Structure and expression of murine *mgcRacGAP*: its developmental regulation suggests a role for the Rac/MgcRacGAP signalling pathway in neurogenesis

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Rho-family GTPases regulate a wide range of biological functions including cell migration, cell adhesion and cell growth. Recently, results from studies *in vivo* in *Drosophila*, mouse and humans have demonstrated the involvement of these GTPases in mechanisms controlling neuronal differentiation and the development of the central nervous system (CNS). However, the signalling pathways underlying these functions and the proteins directly regulating RhoGTPases in developing neurons are poorly defined. Here we report the structure and expression pattern of the murine orthologue of *mgcRacGAP*, a human gene encoding a RacGTPase partner expressed in male germ cells [Touré, Dorseuil, Morin, Timmons, Jegou, Reibel and Gacon (1998) J. Biol. Chem. **273**, 6019–6023]. In contrast with that from humans, murine *mgcRacGAP* encodes two distinct transcripts. Both are developmentally regulated. A 2.2 kb transcript is strongly expressed in mature testis and is up-regulated with spermatogenesis. A 3 kb RNA is predominant in the embryo and is expressed primarily in the CNS during the neurogenic phase, decreasing after birth. *In situ* hybridization analysis in embryonic-day 14.5 mouse embryos demonstrates a preferential expression of *mgcRacGAP* in the proliferative ventricular zone of the cortex. In addition to the expression of *mgcRacGAP* in male germ cells already reported in humans and suggesting an involvement in spermatogenesis, we characterize an embryonic transcript whose expression is closely correlated with neurogenesis. This result addresses the question of the role of Rac/MgcRacGAP pathway in neuronal proliferation.

Key words: embryonic cerebral cortex, neuronal proliferation, RhoGTPases, spermatogenesis.

INTRODUCTION

The Ras-related Rho family GTPases Cdc42, Rac and Rho have been shown to regulate a wide spectrum of cellular functions [1]. From experiments *in vitro* in cultured fibroblasts, RhoGTPases are known to be involved in actin cytoskeleton organization, transcriptional regulation and cell growth control. Recently, evidence has accumulated for a role of Rho-related GTPases in neuronal development and in the regulation of neuritogenesis [2,3]. In both neuronal cell lines and primary neurons, Rho, Rac and Cdc42 have been implicated in the regulation of growth cone behaviour [4–6] and of dendritic growth [7]. The relevance of these functions *in vivo* has also been demonstrated in several animal models. Studies in *Drosophila* have suggested different morphogenetic functions for distinct RhoGTPases: Rac has a preferential effect on axonal outgrowth and Cdc42 has a more general effect in neuronal migration as well as axon and dendrite outgrowth [2]. In *Caenorhabditis elegans*, activated alleles of the *mig-2* gene, which encodes a new Rho family GTPase member, cause misguided axon growth [8]; the UNC-73 gene, a Rac-specific activator, is required for cell and growth cone migration [9]. In mouse, the expression of a constitutively active form of Rac1 in Purkinje cells leads to a reduction of axons and abnormal dendritic spines [10].

Besides this role in neuritogenesis and neuronal morphology, recent work suggests the involvement of the Rho family GTPases in neuronal proliferation. The Drosophila *mbt* gene, which encodes a protein related to the PAK family of Rho-dependent kinase, was shown to control the generation and survival of neuroblasts in central brain structures [11]. Finally, mutations in two genes encoding Rho-interacting proteins [PAK-3 and a Rho GTPase-activating protein (GAP), oligophrenin] have been recently found to be responsible for mental retardation in humans, suggesting a role in brain development [12,13].

Although all these results demonstrate that RhoGTPases are clearly involved in many aspects of nervous system development, the underlying signalling pathways and their regulatory mechanisms remain to be elucidated. Analysis of the expression of RhoGTPase partners during specific phases of central nervous system (CNS) development and/or in specific regions of the developing brain might help to define RhoGTPase-dependent pathways controlling neuronal cell proliferation, migration and morphogenesis.

In the present study we characterized the mouse *mgcRacGAP* orthologue and analysed its pattern of expression during development. MgcRacGAP was initially described in humans as a Rac/Cdc42 GAP preferentially expressed in male germ cells and belonging to the chimaerin family [14]. Proteins of this family have the ability to down-regulate Rho/GTPases through their GAP activity, and may have an additional role in signal transduction downstream of RhoGTPase, as has been demonstrated for other members of the chimaerin family [15]. We show here that the murine *mgcRacGAP* gene encodes two transcripts, both of which are developmentally regulated. A 2.2 kb RNA is preferentially expressed in adult testis and is up-regulated with the onset of spermatogenesis. A 3 kb transcript is predominant in the embryonic nervous system and is regulated during mouse CNS development. Our results indicate that mouse *mgcRacGAP* gene expression is concomitant with the timing and sites of neurogenesis.

