Rho-family GTPases: it's not only Rac and Rho (and I like it)

Krister Wennerberg* and Channing J. Der

Department of Pharmacology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295, USA

*Author for correspondence (e-mail: krister@med.unc.edu)

Journal of Cell Science 117, 1301-1312 Published by The Company of Biologists 2004
doi:10.1242/jcs.01118

Summary

The Rho-family proteins make up a major branch of the Ras superfamily of small GTPases. To date, 22 human genes encoding at least 25 proteins have been described. The best known 'classical' members are RhoA, Rac1 and Cdc42. Highly related isoforms of these three proteins have not been studied as intensively, in part because it has been assumed that they are functionally identical to their better-studied counterparts. This now appears not to be the case. Variations in C-terminal-signaled modifications and subcellular targeting cause otherwise highly biochemically related isoforms (e.g. RhoA, RhoB and RhoC) to exhibit surprisingly divergent biological activities. Whereas the classical Rho GTPases are regulated by GDP/GTP cycling, other Rho GTPases are also regulated by other mechanisms, particularly by transcriptional regulation. Newer members of the family possess additional sequence elements beyond the GTPase domain, which suggests they exhibit yet other mechanisms of regulation.

Key words: Rho, Rac, Cdc42, GTPase, Cytoskeleton

Introduction

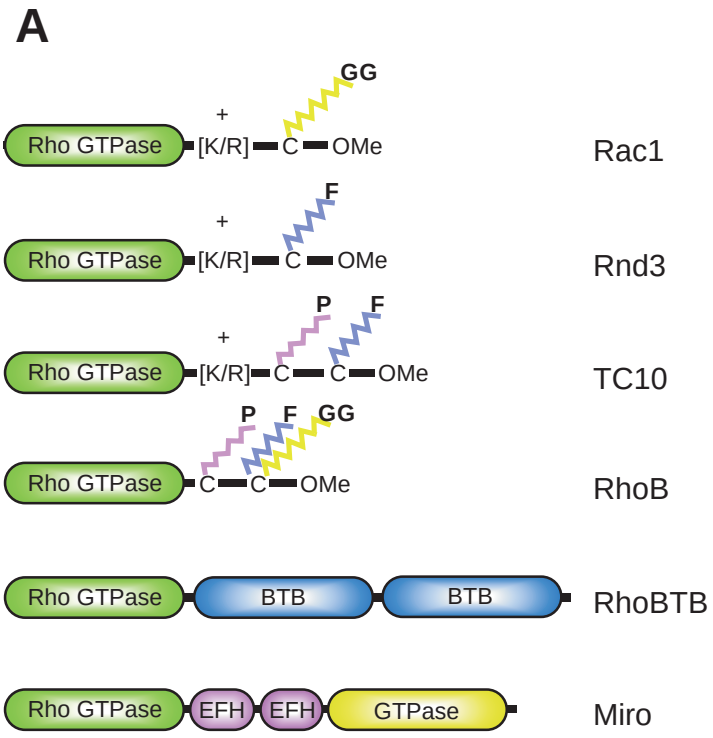
The Rho-family proteins are defined by the presence of a Rho-type GTPase-like domain. A structural feature that distinguishes the Rho proteins from other small GTPases is the so-called Rho insert domain located between the fifth β strand and the fourth α helix in the small GTPase domain (Valencia et al., 1991). Most typical Rho proteins are small (190-250 residues) and consist only of the GTPase domain and short N- and C-terminal extensions. However, some of the more atypical family members contain additional well-defined domains and can be >700 amino acids long. Within their GTPase domains, they share approximately 30% amino acid identity with the Ras proteins and 40-95% identity within the family. All members contain the sequence motifs characteristic of all GTP-binding proteins, bind to GDP and GTP with high affinity, and are thought to cycle between active, GTP-bound and inactive, GDP-bound states. In addition, the majority of members undergo C-terminal post-translational modification by isoprenoid lipids (Fig. 1). Together with other C-terminal modifications or sequences, isoprenoid addition facilitates their subcellular location and association with specific membranes, which is crucial for their functions. However, the Chp, RhoBTB and Miro proteins lack this mode of membrane targeting.

Most of the functional information on Rho-family proteins has come from studies of RhoA, Rac1 and Cdc42. All share common growth-promoting and anti-apoptotic functions, as well as regulation of gene expression, through activation of signaling molecules such as serum response factor, NF-κB, the stress-activated protein kinases and cyclin D1 (Pruitt and Der, 2001; Van Aelst and D'Souza-Schorey, 1997). All three promote actin cytoskeleton reorganization, but have distinct effects on cell shape and movement (Hall, 1998; Schmitz et al., 2000). RhoA promotes actin-myosin contractility and, thereby, the formation of stress fibers and focal adhesions, regulating cell shape, attachment and motility. Rac1 promotes actin polymerization and the formation of lamellipodia, which are curtain-like extensions that consist of thin protrusive actin sheets at the leading edge of migrating cells. Cdc42 causes formation of filopodia, which are thin, finger-like cytoplasmic extensions that contain tight actin bundles and might be involved in the recognition of the extracellular environment.

In common with Ras and other small GTPases, the GDP- and GTP-bound states of the classical Rho GTPases differ in conformation primarily in two localized regions, switch I and II, and this feature is probably shared by all Rho GTPases (Vetter and Wittinghofer, 2001). The conformation of the GTP-bound protein results in increased binding affinity for downstream effector proteins. Each Rho-family protein recognizes multiple effectors, and some effectors are recognized by multiple family members. Interaction with and activation of the effector function stimulates signaling pathways that mediate the diverse functions of Rho-family proteins. The multitude of effectors identified for RhoA, Rac1, Cdc42 and other family members (Aspenström, 1999; Bishop and Hall, 2000) reflects the complex and diverse functional properties of these proteins.

The Rho-family of proteins

On the basis of primary amino acid sequence identity, structural motifs and biological function, the Rho family can be divided into six subfamilies that exhibit similar, but not identical, properties (Figs 2, 3). These are: the RhoA-related subfamily (RhoA, RhoB and RhoC); the Rac1-related subfamily [Rac1 (and its splice variant Rac1b), Rac2, Rac3 and



B

	Expression pattern	Regulated expression	Prenylation	Palmitoyl-ation
RhoA	Ubiquitous		GG	
RhoB	Ubiquitous	Yes	GG/F	Yes
RhoC	Ubiquitous		GG	
Rac1	Ubiquitous		GG	
Rac2	Hp		GG	
Rac3	Br, He, Pl., Pa	Yes	GG	
RhoG	Ubiquitous	Yes	GG	
Cdc42	Ubiquitous		GG	
TC10	He, Sm		F	Yes
TCL	He, Lu, Li		F?	Yes?
Chp/Wrch-2	Br, Sp, Lu, Te		No	
Wrch-1	?	Yes	GG?	
Rnd1	Br, Li		F	
Rnd2	Br, Te		F	
RhoE/Rnd3	Ubiquitous	Yes	F	
RhoD	?		F	
Rif	Ubiquitous		GG?	
RhoH/TTF	Hp	Yes	GG	
RhoBTB-1	Ubiquitous		No	
RhoBTB-2	Br		No	
Miro-1	Ubiquitous		No	
Miro-2	Ubiquitous		No	

Fig. 1. Post-translational modification and expression patterns of Rho proteins. (A) Representation of the different C-termini, the post-translational lipid modifications that occur at these sites in Rho proteins, and the additional membrane targeting signals. The first four represent variations seen in CAAX motif-terminating Rho GTPases. The CAAX sequence signals for either farnesyl (F) or geranylgeranyl (GG) isoprenoid modification of the cysteine residue, followed by proteolytic removal of the AAX residues, and carboxylmethylation (OMe) of the now-terminal cysteine residue. Rac1 (also Rac2, Rac3, RhoA, RhoC and Cdc42) has a polybasic [K/R] sequence followed by a GG-modified cysteine. Rnd proteins contain a polybasic sequence followed by an F modification of the cysteine. TC10 (and probably TCL) is modified by both F and a palmitoyl (P) group. RhoB is modified by a P group at one cysteine and either a F or GG group at the other cysteine. RhoD is also F-modified, whereas Rif is expected to be GG-modified. These modifications have been verified by direct or indirect analyses (Adamson et al., 1992b; Ando et al., 1992; Foster et al., 1996; Michaelson et al., 2001; Murphy et al., 1996; Yamane et al., 1991). RhoBTB, which does not undergo any known post-translational modifications, contains two BTB domains C-terminal of the GTPase domain, and does not terminate with a CAAX motif. Miro contains two EF-hand (EFH) motifs and one additional GTPase domain that are C-terminal of the Rho GTPase-like domain, and does not terminate with a CAAX motif. (B) A summary of expression patterns, regulated expression and post-translational modifications of the Rho-family proteins. The post-translational modifications that have not been experimentally verified are marked with a '?'. Abbreviations: Hp, hematopoietic; Br, brain; He, heart; Pl, placenta; Pa, pancreas; Sm, skeletal muscle; Lu, lung; Li, liver; Sp, spleen; Te, testis.

RhoG]; the Cdc42-related subfamily [Cdc42 (and its brain-specific C-terminal splice variant G25K), TC10, TCL, Chp/Wrch-2 and Wrch-1]; the Rnd subfamily (Rnd1, Rnd2, and RhoE/Rnd3); the RhoBTB subfamily; and the recently described Miro subfamily. In addition, RhoD, Rif and TTF/RhoH do not obviously fall into any of these subfamilies.

RhoA-related proteins

RhoA, RhoB and RhoC exhibit significant amino acid sequence identity (~85%), all stimulate actin-myosin contractility, and all are thought to interact with the same guanine nucleotide exchange factors (GEFs) and effectors. However, they clearly have some functional differences (see below). In large part, these are likely to be a consequence of divergence in their C-terminal 15 amino acids (where the highest degree of difference is found; Fig. 2), which dictate distinct subcellular locations. When overexpressed in cells as epitope-tagged proteins, RhoA is cytosolic and to a certain degree bound to the plasma membrane, RhoB is associated with the plasma membrane and endomembrane vesicles, and RhoC is cytosolic and associated with undefined perinuclear structures (Adamson et al., 1992a; Michaelson et al., 2001; Wang et al., 2003).

