene spans 225 kb in chromosome region 11p14. Defects in this region are considered to be responsible for the USH1C syndrome and for the recessive non-syndromic sensorineural deafness DFNB18. Genetic linkage analysis in the French-Acadian population of Louisiana mapped the *USH1C* gene between markers D11S902 and D11S1310 [21], the latter marker being located in intron 9 of *DELGEF*. Recently, the disease locus was thought to be more distal between the flanking markers D11S902 and D11S1890 [20,22]. The locus for a recessive non-syndromic sensorineural deafness, *DFNB18*, has also been mapped to this region [16], with a maximum lod score of 4.4 for the microsatellite marker D11S1888, 32 kb from the 3′ end of *DELGEF*. Thus, mutations in *DELGEF* may be involved in both types of deafness.

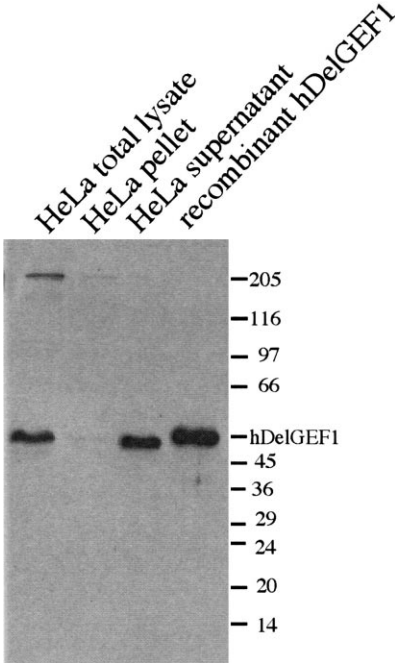

Fig. 5. Immunodetection of hDelGEF1 protein in cell lysates. 15 ng of recombinant His-tagged hDelGEF protein expressed in *E. coli* and 30 μg of HeLa total lysate, HeLa 100 000×*g* pellet and 100 000×*g* supernatant were run on a SDS-PAGE gel and blotted onto nitrocellulose. For immunodetection affinity-purified antibodies to the C-terminus of hDelGEF1 were used.

DelGEF belongs to the RCC1-related family of proteins, which shares a structure of seven sequence repeats of 40–70 residues each, characterized by highly conserved glycines (Fig. 3). RCC1 is the nucleotide exchange factor for the small nuclear GTPase Ran which regulates nucleocytoplasmic transport. Its three-dimensional structure resembles a propeller with seven blades. The conserved glycines mark positions where no side chain is permitted because of sterical restraints. A WG motif in each blade except in blade two is involved in forming the hydrophobic core, and most of the blades display a conserved (*cis*)Pro. Five histidine residues in RCC1 are conserved, in positions 78 (corresponding to His-60 in human and murine DelGEF), 130, 183 (His-163 in DelGEF), 305 and 410. These residues occupy analogous positions in individual blades of the propeller and are believed to stabilize the structure. hDelGEF1 carries the characteristic GXXH sequence in the N-terminal part of all blades except blade three. Mutations in H78 or in H304 of RCC1 resulted in a markedly reduced nucleotide exchange activity [23,24].

Conservation of the blade structure is essential. For example, loss of function in another family member, Rpgr, involved in X-linked retinitis pigmentosa, is believed to result from mutations in conserved residues. In patients, conserved glycines (codons 60 and 215, corresponding to 41 and 197 in RCC1 and to 21 and 178 in DelGEF) in the RCC1-related region were found to be mutated to valine, and a conserved histidine (codon 98, corresponding to H78 in RCC1 and to H60 in DelGEF) was substituted by a glutamine [7,9,10]. In other patients, nonsense mutations resulted in a premature stop codon within the RCC1 homology region [7].

Specific structural elements for individual functions appear to be superimposed on the propeller scaffold, e.g. an extra β-sheet protruding from blade three in RCC1 is thought to be responsible for its Ran-specific guanine nucleotide exchange factor activity [3]. In human and murine DelGEF, the unique