Abbreviations used: CNS, central nervous system; EST, expressed sequence tag; GAP, GTPase-activating protein; MgcRacGAP, male-germ-cell RacGAP; UTR, untranslated region.

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MATERIALS AND METHODS

Identification of mouse MgcRacGAP

Database searches were performed with the cDNA sequence of human *mgcRacGAP* by using BLASTn algorithms against the mouse dbEST database at the National Center for Biotechnology Information (NCBI). Overlapping segments of expressed sequence tag (EST) sequences were compiled. The longest inserts and the most 5′ cDNA clones were obtained from HMGP Resource Centre (Cambridge, U.K.). All ESTs obtained were sequenced in both strands.

A homology search for functional protein domains was performed by BLAST analysis against the Prodom protein domain database at the Institut National de Recherche Agronomique (Toulouse, France) [16]. Prediction of coil-coiled regions was done with the COIL program [17].

Primer extension

Total RNA was extracted by using the RNAxel kit (Eurobio, Les Ulis, France) and treated with DNase RQ1 (Promega, Madison, WI, U.S.A.). A 1 μg portion of each RNA sample [total RNA embryonic-day 10.5 (E10.5) embryo and mature testis] was annealed with 10 pmol of specific primers (PE1 or PE2, Figure 1) for 90 min at 55 °C and incubated for 1 h at 42 °C in a final volume of 30 μl containing 200 units of Superscript reverse transcriptase (Life Technology, Paisley, Strathclyde, U.K.). Primers were previously end-labelled with T4 polynucleotide kinase and purified on a NAP-5 column (Pharmacia, Uppsala, Sweden). Reverse transcription products were loaded on a 7 M urea/6 % (w/v) polyacrylamide sequencing gel and run next to a primer extension specific ladder.

Western blot analysis

Total proteins from human and mouse testis were extracted in Laemmli sample buffer, resolved by SDS/PAGE [12 % (w/v) gel] and electroblotted to nitrocellulose. Filters were incubated sequentially with purified chicken anti-(human MgcRacGAP) antibodies [14] and peroxidase-labelled rabbit anti-chicken immunoglobulins (Dako Ltd., High Wycombe, Bucks., U.K.). Proteins were detected by enhanced chemiluminescence (ECL®, Amersham International, Little Chalfont, Bucks., U.K.).

Northern blot analysis

Total RNA was extracted with an RNAxel kit (Eurobio). RNA (25 μg) from each sample was resolved by electrophoresis and transferred to nylon membrane in accordance with standard techniques [18]. Mouse *mgcRacGAP* full-length cDNA was ^{32}P-labelled with a random priming labelling kit (Amersham International) and used to probe blots at 65 °C in Church buffer [19].

In situ hybridization

Mouse embryos were fixed overnight in 4 % (w/v) paraformaldehyde in PBS, dehydrated, embedded in wax and processed to 5–7 μm sections on Superfrostplus slides (Erie Scientific Company, Portsmouth, Hants., U.K.). ^{35}S-UTP-labelled sense and anti-sense *mgcRacGAP* cRNA probes were generated with the Riboprobe system (Promega) from linearized pT7/T3 plasmids (Pharmacia). Two probes were used: the inserts of mouse *mgcRacGAP* cDNA were 800 bp long (bases 1240–2068) for the EST-1 probe and 520 bp long (bases 2390–2954) for EST-6 (see Figure 3). The sense probe was transcribed with T7 RNA polymerase and the anti-sense probe with T3 RNA polymerase. Sections were hybridized overnight at 55 °C, washed as described by Ott et al. [20] and exposed to Ilford K5 emulsion for 1–5 days. Counterstaining was performed with Toluidine Blue.

RESULTS AND DISCUSSION

Mouse *mgcRacGAP* RNA and protein

To obtain the mouse orthologue of *mgcRacGAP*, we performed a BLAST search of the mouse EST database (dbEST) with