RhoB (Chardin et al., 1988) contrasts with RhoA and is also transcriptionally regulated. Both the RhoB mRNA and protein are unstable molecules and are upregulated by growth factors and during the G1 and S phases of the cell cycle (Jahner and Hunter, 1991; Zalcman et al., 1995), which indicates that RhoB has a role in cell proliferation. RhoB can be prenylated by either a geranylgeranyl (GG), like most other Rho proteins, or a farnesyl (F) isoprenoid group, and is modified additionally by the palmitate fatty acid (Adamson et al., 1992b) (Fig. 1). As opposed to RhoA and RhoC, RhoB seems to have a growth-inhibitory effect (Chen et al., 2000; Du and Prendergast, 1999). Expression of RhoB has been reported to be downregulated in tumors (Adnane et al., 2002). RhoB-deficient mice, which develop normally, show enhanced carcinogen-induced skin tumor formation (Liu et al., 2001). However, cell-type differences are seen and, like constitutively activated RhoA,

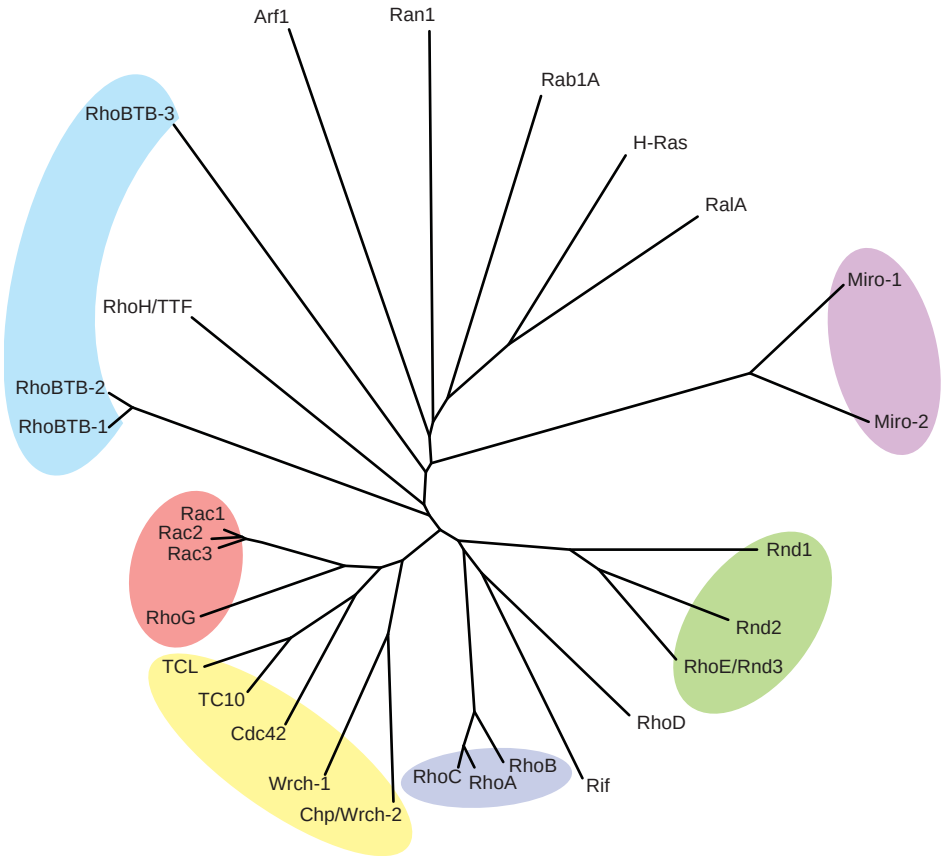


Fig. 2. Phylogenetic tree of the Rho-family GTPases and representatives of other Ras-superfamily GTPases. A phylogenetic analysis of the amino acid sequences of the Rho domains of the 22 Rho-family members made with ClustalW, together with functional data (see text) shows that the family can be roughly divided into six major branches: RhoA-related, Rac-related, Cdc42-related, Rnd proteins, RhoBTB proteins and Miro proteins.

constitutively activated RhoB can transform rodent fibroblasts (Prendergast et al., 1995; Wang et al., 2003). In addition, RhoB, but not RhoA or RhoC, can regulate the transport of late endosomes (Gampel et al., 1999; Mellor et al., 1998). Finally, RhoB might be targeted by farnesyltransferase inhibitors (FTIs). Although developed originally as anti-Ras drugs, the FTIs might exert their anti-tumor activity by forcing a shift of RhoB from the F to the GG form (Prendergast, 2000).

RhoC (Chardin et al., 1988) has also been connected to cancer development. It is upregulated in malignant pancreatic ductal carcinomas (Suwa et al., 1998), inflammatory breast cancer tumors (van Golen et al., 2000a) and highly metastatic melanomas (Clark et al., 2000). Ectopic overexpression of RhoC increases the tumorigenic and metastatic properties of tumor progenitor cells (Clark et al., 2000; van Golen et al., 2000a). In addition, RhoC induces the expression of angiogenic factors in human mammary epithelial cells (van Golen et al., 2000b), possibly facilitating the vascularization of tumors in which it is expressed. By contrast, activated RhoC does not transform NIH 3T3 mouse fibroblasts (Wang et al., 2003). Interestingly, we have found a GEF that interacts with RhoA and RhoB but not with RhoC (Arthur et al., 2002), and Sahai and Marshall have reported that RhoC binds more efficiently to Rho kinase, a major effector for the RhoA-like proteins, than does RhoA (Sahai and Marshall, 2002).

Rac1-related proteins

All the Rac-related proteins stimulate the formation of lamellipodia and membrane ruffles, presumably through interaction with the PIR121-Nap125-HSPC300-WAVE complex (Eden et al., 2002). Within the subfamily, Rac1, Rac2 and Rac3 share significant sequence identity (~88%). These three diverge primarily in the C-terminal 15 residues, whereas RhoG is somewhat more divergent overall (72% identical to Rac1). A splice variant of Rac1, Rac1b, generated by alternative exon usage, contains an additional 19-residue insert directly C-terminal to the switch II region. Rac1b is expressed preferentially in breast and colon cancers (Jordan et al., 1999; Schnelzer et al., 2000), possesses an increased intrinsic guanine nucleotide exchange rate and decreased intrinsic GTPase activity (Fiegen et al., 2004; Matos et al., 2003). Rac1b is also unable to interact with Rho guanine nucleotide dissociation inhibitor (RhoGDI) and, thus, it exhibits enhanced association with the plasma membrane (Matos et al., 2003). Consequently, it exhibits properties consistent with a constitutively activated protein. However, Rac1b also has differential signaling properties. It is unable to activate the JNK stress-activated kinase, but retains the ability to activate the NF-κB transcription factor (Matos et al., 2003). This might be explained by a differential binding to effectors, as highlighted by its strongly reduced affinity for the PAK serine/threonine kinase (Fiegen et al., 2003; Matos et al., 2003).

Rac1 expression is ubiquitous, whereas Rac2 (Didsbury et al., 1989) is expressed only in hematopoietic cells, where it seems to have specialized functions. A lack of Rac1 results in embryonic lethality (Sugihara et al., 1998), whereas a Rac2 deficiency allows normal development but results in mice that have hematopoietic cell defects (Roberts et al., 1999). Rac2 downregulation, deletion or inactivation correlates with several

neutrophilic, phagocytic and lymphocytic defects (Ambruso et al., 2000; Kasper et al., 2000; Li et al., 2000; Roberts et al., 1999; Williams et al., 2000). Many of these defects are probably caused by the Rac2-specific activation of NADPH oxidase (Dorseuil et al., 1996; Mizuno et al., 1992) and generation of reactive oxygen species (ROS) in hematopoietic cells (Werner, 2004). ROS production is important both in host defense mechanisms (Roberts et al., 1999) and in blood cell differentiation signaling (Li et al., 2000). The additional defects in hematopoietic cell function observed with a

