Minireview

Human RhoGAP domain-containing proteins: structure, function and evolutionary relationships

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Abstract Proteins containing a RhoGAP (Rho GTPase activating protein) domain usually function to catalyze the hydrolysis of GTP that is bound to Rho, Rac and/or Cdc42, inactivating these regulators of the actin cytoskeleton. Using database searches, at least 53 distinct RhoGAP domain-containing proteins are likely to be encoded in human DNA. Phylogenetic analysis of only the RhoGAP domains divides these proteins into distinct families that appear to be functionally related. We also review the current understanding of the structure and likely functions of these human proteins. The presence of RhoGAP domains in a number of different human proteins suggests that cytoskeletal changes, regulated by Rho GTPase, may be integrated with many different signaling pathways. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Rho GTPase activating protein; BH domain; Rho GTPase; Actin cytoskeleton

1. Introduction

The Rho family of GTPases have emerged as key players in regulating a diverse set of biological activities including roles in actin polymerization, focal complex and focal adhesion assembly, cell cycle progression, membrane trafficking, cell adhesion and cell polarity [1]. Although there are at least 21 distinct Rho GTPase members, most studies have focused on only three: Cdc42, Rac1 and RhoA. The ability of these GTPases to regulate a myriad of cellular processes stems from their interaction with multiple effector proteins. These interactions are generally GTP-dependent, whereby the interaction only occurs when these GTPases (Rho, Rac or Cdc42) are bound to GTP [2,3]. Consequently, the ability of the Rho GTPases to regulate or affect signaling pathways is ultimately determined by their nucleotide-bound state, which is controlled by an intrinsic GTPase activity and by additional types of regulatory proteins. These additional regulatory proteins include guanine dissociation inhibitors, which stabilize either the GDP- or GTP-bound state; guanine nucleotide exchange factors (GEFs), proteins that promote the active GTP-bound state by facilitating the exchange of GDP by GTP; and GTPase activating proteins (GAPs) that inactivate the Rho GTPases by promoting GTP hydrolysis [4,5]. This review focuses on one class of these regulatory proteins, the GAPs.

The first identified GAP protein, p50 rhoGAP, was purified from human spleen extracts and found to stimulate the intrinsic GTPase activity of Rho, Rac and Cdc42 [6]. Initial cloning of p50 rhoGAP revealed that it shared homology with two other proteins, BCR and N-chimaerin, both of which also stimulated hydrolysis of GTP bound to Rac and Cdc42 [7]. Subsequent molecular cloning and biochemical studies revealed that a region of approximately 170 amino acids, designated the RhoGAP domain (also known as BH domain), common to these proteins, was necessary and usually sufficient for the GAP activity [8]. Since then a large and diverse collection of proteins has been found to contain sequences encoding a similar domain, making each of these proteins a potential candidate GAP for the Rho GTPases. The availability of structural data from three different RhoGAP domains [9–11] and complexes of one of these, p50 rhoGAP, with both Cdc42 [12,13] and RhoA [14] provides important insights into the mechanisms underlying RhoGAP-mediated GTP hydrolysis and provides a rational basis for making selected genetic mutations. In particular, a conserved arginine residue, present in most RhoGAP-containing proteins, is inserted into the active site of all Rho GTPases and functions to stabilize a conformation needed for hydrolysis [12–14]. While mutational analysis has confirmed the importance of this conserved arginine residue [12,15], additional amino acids are required for orientating the hydrolytic machinery of the Rho GTPases [12,14] and are lacking in some inactive RhoGAP domain-containing proteins such as p85α [10,11]. In this review, we focus only on RhoGAP domains found in human proteins and review what is known about these molecules including possible mechanisms for their activation and summarize some of the published speculations about the functional significance of the various RhoGAP domains. An in-depth analysis of 3D structures and details on the chemistry of catalysis by RhoGAP proteins can be found in two recent reviews [16,17].

2. Diverse human RhoGAP-containing proteins

We analyzed RhoGAP-containing proteins in the human genome by searching current cDNA, expressed sequence tags (ESTs) and genomic sequences at the National Center for Biotechnology Information (NCBI) GenBank databases using BLASTP and TBLASTN searches. In total, 53 distinct

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human cDNAs encoding potential RhoGAP domain-encoding proteins were identified from publicly available databases (Table 1). These results are in conflict with the first draft of the human genome sequence, which claimed 77 different human genes contain RhoGAP domains [18], many of which were identified from duplicate database entries, from partial sequences and as splice variants. Our analysis also revealed that human RhoGAP-containing proteins are encoded throughout the genome and rarely cluster in specific chromosomal regions (Table 1).