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                PE1                                               PE2
ggcacgaggcgctggtagacagcagccctcctggggcgcggcggccggagagtgagcgccgcgggccggacg   72
                                 M   D   T   T   M   V   N   L   W          9
gggttgcgtgtgcgcgggccgggagcctcgaaag ATG GAT ACT ACA ATG GTG AAT TTG TGG    134
 T   L   F   E   Q   L   V   R   R   M   E   I   I   N   E   G   N   E     27
ACT CTG TTT GAG CAG CTT GTG CGC CGG ATG GAG ATT ATC AAT GAA GGA AAT GAA   188
 S   I   E   F   I   Q   V   V   K   D   F   E   D   F   R   K   K   Y     45
AGC ATT GAA TTC ATC CAG GTT GTG AAG GAC TTC GAG GAC TTC CGA AAG AAG TAT   242
 Q   R   T   N   Q   E   L   E   K   F   K   D   L   L   L   K   A   E     63
CAA AGA ACC AAC CAG GAG CTG GAG AAA TTC AAA GAC CTA TTG TTG AAA GCA GAG   296
 T   G   R   S   A   L   D   V   K   L   K   H   A   R   N   Q   V   D     81
ACT GGG CGG AGC GCC CTG GAC GTG AAG CTG AAG CAT GCC CGT AAT CAA GTG GAC   350
 V   E   I   K   R   R   Q   R   A   E   A   E   C   A   K   L   E   Q     99
GTG GAG ATC AAG CGG AGG CAG CGC GCT GAG GCA GAG TGT GCA AAG CTG GAA CAA   404
 Q   I   Q   L   I   R   D   I   L   M   C   D   T   S   G   S   I   Q    117
CAG ATT CAG CTG ATT CGA GAC ATA CTC ATG TGT GAC ACA TCT GGC AGT ATT CAG   458
 L   S   E   E   Q   K   S   A   L   A   F   L   N   R   G   Q   A   S    135
CTG AGC GAG GAA CAA AAA TCA GCT CTC GCT TTC CTC AAC CGA GGC CAA GCA TCC   512
 S   G   H   A   G   N   N   R   L   S   T   I   D   E   S   G   S   I    153
AGT GGC CAC GCC GGC AAC AAT AGA CTG TCA ACG ATT GAT GAA TCT GGT TCC ATT   566
 L   S   D   I   S   F   D   K   T   D   E   S   L   D   W   D   S   S    171
TTA TCA GAT ATC AGC TTT GAC AAG ACT GAT GAA TCA CTG GAC TGG GAT TCT TCT   620
 L   V   K   N   F   K   M   K   K   R   E   K   R   R   S   N   S   R    189
TTG GTG AAG AAT TTC AAA ATG AAG AAA CGA GAG AAG AGG CGC TCC AAC AGT AGA   674
 Q   F   I   D   G   P   P   G   P   V   K   K   T   C   S   I   G   S    207
CAG TTC ATC GAC GGC CCT CCT GGG CCT GTG AAG AAA ACT TGT TCC ATT GGC TCT   728
 T   V   D   Q   A   N   E   S   I   V   A   K   T   T   V   T   V   P    225
ACA GTA GAC CAG GCA AAT GAA TCA ATA GTT GCA AAA ACT ACA GTG ACT GTT CCC   782
 S   D   G   G   P   I   E   A   V   S   T   I   E   T   L   P   S   W    243
AGT GAT GGG GGA CCC ATT GAA GCT GTG TCT ACT ATT GAG ACA TTG CCG TCC TGG   836
 T   R   S   R   G   K   S   G   P   L   Q   P   V   N   S   D   S   A    261
ACC AGG AGT CGC GGG AAG TCA GGA CCT TTA CAA CCT GTG AAC AGT GAC TCC GCT   890
 L   N   S   R   P   L   E   P   R   T   D   T   D   N   L   G   T   P    279
CTG AAC AGC AGG CCA CTG GAG CCA AGA ACT GAC ACA GAC AAT TTG GGC ACA CCT   944
 Q   N   T   G   G   M   R   L   H   D   F   V   S   K   T   V   I   K    297
CAG AAT ACT GGA GGC ATG CGC TTG CAC GAC TTC GTC TCA AAG ACG GTT ATT AAG   998
 P   E   S   C   V   P   C   G   K   R   I   K   F   G   K   L   S   L    315
CCT GAA TCT TGT GTT CCG TGT GGA AAG CGG ATC AAG TTT GGC AAG CTG TCT CTG  1052
 K   C   R   D   C   R   L   V   S   H   P   E   C   R   D   R   C   P    333
AAG TGT CGA GAC TGT CGT TTG GTC TCC CAT CCA GAA TGT CGG GAC CGA TGT CCC  1106
 L   P   C   I   P   P   L   V   G   T   P   V   K   I   G   E   G   M    351
CTT CCC TGC ATC CCC CCC CTG GTG GGG ACA CCG GTT AAG ATT GGA GAG GGC ATG  1160