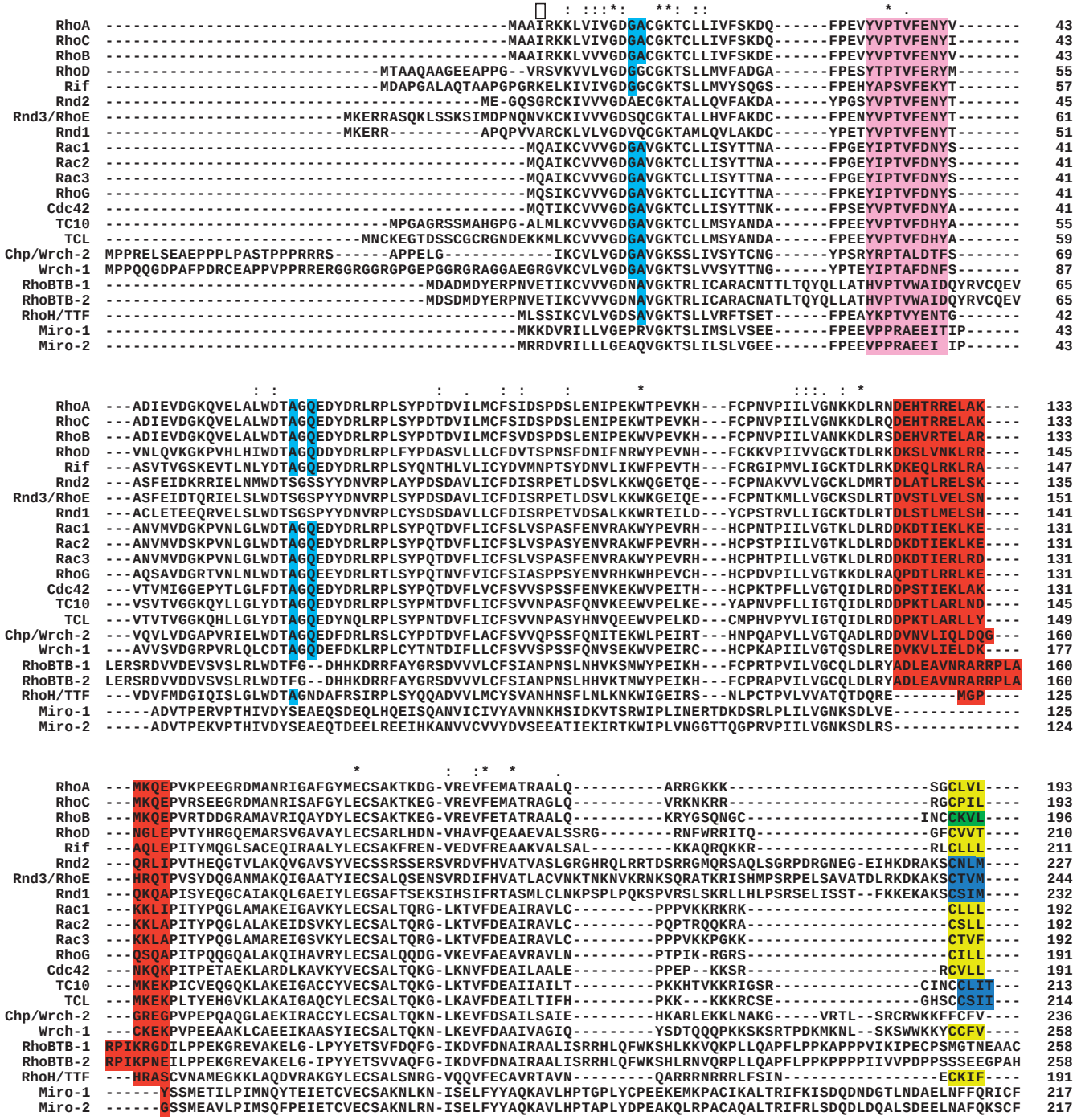


Fig. 3. Sequence alignment of the Rho family. The amino acid sequences of the 22 described Rho-family gene members (main isoforms) were aligned by ClustalW. Highlighted are residues important for GTPase activity (cyan), the core effector domain (pink), the Rho insert domain (red), and prenylation motifs (yellow: geranylgeranyl; blue: farnesyl; green: geranylgeranyl or farnesyl). Note that the amino acid sequences of RhoBTB-1, RhoBTB-2, Miro-1 and Miro-2 have been truncated in the C-termini. The protein sequences used correspond to the following accession numbers (from top to bottom): NP_001655, NP_786886, NP_004031, NP_055393, NP_061907, NP_005431, NP_005159, NP_055285, NP_008839, NP_002863, NP_005043, NP_001656, NP_001782, NP_036381, NP_065714, NP_598378, NP_067028, NP_055651, NP_055993, NP_004301, NP_060777 and NP_620124.

combined Rac1 and Rac2 deficiency further emphasize the overlapping and distinct roles of these two related GTPases in hematopoietic cells (Gu et al., 2003).

Rac3 is most highly expressed in brain but is upregulated upon serum stimulation of fibroblasts (Haataja et al., 1997). It is localized to the membrane to a higher degree than is Rac1 (Mira et al., 2000). Furthermore, it is hyperactive in breast cancers (Mira et al., 2000) and the *Rac3* gene is close to a region of chromosome 17 that is often deleted in breast cancers (Morris et al., 2000), which might deregulate its expression.

Vincent et al. first described *RhoG* as a late-response gene induced after serum stimulation of starved fibroblasts, suggesting that it is a transcriptionally regulated gene whose product is involved in regulation of the cell cycle (Vincent et al., 1992). Activated mutants of RhoG induce lamellipodia formation, membrane ruffling and to some extent formation of filopodia (Gauthier-Rouviere et al., 1998). How it signals is controversial; some reports say that it mediates its cellular effects by activating Rac1 and Cdc42 (Gauthier-Rouviere et al., 1998; Katoh and Negishi, 2003; Katoh et al., 2000), whereas others argue that it signals independently of them (Bellanger et al., 2000; Movilla and Bustelo, 1999; Prieto-Sanchez and Bustelo, 2003; Schuebel et al., 1998; Wennerberg et al., 2002). However, a recent report provides a mechanism for how RhoG can activate Rac1, implicating the DOCK180 RhoGEF (Katoh and Negishi, 2003). Katoh et al. have also reported that RhoG plays a specific role in nerve growth factor (NGF)- and/or Ras-mediated neurite outgrowth in PC12 pheochromocytoma cells (Katoh et al., 2000).

Cdc42-related proteins

The Cdc42-related proteins all stimulate the formation of filopodia, probably through their association with Wiscott-Aldrich syndrome protein (WASP) or N-WASP (Machesky and Insall, 1998; Miki et al., 1998); the possible exception to this is Wrch-1, which does not bind either WASP or N-WASP (Aspenström et al., 2004). Two isoforms of human (and mouse) Cdc42 arise from alternative exon splicing of the same gene (Marks and Kwiatkowski, 1996; Nicole et al., 1999). Both are 191-residue proteins that differ at residue 163 and in their C-terminal 10 amino acids. The most commonly studied form (sometimes referred to as placental Cdc42p, Cdc42Hs or Cdc42a) is expressed ubiquitously, whereas the other form (brain Cdc42p, Cdc42b or G25K) is restricted to the brain.

TC10 (Drivas et al., 1990) and TCL (also known as TC10β and RhoT; Vignal et al., 2000) are very closely related and have functions highly similar to that of Cdc42. TC10- and TCL-induced filopodia are longer than those promoted by activated Cdc42 (Murphy et al., 1999; Neudauer et al., 1998). TC10 and TCL interact with most Cdc42 effectors, but differences clearly exist (Murphy et al., 1999; Neudauer et al., 1998). In contrast to the exclusively geranylgeranylated C-terminus of Cdc42, the C-terminal CAAX motifs of TC10 and TCL suggest that they can be either farnesylated or geranylgeranylated. TC10 is also modified by palmitate, which prevents its recognition by RhoGDI-1 (Michaelson et al., 2001). The activities of TC10 and TCL seem to be regulated by extracellular stimuli different from those that activate Cdc42. TC10 and TCL have yet to be to found to be regulated by GEFs of the Dbl family (Schmidt and Hall, 2002), which are thought to be the most common activators of Rho-family proteins. Instead, Chiang et al. has suggested that TC10 is activated by the Rap GEF C3G upon insulin stimulation of adipocytes (Chiang et al., 2002). TC10 activation then mediates GLUT-4 translocation to the plasma membrane and is necessary for adipocyte differentiation (Nishizuka et al., 2003). In addition, TC10 expression and TCL expression are upregulated during nerve regeneration (Tanabe et al., 2000) and neurite differentiation (Abe et al., 2003), and activation of these GTPases stimulates neurite extension. Two different splice isoforms of TCL have been described (Chiang et al., 2002). One has a 10 amino acid extension at the N-terminus.

Aronheim et al. identified the rat protein Chp (or, as its human version is called, Wrch-2) as a Pak2-serine/threonine-kinase-interacting protein in a yeast two-hybrid screen (Aronheim et al., 1998). In common with activated versions of Cdc42, activated Chp binds Pak and N-WASP and stimulates formation of filopodia (Aronheim et al., 1998). Chp differs from other Rho-family members in that it lacks a conventional C-terminal CAAX prenylation sequence. Nevertheless, the C-terminus is needed for its biological effects (Aronheim et al., 1998).

The gene encoding Wrch-1 is upregulated by the Wnt-1 signaling pathway (Tao et al., 2001). Wnt-1 stimulates the Frizzled cell-surface receptor, leading to inactivation of the APC tumor suppressor and activation of β-catenin transcriptional activity and transformation. Expression of activated Wrch-1 in mammary epithelial cells mimics the morphological effects of Wnt-1 transformation (Tao et al., 2001), indicating that Wrch-1 is a crucial signaling component in Wnt-dependent oncogenesis. To date, it has not been determined whether known RhoGEFs, GTPase-activating proteins (GAPs) or GDIs are regulators of Chp and Wrch-1. However, the ability of a dominant-negative S45N version of Chp (based on analogous mutants of Ras and Rho GTPases that block GEF function) to block tumor necrosis factor α (TNF-α)-mediated signaling argues that Chp GEFs do exist.

Both Chp and Wrch-1 possess additional N-terminal and C-terminal sequences that flank their GTPase domains (Fig. 3). Interestingly, their N-terminal sequences contain proline-rich sequences that might serve as Src-homology 3 (SH3) domain recognition sequences. This suggests that SH3-domain-containing proteins could recognize and regulate their functions. Similarly, their additional C-terminal sequences might distinguish their functions and regulation from those of the more classical Cdc42-related proteins.

The Rnd subfamily

The three Rnd-family members show a fairly high degree of sequence identity to RhoA (45-49%) but nevertheless exhibit biochemical and functional features distinct from those of RhoA. First, they are considered to be GTPase deficient, they are not stable in either a GDP-bound or nucleotide-free form (Nobes et al., 1998), and RhoE/Rnd3 (also known as Rho8) is constitutively GTP bound in vivo (Foster et al., 1996). They also contain a substitution leading to low affinity for nucleotides. Second, they are farnesylated instead of geranylgeranylated (Foster et al., 1996; Nobes et al., 1998). Third, the striking functional feature of at least Rnd1 (also known as Rho6) and RhoE/Rnd3 is that, in contrast to the

stimulation of contractility and the subsequent formation of actin stress fibers and focal adhesions caused by the related RhoA GTPase, they inhibit these effects and cause cell rounding (Guasch et al., 1998; Nobes et al., 1998). The mechanism for this seems to be a combination of both binding and activating p190RhoGAP (Wennerberg et al., 2003) and, at least in the case of RhoE/Rnd3, binding and inactivating the RhoA effector ROCK1 (Riento et al., 2003). Another Rnd effector that could be involved in this effect is Socius (Katoh et al., 2002), but it is unclear how this contributes to the rounding activity of these proteins.