Although the in vitro specificity of the GAP activity of approximately half of the human proteins reviewed here is known for RhoA, Rac1 and Cdc42 (Table 1), these results may have limited physiological relevance because it has been established that at least some of these molecules, for example p190-A [19,20] and Myosin IXb [21], have different GAP specificity in vitro and in vivo. Additional studies are also needed to determine the specificity of these RhoGAPs towards the less well-known members of the Rho GTPase family such as RhoG and Wrch-1.

Table 1
Human RhoGAP-containing genes, chromosomal localization and GAP specificity[a]

Name and synonym(s)	SwissProt TrEMBL	PIR	GenPept/RefSeq	Chr	Rho	Rac	Cdc42	Ref.
KIAA1501	Q9P227	A59434	BAA96025.1	16p11.2				
KIAA1424; ArhGAP10	Q9P2C3	A59438	BAA92662.1	10p12				
α-Chimaerin; N-chimaerin; chimerin-1		S08242	CAA35769.1	2q31	−	++	−	[7]
β-Chimaerin; RhoGAP3		A53764	AAA16836.1	7p15.3	−	++	−	[24] rat
p190-B, ARHGAP5; RhoGAP5	Q13017	B59431	NP_001164	14q12	++	+	+	[28]
p190-A	Q9NRY4	A40971	AAF80386.1	19q13.3	++	−	−	[20,29] rat
Oligophrenin-1	O60890	H59434	NP_002538	Xq12	++	++	++	[36]
GRAF; oligophrenin-1 like protein	Q9UNA1	F59430	NP_055886	5q31	++	−	++	[33] chicken
GRAF-2	Q96S75		BAB61771.1	4q31.22	++	−	++	[76]
KIAA0013	Q9Y3S6	A59431	NP_055598	15q24				
KIAA0189	Q92502	B59430	NP_055540	Xq11.2				
CAB42562; GT650	Q9Y3M8	H59432	NP_443083	13q12				
DLC-1 (deleted in liver cancer), p122	O43199	G59435	NP_006085	8p22	++	−	ND	[39,41] rat
ArhGAP6; RHG6; rhogap-x	O43182	E59434	NP_038286	Xp22.3	++	−	−	[42] mouse
LOC118743; FLJ00194			XP_058340	10q24				
KIAA1314; FLJ10312	Q9P2N2	E59436	BAA92552.1	18p11.3				
CAC17688; C20orf95	Q9H1G7	H59433	CAC17688.2	20q11				
MacGAP	Q96S64	G59432	NP_277050	6q23.1				
KIAA1391	Q9P2F6	C59436	BAA92629.1	11q23.2				
RhoGAP8	Q9NSG0	B59436	CAB90248.1	22q13.3				
p50 RhoGAP; Cdc42 RhoGAP	Q07960	A49678	NP_004299	11	+	+	++	[20,43,44]
RHG4; p115; C1	P98171	I38100	CAA55394.1	Xq28				
srGAP-3; KIAA0411		G59434	CAC22407.1	3p25.3				
srGAP-1; KIAA1304	Q9P2P2	G59436	BAA92542.1	12q14.1	+	−	++	[46]
srGAP-2; KIAA0456; ARHGAP14	O75044	C59437	BAA32301.1	1q21.2				
3BP-1	Q9Y3L3	T46916		22q13.1	−	++	+	[48]
KIAA0672	O75160	A59433	NP_055674	17p11.2				
RICH-1; RhoGAP (NADRIN)	Q96KS3	F59433	CAC37948.1	16p11.2	−	++	++	[49]
GMIP; LOC51291	Q9P107	D59435	NP_057657	19p11				
PARG1	O15463	E59430	NP_004806	1p21.3	++	+	+	[51]
HA-1; KIAA0233	Q92619	D59433	BAA13212.1	19p13.3				
p85-α; p85-alpha	P27986	A38748		5q12	−	−	−	[8]
p85-β; p85-beta	O00459	H59435	NP_005018	19q13.2				
INPP5B	P32019	A41075	AAA79207.1	1p34				
OCRL-1		G59431	NP_001578	Xq25–26				
CHR 5 ORF	Q9NYF5	B59433	NP_057687	5q31				
KIAA1688	Q9C0H5	C59434	BAB21779.1	8q24.3				
HYPO DKFZP56	Q9H0T6	A59430	NP_112595	4q21.3				
KIAA0053	P42331	C59430	NP_055697	2p13.3				
Myosin IXB; MYO9B		A59256	NP_004136	1q13.1	++	ND	ND	[21,57]
Myosin IXA	Q9UNJ2	E59435	NP_008832	15q22				
FLJ13511	O60432	E59437	AAC18917.1	19p13.3				
ARAP1; CENTAURIN DELTA2	Q96L71	C59431	NP_056057	11q13.1				[58]
ARAP2; KIAA0580	Q9Y4E4	T00342	BAA25506.1	4p14				
ARAP3; FLJ21065	Q8WWN8	E59431	CAC83946.1	5q31.3				[45]
KIAA1204; homolog of mouse CdGAP	Q9ULL6	A59437	BAA86518.1	3q13				
N-chimaerin homolog; LOC126397	O14560	T00705	AAB81198.1	19q13				
MgcRacGAP; RacGAP-1; KIAA1478	Q9P2W2	D59430	NP_037409	12p13.2	−	++	++	[59]
RALBP1; RLIP76	Q15311	F59435	NP_006779	18p11.3	−	+	++	[61,62]
BCR	P11274	TVHUBR	NP_004318	22q11	−	++	+	[7,20]
ABR	Q12979	A49307	NP_068781	17p13.3	−	++	++	[68,69]
ARHGAP9; RhoGAP9; MGC12959	Q96S74	JC7701	BAB56159.1	12q14	+	++	++	[72]
ARHGAP12, RhoGAP-12; FLJ10971	Q9NT76	T46471	NP_060757	10p12				