 L   A   D   F   V   S   Q   A   S   P   M   I   P   A   I   V   V   S    369
CTG GCC GAC TTC GTG TCG CAG GCT TCT CCC ATG ATC CCT GCC ATT GTC GTC AGC  1214
 C   V   N   E   I   E   Q   R   G   L   T   E   A   G   L   Y   R   I    387
TGT GTC AAT GAG ATC GAG CAG CGA GGC CTG ACT GAG GCA GGC TTG TAC AGG ATC  1268
 Y   G   C   D   R   T   V   K   E   L   K   E   K   F   L   K   V   K    405
TAC GGC TGT GAC CGC ACA GTG AAA GAA CTG AAA GAA AAA TTC CTT AAG GTG AAA  1322
 T   V   P   L   L   S   K   V   D   D   I   H   V   I   C   S   L   L    423
ACT GTG CCC CTC CTC AGC AAA GTG GAC GAT ATC CAC GTC ATC TGC AGC CTC CTG  1376
 K   D   F   L   R   N   L   K   E   P   L   L   T   F   W   L   S   K    441
AAG GAC TTC CTG CGC AAC CTC AAA GAG CCC CTC CTG ACC TTC TGG CTG AGC AAA  1430
 A   F   M   E   A   A   E   I   T   D   E   D   N   S   T   A   A   M    459
GCC TTC ATG GAG GCA GCA GAG ATA ACA GAT GAA GAC AAC AGC ACA GCC GCC ATG  1484
 Y   Q   A   V   S   E   L   P   Q   A   N   R   D   T   L   A   F   L    477
TAC CAG GCT GTC AGT GAG CTG CCC CAG GCC AAC AGG GAC ACG CTA GCC TTC CTT  1538
 M   I   H   L   Q   R   V   S   Q   S   P   D   T   K   M   D   I   A    495
ATG ATC CAC CTA CAG AGA GTG TCT CAG AGT CCA GAC ACT AAG ATG GAT ATT GCC  1592
 N   L   A   K   V   F   G   P   T   I   V   A   H   T   V   P   N   P    513
AAT CTA GCT AAA GTC TTT GGC CCT ACA ATA GTT GCC CAT ACT GTG CCC AAT CCA  1646
 D   P   V   T   M   F   Q   D   I   K   R   Q   L   K   V   V   E   R    531
GAT CCA GTG ACA ATG TTC CAG GAC ATC AAA CGT CAG CTC AAG GTG GTG GAG CGC  1700
 L   L   S   L   P   L   E   Y   W   N   Q   F   M   M   V   D   Q   E    549
CTA CTC TCT CTC CCC TTG GAG TAC TGG AAT CAG TTC ATG ATG GTG GAC CAA GAG  1754
 N   I   D   S   Q   R   G   N   G   N   S   T   P   R   T   P   D   V    567
AAC ATA GAC AGC CAG CGA GGC AAT GGA AAC TCA ACA CCA CGC ACA CCA GAC GTT  1808
 K   V   S   L   L   G   P   V   T   T   P   E   F   Q   L   V   K   T    585
AAA GTG AGC TTA CTG GGG CCT GTG ACC ACT CCT GAA TTC CAG CTT GTC AAG ACT  1862
 P   L   S   S   S   L   S   Q   R   L   Y   N   L   S   K   S   T   P    603
CCT TTA TCA AGT TCC CTG TCA CAG AGG TTG TAC AAC CTC TCC AAG AGC ACA CCC  1916
 R   F   G   N   K   S   K   S   A   T   N   L   G   Q   Q   G   K   F    621
AGA TTT GGG AAC AAG AGC AAG TCT GCC ACC AAC TTA GGT CAA CAG GGC AAA TTT  1970
 F   P   A   P   Y   L   K   *                                            629
TTC CCT GCT CCG TAC CTC AAG TAA  agctgtgtctgcctgtgtttactgcacgagacacccctg 2033
tctgctcttcagcctcctgtgtaatgactacttttagcattttccagactttaaataaagttgaacgcgtat 2105
gagaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa                                    2145
```

Figure 1 Nucleotide and predicted amino acid sequences of full-length 2.2 kb cDNA

Reverse primers PE1 and PE2 used in the primer extension analysis are underlined by an arrow. The stop codon is denoted by an asterisk and the polyadenylation signal is underlined.

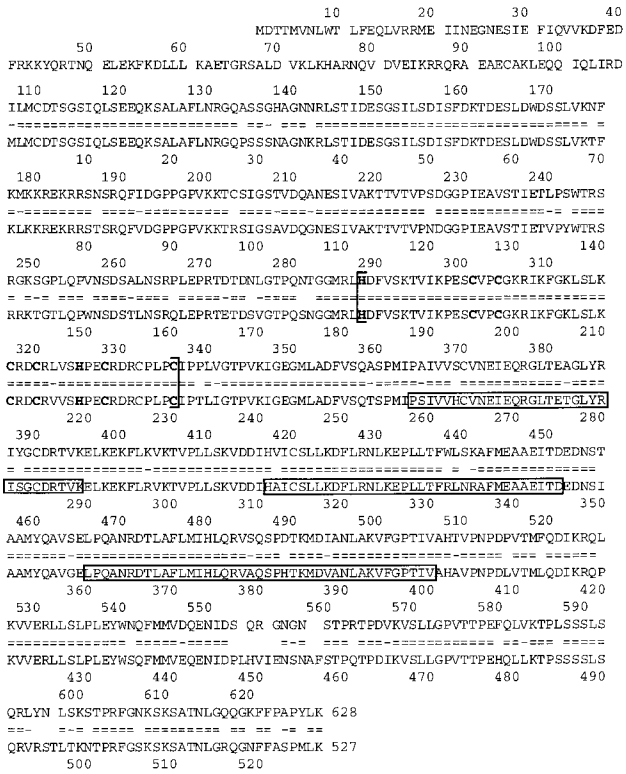

Figure 2 Amino acid sequence comparison of mouse and human MgcRacGAP proteins

The upper sequence is the mouse MgcRacGAP protein; the lower sequence is that of human MgcRacGAP. Identical amino acids are represented by equals signs; a dash indicates a conservative change. The cysteine-rich domain is delimited by brackets. Boxed sequences correspond to the three blocks conserved in RhoGAP domains.