Rnd1 (Nobes et al., 1998) is predominantly expressed in adult brain and liver. However, in fetal tissue, it has a more ubiquitous expression pattern (Nobes et al., 1998) and, in *Xenopus* embryos, it is highly expressed in tissues that undergo extensive morphogenetic changes (Wunnenberg-Stapleton et al., 1999). Ectopic overexpression of Rnd1 in PC12 cells stimulates neurite extension (Aoki et al., 2000) and inhibits Ca^{2+} sensitization of smooth muscle cells (Loirand et al., 1999), but it is unclear whether these are physiologically significant activities of Rnd1.

Rnd2 (also known as Rho7 and RhoN) (Nobes et al., 1998) is most highly expressed in testis, brain and liver (Nishi et al., 1999; Nobes et al., 1998). Little is known of its function, but it has been shown to bind to three proteins: Vps4-A, a protein involved in endosome sorting (Tanaka et al., 2002); rapostlin, a protein that induces neurite branching (Fujita et al., 2002); and MgcRacGAP, a RhoGAP that has been implicated in cytokinesis (Naud et al., 2003).

Foster et al. initially identified RhoE/Rnd3 as a protein that interacts with p190RhoGAP (Foster et al., 1996), but the significance of this interaction was only clarified recently (Wennerberg et al., 2003) by the finding that p190RhoGAP is an effector in Rnd-mediated inhibition of RhoA function. RhoE/Rnd3 mRNA is ubiquitously expressed, but the protein seems to be sparsely present (Foster et al., 1996). Raf transformation of Madin-Darby canine kidney (MDCK) cells causes upregulation of RhoE/Rnd3, which mediates the stress fiber loss and multilayer growth that accompanies this transformation (Hansen et al., 2000). Similarly, in human epithelial cells, Ras transformation causes an upregulation of RhoE/Rnd3 protein levels (X. Singh and C.J.D., unpublished). Platelet-derived growth factor (PDGF)-stimulation of fibroblasts transiently stimulates synthesis of RhoE/Rnd3 protein, which accompanies the observed transient loss of stress fibers and rounding of the cell body (Riento et al., 2003). Thus, rather than depending on GDP/GTP cycling, RhoE/Rnd3 might be regulated at the level of transcription or translation.

RhoD and Rif

Although RhoD (also called RhoHP1) and Rif are structurally related (48% sequence similarity) and possess additional N-terminal sequences, initial studies indicated that they are functionally divergent with regards to effects on actin organization (Murphy et al., 1996; Ellis and Mellor, 2000). However, more-recent evidence suggests that they have common functions in the regulation of actin organization (Aspenström et al., 2004). RhoD has been implicated in regulating two distinct actin-associated cellular processes. First, it regulates the actin-dependent transport of early endosomes (Murphy et al., 1996) through binding to the Diaphanous-related formin protein hDia2C and subsequently activating Src (Gasman et al., 2003). Second, it promotes the disassembly of actin stress fibers and disruption of focal adhesions and inhibits cell motility (Murphy et al., 2001; Tsubakimoto et al., 1999). At this point, it is unclear whether the two processes are related. Another study showed that transient expression of a GTPase-deficient mutant of RhoD induces formation of filopodia, an activity similar to that seen with GTPase-deficient Rif in endothelial cells (Aspenström et al., 2004). This suggests that regulation of actin organization by RhoD depends on the cellular context.

Rif is widely expressed and promotes the formation of filopodia (Ellis and Mellor, 2000) even though it is only remotely related to Cdc42 (43% identity) and, on the basis of its effector domain sequence, one would not expect it to interact with Cdc42 effectors involved in actin reorganization (N-WASP and WASP). Interestingly, Rif-dependent filopodia formation is independent of Cdc42 and vice versa (Aspenström et al., 2004; Ellis and Mellor, 2000), and instead the two GTPases can cooperate in the generation of these structures.

RhoH/TTF

RhoH/TTF ('translocation three four') was identified originally as a chimeric transcript from a chromosome 3:4 translocation (Dallery et al., 1995) and subsequently renamed RhoH because of its hematopoietic-cell-specific expression. The *RhoH/TTF* gene is rearranged and deregulated or fused to other genes in both non-Hodgkin's lymphoma and multiple myeloma (Preudhomme et al., 2000). RhoH/TTF possesses a Rho insert sequence, albeit shorter than that seen in other Rho GTPases. Like the Rnd proteins, it lacks the conserved residues corresponding to G12 and Q61 found in other Rho GTPases and consequently is likely to be GTPase deficient and hence not regulated by GDP/GTP cycling (Li et al., 2002). Instead, the downregulation of RhoH/TTF expression by phorbol myristic acid suggests it is transcriptionally regulated. RhoH/TTF also interacts with all three RhoGDIs, which suggests that regulation of subcellular location is also another important mode of regulation. As opposed to most other Rho proteins, ectopic overexpression of activated RhoH/TTF has no effects on the actin cytoskeleton (Aspenström et al., 2004) but, instead, inhibits Rac1, RhoA and Cd42 signaling in T cells (Li et al., 2002).

The RhoBTB subfamily

Rivero et al. initially identified the RhoBTB subfamily of proteins in a database search for Rho-related GTPases in *Dictyostelium*, finding orthologs in *Drosophila* and vertebrates (Rivero et al., 2001). These atypical Rho GTPases are structurally different from the other Rho-family members and possess significant additional sequences following their Rho GTPase domain (Fig. 1). The additional C-terminal sequences include a tandem repeat of BTB domains and a lack of C-terminal CAAX prenylation signals. The BTB domain was identified initially in the *Drosophila* transcriptional repressors Broad complex, Tramtrack and Bric-a-brac, and can be found in ~200 human proteins (Collins et al., 2001). This family consists of three members in mammals: RhoBTB-1, RhoBTB-

2 and RhoBTB-3. RhoBTB-1 and RhoBTB-2 possess the Rho insert sequence that distinguishes Rho GTPases from all other Ras superfamily proteins. However, the GTPase domain of RhoBTB-3 is poorly conserved and not a typical Rho domain, and RhoBTB-3 should therefore not be considered a Rho-family protein.

RhoBTB2 was identified independently as a gene (*DBC2*) that is deleted, mutated or not expressed in many breast cancer cell lines and tissue samples (Hamaguchi et al., 2002). Expression of the wild-type protein, but not of mutant forms found in breast cancers, inhibits proliferation of T47D human breast cancer cells (Hamaguchi et al., 2002). Ectopic expression of RhoBTB-1 or RhoBTB-2, unlike most other Rho GTPases, does not cause alterations in actin organization or cell morphology (Aspenström et al., 2004).

The Miro subfamily

The Miro subfamily consists of two proteins: Miro-1 and Miro-2 (Fransson et al., 2003). These proteins contain two putative GTPase domains and two EF-hand motifs. The N-terminal GTPase domain bears most sequence similarity to other Rho GTPases. Because these proteins were found in a database search for proteins containing a Rho consensus domain, they have been referred to as atypical Rho proteins (Fransson et al., 2003). However, given their overall sequence divergence from other Rho GTPases, together with the lack of the Rho-specific insert sequences (Fig. 3), perhaps these should not be considered Rho proteins. Instead, they could be seen as a new family of Ras superfamily GTPases. The Miro GTPases localize to mitochondria and might promote apoptosis (Fransson et al., 2003). They do not have any effects on the actin cytoskeleton or cell morphology (Aspenström et al., 2004).

Regulation of Rho protein activity

Traditionally, the activities of Rho GTPases are considered to be regulated by the switching between an inactive, GDP-bound form and an active, GTP-bound form. However, recent findings have indicated that their activities are regulated in several additional ways. These alternative modes of regulation seem to be especially important for many of the less studied Rho GTPases.

Regulation by nucleotide binding

The GDP/GTP cycling of Rho-family proteins is controlled mainly by three distinct functional classes of regulatory protein: (1) GEFs, which stimulate the weak intrinsic exchange activity of Rho-family proteins to promote an exchange of the bound GDP for GTP and thus formation of active Rho-GTP (Schmidt and Hall, 2002) and association with downstream effectors; (2) GAPs, which stimulate the intrinsic GTP hydrolysis activity of Rho-family proteins and thereby promote formation of the inactive GDP-bound protein (Moon and Zheng, 2003); and (3) GDIs, which inhibit Rho proteins by blocking nucleotide exchange, and thus the binding of effectors and GAPs to GTP-bound Rho GTPases (Olofsson, 1999). RhoGDIs also regulate association of Rho GTPases with membranes.

With a few exceptions, the regulation of these three families is unclear. The RhoGEF family is large, consisting of ~60 members belonging to the Dbl family of proteins (Schmidt and Hall, 2002) and ~10 members belonging to the more recently discovered Dock family (Côté and Vuori, 2002). The RhoGAP family is also vast: ~80 distinct members are encoded in the human genome, and their modes of regulation are defined even less well than those of the RhoGEFs. Outside their RhoGEF or RhoGAP domains, these proteins are highly diverse in sequence and domain structure. This diversity probably reflects the fact that Rho GTPase activity can be regulated in a multitude of fashions (Moon and Zheng, 2003; Schmidt and Hall, 2002).

Regulation by lipid modification and subcellular localization

Rho GTPase function is critically dependent on association of the GTPases with membranes and their subcellular locations (Fig. 4). These properties are influenced by C-terminal lipid modifications and sequences (Fig. 1), as well as by interaction with RhoGDIs. The diversity of the cellular distributions of Rho GTPases reflects the important role that location has in their function.

