Review

The Rho small GTPase: Functions in health and disease

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Summary. Cell shape changes, contractility, adhesion, migration, gene transcription, cytokinesis, membrane trafficking, and growth, require Rho small GTPase function. The basis for this is that Rho regulates actin filament assembly, and serum response factor (SRF)-mediated gene transcription. Upon activation by serum or cell adhesion, Rho stimulates a distinct signal transduction pathway that induces cytoskeletal and transcriptional responses through diverse effectors. Rho activity is tightly controlled by guanine nucleotide exchange factors, GTPase activating proteins, and guanine dissociation inhibitors. Dysregulation of the Rho pathway is implicated in multiple pathological conditions including cancer and metastasis, cardiovascular disease, bacterial and viral pathogenesis, hepatic disease, and developmental disorders.

Key words: Rho, disease, small GTPase

Introduction

Regulation of cell shape, contraction, adhesion, motility, and proliferation is essential during development and for adult tissue function, and the ubiquitous Rho family of small GTPases play key roles in these processes (reviewed by Van Aelst and D'Souza-Schorey, 1997; reviewed by Bishop and Hall, 2000). The three main categories of Rho family GTPases, Rho, Rac, and Cdc42, are part of the Ras small GTPase superfamily. A recent analysis reveals six related classes in mammalian cells consisting of at least 16 distinct proteins: Rho (RhoA, RhoB, RhoC), Rnd (Rnd1/Rho6, Rnd2/Rho7, Rnd3/Rho8, RhoE), RhoD, RhoH/TTF, Rac (Rac1, Rac2, Rac3, RhoG), and Cdc42 (Cdc42Hs, G25K, TC10) (reviewed by Kjoller and Hall, 1999). Given the extensive literature on this family, this review focuses on the most well understood Rho members, RhoA, RhoB, and RhoC, and overviews recent progress in understanding their normal functions and discoveries concerning their role in disease.

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Rho-dependent cellular functions

Actin-myosin filament assembly

In response to many stimuli, cells rapidly form dynamic actin filamentous structures which allow the cell to perform particular functions such as contraction or motility. It is now evident that in virtually all cells studied, Rho, Rac, and Cdc42 each control the formation of distinct actin filament-based structures. Discoveries in the early 1990's revealed that, in response to serum, Rho regulates actin stress fiber formation (Ridley and Hall, 1992), Rac regulates lamellipodia formation (Ridley et al., 1992), and Cdc42 regulates filopodia formation (Kozma et al., 1995; Nobes and Hall 1995) in fibroblasts. Thus, in cells such as fibroblasts (Ridley and Hall, 1992) and endothelial cells (Wojciak-Stothard et al., 1998), Rho stimulation leads to rapid assembly of stress fibers composed of long contractile bundles of actin filaments interspersed with myosin; these traverse the cell and promote cell tension. The *Clostridium botulinum* C3 transferase toxin, a specific Rho inhibitor, blocks actin stress fiber formation (Chardin et al., 1989), and is a valuable reagent for analyzing Rho-dependent effects. In neuronal and astroglial cells, Rho activity induces retraction of cell processes and rounding of the cell body (Jalink et al., 1994; Katoh et al., 1996; Majumdar et al., 1998). Rho-dependent actin filament assembly plays a key role in smooth muscle contraction, neurite retraction, and cytokinesis (reviewed by Narumiya et al., 1997).

Two routes of Rho-induced actin filament assembly have been proposed which may act synergistically. A major route, shown in Figure 1, induces activation of myosin II, which is the motor protein for actin filaments. This route occurs via regulation of the phosphorylation state of myosin-binding subunit (MBS), the regulatory subunit of myosin light chain (MLC) phosphatase, by the serine-threonine Rho kinase family of Rho effectors. This family includes the p164 Rho-associated kinase, or ROKα (Matsui et al., 1996), and the closely related p160 Rho-associated coiled-coil containing protein kinase, or ROKβ/p160 ROK (Leung et al., 1995; Ishizaki et al., 1997). Upon binding to active Rho, ROK is activated and phosphorylates MBS, resulting in myosin

phosphatase inactivation, and subsequent inhibition of MLC dephosphorylation (Kimura et al., 1996). Accumulation of active, phosphorylated myosin causes increased acto-myosin assembly, contraction, and stress fiber formation (Kimura et al., 1996). ROK can also directly phosphorylate MLC (Amano et al., 1996a), providing another possible path to myosin activation.