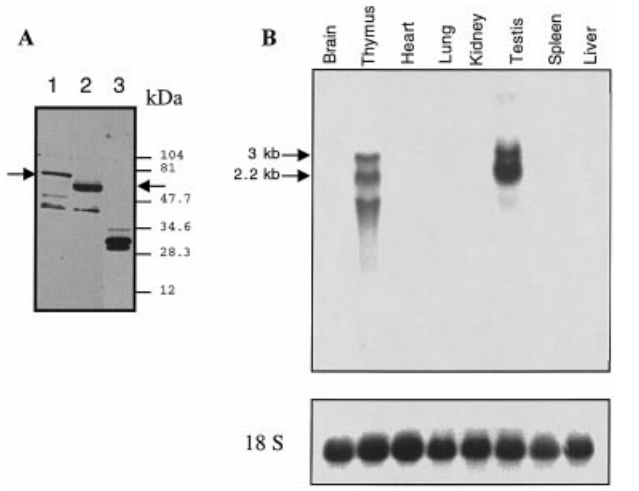

Figure 3 Tissue distribution of mouse *mgcRacGAP* mRNA and protein

(**A**) Immunodetection of MgcRacGAP in mouse and human testis. Equal amounts of protein extract from mouse testis (lane 1) and human testis (lane 2) were run in parallel with the recombinant GAP domain of human MgcRacGAP (residues 234–512) (lane 3). MgcRacGAP was detected by using affinity-purified chicken antibodies. Arrows indicate human and mouse MgcRacGAP proteins. The positions of molecular mass markers are indicated at the right. (**B**) Northern blot analysis. Mouse *mgcRacGAP* cDNA was used as a probe. Each lane contained 25 μg of total RNA from the indicated tissues. Loading is indicated in the bottom panel by 18 S ribosomal RNA.

human *mgcRacGAP* cDNA sequence. Among others, a 2.2 kb EST cDNA was obtained. It was sequenced and shown to match the complete coding sequence of human *mgcRacGAP*. Within the 2145 bp established sequence, a unique long open reading frame from base 108 to base 1994 was detected (Figure 1). The predicted amino acid sequence contains 628 residues. Alignment of human and mouse proteins (Figure 2) shows an overall 86% identity. The two proteins are almost identical in the GAP domain (90%) and the cysteine-rich region (98%), previously defined as functional regions of MgcRacGAP [14]. The similarity is slightly lower (71% identity) in the C-terminal region, suggesting that this domain might be functionally less important.

Strikingly, the mouse cDNA encodes an additional N-terminal polypeptide of 106 residues compared with the human orthologue. The presence of this additional domain is confirmed by Western blot analysis. Indeed, the mouse protein detected in testis extract with anti-MgcRacGAP antibodies shows an apparent molecular mass of 70 kDa, in good agreement with the predicted size of the protein (70144 Da). The mouse protein is approx. 10 kDa longer than human MgcRacGAP protein (58 kDa) (Figure 3A). This additional domain is predicted to be superfolded as a coiled-coil structure, which is consistent with a role in protein–protein interaction [17,21]. It has yet to be determined whether this region can engage in homodimer or heterodimer formation, and the role of this domain in mouse MgcRacGAP is as yet unknown. However, this indicates that functional differences between the human and the mouse proteins might exist.

Expression and RNA characterization of murine *mgcRacGAP*

To investigate the expression of *mgcRacGAP* in mouse, we performed Northern blot analysis of several adult tissues. Figure 3(B) shows that *mgcRacGAP* is expressed in testis and thymus. A weak signal was also detected in brain after a longer exposure (see Figure 5B). Several transcripts of different lengths were detected in mice. A 2.2 kb transcript is highly expressed in testis, whereas a 3 kb transcript is predominant in embryos and adult organs other than testis. We also observed one other transcript form (1 kb) in thymus. These results are in contrast with *mgcRacGAP* expression in humans, where it is widespread and only one mRNA form has been reported [14].