As opposed to RhoGEFs and RhoGAPs, there are only a few known RhoGDIs. Three well-defined RhoGDIs have been identified: RhoGDI-1 (α), RHoGDI-2 (D4/LyGDI/β), and RhoGDI-3 (γ), and a few other proteins are suggested to have GDI activity towards Rho proteins (Anastasiadis et al., 2000; Degani et al., 2002). The three conventional GDIs seem to have different Rho-binding specificites. Whereas RhoGDI-1 binds well to RhoA, Rac1 and Cdc42, RhoGDI-3 binds well to RhoB and RhoG, and seemingly with a lower affinity to RhoA and Cdc42, but not to Rac1 and Rac2 (Adra et al., 1997; Zalcman et al., 1996). The targets for RhoGDI-2 remain unclear, but it does not bind well to RhoA, Rac1 or Cdc42 (Olofsson, 1999). Interestingly, the RhoGDI-1 concentration in the cell compares with that of the target GTPases (Michaelson et al., 2001). This stoichiometry indicates that a large fraction of Rho proteins in a cell are bound and inactivated by GDIs and therefore that binding of Rho to Rho-GDI is a potent regulatory mechanism. Several proteins bind to RhoGDIs and might thereby regulate their association with Rho proteins (Takahashi et al., 1997; Yamashita and Tohyama, 2003), for example, by releasing Rho proteins at membrane sites where they are needed. Changes in RhoGDI expression have been reported in some cancers (Gildea et al., 2002; Jones et al., 2002).

The majority of Rho GTPases terminate with CAAX tetrapeptide sequences that are isoprenylated. Some Rho GTPases are modified by the C15 F isoprenoid, and others are modified by the more hydrophobic C20 GG isoprenoid modification (Ghomashchi et al., 1995; Silvius and l'Heureux, 1994) (Fig. 1). Despite the fact that the GG modification promotes stronger membrane association, the type of isoprenoid does not seem to contribute to functional distinctions, except perhaps in the case RhoB (Allal et al., 2000; Joyce and Cox, 2003; Michaelson et al., 2001; Solski et al., 2002). Isoprenylation alone is not sufficient to determine the subcellular location of Rho GTPases. As in the case of Ras proteins, a second C-terminal signal is required. Some members are modified additionally by palmitate at C-terminal

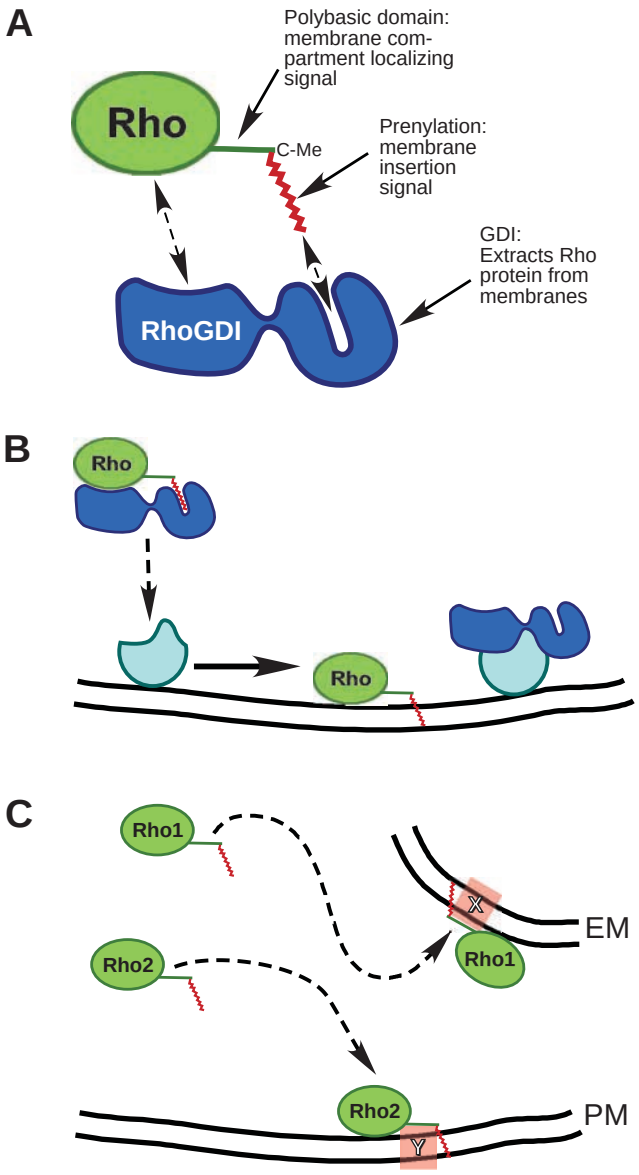


Fig. 4. Examples of Rho regulation by membrane localization. (A) Schematic presentation of elements in Rho that dictate membrane targeting. (B) Membrane delivery of Rho protein by Rho-complexed RhoGDI binding to target molecules at specific membrane sites followed by release of Rho protein to the membrane. (C) The polybasic domain found in some Rho-family proteins provides a second signal that dictates membrane localization. The components in the membranes (here depicted as 'X' and 'Y') that specifically bind to and localize each Rho protein (here examplified by the names 'Rho1' and 'Rho2') are unknown. Abbreviations: PM, plasma membrane; EM, endomembrane.

cysteine residues immediately upstream of the CAAX motif, whereas some contain lysine/arginine-rich sequences, and others contain both sequence elements. Palmitoylation can also influence RhoGDI recognition (Michaelson et al., 2001). For example, the palmitoylation of RhoB prevents RhoGDI-1 recognition and hence further influences subcellular localization (Michaelson et al., 2001). Some Rho GTPases lack conventional CAAX motifs (Chp, RhoBTB and Miro); whether their C-terminal sequences are still important for subcellular localization is not known.

This diversity in C-terminal sequences contributes to the diversity of functions seen with otherwise highly related Rho GTPases. Studies of the subcellular localization of green fluorescent protein (GFP)-tagged Rho proteins in living cells (Michaelson et al., 2001) concluded that even closely related Rho proteins could be localized to very different subcellular compartments in the cell. For example, whereas Rac1 is largely located at the plasma membrane, its close relative Rac2 (92% identity) mainly localizes to endomembranes. Distinct subcellular localization might dictate distinct upstream activation/inactivation and the downstream effector signaling. The same is true for the highly related RhoA, RhoB and RhoC proteins. A recent study developed an innovative approach to demonstrate this diversity of targeting. Zheng and colleagues showed that the GAP domain of the RhoA, RhoB and RhoC GAP p190 RhoGAP, when fused to the C-terminus of either RhoA, RhoB or RhoC, can specifically inhibit the function of each of the three GTPases, respectively (Wang et al., 2003). This, together with their association with distinct membrane compartments, emphasizes that these structurally and biochemically related Rho isoforms have distinct cellular functions. Further evidence came from the recent work of Hordijk and colleagues. They showed that peptides corresponding to the polybasic regions of RhoA, Cdc42, Rac1 and Rac2 could specifically inhibit the corresponding GTPase in cells, concluding that the these motifs bind to saturatable sites and that this is important for function (van Hennik et al., 2003).

In addition, RhoGDIs play an important role in regulating subcellular localization of Rho. For example, Del Pozo et al. have shown that RhoGDI delivers activated Rac1 and Cdc42 at new sites of integrin adhesion to allow protrusion at the leading edge of a migrating cell (Del Pozo et al., 2002). RhoG is localized to the Golgi complex by RhoGDI-3 (Brunet et al., 2002). RhoA and RhoB localize very differently in the cell (Gampel et al., 1999; Michaelson et al., 2001), potentially because of their differential GDI binding. Binding of RhoA to GDI can be regulated through phosphorylation of a C-terminal serine in RhoA by protein kinase A (Lang et al., 1996) and binding of other Rho proteins to GDIs might be regulated in similar ways (Ellerbroek et al., 2003). RhoGDI-2 is downregulated in many metastatic tumor samples, and re-expression of RhoGDI-2 in metastatic cell lines that do not express it can inhibit their metastatic and migratory behavior (Gildea et al., 2002). However, as the GTPase(s) targeted by RhoGDI-2 is not known, which RhoGTPases the downregulation affects is unclear.

Regulation by gene expression

In addition to being regulated by GEFs, GAPs and GDIs, many Rho GTPases seem to be highly regulated at the level of their expression (Fig. 1). Whereas the mRNA and protein levels of Rho GTPases such as RhoA, Rac1 and Cdc42 are relatively stable in essentially all tissues, those of the majority are not. Several Rho proteins are restricted to certain tissues, which indicates that they perform specialized functions in these cells. Rac2, for example, has a specialized role in the production of oxygen radicals in host defense mechanisms in hematopoietic

cells (Roberts et al., 1999). Furthermore, several of the less studied Rho GTPases seem to be transcriptionally regulated, which suggests that their genes are only transcribed when needed (see above). For example, *RhoG* is a growth-stimulus-induced early response gene (Vincent et al., 1992). Since members of the Rnd subfamily do not seem to be regulated by GTP/GDP loading (see above), and instead their levels are upregulated by mitogenic stimuli (Hansen et al., 2000; Riento et al., 2003), these proteins might also be controlled at the level of transcription or translation. The importance of transcriptional regulation is further strengthened by the fact that some Rho GTPases (e.g. RhoB) are unstable and therefore have short half-lifes in the cell (Lebowitz et al., 1995).

Conclusions

Although 22 distinct genes encode at least 25 human Rho-family GTPases, our current knowledge of Rho GTPase function is based predominantly on what we know about RhoA, Rac1 and Cdc42. The emphasis on these three proteins is in part because of the availability of reagents to study their function and perhaps in part because of their crucial roles in cell physiology. Nevertheless, as the other Rho GTPases are further studied, some are likely to increase in stature in cell biology. Some of these clearly have functions distinct from the three 'classical' Rho proteins. Others have signaling properties and effects on the cytoskeleton that seem to overlap those of RhoA, Rac1 and Cdc42. Furthermore, they might be involved in many biological phenomena in which it had been concluded that RhoA, Rac1 or Cdc42 might play a role, solely because these three were the only Rho proteins considered and the tools used, such as dominant-negative mutants or protein overexpression, were not truly specific. This situation is further complicated because many of the commercially available antibodies towards Rho proteins crossreact with several family members. Other less-studied Rho proteins will probably also be shown to play roles in processes where Rho-family members have not been implicated before. Future studies with awareness of the large number of similar Rho proteins, their cellular expression patterns and subcellular localizations, together with the use of more-specific reagents, will hopefully clarify the individual roles of each Rho protein.