Another route to Rho-induced stress fiber formation targets actin itself. This is mediated by several Rho effectors such as the group of formin homology proteins, the Dia family, which include Dia1 and Dia2 (reviewed by Wasserman, 1998). Dia members mediate actin reorganization through binding to profilin, which promotes monomeric actin filament assembly (Watanabe et al., 1997). Moreover, Dia- and ROK-mediated effects likely synergize to induce the extensive actin filament changes observed upon Rho activation (Nakano et al., 1999; reviewed by Ridley, 1999; Watanabe et al., 1999). Other components which mediate Rho-dependent actin effects are moesin (Mackay et al., 1997), a membrane-cytoskeleton linker protein which binds F-actin. While moesin does not interact with Rho, it may be positively regulated by ROK (Matsui et al., 1998), thus promoting actin assembly. Similarly, adducin may be a ROK substrate, leading to its interaction with F-actin, and actin polymerization (Kimura et al., 1998). Additionally,

Rho-induced phosphatidylinositol 4-phosphate 5-kinase (PI(4)P5K) activity (Chong et al., 1994), leading to phosphatidylinositol 4,5 bisphosphate (PI(4,5)P2) production, promotes actin polymerization (Gilmore and Burridge, 1996; reviewed by Toker, 2000). Furthermore, Rho-induced stimulation of the Na^+/H^+ exchange protein, NHE1 (Vexler et al., 1996), may promote actin polymerization via ROK (Tominaga et al., 1998).

Cell adhesion

Rho regulates cell adhesion by inducing formation of focal adhesions, or complexes of cytoskeletal proteins which associate with the integrin cytoplasmic tail (Ridley and Hall, 1992). In response to stimuli such as serum, focal adhesions form at the ends of stress fibers (reviewed by Burridge and Chrzanowska-Wodnicka, 1996), and this is required for integrin-mediated cell-extracellular matrix adhesion. Focal adhesions play a key role in cell migration, which is necessary for morphogenesis, wound repair, and metastasis. During cell migration, detachment from the matrix in the rear of the cell and re-adhesion in the front must occur. Rho-dependent focal adhesion formation and turnover play a key role in providing cell tension and points of traction that allow the cell to move, in addition to Rac and Cdc42-mediated events (reviewed by Horwitz and Parsons, 1999). While the requirement for Rho in focal adhesion formation is well established, the precise mechanism of Rho action on this multimolecular complex is partially understood. As with stress fibers, ROK activity plays an inductive role (Leung et al., 1996; Amano et al., 1997), although stress fiber and focal adhesion formation appear to be separable (Nobes and Hall, 1995).

In addition, Rho is required for cadherin-dependent cell-cell adhesion and subsequent adherens junction formation, but not desmosome formation, in epithelial cells. Inhibition of endogenous RhoA by microinjection of the C3 transferase blocks cadherin-mediated cell-cell adhesion in epithelial cells; the basis for this is thought to be Rho-mediated stimulation of rapid actin filament accumulation at the cell-cell contacts induced by cadherin adhesion (Braga et al., 1997; Takaishi et al., 1997). Rac function is also required for adherens junction formation, and the precise mechanism(s) by which Rho family members exert these effects is under investigation. Rho pathway components have been proposed to interact with catenins which associate with the cadherin cytoplasmic tail (Braga et al., 1997). Indeed, an effector for Cdc42 and Rac1 called IQGAP1 associates with beta-catenin (reviewed by Kaibuchi et al., 1999). Specific components that link the Rho pathway to this cell adhesion complex remain to be defined.

Gene transcription and cell proliferation

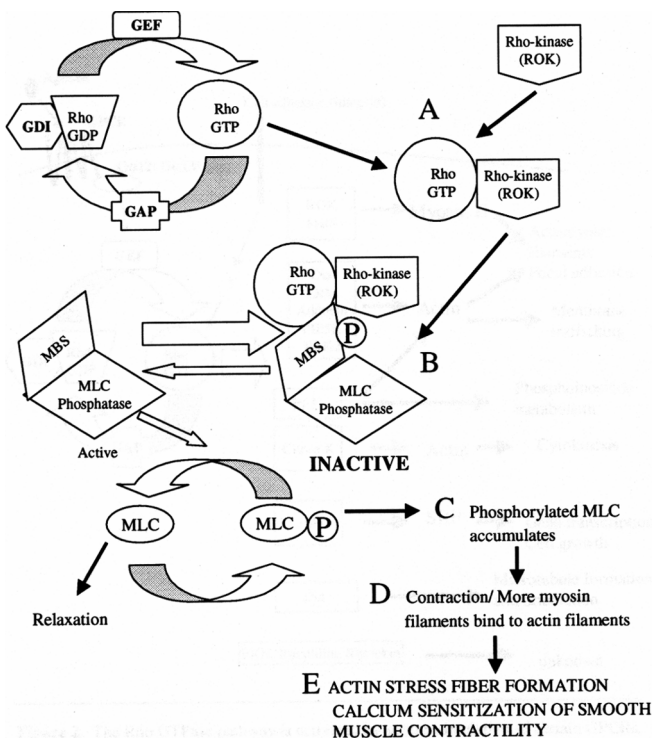In addition to cytoskeletal responses, Rho plays a key role in regulating gene transcription and cell cycle