The cDNA that we first characterized is 2145 bp long with a poly(A) site (nt 2087–2092) and a poly(A) tail 16 nt downstream of the poly(A) site. The size of this cDNA is in agreement with the 2.2 kb mRNA transcript that is preferentially expressed in the mature testis (male germ cells). The mouse *mgcRacGAP* gene encodes a second RNA species that is predominant in the embryo and adult organs. 5′ primer extension experiments with PE1 and PE2 oligonucleotides (Figure 1) gave the same results whether RNA was extracted from E10.5 embryo or adult testis, showing a unique transcription initiation site approx. 10 bases upstream of the 5′ end of the sequenced cDNA. Furthermore, reverse-transcriptase-mediated PCR amplification from embryonic or mature testis RNA with different sets of primers localized in the coding region gave identical PCR products (results not shown). Taken together, these results suggest that the two *mgcRacGAP* transcripts contain identical 5′ untranslated region (UTR) and coding regions.

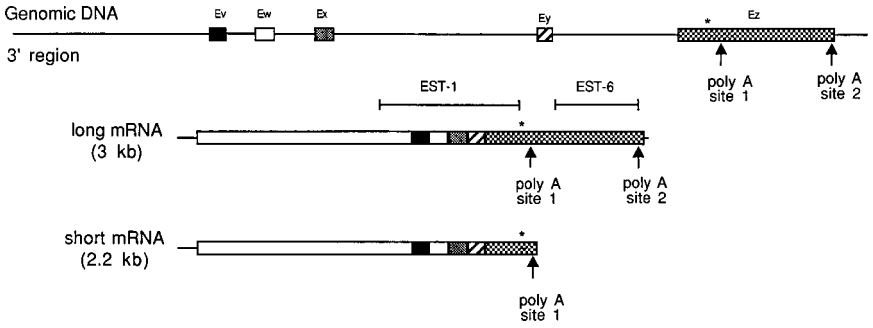

Figure 4 Schematic representation of the 3′ end of the murine *mgcRacGAP* gene and mRNA species

The 3′ portion of mouse *mgcRacGAP* gene contains two alternative poly(A) sites. Short (2.2 kb) and long (3 kb) transcripts result from the use of poly(A) sites 1 and 2 respectively. EST-1 and EST-6 are the two probes used for *in situ* hybridization experiments. An asterisk indicates the stop codon.

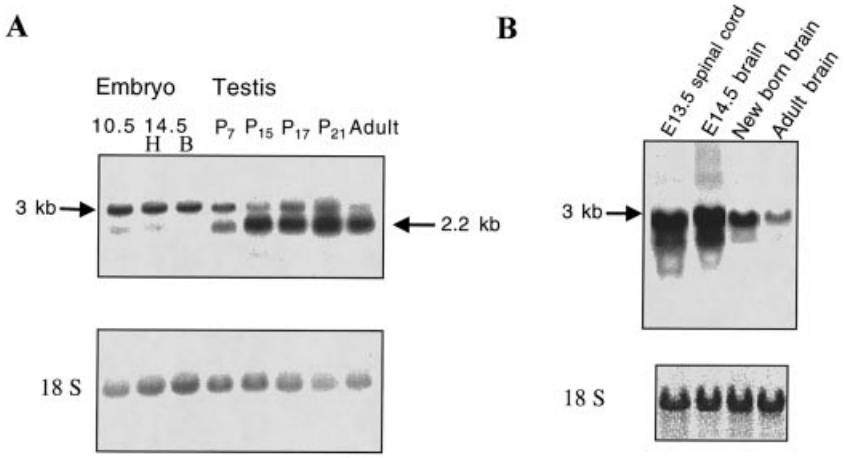

Figure 5 Developmental expression of *mgcRacGAP*

Northern blots of total RNA (25 μg) were probed with mouse *mgcRacGAP* cDNA. (**A**) Total RNA species were extracted from whole embryo at stage E10.5, from head (H) or body (B) of an E14.5 embryo and from testis of mice aged between day 7 and 70 (P7, P15, P21, adult). (**B**) Total RNA species from spinal cord of E13.5 embryo and from brain of E14.5 embryo, newborn mouse and adult mouse. Loading is indicated in the bottom panels by 18 S ribosomal RNA.

In the course of genomic cloning of the mouse *mgcRacGAP* gene, we characterized a genomic DNA fragment that includes five exons and six introns. The last exon contains two potential poly(A) sites in tandem, separated by 775 bp (Figure 4). Analysis of the last exon with the BLAST algorithm against the mouse EST database revealed the presence of several ESTs (Figure 4) matching the genomic region located 5′ and 3′ to the first poly(A) site. The use of the first or second poly(A) sites could generate two mRNA species differing in their 3′ UTR by 775 bp (Figure 4), which is consistent with the difference in length observed by Northern blot analysis. This suggests that the two mRNA species differ from each other by the length of their 3′ UTR, as a result of the alternative use of poly(A) sites. The 3′ UTR of mRNA has critical regulatory roles in various processes such as mRNA transport, localization and translation, particularly during embryonic development and differentiation [22,23]. Many genes have already been shown to use a testis-specific poly(A) site, leading to shorter mRNA species in this tissue [24]. In agreement with these previous observations, the use of the first poly(A) site for murine *mgcRacGAP* in male germ cells leads to a transcript with a very short 3′ UTR (150 bp). However, the biological significance of alternative polyadenylation of *mgcRacGAP* is still unknown.

mgcRacGAP expression during mouse testis development