We apologize for not being able to cite original work of many colleagues owing to space constraints. We thank Kent Rossman for many valuable discussions and suggestions. Our studies were supported by grants from the National Institutes of Health (CA63071, CA67771 and CA92240). K.W. was supported by a fellowship from the Swedish Foundation for International Cooperation in Research and Higher Education.

References

Abe, T., Kato, M., Miki, H., Takenawa, T. and Endo, T. (2003). Small GTPase Tc10 and its homologue RhoT induce N-WASP-mediated long process formation and neurite outgrowth. *J. Cell Sci.* **116**, 155-168.

Adamson, P., Paterson, H. F. and Hall, A. (1992a). Intracellular localization of the P21rho proteins. *J. Cell Biol.* **119**, 617-627.

Adamson, P., Marshall, C. J., Hall, A. and Tilbrook, P. A. (1992b). Post-translational modifications of p21rho proteins. *J. Biol. Chem.* **267**, 20033-20038.

Adnane, J., Muro-Cacho, C., Mathews, L., Sebti, S. M. and Munoz-Antonia, T. (2002). Suppression of rho B expression in invasive carcinoma from head and neck cancer patients. *Clin. Cancer Res.* **8**, 2225-2232.

Adra, C. N., Manor, D., Ko, J. L., Zhu, S., Horiuchi, T., van Aelst, L., Cerione, R. A. and Lim, B. (1997). RhoGDIgamma: a GDP-dissociation inhibitor for Rho proteins with preferential expression in brain and pancreas. *Proc. Natl. Acad. Sci. USA* **94**, 4279-4284.

Allal, C., Favre, G., Couderc, B., Salicio, S., Sixou, S., Hamilton, A. D., Sebti, S. M., Lajoie-Mazenc, I. and Pradines, A. (2000). RhoA prenylation is required for promotion of cell growth and transformation and cytoskeleton organization but not for induction of serum response element transcription. *J. Biol. Chem.* **275**, 31001-31008.

Ambruso, D. R., Knall, C., Abell, A. N., Panepinto, J., Kurkchubasche, A., Thurman, G., Gonzalez-Aller, C., Hiester, A., deBoer, M., Harbeck, R. J. et al. (2000). Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation. *Proc. Natl. Acad. Sci. USA* **97**, 4654-4659.

Anastasiadis, P. Z., Moon, S. Y., Thoreson, M. A., Mariner, D. J., Crawford, H. C., Zheng, Y. and Reynolds, A. B. (2000). Inhibition of RhoA by p120 catenin. *Nat. Cell Biol.* **2**, 637-644.

Ando, S., Kaibuchi, K., Sasaki, T., Hiraoka, K., Nishiyama, T., Mizuno, T., Asada, M., Nunoi, H., Matsuda, I., Matsuura, Y. et al. (1992). Post-translational processing of rac p21s is important both for their interaction with the GDP/GTP exchange proteins and for their activation of NADPH oxidase. *J. Biol. Chem.* **267**, 25709-25713.

Aoki, J., Katoh, H., Mori, K. and Negishi, M. (2000). Rnd1, a novel rho family GTPase, induces the formation of neuritic processes in PC12 cells. *Biochem. Biophys. Res. Commun.* **278**, 604-608.

Aronheim, A., Broder, Y. C., Cohen, A., Fritsch, A., Belisle, B. and Abo, A. (1998). Chp, a homologue of the GTPase Cdc42Hs, activates the JNK pathway and is implicated in reorganizing the actin cytoskeleton. *Curr. Biol.* **8**, 1125-1128.

Arthur, W. T., Ellerbroek, S. M., Der, C. J., Burridge, K. and Wennerberg, K. (2002). XPLN, a guanine nucleotide exchange factor for RhoA and RhoB, but not RhoC. *J. Biol. Chem.* **277**, 42964-42972.

Aspenström, P. (1999). Effectors for the Rho GTPases. *Curr. Opin. Cell Biol.* **11**, 95-102.

Aspenström, P., Fransson, A. and Saras, J. (2004). Rho GTPases have diverse effects on the organization of the actin filament system. *Biochem. J.* **377**, 327-337.

Bellanger, J. M., Astier, C., Sardet, C., Ohta, Y., Stossel, T. P. and Debant, A. (2000). The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nat. Cell Biol.* **2**, 888-892.

Bishop, A. L. and Hall, A. (2000). Rho GTPases and their effector proteins. *Biochem. J.* **348**, 241-255.

Brunet, N., Morin, A. and Olofsson, B. (2002). RhoGDI-3 regulates RhoG and targets this protein to the Golgi complex through its unique N-terminal domain. *Traffic* **3**, 342-357.

Chardin, P., Madaule, P. and Tavitian, A. (1988). Coding sequence of human rho cDNAs clone 6 and clone 9. *Nucleic Acids Res.* **16**, 2717.

Chen, Z., Sun, J., Pradines, A., Favre, G., Adnane, J. and Sebti, S. M. (2000). Both farnesylated and geranylgeranylated RhoB inhibit malignant transformation and suppress human tumor growth in nude mice. *J. Biol. Chem.* **275**, 17974-17978.

Chiang, S. H., Hou, J. C., Hwang, J., Pessin, J. E. and Saltiel, A. R. (2002). Cloning and functional characterization of related TC10 isoforms, a subfamily of Rho proteins involved in insulin-stimulated glucose transport. *J. Biol. Chem.* **277**, 13067-13073.

Clark, E. A., Golub, T. R., Lander, E. S. and Hynes, R. O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* **406**, 532-535.

Collins, T., Stone, J. R. and Williams, A. J. (2001). All in the family: the BTB/POZ, KRAB, and SCAN domains. *Mol. Cell. Biol.* **21**, 3609-3615.

Côté, J. F. and Vuori, K. (2002). Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. *J. Cell Sci.* **115**, 4901-4913.

Dallery, E., Galiegue-Zouitina, S., Collyn-d'Hooghe, M., Quief, S., Denis, C., Hildebrand, M. P., Lantoine, D., Deweindt, C., Tilly, H., Bastard, C. et al. (1995). TTF, a gene encoding a novel small G protein, fuses to the lymphoma-associated LAZ3 gene by t(3;4) chromosomal translocation. *Oncogene* **10**, 2171-2178.

Degani, S., Balzac, F., Brancaccio, M., Guazzone, S., Retta, S. F., Silengo, L., Eva, A. and Tarone, G. (2002). The integrin cytoplasmic domain-associated protein ICAP-1 binds and regulates Rho family GTPases during cell spreading. *J. Cell Biol.* **156**, 377-387.

Del Pozo, M. A., Kiosses, W. B., Alderson, N. B., Meller, N., Hahn, K. M. and Schwartz, M. A. (2002). Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. *Nat. Cell Biol.* **4**, 232-239.

Didsbury, J., Weber, R. F., Bokoch, G. M., Evans, T. and Snyderman, R. (1989). rac, a novel ras-related family of proteins that are botulinum toxin substrates. *J. Biol. Chem.* **264**, 16378-16382.

Dorseuil, O., Reibel, L., Bokoch, G. M., Camonis, J. and Gacon, G. (1996). The Rac target NADPH oxidase p67phox interacts preferentially with Rac2 rather than Rac1. *J. Biol. Chem.* **271**, 83-88.

Drivas, G. T., Shih, A., Coutavas, E., Rush, M. G. and D'Eustachio, P. (1990). Characterization of four novel ras-like genes expressed in a human teratocarcinoma cell line. *Mol. Cell. Biol.* **10**, 1793-1798.

Du, W. and Prendergast, G. C. (1999). Geranylgeranylated RhoB mediates suppression of human tumor cell growth by farnesyltransferase inhibitors. *Cancer Res.* **59**, 5492-5496.

Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M. and Kirschner, M. W. (2002). Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* **418**, 790-793.

Ellerbroek, S. M., Wennerberg, K. and Burridge, K. (2003). Serine phosphorylation negatively regulates RhoA in vivo. *J. Biol. Chem.* **278**, 19023-19031.

Ellis, S. and Mellor, H. (2000). The novel Rho-family GTPase rif regulates coordinated actin-based membrane rearrangements. *Curr. Biol.* **10**, 1387-1390.

Fiegen, D., Haeusler, L. C., Blumenstein, L., Herbrand, U., Dvorsky, R., Vetter, I. R. and Ahmadian, M. R. (2004). Alternative splicing of Rac1 generates Rac1b, a self-activating GTPase. *J. Biol. Chem.* **279**, 4743-4749.

Foster, R., Hu, K. Q., Lu, Y., Nolan, K. M., Thissen, J. and Settleman, J. (1996). Identification of a novel human Rho protein with unusual properties: GTPase deficiency and in vivo farnesylation. *Mol. Cell. Biol.* **16**, 2689-2699.

Fransson, A., Ruusala, A. and Aspenstrom, P. (2003). Atypical Rho GTPases have roles in mitochondrial homeostasis and apoptosis. *J. Biol. Chem.* **278**, 6495-6502.

Fujita, H., Katoh, H., Ishikawa, Y., Mori, K. and Negishi, M. (2002). Rapostlin is a novel effector of Rnd2 GTPase inducing neurite branching. *J. Biol. Chem.* **277**, 45428-45434.

Gampel, A., Parker, P. J. and Mellor, H. (1999). Regulation of epidermal growth factor receptor traffic by the small GTPase rhoB. *Curr. Biol.* **9**, 955-958.

Gasman, S., Kalaidzidis, Y. and Zerial, M. (2003). RhoD regulates endosome dynamics through Diaphanous-related formin and Src tyrosine kinase. *Nat. Cell Biol.* **5**, 195-204.

Gauthier-Rouviere, C., Vignal, E., Meriane, M., Roux, P., Montcourier, P. and Fort, P. (1998). RhoG GTPase controls a pathway that independently activates Rac1 and Cdc42Hs. *Mol. Biol. Cell* **9**, 1379-1394.