Fig. 1. Mechanism of Rho-induced acto-myosin contractility via ROK. **A.** Rho activates its effector, ROK, which then **B.** inactivates the MLC phosphatase through phosphorylation. **C.** This allows phosphorylated MLC to accumulate, leading to **D.** increased binding of myosin filaments to actin filaments, and **E.** Subsequent contraction and actin stress fiber formation.

progression. Rho is reported to stimulate the transcriptional pathways mediated by the serum response factor (SRF) (Hill et al., 1995), nuclear factor κB (NFκB) (Perona et al., 1997), and the c-jun amino-terminal kinase/stress-activated protein kinase cascade (JNK/SAPK) (Teramoto et al., 1996). The intermediate effectors between Rho and the target transcription factors are poorly understood. Nevertheless, the functional link between Rho and SRF-mediated transcription is now well-established, indicating that Rho is required for serum-induced transcription from many promoters that encode a serum response element (SRE), including immediate early genes such as c-fos (Hill et al., 1995), ß- actin (Hill et al., 1995), and striated alpha-actin which is necessary during myogenesis (Wei et al., 1998). This finding has led to the common use of SRE transcriptional reporter constructs to assay Rho-induced signals, in vivo. A study aimed at delineating the mechanism of serum-induced SRF activation identified LIM-kinase-1, a substrate of ROK involved in F-actin aggregation, as an intermediate (Sotiropoulos et al., 1999). These findings indicate an intriguing convergence between Rho-induced gene transcription and actin dynamics. In addition to gene transcription, Rho GTPases regulate progression through the cell cycle (Olson et al., 1995), and Rho is required for Ras-mediated cell cycle progression and transformation (Qiu et al., 1995).

Membrane trafficking

Complex processes such as endocytosis, phagocytosis, and exocytosis involve the shuttling of intracellular membrane vesicles which is accompanied by reorganization of the actin cytoskeleton. On this basis, Rho has been implicated in these cellular trafficking processes (reviewed by Ellis and Mellor, 2000). Activated RhoA blocks clathrin-dependent endocytic internalization of the transferrin receptor (Lamaze et al., 1996) and the muscarinic acetylcholine receptor (Vogler et al., 1999), but increases clathrin-independent endocytosis (Schmalzing et al., 1995). Two other Rho isoforms, RhoB and RhoD, localize to endosomes (Adamson et al., 1992b; Murphy et al., 1996) and play a role in cellular trafficking. RhoB retards transport of activated EGF receptor from the endosome to the lysosome (Gampel et al., 1999), and RhoD regulates the rate of vesicle movement along cytoskeletal tracks (Murphy et al., 1996; reviewed by Allan and Schroer, 1999). RhoA also plays a role in secretion in rat mast cells (Price et al., 1995). Precise mechanisms of Rho-mediated membrane trafficking events remain to be defined.

Rho signaling pathway

Rho family GTPases are activated by distinct extracellular stimuli leading to their own separate intracellular signaling pathways and final outputs. Like all GTPases, Rho signaling is dependent on its cycling between an inactive, GDP-bound form and an active, GTP-bound form. Proper subcellular localization of Rho is necessary, and Rho cycles between the cytosol and membrane (Adamson et al., 1992b), depending on whether it is inactive or active, respectively. These two key processes are controlled by three types of Rho regulators: guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs), reviewed below. Multiple GEFs and GAPs for Rho have been identified; these regulators likely perform distinct functions by integrating different signal inputs onto the Rho target; in most cases, their own regulation is poorly understood.

Rho GEFs

Rho family GEFs were originally discovered based on their potent oncogenic activities when mutated (reviewed by Cerione and Zheng, 1996), indicating the key role of Rho GTPases in growth control. Rho family GEFs invariably contain a DH (Dbl homology) and PH (pleckstrin homology) domain cassette. The DH domain confers guanine nucleotide exchange activity by catalyzing the exchange of GDP for GTP, specifically for Rho GTPases (reviewed by Cerione and Zheng, 1996). The PH domain has multiple functions (reviewed by Lemmon and Ferguson, 1998). In addition, GEFs contain distinct signaling domains in their regulatory regions that potentially allow GEFs to transduce diverse signal inputs from cell surface receptors (reviewed by Cerione and Zheng, 1996).