[a]The order of the list is the same as given in the phylogenetic analysis (Fig. 1). Protein sequence IDs are included from the Swiss-Prot/TrEMBL (http://www.ebi.ac.uk/swissprot/), PIR Protein Sequence (http://pir.georgetown.edu) and GenPept and RefSeq (http://www.ncbi.nlm.nih.gov/LocusLink/) databases. GAP activity, if known, is as follows: ++, high activity; +, less activity; −, no activity and ND, not determined. Unless otherwise indicated, the references are to the earliest in vitro and in vivo descriptions of GAP activity by human proteins.

3. Evolutionary relationships of human RhoGAP-containing proteins

In order to identify potential functional relationships among the RhoGAP domains in these 53 human proteins, we first generated a multiple sequence alignment of only their RhoGAP domains (available at http://lombardi.georgetown.edu/research/areas/growthregulation/rgalign2.htm). This alignment shows that the amino acids of all of these RhoGAP domains are sufficiently homologous to justify concluding that their 3D structures are also likely to be similar to the RhoGAP domains found in p50 rhoGAP [9], p85 [10] and GRAF [11]. Inspection of this pileup also showed that four of these proteins, OCRL-1, INPP5B, CHR 5 ORF and ARAP2, do not contain the conserved arginine residue suggesting that these proteins do not have RhoGAP activity. Using this alignment to define the N- and C-terminal ends of the GAP domains, we determined the likely phylogenetic relationship amongst these 53 RhoGAP domains (Fig. 1). This phylogenetic analysis offers evidence for frequent domain duplication and for duplication of the entire genes containing

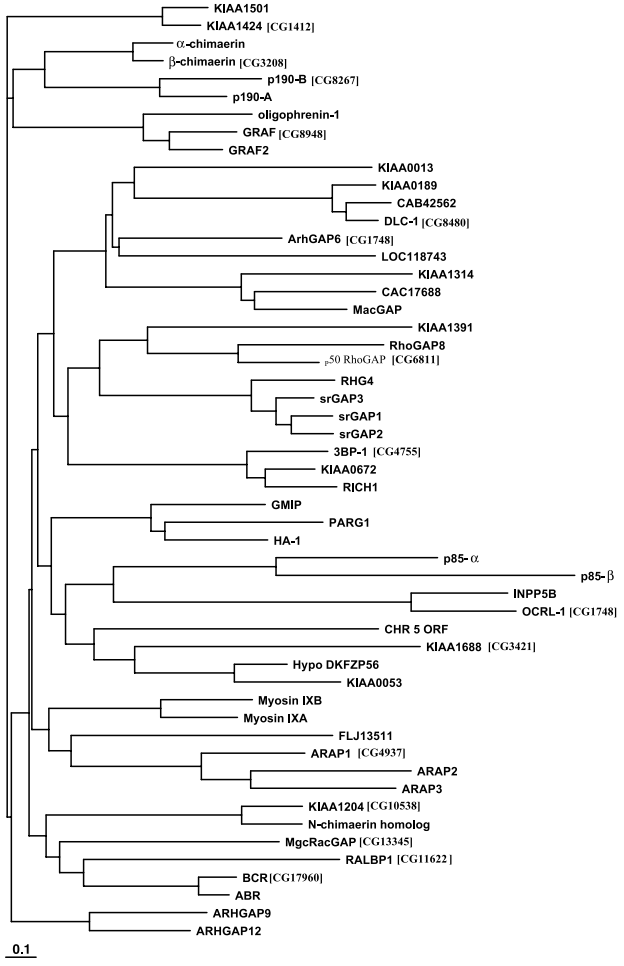

Fig. 1. An unrooted phylogenetic tree of human RhoGAP-containing proteins. The tree was produced by amino acid sequence alignment of the 53 human RhoGAP-containing proteins (available at http://bc.georgetown.edu/pb/RhoGAP) using the neighbor joining method based on CLUSTAL W multiple sequence alignments. A bar represents the number of substitution events (10 per 100 amino acids). The *Drosophila* orthologs to the various human genes are indicated in parentheses.