Ghomashchi, F., Zhang, X., Liu, L. and Gelb, M. H. (1995). Binding of prenylated and polybasic peptides to membranes: affinities and intervesicle exchange. *Biochemistry* **34**, 11910-11918.

Gildea, J. J., Seraj, M. J., Oxford, G., Harding, M. A., Hampton, G. M., Moskaluk, C. A., Frierson, H. F., Conaway, M. R. and Theodorescu, D. (2002). RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. *Cancer Res.* **62**, 6418-6423.

Gu, Y., Filippi, M. D., Cancelas, J. A., Siefring, J. E., Williams, E. P., Jasti, A. C., Harris, C. E., Lee, A. W., Prabhakar, R., Atkinson, S. J. et al. (2003). Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases. *Science* **302**, 445-449.

Guasch, R. M., Scambler, P., Jones, G. E. and Ridley, A. J. (1998). RhoE regulates actin cytoskeleton organization and cell migration. *Mol. Cell. Biol.* **18**, 4761-4771.

Haataja, L., Groffen, J. and Heisterkamp, N. (1997). Characterization of RAC3, a novel member of the Rho family. *J. Biol. Chem.* **272**, 20384-20388.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279**, 509-514.

Hamaguchi, M., Meth, J. L., von Klitzing, C., Wei, W., Esposito, D., Rodgers, L., Walsh, T., Welcsh, P., King, M. C. and Wigler, M. H. (2002). DBC2, a candidate for a tumor suppressor gene involved in breast cancer. *Proc. Natl. Acad. Sci. USA* **99**, 13647-13652.

Hansen, S. H., Zegers, M. M., Woodrow, M., Rodriguez-Viciana, P., Chardin, P., Mostov, K. E. and McMahon, M. (2000). Induced expression of Rnd3 is associated with transformation of polarized epithelial cells by the Raf-MEK-extracellular signal-regulated kinase pathway. *Mol. Cell. Biol.* **20**, 9364-9375.

Jahner, D. and Hunter, T. (1991). The ras-related gene rhoB is an immediate-early gene inducible by v-Fps, epidermal growth factor, and platelet-derived growth factor in rat fibroblasts. *Mol. Cell. Biol.* **11**, 3682-3690.

Jones, M. B., Krutzsch, H., Shu, H., Zhao, Y., Liotta, L. A., Kohn, E. C. and Petricoin, E. F., III (2002). Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. *Proteomics* **2**, 76-84.

Jordan, P., Brazao, R., Boavida, M. G., Gespach, C. and Chastre, E. (1999). Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. *Oncogene* **18**, 6835-6839.

Joyce, P. L. and Cox, A. D. (2003). Rac1 and Rac3 are targets for geranylgeranyltransferase I inhibitor-mediated inhibition of signaling, transformation, and membrane ruffling. *Cancer Res.* **63**, 7959-7967.

Kasper, B., Tidow, N., Grothues, D. and Welte, K. (2000). Differential expression and regulation of GTPases (RhoA and Rac2) and GDIs (LyGDI and RhoGDI) in neutrophils from patients with severe congenital neutropenia. *Blood* **95**, 2947-2953.

Katoh, H., Yasui, H., Yamaguchi, Y., Aoki, J., Fujita, H., Mori, K. and Negishi, M. (2000). Small GTPase RhoG is a key regulator for neurite outgrowth in PC12 cells. *Mol. Cell. Biol.* **20**, 7378-7387.

Katoh, H., Harada, A., Mori, K. and Negishi, M. (2002). Socius is a novel Rnd GTPase-interacting protein involved in disassembly of actin stress fibers. *Mol. Cell. Biol.* **22**, 2952-2964.

Katoh, H. and Negishi, M. (2003). RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo. *Nature* **424**, 461-464.

Lang, P., Gesbert, F., Delespine-Carmagnat, M., Stancou, R., Pouchelet, M. and Bertoglio, J. (1996). Protein kinase A phosphorylation of RhoA mediates the morphological and functional effects of cyclic AMP in cytotoxic lymphocytes. *EMBO J.* **15**, 510-519.

Lebowitz, P. F., Davide, J. P. and Prendergast, G. C. (1995). Evidence that farnesyltransferase inhibitors suppress Ras transformation by interfering with Rho activity. *Mol. Cell. Biol.* **15**, 6613-6622.

Li, B., Yu, H., Zheng, W., Voll, R., Na, S., Roberts, A. W., Williams, D. A., Davis, R. J., Ghosh, S. and Flavell, R. A. (2000). Role of the guanosine triphosphatase Rac2 in T helper 1 cell differentiation. *Science* **288**, 2219-2222.

Li, X., Bu, X., Lu, B., Avraham, H., Flavell, R. A. and Lim, B. (2002). The hematopoiesis-specific GTP-binding protein RhoH is GTPase deficient and modulates activities of other Rho GTPases by an inhibitory function. *Mol. Cell. Biol.* **22**, 1158-1171.

Liu, A. X., Rane, N., Liu, J. P. and Prendergast, G. C. (2001). RhoB is dispensable for mouse development, but it modifies susceptibility to tumor formation as well as cell adhesion and growth factor signaling in transformed cells. *Mol. Cell. Biol.* **21**, 6906-6912.

Loirand, G., Cario-Toumaniantz, C., Chardin, P. and Pacaud, P. (1999). The Rho-related protein Rnd1 inhibits Ca^{2+} sensitization of rat smooth muscle. *J. Physiol.* **516**, 825-834.

Machesky, L. M. and Insall, R. H. (1998). Scar1 and the related Wiskott-Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* **8**, 1347-1356.

Marks, P. W. and Kwiatkowski, D. J. (1996). Genomic organization and chromosomal location of murine Cdc42. *Genomics* **38**, 13-18.

Matos, P., Collard, J. G. and Jordan, P. (2003). Tumor-related alternative spliced Rac1b is not regulated by Rho-GDI and exhibits selective downstream signaling. *J. Biol. Chem.* **278**, 50442-50448.

Mellor, H., Flynn, P., Nobes, C. D., Hall, A. and Parker, P. J. (1998). PRK1 is targeted to endosomes by the small GTPase, RhoB. *J. Biol. Chem.* **273**, 4811-4814.

Michaelson, D., Silletti, J., Murphy, G., D'Eustachio, P., Rush, M. and Philips, M. R. (2001). Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. *J. Cell Biol.* **152**, 111-126.

Miki, H., Sasaki, T., Takai, Y. and Takenawa, T. (1998). Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature* **391**, 93-96.

Mira, J. P., Benard, V., Groffen, J., Sanders, L. C. and Knaus, U. G. (2000). Endogenous, hyperactive Rac3 controls proliferation of breast cancer cells by a p21-activated kinase-dependent pathway. *Proc. Natl. Acad. Sci. USA* **97**, 185-189.

Mizuno, T., Kaibuchi, K., Ando, S., Musha, T., Hiraoka, K., Takaishi, K., Asada, M., Nunoi, H., Matsuda, I. and Takai, Y. (1992). Regulation of the superoxide-generating NADPH oxidase by a small GTP-binding protein and its stimulatory and inhibitory GDP/GTP exchange proteins. *J. Biol. Chem.* **267**, 10215-10218.

Moon, S. Y. and Zheng, Y. (2003). Rho GTPase-activating proteins in cell regulation. *Trends Cell Biol.* **13**, 13-22.

Morris, C. M., Haataja, L., McDonald, M., Gough, S., Markie, D., Groffen, J. and Heisterkamp, N. (2000). The small GTPase RAC3 gene is located within chromosome band 17q25.3 outside and telomeric of a region commonly deleted in breast and ovarian tumours. *Cytogenet. Cell Genet.* **89**, 18-23.

Movilla, N. and Bustelo, X. R. (1999). Biological and regulatory properties of Vav-3, a new member of the Vav family of oncoproteins. *Mol. Cell. Biol.* **19**, 7870-7885.

Murphy, C., Saffrich, R., Grummt, M., Gournier, H., Rybin, V., Rubino, M., Auvinen, P., Lutcke, A., Parton, R. G. and Zerial, M. (1996). Endosome dynamics regulated by a Rho protein. *Nature* **384**, 427-432.

Murphy, C., Saffrich, R., Olivo-Marin, J. C., Giner, A., Ansorge, W., Fotsis, T. and Zerial, M. (2001). Dual function of rhoD in vesicular movement and cell motility. *Eur. J. Cell Biol.* **80**, 391-398.

Murphy, G. A., Solski, P. A., Jillian, S. A., Perez de la Ossa, P., D'Eustachio, P., Der, C. J. and Rush, M. G. (1999). Cellular functions of TC10, a Rho family GTPase: regulation of morphology, signal transduction and cell growth. *Oncogene* **18**, 3831-3845.

Naud, N., Toure, A., Liu, J., Pineau, C., Morin, L., Dorseuil, O., Escalier, D., Chardin, P. and Gacon, G. (2003). Rho family GTPase Rnd2 interacts and co-localizes with MgcRacGAP in male germ cells. *Biochem. J.* **372**, 105-112.

Neudauer, C. L., Joberty, G., Tatsis, N. and Macara, I. G. (1998). Distinct cellular effects and interactions of the Rho-family GTPase TC10. *Curr. Biol.* **8**, 1151-1160.

Nicole, S., White, P. S., Topaloglu, H., Beigthon, P., Salih, M., Hentati, F. and Fontaine, B. (1999). The human CDC42 gene: genomic organization, evidence for the existence of a putative pseudogene and exclusion as a SJS1 candidate gene. *Hum. Genet.* **105**, 98-103.

Nishi, M., Takeshima, H., Houtani, T., Nakagawara, K., Noda, T. and Sugimoto, T. (1999). RhoN, a novel small GTP-binding protein expressed predominantly in neurons and hepatic stellate cells. *Brain Res. Mol. Brain Res.* **67**, 74-81.

Nishizuka, M., Arimoto, E., Tsuchiya, T., Nishihara, T. and Imagawa, M. (2003). Crucial role of TCL/TC10beta L, a subfamily of Rho GTPase, in adipocyte differentiation. *J. Biol. Chem.* **278**, 15279-15284.