The multiple Rho GEFs fall into two broad categories: Rho-specific GEFs that exclusively act on Rho, and broad-specificity GEFs that activate Rac and/or Cdc42 in addition to Rho. Lbc, the earliest reported Rho-specific GEF, activates Rho A, B, C, but not Rac or Cdc42 (Zheng et al., 1995). Lbc contains a putative Ca^{2+}-binding EF hand (Toksoz and Williams, 1994), while Brx, an Lbc isoform implicated in nuclear hormone receptor modulation, contains an additional putative diacylglycerol binding site (Rubino et al., 1998), and AKAP-Lbc, a longer splice variant, contains a protein kinase A anchoring site (Diviani et al., 2001). Additional Rho-specific GEFs include p115 Rho GEF (Hart et al., 1996), its mouse homologue Lsc (Whitehead et al., 1995; Glaven et al., 1996), and PDZ Rho GEF (Fukuhara et al., 1999). These Rho GEFs contain a recently defined domain, the LH (Lsc homology) domain (Fukuhara et al., 1999), which has distant homology to RGS (Regulator of G protein signaling) domains involved in heterotrimeric Gα subunit inactivation (Kozasa, 1998). p190 Rho GEF contains a zinc finger motif and is involved in the control of neuronal morphology (Gebbink et al., 1997), and NET1 (Chan et al., 1996) induces the SAPK/JNK pathway (Alberts and Treisman, 1998). More recently, LARG GEF, discovered as part of a human leukemia breakpoint

fusion product (Kourlas et al., 2000), has been identified as a Rho-specific GEF containing an LH domain (Reuther et al., 2001). The distinct signaling domain composition, subcellular localization, and tissue distribution of Rho-specific GEFs make it likely that they perform distinct functions.

Broad-specificity Rho GEFs include Dbl, the earliest discovered Rho family GEF which targets Rho and Cdc42 (Eva et al., 1988; Hart et al., 1994); Vav2, which can activate Rho, Rac and Cdc42, and plays a critical role in cell-mediated killing by cytotoxic lymphocytes (Abe et al., 2000; Billadeau et al., 2000); Trio (Debant et al., 1996), which contains separate Rho and Rac GEF domains (Bellanger et al., 1998); and Ect2, a GEF for Rho, Rac and Cdc42 (Tatsumoto et al., 1999) involved in cytokinesis (Kimura et al., 2000). The physiologic role of these GEFs is likely to be complex, since they activate more than one signal transduction pathway, and induce subsequent synergistic responses.

Rho GAPs

GAPs stimulate intrinsic GTPase activity by catalyzing the hydrolysis of GTP to GDP on Rho, leading to its inactivation (reviewed by Scheffzek et al., 1998). Similar to the situation with GEFs, multiple GAPs exist with varying specificity for Rho. One of the first Rho GAPs to be identified is the Rho-specific p190GAP which is also the major RasGAP-associated protein (Settleman et al., 1992; Foster et al., 1994). A screen to identify PLC-δ binding partners identified p122 RhoGAP (Homma and Emori, 1995) as a Rho-specific GAP. A recently identified protein with homology to the rat p122 protein, DLC-1, is often deleted in human liver cancer (Yuan et al., 1998). Finally, two of the myosin family members, myr 5 and myr 7, possess a GAP activity primarily on Rho, and, thus provide a direct link between Rho and the actin cytoskeleton (Reinhard et al., 1995; Chieregatti et al., 1998; Post et al., 1998). These myosin members appear to play important roles in regulating neuronal morphology and function.

GAPs with a broader specificity range include one of the earliest Rho family GAPs identified, the product of the breakpoint cluster region gene, Bcr. Bcr was originally reported to act on Rac, and not Rho (Ridley et al., 1993), and more recently, on RhoA, but at a much higher concentration (Zhang and Zheng, 1998). Similarly, the 3BP-1 protein was originally thought to be inactive towards Rho, but was later shown to act at higher concentrations (Zhang and Zheng, 1998). The p50 Rho GAP acts equally efficiently on Rho, Rac, and Cdc42 (Lancaster et al., 1994; Zhang and Zheng, 1998). A screen for binding partners of focal adhesion kinase identified Graf (GTPase regulator associated with FAK), a GAP for Rho and Cdc42 (Hildebrand et al., 1996). In addition, two separate X-linked, disease associated Rho GAPs, C1 and ARHGAP6, have been cloned (Tribioli et al., 1996; Schaefer et al., 1997) whose specificities

towards Rho family members remain to be determined.