these domains. That is, when two RhoGAP domains are closely related, we found that the entire proteins are more closely related to each other than the entire protein having RhoGAP domains from different families (Fig. 1). For example, the similarity between the RhoGAP domains in Myosin IXa and Myosin IXb was sufficient to predict that these two proteins are more similar to one another than to any of the other proteins analyzed. This analysis also showed that the sequence homologies among the 53 different domains did not strongly correlate with the specific GAP activity towards Cdc42, Rac and/or Rho. One possible explanation is that several amino acids in these subdomains are involved in determining RhoGAP specificity. Additional comparative studies are needed to determine which amino acid differences are involved in determining specificity and how these changes affect the 3D structures and/or interactions with the Rho GTPases. Finally, the two RhoGAP-containing families that presumably lack functional GAP activity, OCRL and the p85 subunits of phosphoinositide 3′-kinase families [8], appear phylogenetically the most distant from the majority of the GAPs (Fig. 1).

To further understand the evolution of the human RhoGAP-containing proteins, we compared human and *Drosophila melanogaster* RhoGAP-containing proteins. Based on previous studies [22] and additional database searches, a total of 21 unique *D. melanogaster* RhoGAP-containing sequences were detected. As in the human genome, genes encoding *D. melanogaster* RhoGAP-containing proteins are widely dispersed in the genome (data not shown). Although six *D. melanogaster* proteins are unique to this species, 15 have human orthologs, including some involved in human diseases such as BCR, GRAF, and OCRL-1 (Fig. 1). Interestingly, orthologs for several human RhoGAP-containing families including those for Myosin IX and srGAP were not found. The mere existence of *Drosophila* and human orthologs suggests that these conserved proteins may play important roles in these organisms.

4. Overview of human RhoGAP-containing protein families

Since a previous review article on RhoGAP-containing proteins by Lamarche and Hall in 1994 [4], the number of eukaryotic RhoGAP proteins identified and characterized has increased immensely. Here we have focused on providing a summary of what is known about human RhoGAP-containing proteins.

4.1. α-Chimaerin and β-chimaerin

α-Chimaerin and β-chimaerin both contain a phorbol ester-C1 binding domain and a C-terminal RhoGAP domain (Fig. 2), while splice variants for these genes contain additional N-terminal SH2 domains. Analysis of the RhoGAP domain of α-chimaerins reveals GAP activity only for Rac [7]. The C1 sequences in β2-chimaerin, like those in protein kinase C members, bind phorbol esters and regulate its accumulation both at the plasma membrane and in the perinuclear compartment [23]. While β-chimaerin and α-chimaerin show restricted expression in the brain and testes, α2-chimaerin and β2-chimaerin splice variants are ubiquitously expressed [24,25]. Interestingly, β2-chimaerin mRNA expression is markedly down-regulated in malignant gliomas compared to normal brain and low grade astrocytoma [26].