It was reported previously that the closest homologue to human MgcRacGAP is the *Drosophila* protein RnRacGAP, encoded by the *rotund* locus [14]. This protein has been shown to be essential to the differentiation of male germ cells, because mutation of the *rotund* locus leads to sterility in male flies [25]. *In situ* hybridization analysis in testis has also demonstrated a similar expression of *RnRacGAP* and *mgcRacGAP* in spermatocytes in both species [14,25]. In this context, we examined *mgcRacGAP* expression in testis at different postnatal stages in mice, relative to the time course of spermatogenesis (Figure 5A). Transcripts are detected in testis at postnatal day 7 (P7) before the onset of meiosis in spermatogonia. The predominant mRNA expressed at this stage is the 3 kb form. At P15, when the first spermatocytes begin to appear, the 2.2 kb transcript is highly expressed. Analysis of the subsequent stages and adult testis showed that the 2.2 kb RNA level increased, whereas the 3 kb transcript decreased. On the

postnatal onset of spermatogenesis, the ratio of somatic cells to germ cells in the testis is greatly decreased and could account for the decrease in the 3 kb RNA. This observation suggests that the 3 kb transcript could be specific for somatic tissues of adult and embryo, whereas the shorter form is specific to germ cells and accumulates in spermatocytes.

Therefore, as observed in humans and *Drosophila*, the expression of murine *mgcRacGAP* is restricted to late stages of spermatogenesis (corresponding to meiosis and spermiogenesis). This has been recently confirmed by Western blot analysis of rat elutriated testis cell extracts, showing the presence of the protein in spermatocytes and spermatids (A. Touré, unpublished work). The conserved pattern of *mgcRacGAP* expression at the time of spermatogenesis among mammals and *Drosophila*, and the mutant phenotype observed in *Drosophila*, strongly suggest the importance of MgcRacGAP in the development and/or function of male germ cells.

mgcRacGAP expression during mouse brain development

We first investigated the expression of *mgcRacGAP* by Northern blot analysis during mouse development. By E14.5, germ cells are mitotically blocked at the spermatogonia stage when *mgcRacGAP* is not expressed [14]. Surprisingly, MgcRacGAP is highly expressed in whole embryos at E10.5 and in both the head and the trunk of E14.5 embryos (Figure 5A). This means that the embryonic expression of *mgcRacGAP* is thus contributed by other tissues. The strong signal detected in the head and trunk of E14.5 embryos suggests an expression in a common component such as the CNS. Indeed, *mgcRacGAP* was found expressed in the brain and spinal cord of the embryo (Figure 5B). Northern blot analysis demonstrated that *mgcRacGAP* expression is very high in embryonic brain and decreases drastically after birth to a very low level in the adult (Figure 5B). This result indicates that *mgcRacGAP* expression is correlated with the active phase of neuronal precursor proliferation.