Rho GDIs

GDP dissociation inhibitors are thought to regulate Rho in two ways. Firstly, they inhibit dissociation of the guanine nucleotide bound to Rho; secondly, they sequester the GDP-bound form of Rho in the cytoplasm by binding to the prenylated C-terminus which is important for membrane localization of Rho GTPases (reviewed by Olofsson, 1999). One proposed mechanism of RhoGDI function is that an interaction between RhoGDI and ezrin leads to release of Rho from the GDI, allowing Rho to be activated by a Rho GEF (Takahashi et al., 1997).

To date, three GDIs have been found which act on Rho: RhoGDI-1 (RhoGDIα) (Fukumoto et al., 1990; Gorvel et al., 1998), RhoGDI-2 (D4/Ly-GDI or RhoGDIß) (Lelias et al., 1993; Scherle et al., 1993), and RhoGDI-3 (RhoGDIδ) (Zalcman et al., 1996; Adra et al., 1997). The first GDI discovered, RhoGDI-1, appears to complex with, and inhibit, RhoA, Cdc42, Rac1, and Rac2 (Fukumoto et al., 1990; Gorvel et al., 1998). Subsequently, RhoGDI-2 was identified as a lymphocyte-specific protein (Lelias et al., 1993; Scherle et al., 1993). There is conflicting evidence for the GTPase specificity of RhoGDI-2, also referred to as D4 or LyGDI. While RhoGDI-2 was originally reported to inhibit GDP dissociation from RhoA (Scherle et al., 1993), more recently it was found that D4/LyGDI could not interact with RhoA (Gorvel et al., 1998). Finally, RhoGDI-3 (Zalcman et al., 1996; Adra et al., 1997) does not interact with RhoA, but does bind to RhoB and RhoG, two growth regulated GTPases (Zalcman et al., 1996).

Rho regulation by serum

A major pathway of Rho activation is through serum stimulation of certain G protein coupled-receptors (GPCRs). The first serum component shown to activate Rho responses was lysophosphatidic acid (LPA), a GPCR agonist which rapidly induces actin stress fiber formation in fibroblasts (Ridley and Hall, 1992). Subsequent studies have identified multiple other GPCR agonists which activate Rho signals such as thrombin, sphingosine 1-phosphate, bombesin, endothelin, and formyl-methionyl-leucyl-phenylalanine (FMLP) (reviewed by Seasholtz et al., 1999a). Elucidation of mechanisms of GPCR-induced Rho stimulation is an area of considerable interest. The Johnson lab first demonstrated that activated forms of heterotrimeric Gα12 and Gα13 subunits induce Rho-dependent stress fiber formation in fibroblasts (Buhl et al., 1995). Subsequent studies showed that activated Gα12/13 and Gαq stimulate Rho-dependent neurite retraction (Katoh et al., 1998) and astrocytoma cell rounding (Majumdar et al., 1999; Sagi et al., 2001). In addition to cytoskeletal effects, Gα12, Gα13, and each of the Gαq family

members induce SRF-mediated transcription via Rho (Mao et al., 1998). These studies establish that Gα12, Gα13, and Gαq, rather than Gαs or Gαi/o, induce Rho signals. While the mechanisms by which Gα12 and Gαq signals activate Rho are currently poorly understood, a mechanism of Gα13-induced Rho activation has been elucidated, at least in vitro. Hart et al. (1998) demonstrated that Gα13 directly stimulates the p115 Rho GEF via the GEF LH domain. In contrast, Gα12 and Gαq do not directly stimulate p115 Rho GEF (Hart et al., 1998), and it is possible that they transduce signals via another Rho GEF(s), as supported by several studies (Fukuhara et al., 1999; Majumdar et al., 1999; Sagi et al., 2001).

Rho regulation by cell adhesion

The second pathway of Rho stimulation is via adhesion receptors, in particular, the integrins. Ren et al. (1999) first showed that plating fibroblasts onto fibronectin coated plates results in a transient inhibition of Rho activity, followed by Rho activation; the latter is enhanced by serum. This indicates that adhesion and serum synergize to activate Rho. Another study demonstrates Rho activation by integrins in macrophages, which occurs through crosslinking of the complement receptor CR3 (Caron and Hall, 1998). Rac and Cdc42 are also activated by integrins, but Rho activation via integrins is only partially dependent on Rac and Cdc42 indicating that Rho may be regulated by a separate pathway (Clark et al., 1998). In addition to integrin-stimulated Rho activity, cadherin-mediated cell-cell adhesion may regulate Rho activity. For example, p120 catenin, which binds to cadherin, inhibits RhoA activity (Anastasiadis et al., 2000). More recently, cadherin engagement has been shown to inhibit Rho activity (Noren et al., 2001).