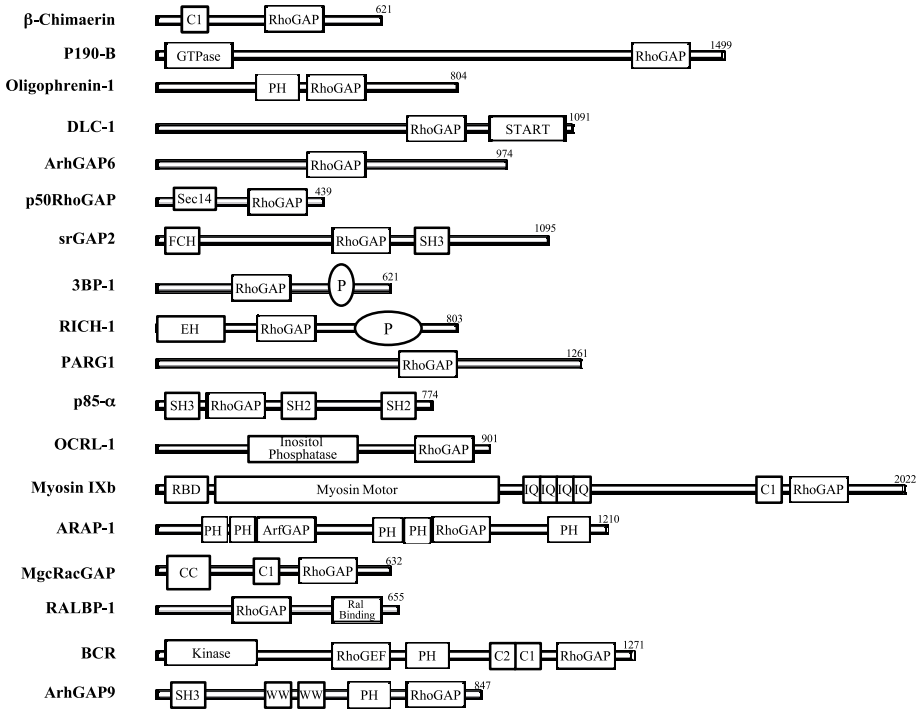

Fig. 2. Schematic representation of selected human RhoGAP-containing proteins. Representative members of each cluster are shown. Domain name abbreviations: C1, cysteine-rich phorbol ester binding; C2, calcium-dependent lipid binding; CC, coiled-coil; DH, Dbl homology; EH, Eps15 homology; FCH, Fes/CIP4 homology; IQ, calmodulin binding motif; P, proline-rich; PH, pleckstrin homology; RBD, Ras binding domain; RhoGAP, Rho GTPase activating protein; SH3, Src homology 3; Sec14, sec14-like.

4.2. p190-A and p190-B

p190-A was originally identified as a rat p120 RasGAP-interacting protein [19]. In humans, two homologs, p190-A [27] and p190-B [28], contain N-terminal GTPase, and C-terminal RhoGAP domains (Fig. 2). While in vitro assays demonstrated that p190-A has equal GAP activity for Rho, Rac and Cdc42 [29], in vivo experiments with p190-A demonstrated a preference for Rho [20]. Studies on p190 also show that its RhoGAP activity is regulated by phosphorylation. Upon stimulation by growth factors or cell attachment, tyrosine residues in the central portion of p190 are phosphorylated by kinases such as Src [30]. This phosphorylation causes a conformational change in the p190 molecule resulting in the activation of its GAP activity, which decreases Rho GTP levels and inhibits signaling to effector proteins, leading to a net loss of stress fibers. Mouse knockout studies indicate that p190-A mediates Src-dependent adhesion signals involved in neuritogenesis through its effect on the intrinsic GTPase activity of Rho and are consistent with it being the major tyrosine phosphorylated protein in the brain [31]. These results are also supported by genetic analysis in *Drosophila* showing that a p190 homolog regulates axon branch stability [22]. Interestingly, knockout of the mouse p190-B homolog dramatically decreases the size of mice, an effect that appears to be mediated through a transcription factor, CREB [32].

4.3. GRAF, GRAF-2 and Oligophrenin-1

The three human members of this family, GRAF, GRAF-2 and Oligophrenin-1, all contain a PH domain and a RhoGAP domain (Fig. 2). In addition, GRAF and GRAF-2 contain C-terminal SH3 domains. The first known member of this family, designated GRAF, was originally identified as a chicken cDNA encoding a protein that specifically interacted with focal adhesion kinase and possessed GAP activity for Rho and Cdc42 in vitro [33]. More recently, human GRAF was detected as a fusion partner with the mixed-lineage leukemia (MLL) gene and inactivation of both alleles was detected in several patients with myelodysplastic syndrome [34]. Another family member, human GRAF-2, was identified in a yeast two-hybrid screen as being able to interact with the Rho effector protein, PKN-β (a novel isoform of PKN; also known as PRK) [35]. In vitro studies with the GAP domain of GRAF-2 reveal that it is active towards Cdc42 and Rho, but not Rac. Current thinking suggests that these molecules may act as adapters to coordinate Rho activity with kinase signaling pathways.

Oligophrenin-1 was first identified as a gene deleted in X-linked mental retardation [36]. Oligophrenin-1 has equal GAP activity for all three Rho GTPases. A high level of expression of Oligophrenin-1 in the fetal brain suggests that this protein may function in regulating Rho GTPases during neuritogenesis. In addition to its role in normal brain development, gene array studies indicate that Oligophrenin-1 is overexpressed in some cancers including colon cancer [37] and in glial tumors [38].

4.4. DLC-1

DLC-1, the human analog of the rat p122 protein [39], is frequently deleted in hepatocellular carcinoma [40]. The RhoGAP domain of DLC-1 is located in its C-terminus (Fig. 2) and studies with the rat homolog reveal RhoGAP activity towards Rho in vitro and in vivo [39,41]. While this molecule can also interact and activate phospholipase C δ1 activity, transfection studies indicate that the ability of p122 to induce cell detachment is only due to its RhoGAP activity [41].