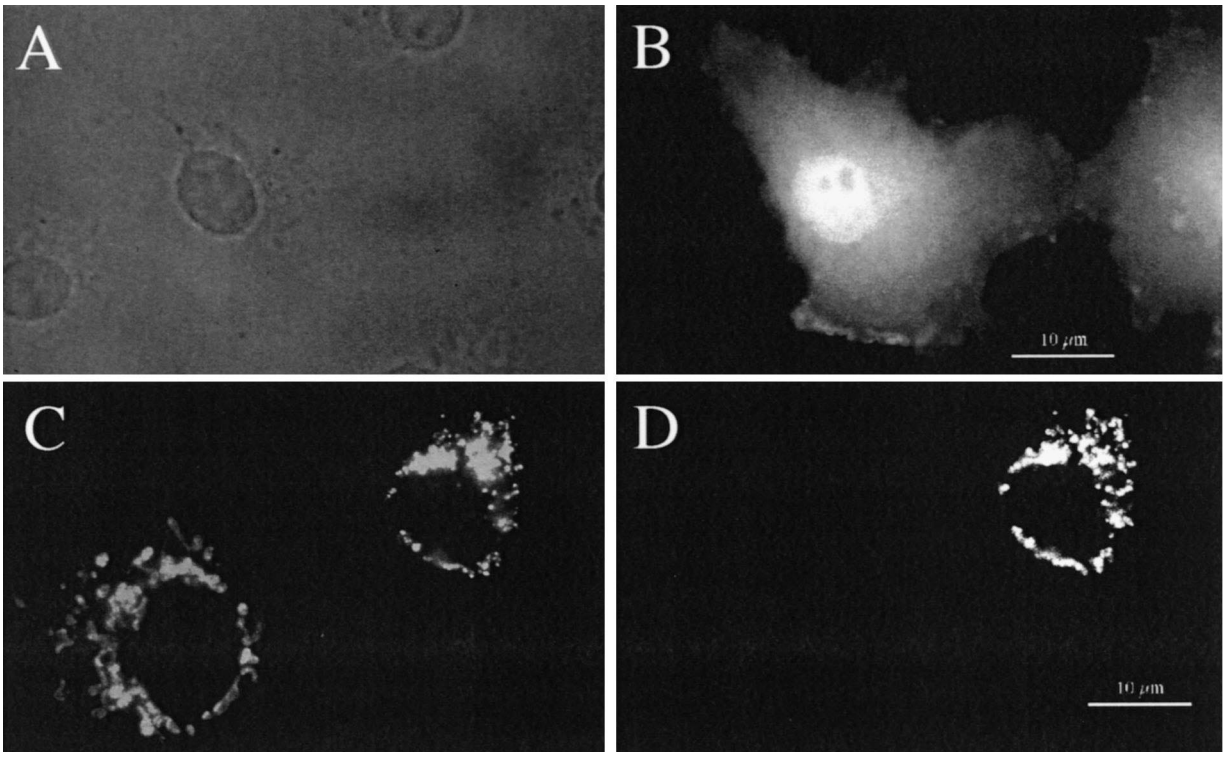

Fig. 6. Immunolocalization of hDelGEF in COS-7 cells. A: Phase contrast of cells transfected with an expression vector encoding DelGEF1 with an additional C-terminal flag epitope. B: Corresponding immunofluorescence using affinity-purified antibody to the C-terminus of hDelGEF1. C: Mitochondrial staining using Mitotracker Red CMXRos of cells transfected with an expression vector encoding DelGEF2 with an additional C-terminal flag epitope. D: Corresponding immunofluorescence using affinity-purified antibody to the N-terminus of hDelGEF.

insert in blade six (residues 303–318) may be functionally important.

The amount of DelGEF protein in cells is low. DelGEF1 can be detected in immunoblots of cell lysates (Fig. 5), but in immunofluorescence studies it is seen only upon overexpression in transfected cells (Fig. 6B). The existence of *DELGEF2* is indicated at the cDNA level, but has not been demonstrated by immunostaining of protein bands on blots of cell lysates. Notably, the last two of the seven putative propeller blades are deleted from this smaller protein. A similar situation was observed with murine Rpgr, where the last two repeats of the RCC1 homology region are deleted in one splice variant [25]. The truncation of DelGEF results in an altered location of overexpressed DelGEF2 at the mitochondria (Fig. 6D).

The closest homologue to DelGEF in *Saccharomyces cerevisiae* is the alpha tubulin suppressor ATS1p [26], which also lacks the C-terminal two blades. This protein appears to influence microtubule stability, since overexpression of the non-essential *ATS1* gene rescues both the growth and microtubule phenotypes of a specific class of α-tubulin mutations, class 2 *tub1*, that cause growth arrest with excess microtubules [27]. Overexpression of the yeast RCC1 homologue *SRM1/PRP20* also suppresses class 2 mutants. Similarly, the readily soluble DelGEF proteins could modulate mammalian microtubules, perhaps by interaction with a microtubule-associated protein. Yet as with *ATS1* in yeast, no visible effect on the number and structure of normal microtubules in COS-7 cells was observed upon overexpression of h*DELGEF1* or *2* (not shown). Mutation analysis of the *DELGEF* gene or of cDNA from well characterized patients suffering from USH 1C or DFNB18 deafness and knockout of the murine *DELGEF* gene may help to clarify its role.

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