Rho effectors

Many different effectors for Rho have been identified which allow this single, small GTPase to regulate the multiple cellular processes described above (Fig. 2). One of the main functions of Rho is regulation of actin filament assembly. This process involves Rho effectors such as the Rho-associated kinases (p164ROKα and p160ROKß) and the myosin binding subunit (MBS) of the myosin light chain (MLC) phosphatase, both of which are important in actin stress fiber and focal adhesion formation, as reviewed above. ROK inhibits the myosin light chain phosphatase by phosphorylation, allowing accumulation of phosphorylated MLC required for actin filament assembly; ROK can also directly activate MLC by phosphorylation (Fig. 1). The diaphanous-related formin, p140 mDia (Watanabe et al., 1997; Nakano et al., 1999), and protein kinase C-related kinase (PRK2) (Vincent and Settleman, 1997), also play roles in actin reorganization events. Additionally, Dia 1 and Dia2 are reported to regulate the formation and orientation of stable

microtubules, independent of ROK (Palazzo et al., 2001). Rho is also involved in cytokinesis through the effector CitronK (Madaule et al., 1998). Phospholipase D, an enzyme that hydrolyzes phosphatidylcholine to choline and phosphatidic acid, is another Rho effector (Hess et al., 1997; Bae et al., 1998) implicated in actin stress fiber formation (Kam and Exton, 2001). In addition, Rho activity may modulate phosphoinositide metabolism via PI(4)P5K (Chong et al., 1994). Generation of PI(4,5)P2 by this enzyme may be involved in Rho-mediated, actin cytoskeletal rearrangements, since overexpression of PI(4)P5K in Cos-7 cells results in massive actin polymerization (Shibasaki et al., 1997). Furthermore, interaction between PI(4)P5K and Rho has been shown in Swiss-3T3-cell lysates (Ren et al., 1996). In terms of Rho-induced SRF-mediated transcription, the LIM-1 kinase appears to be an effector (Sotiropoulos et al., 1999). Other potential effectors which bind to Rho, but whose function remains to be determined include protein kinase N (PRK1) (Amano et al., 1996b; Watanabe et al., 1996), Rhophilin (Watanabe et al., 1996) and Rhotekin (Reid et al., 1996). Moreover the tumour suppressor Hamartin leads to actin stress fiber assembly via Rho (Lamb et al., 2000).

Rho and human disease

Transformation and tumorigenesis
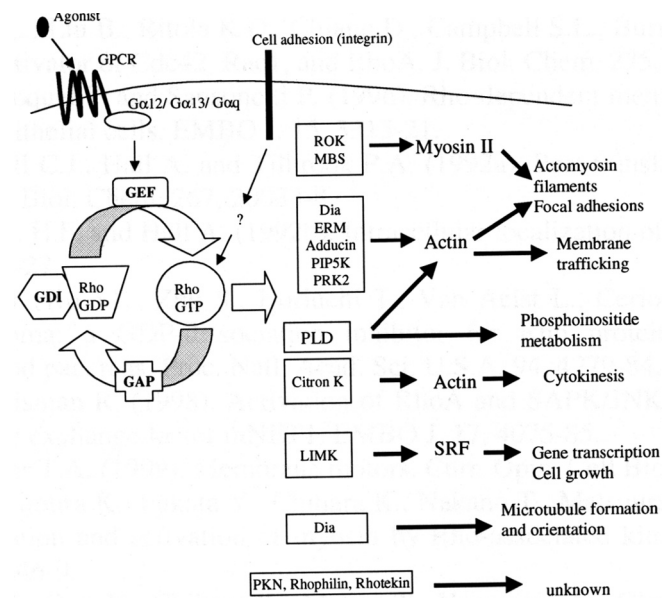
Rho pathway dysfunction is extensively linked to

Fig. 2. The Rho GTPase pathway is activated by serum stimulation of certain GPCRs, and by adhesion receptors. Rho activity is regulated by intracellular GRFs, GAPs, and GDIs. Activated Rho stimulates various Rho effectors (in rectangles) that mediate many different responses including formation of actin stress fibers and focal adhesions, SRF-mediated gene transcription, phosphoinositide metabolism, cytokinesis, and intracellular trafficking.