4.5. ArhGAP6

ArhGAP6 contains a central RhoGAP domain (Fig. 2) and several potential SH3 domain binding sequences [42]. ArhGAP6 was originally identified as a potential cause of microophthalmia with linear skin defects syndrome (MLS) characterized by eye, skin and central nervous system malformations [42]. Although the ArhGAP6 gene is commonly deleted in MLS, additional studies using transgenic mice do not support a role for a mutant ArhGAP6 gene as being causative in MLS. While the GAP domain of ArhGAP6 has activity for RhoA-GTP but not for GTP bound to Rac1 or Cdc42, transfection studies suggest that it can also regulate actin changes independent of the RhoGAP domain [42].

4.6. p50 rhoGAP

p50 rhoGAP, the first RhoGAP protein identified [43,44], is expressed ubiquitously and is the smallest member of the RhoGAP family (499 amino acids). p50 rhoGAP contains an N-terminal Sec14 domain and a C-terminal RhoGAP domain (Fig. 2). In vitro and in vivo studies show that p50 rhoGAP preferentially binds Cdc42 over Rho and Rac [20,43,44]. Phospholipid affinity chromatography studies have recently added p50 rhoGAP to the list of proteins that can bind phosphatidylinositol 3,4,5-trisphosphate (PtdIns-(3,4,5)P_3) [45]. The interaction of p50 rhoGAP with PtdIns(3,4,5)P_3 likely involves the Sec14 domain [45], a domain originally found in the yeast phosphoinositol transfer protein Sec14p. Although not yet tested, p50 rhoGAP interactions with PtdIns(3,4,5)P_3 might result in its recruitment to the plasma membrane and/or conformational changes that regulate its GAP activity.

4.7. srGAP-1, srGAP-2 and srGAP-3

The srGAP family contains an N-terminal FCH domain, a central RhoGAP domain and a C-terminal SH3 domain (Fig. 2). The three members, srGAP-1, srGAP-2 and srGAP-3, are all binding partners for the intracellular tail of the Roundabout receptor (Robo), a receptor that binds the Slit secreted protein and mediates signaling pathways involved in cellular repulsion [46]. While srGAP-2 protein is ubiquitously expressed, srGAP-1 is highly expressed in the lung (less so in the brain, kidney and testes) and srGAP-3 is highly expressed in brain, lung and spleen. Mapping experiments showed that a proline-rich sequence (PPPPVPPP) in the Robo receptor intracellular tail interacts with the SH3 domain of the srGAPs [46]. Based on these interactions, Slit ligand binding to Robo is thought to induce a conformational change in the intracellular tail of Robo, exposing a buried SH3 binding sequence. srGAPs contain SH3 domains that interact with the now exposed proline-rich SH3 binding site in Robo. This interaction then activates the RhoGAP activity of srGAPs and causes a local down-regulation of Cdc42 [46]. The resulting decrease in Cdc42 GTP levels leads to a localized loss of actin polymerization and actin-associated structures including filopodia and lamellipodia. A localized loss of the ability to increase or maintain a normal level of actin polymerization may explain the inability of certain cells to move in the direction of the Slit ligand gradient [46].

4.8. 3BP-1

3BP-1 was originally identified by far Western screening as a protein capable of interacting with the SH3 domain of c-Abl [47]. 3BP-1 contains a central RhoGAP domain and a proline-rich SH3 binding domain in its C-terminus (Fig. 2). In vitro studies have shown that 3BP-1 has GAP activity towards Rac, but not for Rho or Cdc42 [48]. 3BP-1 may normally function to limit Rac signaling, since microinjection of 3BP-1 into serum-starved fibroblasts inhibited PDGF-stimulated Rac-mediated membrane ruffling [48].

4.9. Rich-1

Rich-1 was identified in a yeast two-hybrid screen using CIP4, a Cdc42 effector protein, as bait [49]. Rich-1 contains an N-terminal endophilin homology (EH) domain, a RhoGAP domain and several C-terminal proline-rich regions (Fig. 2). Domain mapping studies show that the proline-rich region of Rich-1 interacts with the SH3 domain of CIP4. Although not yet tested, the EH domain in CIP4, as the EH domain in endophilin [50], may function in tubulovesicular membrane trafficking by binding and evaginating lipid bilayers at the clathrin-coated pit. In vitro experiments show that Rich-1 has GAP activity for Cdc42 and Rac but not for RhoA [49]. These in vitro results are consistent with the known in vivo effects of Rich-1, where overexpression inhibits Rac-mediated membrane ruffling, but has no effect on Rho-induced stress fiber formation [49].