To define precisely the expression sites of *mgcRacGAP*, we undertook an analysis of embryo sections by *in situ* hybridization. Two different RNA probes were used: EST-1, corresponding to a fragment of the coding sequence, and EST-6, which is specific for the 3′ UTR end of the long transcript (Figure 4). The two probes gave qualitatively the same results. Strikingly, hybridization on sections of E14.5 embryos reveals a strong signal restricted to the developing brain (Figures 6A and 6B). The expression is restricted to the developing cerebral cortex of the telencephalon and the medial and lateral ganglionic eminences. Figure 6(C) shows that mRNA accumulation is predominant in the subventricular and ventricular zones of the embryonic cortex at the inner surface of the telencephalon, which are known to be regions of neuronal proliferation during neurogenesis. We also observed a specific signal in the inner surface of the mesencephalon at the level of the cerebellum primordium (Figures 6A and 6B). In mouse development, most cortical neurons are generated between E12 and E16 [26]. Neurons from the neocortex are generated from proliferating cells of the telencephalic ventricular zone. The laminar structure of the adult cerebral cortex is established by the migration of neurons from the ventricular zone towards the upper layers in an inside-out manner [27]. The restricted and strong expression of *mgcRacGAP* in regions of neuronal proliferation by E14.5 (Figure 6C) and the decrease of expression during brain development (Figure 5B) are consistent with the site and timing of neurogenesis. Further examination will be required to establish a precise time course of *mgcRacGAP* expression in the mouse embryonic nervous system but our observations already suggest a role for this protein in neurogenesis in addition to its putative role in spermatogenesis.

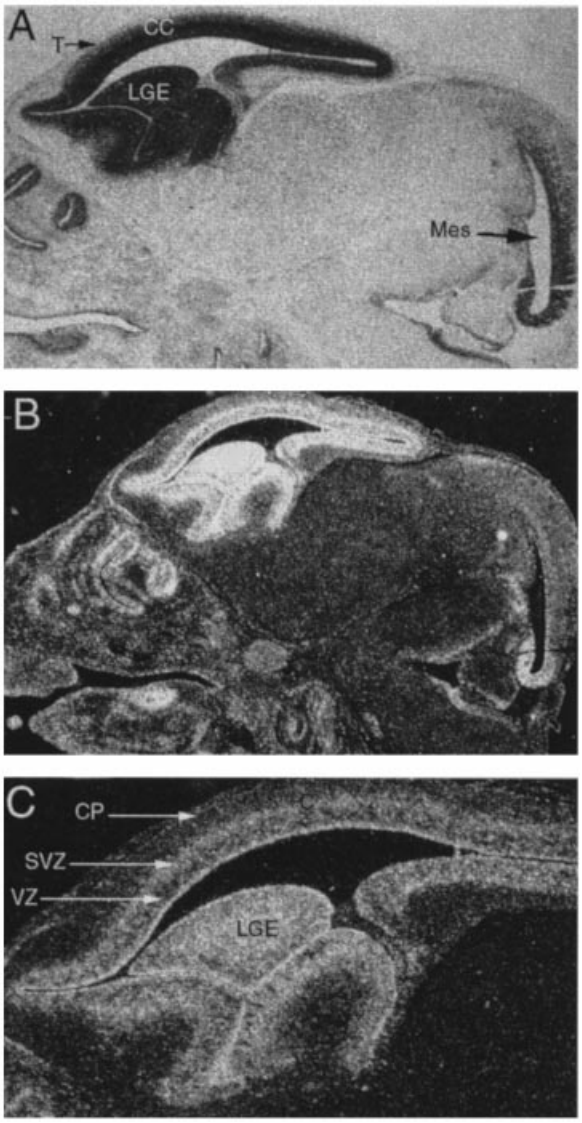

Figure 6 Expression of *mgcRacGAP* mRNA species in the developing CNS

In situ hybridization of a parasagittal section of an E14.5 mouse embryo. (**A**) Bright-field picture of the head; (**B**) dark-field view showing hybridization to the developing cortex of the telencephalon; (**C**) a higher magnification of (**B**), showing that the signal is restricted to the subventricular and ventricular zones of the developing brain. Abbreviations: CC, cerebral cortex; CP, cortical plate; LGE, lateral ganglionic eminence; Mes, mesencephalum; T, telencephalum; SVZ, subventricular zone; VZ, ventricular zone.

RhoGTPases are already known to be involved in neuritogenesis and axonal guidance [3] but MgcRacGAP is the first RhoGTPase-regulating protein whose expression is correlated with the neuroblast cell division process. Both neuroblast generation and migration might be regulated by Rho-dependent pathways. The involvement of RhoGTPases in the mechanism of cell migration is well documented [8,28,29] and multiple lines of evidence have demonstrated the role of RhoGTPases in regulating cell proliferation. Indeed, RhoGTPases are known to be regulators of cell cycle progression and have transforming and oncogenic potential [30–32]. Although the signalling pathways involved in these regulatory mechanisms are poorly defined,

RhoGTPases, and especially Rac, seem to be key regulators of cell division. Whether MgcRacGAP is involved in cell proliferation itself or in cytokinesis and whether Rac-Cdc42/MgcRacGAP signalling pathways might be involved in regulating mitosis in neuroblasts and/or meiosis in germ cells are questions that we are addressing currently.

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