cell transformation and tumorigenesis. Early studies reported that RhoA gene amplification promotes fibroblast transformation (Avraham and Weinberg, 1989; Avraham, 1990). In addition, constitutively active, mutant forms of Rho altered at the homologous codons to Ras, such as RhoV14 and RhoL63, are transforming in vitro, and tumorigenic in vivo (Perona et al., 1993). However, in contrast to Ras, which is found mutated at the homologous codons in ~30% of human cancers (reviewed by Bos, 1989), to date there is no report of activating mutations in Rho at these codons in human cancer. Recently, however, it was observed that thirteen of twenty-eight (46%) B-cell diffuse large cell lymphoma (DLCL) samples contain mutations throughout the first 1.6 kb of the RhoH/TTF gene (Pasqualucci et al., 2001). All mutations identified are within non-coding regions of the gene suggesting that these mutations may have an effect on the regulation of RhoH expression. In rare leukemia-associated translocations, RhoH coding sequence is also found to be fused to the immunoglobulin locus, suggesting that RhoH may be a proto-oncogene analogous to c-myc (Preudhomme et al., 2000). This finding raises the possibility that Rho family GTPases may play a role in human disease through mutation at sites which differ from the mutation hot-spots historically documented for Ras (reviewed by Bos, 1989).

Additional Rho pathway components implicated in transformation and oncogenesis include several Rho GEFs, which are potent oncogenes when mutated, usually by truncation of regulatory regions (reviewed by Cerione and Zheng, 1996). Whether mutant Rho GEFs are implicated in human cancer is a possibility supported by the recent finding that the LARG Rho-specific GEF is part of the translocation breakpoint in an acute myeloid leukemia sample resulting in deletion of its N-terminal coding region (Kourlas et al., 2000). The pathologic effect of this mutation remains to be determined. Another implicated component is the PKN Rho effector which may promote breast epithelial cell immortalization by binding to, and phosphorylating, the immortalizing E6 protein of papilloma virus (Gao et al., 2000). Rho also plays an essential role in transformation via other signaling pathways; for example, Rho signals are required for ligand-independent oncogenic signaling by a mutant epidermal growth factor receptor (Boerner et al., 2000). In addition, Rho is required for transformation by mutant forms of the Ras GTPase (Prendergast et al., 1995; Qiu et al., 1995). Moreover, links of RhoA with apoptotic signaling pathways (reviewed by Aznar and Lacal, 2001) lend support to the notion that Rho may exhibit both transforming and tumor suppressor functions.

Metastasis

The key role played by Rho in cell migration, discussed above, provides the basis for its links to increased cancer invasion and metastasis (reviewed by Evers et al., 2000; Schmitz et al. 2000). Clark et al.

(2000) found selective upregulation of RhoC by gene expression profiling of both human and mouse metastatic melanoma cells, and showed that exogenous RhoC overexpression induces potent metastasis in poorly metastatic melanoma cells. In these studies, RhoA expresssion was not changed, and RhoC overexpression induced a stronger migratory and invasive response than RhoA (Clark et al., 2000), indicating functional differences between these otherwise highly similar Rho members. RhoC overexpression did not alter the cell proliferation rate, suggesting that the mechanism involves Rho effects on the actin cytoskeleton. This possibility is supported by Somlyo et al. (2000), who showed that the Y-27632 ROK inhibitor decreases migration and metastasis of the PC3 prostate carcinoma cell line. These and additional studies (Itoh et al., 1999; Wicki and Niggli, 2001) suggest the possible therapeutic value of inhibitors of Rho pathway components in controlling malignancy. These experimental data are in keeping with findings in primary human malignancies; for example, RhoC overexpression correlates with increased metastasis in pancreatic carcinomas (Suwa et al., 1998). In addition, RhoC overexpression is observed in a high proportion of inflammatory breast cancer (van Golen et al., 1999), and may contribute to the highly aggressive and invasive phenotype associated with this breast cancer type (van Golen et al., 2000). Furthermore, analysis of RhoA expression levels in breast, colon, and lung tumors (Fritz et al. 1999), reveals a correlation between increased levels of RhoA protein and increased tumor malignancy, particularly in breast tumors. The mechanism(s) leading to RhoA and RhoC overexpression in malignancy remains to be determined. Although inhibition of fibroblast invasion by dominant negative forms of Rho, Rac, and Cdc42 has shown the importance of these GTPases in migration and invasion (Banyard et al., 2000), expression of constitutively active forms of Rho or Cdc42 in fibroblasts may also inhibit invasion, suggesting that there is a key range of activation of the small GTPases required for invasive processes (Banyard et al., 2000).