4.10. PARG1

PARG1 contains a RhoGAP domain and a cysteine-rich domain related to a phorbol ester/diacylglycerol binding domain (Fig. 2). PARG1 was identified by affinity chromatography as a protein whose C-terminal sequence DYQCTIL interacts with the fourth PDZ domain of the protein tyrosine phosphatase PTPL1. Although the RhoGAP domain of PARG1 shows preferential GAP activity towards Rho in vivo, little else is known about the normal function of this protein [51].

4.11. p85-α and p85-β

p85-α and p85-β subunits are highly homologous proteins characterized by an N-terminal SH3 domain, two SH2 domains and a region with only partial homology to the consensus RhoGAP domain (Fig. 2). The p85-α and p85-β proteins are known to function as regulatory subunits for the 110 kDa catalytic subunit of phosphatidylinositol 3-kinase [52,53], which phosphorylates the inositol ring at the 3-position. Unlike other RhoGAP-containing proteins, p85-α does not have catalytic activity [8]. Nevertheless, the RhoGAP domains in these subunits may have functional significance, as p85-α can interact with GTP-bound Cdc42 and Rac in vitro and co-immunoprecipitates with Cdc42 in a GTP-dependent fashion [54]. Thus, p85 subunits may act as adapter proteins to localize phosphatidylinositol 3-kinase activity to sites of Cdc42 activation.

4.12. OCRL-1 and INPP5B

OCRL-1 was originally discovered because mutations in this gene are responsible for Lowe's Syndrome (also known as oculocerebrorenal syndrome). This syndrome is characterized by eye and renal abnormalities and severe mental retardation [55]. OCRL-1 and a related protein, INPP5B (type II Ins-polyphosphate 5-phosphatase), have a central inositol polyphosphate 5-phosphatase catalytic domain and a C-terminal RhoGAP domain (Fig. 2). OCRL-1 appears to normally func-

tion in protein trafficking [56]. Although the RhoGAP specificity of OCRL-1 and INPP5B has not been reported, both proteins are expected to be inactive because they contain a glutamine instead of the conserved arginine in the active site.

4.13. Myosin IXa and Myosin IXb

Myosins are highly diverse proteins consisting of at least 14 different classes. The Myosin IX class contain a typical myosin-like head domain, an actin binding region, IQ-calmodulin binding repeats, a protein kinase C regulatory domain and a RhoGAP domain in the C-terminus (Fig. 2). To date only human Myosin IXb has been characterized in vitro and in vivo for GAP activity. Myosin IXb is highly expressed in peripheral blood lymphocytes [21,57] and like other myosins, has active motor activity that is inhibited by calcium [57]. In vitro analysis of the GAP activity in Myosin IXb revealed that it is specific for Rho. It has been postulated that this GAP activity may be involved in transiently inhibiting Rho activity during the early stages of cell spreading.

4.14. ARAP1, ARAP2, and ARAP3

The ARAP subfamily is a recently discovered subclass of Arf GAP-containing proteins [45,58]. All three human ARAP proteins are characterized by having a common set of domains, which include five PH domains, an Arf GAP domain and a RhoGAP domain (Fig. 2). The PH domains in ARAP1 [58] and ARAP3 [45] function to specifically bind PtdIns(3,4,5)P_3 thereby increase Arf GAP activity. In vitro studies showed that recombinant ARAP1 [58] and ARAP3 [45] have equal GAP activity toward Rho, Rac and Cdc42. Unlike the Arf GAP activity of ARAP1 and ARAP3, the RhoGAP activity was neither stimulated nor inhibited by PtdIns(3,4,5)P_3 binding [45,58]. Interestingly, one of the ARAP members, ARAP2, lacks the catalytic arginine in the active site and thus may not have RhoGAP activity. Although the exact function of these molecules is not known, ARAPs, with phosphoinositide binding and Arf GAP and RhoGAP activities, may function normally to coordinate protein trafficking with actin cytoskeletal changes.

4.15. MgcRacGAP

MgcRacGAP contains an N-terminal ERM domain, a central protein kinase C-like cysteine-rich motif and a C-terminal RhoGAP domain (Fig. 2). MgcRacGAP shows equal GAP activity towards Rac1 and Cdc42 and is 30 times less active towards RhoA [59]. MgcRacGAP is mainly expressed in male germ cells and is implicated in mitotic spindle formation. Interestingly, an N-terminal coiled-coil-like domain of MgcRacGAP directly binds microtubules and is required for localization to the central spindle and midbody during late M phase [60].