Nobes, C. D., Lauritzen, I., Mattei, M. G., Paris, S., Hall, A. and Chardin, P. (1998). A new member of the Rho family, Rnd1, promotes disassembly of actin filament structures and loss of cell adhesion. *J. Cell Biol.* **141**, 187-197.

Olofsson, B. (1999). Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. *Cell. Signal.* **11**, 545-554.

Prendergast, G. C. (2000). Farnesyltransferase inhibitors: antineoplastic mechanism and clinical prospects. *Curr. Opin. Cell Biol.* **12**, 166-173.

Prendergast, G. C., Khosravi-Far, R., Solski, P. A., Kurzawa, H., Lebowitz, P. F. and Der, C. J. (1995). Critical role of Rho in cell transformation by oncogenic Ras. *Oncogene* **10**, 2289-2296.

Preudhomme, C., Roumier, C., Hildebrand, M. P., Dallery-Prudhomme, E., Lantoine, D., Lai, J. L., Daudignon, A., Adenis, C., Bauters, F., Fenaux, P. et al. (2000). Nonrandom 4p13 rearrangements of the RhoH/TTF gene, encoding a GTP-binding protein, in non-Hodgkin's lymphoma and multiple myeloma. *Oncogene* **19**, 2023-2032.

Prieto-Sanchez, R. M. and Bustelo, X. R. (2003). Structural basis for the signaling specificity of RhoG and Rac1 GTPases. *J. Biol. Chem.* **278**, 37916-37925

Pruitt, K. and Der, C. J. (2001). Ras and Rho regulation of the cell cycle and oncogenesis. *Cancer Lett.* **171**, 1-10.

Riento, K., Guasch, R. M., Garg, R., Jin, B. and Ridley, A. J. (2003). RhoE binds to ROCK I and inhibits downstream signaling. *Mol. Cell. Biol.* **23**, 4219-4229.

Rivero, F., Dislich, H., Glockner, G. and Noegel, A. A. (2001). The *Dictyostelium discoideum* family of Rho-related proteins. *Nucleic Acids Res* **29**, 1068-1079.

Roberts, A. W., Kim, C., Zhen, L., Lowe, J. B., Kapur, R., Petryniak, B., Spaetti, A., Pollock, J. D., Borneo, J. B., Bradford, G. B. et al. (1999). Deficiency of the hematopoietic cell-specific Rho family GTPase Rac2 is characterized by abnormalities in neutrophil function and host defense. *Immunity* **10**, 183-196.

Sahai, E. and Marshall, C. J. (2002). ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat. Cell Biol.* **4**, 408-415.

Schmidt, A. and Hall, A. (2002). Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev.* **16**, 1587-1609.

Schmitz, A. A., Govek, E. E., Bottner, B. and van Aelst, L. (2000). Rho GTPases: signaling, migration, and invasion. *Exp. Cell Res.* **261**, 1-12.

Schnelzer, A., Prechtel, D., Knaus, U., Dehne, K., Gerhard, M., Graeff, H., Harbeck, N., Schmitt, M. and Lengyel, E. (2000). Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. *Oncogene* **19**, 3013-3020.

Schuebel, K. E., Movilla, N., Rosa, J. L. and Bustelo, X. R. (1998). Phosphorylation-dependent and constitutive activation of Rho proteins by wild-type and oncogenic Vav-2. *EMBO J.* **17**, 6608-6621.

Silvius, J. R. and l'Heureux, F. (1994). Fluorimetric evaluation of the affinities of isoprenylated peptides for lipid bilayers. *Biochemistry* **33**, 3014-3022.

Solski, P. A., Helms, W., Keely, P. J., Su, L. and Der, C. J. (2002). RhoA biological activity is dependent on prenylation but independent of specific isoprenoid modification. *Cell Growth Differ.* **13**, 363-373.

Sugihara, K., Nakatsuji, N., Nakamura, K., Nakao, K., Hashimoto, R., Otani, H., Sakagami, H., Kondo, H., Nozawa, S., Aiba, A. et al. (1998). Rac1 is required for the formation of three germ layers during gastrulation. *Oncogene* **17**, 3427-3433.

Suwa, H., Ohshio, G., Imamura, T., Watanabe, G., Arii, S., Imamura, M., Narumiya, S., Hiai, H. and Fukumoto, M. (1998). Overexpression of the rhoC gene correlates with progression of ductal adenocarcinoma of the pancreas. *Br. J. Cancer* **77**, 147-152.

Takahashi, K., Sasaki, T., Mammoto, A., Takaishi, K., Kameyama, T., Tsukita, S. and Takai, Y. (1997). Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein. *J. Biol. Chem.* **272**, 23371-23375.

Tanabe, K., Tachibana, T., Yamashita, T., Che, Y. H., Yoneda, Y., Ochi, T., Tohyama, M., Yoshikawa, H. and Kiyama, H. (2000). The small GTP-binding protein TC10 promotes nerve elongation in neuronal cells, and its expression is induced during nerve regeneration in rats. *J. Neurosci.* **20**, 4138-4144.

Tanaka, H., Fujita, H., Katoh, H., Mori, K. and Negishi, M. (2002). Vps4-A (vacuolar protein sorting 4-A) is a binding partner for a novel Rho family GTPase, Rnd2. *Biochem. J.* **365**, 349-353.

Tao, W., Pennica, D., Xu, L., Kalejta, R. F. and Levine, A. J. (2001). Wrch-1, a novel member of the Rho gene family that is regulated by Wnt-1. *Genes Dev.* **15**, 1796-1807.

Tsubakimoto, K., Matsumoto, K., Abe, H., Ishii, J., Amano, M., Kaibuchi, K. and Endo, T. (1999). Small GTPase RhoD suppresses cell migration and cytokinesis. *Oncogene* **18**, 2431-2440.

Valencia, A., Chardin, P., Wittinghofer, A. and Sander, C. (1991). The ras protein family: evolutionary tree and role of conserved amino acids. *Biochemistry* **30**, 4637-4648.

Van Aelst, L. and D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. *Genes Dev.* **11**, 2295-2322.

van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L. W. and Merajver, S. D. (2000a). RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. *Cancer Res.* **60**, 5832-5838.

van Golen, K. L., Wu, Z. F., Qiao, X. T., Bao, L. and Merajver, S. D. (2000b). RhoC GTPase overexpression modulates induction of angiogenic factors in breast cells. *Neoplasia* **2**, 418-425.

van Hennik, P. B., ten Klooster, J. P., Halstead, J. R., Voermans, C., Anthony, E. C., Divecha, N. and Hordijk, P. L. (2003). The C-terminal domain of Rac1 contains two motifs that control targeting and signaling specificity. *J. Biol. Chem.* **278**, 39166-39175.

Vetter, I. R. and Wittinghofer, A. (2001). The guanine nucleotide-binding switch in three dimensions. *Science* **294**, 1299-1304.

Vignal, E., de Toledo, M., Comunale, F., Ladopoulou, A., Gauthier-Rouviere, C., Blangy, A. and Fort, P. (2000). Characterization of TCL, a new GTPase of the rho family related to TC10 and Ccdc42. *J. Biol. Chem.* **275**, 36457-36464.

Vincent, S., Jeanteur, P. and Fort, P. (1992). Growth-regulated expression of rhoG, a new member of the ras homolog gene family. *Mol. Cell. Biol.* **12**, 3138-3148.

Wang, L., Yang, L., Luo, Y. and Zheng, Y. (2003). A novel strategy for specifically downregulating individual Rho GTPase activity in tumor cells. *J. Biol. Chem.* **278**, 44617-44625.

Wennerberg, K., Ellerbroek, S. M., Liu, R. Y., Karnoub, A. E., Burridge, K. and Der, C. J. (2002). RhoG signals in parallel with Rac1 and Cdc42. *J. Biol. Chem.* **277**, 47810-47817.

Wennerberg, K., Forget, M. A., Ellerbroek, S. M., Arthur, W. T., Burridge, K., Settleman, J., Der, C. J. and Hansen, S. H. (2003). Rnd proteins

function as RhoA antagonists by activating p190 RhoGAP. *Curr. Biol.* **13**, 1106-1115.

Werner, E. (2004). GTPases and reactive oxygen species: switches for killing and signaling. *J. Cell Sci.* **117**, 143-153.

Williams, D. A., Tao, W., Yang, F., Kim, C., Gu, Y., Mansfield, P., Levine, J. E., Petryniak, B., Derrow, C. W., Harris, C. et al. (2000). Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. *Blood* **96**, 1646-1654.

Wunnenberg-Stapleton, K., Blitz, I. L., Hashimoto, C. and Cho, K. W. (1999). Involvement of the small GTPases XRhoA and XRnd1 in cell adhesion and head formation in early *Xenopus* development. *Development* **126**, 5339-5351.

Yamane, H. K., Farnsworth, C. C., Xie, H. Y., Evans, T., Howald, W. N., Gelb, M. H., Glomset, J. A., Clarke, S. and Fung, B. K. (1991). Membrane-binding domain of the small G protein G25K contains an S-(all-trans-geranylgeranyl)cysteine methyl ester at its carboxyl terminus. *Proc. Natl. Acad. Sci. USA* **88**, 286-290.

Yamashita, T. and Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nat. Neurosci.* **6**, 461-467.

Zalcman, G., Closson, V., Linares-Cruz, G., Lerebours, F., Honore, N., Tavitian, A. and Olofsson, B. (1995). Regulation of Ras-related RhoB protein expression during the cell cycle. *Oncogene* **10**, 1935-1945.

Zalcman, G., Closson, V., Camonis, J., Honore, N., Rousseau-Merck, M. F., Tavitian, A. and Olofsson, B. (1996). RhoGDI-3 is a new GDP dissociation inhibitor (GDI). Identification of a non-cytosolic GDI protein interacting with the small GTP-binding proteins RhoB and RhoG. *J. Biol. Chem.* **271**, 30366-30374.