4.16. RALBP1

RALBP1, also known as RLIP76, contains an N-terminal α-helical region, a central RhoGAP domain, and a C-terminal RAL GTPase binding region (Fig. 2). In vitro RhoGAP assays revealed activity towards Cdc42 and less towards Rac1 but none for RhoA [61,62]. RALBP1 can also interact with Reps1, an Eps homology domain-containing protein, which is tyrosine phosphorylated in response to epidermal growth factor (EGF) stimulation of cells and then binds to the SH3 domains of the adapter proteins Crk and Grb2 [63]. Since the Ral GTPase is known to be involved in protein trafficking, the potential protein interactions involving RALBP1 could possibly coordinate cellular signaling between activated EGF receptors and other small GTPases such as Ras and Cdc42. Other studies with RALBP1 indicate it also may have a role in multidrug resistance because it appears to be active in transmembrane transport of glutathione conjugates and xenobiotics [64].

4.17. BCR and ABR

BCR was originally discovered as a human gene on chromosome 22 that is often translocated along with the c-Abl tyrosine kinase on chromosome 9 [65]. This translocation event results in the production of BCR-cAbl tyrosine kinase fusion proteins present in many patients with chronic myeloid leukemia or acute lymphoblastic leukemia. The BCR protein contains multiple domains including an N-terminal kinase domain, a Dbl domain, a PH domain and a C-terminal RhoGAP domain (Fig. 2). In vitro and in vivo data suggest that BCR has GAP activity primarily for Rac [7,20]. Since the Dbl region of BCR has the most RhoGEF activity for Cdc42, and less activity towards Rac and Rho, it has been proposed that BCR, having both GAP and GEF activities, may function to temporally regulate the activity of these GTPases [66]. Gene knockout studies in mice suggest an important role for BCR in down-regulating Rac-mediated NADPH oxidase activity in neutrophils [67]. A related smaller protein, ABR, is structurally similar to BCR but lacks the N-terminal kinase domain and has GAP activity for both Rac and Cdc42 [68,69]. While knockout mice for ABR do not show an obvious phenotype, double knockouts for ABR and BCR exhibit functional and structural abnormalities of glial cells and show vestibular dysgenesis [70,71].

4.18. ARHGAP9 and ARHGAP12

The ARHGAP9 protein contains an SH3 domain, a PH domain and a WW domain along with the RhoGAP domain (Fig. 2). In vitro studies indicate that ARHGAP9 has GAP activity toward Rac1 and Cdc42, but less towards Rho [72]. Expression studies reveal high levels of ARHGAP9 expression in hematopoietic tissues including cell lines from B-cell leukemias, T-cell leukemias, and myeloid or Hodgkin lymphomas. Transfection analysis suggests that ARHGAP9 may play a role in the regulation of cellular adhesion of hematopoietic cells to extracellular matrix molecules. ARHGAP12, a protein with a domain structure similar to ARHGAP9 but containing two WW domains, is expressed ubiquitously in a wide range of normal tissues and tumor cell lines and is expressed strongly in breast carcinomas [73]. The biological function and specificity of the RhoGAP domain in ARHGAP9 is not known. Furthermore, the SH3 and WW domains, both of which mediate protein–protein interactions with unique proline-rich sequences in target proteins, may regulate the GAP activity of both ARHGAP9 and ARHGAP12 proteins.

5. Conclusions

The 53 human RhoGAP domain-containing proteins are the largest known group of Rho GTPase regulators and significantly outnumber the 21 Rho GTPases they presumably regulate. This excess of GAP proteins probably indicates complex regulation of the Rho GTPases and is consistent with the

existence of almost as many (48) human Dbl domain-containing Rho GEFs [74] that act antagonistically to the RhoGAP proteins by activating the Rho GTPases. While some RhoGAP-containing proteins are involved in several human genetic diseases and/or show altered levels of expression in certain kinds of cancers, the normal functions of most of the domains in these molecules are not known. A major experimental limitation to increasing our understanding of these proteins is that the most frequently used techniques, transfection and microinjection, may not provide reliable information about possible normal functions because introducing excess amounts of these kinds of proteins into cells usually results in constitutive GAP activity. Since unregulated GAP activity dramatically inhibits the Rho GTPases and typically disrupts normal actin dynamics, making correct inferences about the normal biological function of the RhoGAPs may be difficult or impossible with these techniques. Gene knockouts in mice, such as those for BCR [67], p190-A [22], and p190-B [32], may more reliably reveal the normal in vivo function of RhoGAP domain-containing proteins. Targeted knockins, where only the GAP domain is mutated, may also be helpful to determine the functional significance of GAP activity. Finally, studies using RNAi experiments, a rapid method to inhibit gene expression, may also help elucidate the in vivo function of RhoGAP proteins. This method was used recently to examine RhoGEF function [75]. Although current knowledge about the in vivo biological function of most RhoGAP-containing proteins is still rudimentary, we understand even less about how the diverse GAP activities are controlled at the molecular level, including whether these molecules are also involved in integrating multiple signals and if so, how they integrate these signals.

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