In contrast to RhoA and RhoC, RhoB, which is a growth factor inducible gene (Zalcman et al., 1995), appears to be involved in tumor suppression as a target of the anticancer drugs called Farnesyltransferase inhibitors (FTIs), currently in clinical trials (reviewed by Prendergast, 2001). The basis of the RhoB response to FTI is that unlike RhoA and RhoC which are geranylgeranylated, RhoB exists in both a geranylgeranylated (RhoB-GG) and farnesylated (RhoB-F) form (Adamson et al., 1992a). FTI-induced increase in RhoB-GG levels is linked to cell cycle inhibition and reversal of the transformed phenotype. The precise mechanism of RhoB-GG action remains to be determined.

Cardiovascular disease

The Rho pathway has emerged as a novel signaling

pathway implicated in both increased contraction and remodeling of vascular smooth muscle (reviewed by Johns et al., 2000). One of the first studies linking RhoA to vascular hypertension showed that a constitutively active form of Rho, RhoVal14 (RhoV14), induces MLC phosphorylation and increased tension in permeabilized, rabbit mesenteric arteries (Gong et al., 1996). In a different approach, a screen for inhibitors of calcium sensitization of smooth muscle contraction identified the ROK inhibitor, Y27632 (Uehata et al., 1997), indicating that ROK plays a key role in this process through phosphorylating and inhibiting myosin phosphatase (reviewed by Somlyo, 1997) (Fig. 2). Additional studies (Kandabashi et al., 2000; Chrissobolis and Sobey, 2001) emphasize the role of RhoA and its effector, ROK, in vascular hypertension. Whether hypertension in humans involves RhoA and ROK function, and whether their inhibitors control hypertension, remains to be determined.

Aside from controlling vasoconstriction, RhoA is also linked with defects associated with increased vascular smooth muscle cell proliferation and migration, via ROK. Thrombin induces smooth muscle cell proliferation and migration, and Seasholtz et al. (1999b) showed that thrombin-stimulated DNA synthesis and cell migration in rat aortic smooth muscle cells require Rho and ROK function. A related study reported that ROK inhibition by Y27632 reverses the damaging effects of hyperproliferation and migration that occurs in response to balloon injury in rat vascular smooth muscle cells (Sawada et al., 2000).

Angiotensin II (Ang II) plays an important role in the pathogenesis of vascular smooth muscle cell hypertrophy which can lead to a thickening of arterial walls and hypertension (Yamakawa et al., 2000). RhoA overexpression in cardiac myocytes causes a similar phenotype characterized by increased assembly of contractile proteins into organized sarcomeric units, or myofibrillogenesis (Hoshijima et al., 1998). In addition, Rho and ROK appear to play a role in the events leading to this hypertrophy. Use of a dominant negative form of RhoA (Andresen et al., 2001), or treatment with C3 transferase or the ROK inhbitor Y27632 (Hoshijima et al., 1998; Yamakawa et al., 2000), block Angiotensin II-induced hypertrophy of vascular smooth muscle cells found during chronic hypertension.

Bacterial pathogenesis

Bacterial pathogens use two main approaches to infect the host organism. Firstly, the bacteria must avoid internalization and subsequent destruction by phagocytic cells; secondly, they often gain entry into epithelial cells where they are protected from the outside environment and can replicate and thrive. Both of these processes involve endocytic pathways which require Rho (reviewed by Ellis and Mellor, 2000). To avoid endocytosis by phagocytic cells, several pathogens secrete molecules that directly downregulate Rho signaling in the host cell. In addition, several pathogens require Rho function for their entry into epithelial cells, and their pathogenicity increases dramatically when Rho activity is upregulated.

Clostridium botulinum is one of the first pathogens found to secrete a molecule which acts on Rho: the commonly used C3 transferase ADP-ribosylates Rho on Asparagine 41, inactivating the GTPase (Ridley and Hall, 1992). A *Staphylococcus aureus* homologue, C3^{Stau}, ADP-ribosylates RhoA, RhoE and the RhoE isoform, Rnd (Wilde et al., 2001). The Staphylococcus C3, like the botulinum C3 transferase, is also cytotoxic to cultured cell lines and leads to actin filament disassembly. Another well-studied bacterial secretory molecule which modulates Rho function is ExoT from *Pseudomonas aeruginosa*, an opportunistic Gram-negative bacteria often found at sites of pre-existing epithelial cell damage. ExoT, similar to ExoS from the same organism, functions as a GAP to inhibit Rho activity (Krall et al., 2000). The ExoT Rho GAP also modulates phagocytic uptake of the bacteria (Kazmierczak et al., 2001). In addition, *Yersinia pseudotuberculosis*, which can cause acute ileitis, mesenteric lymphadenitis, and septicaemia, secretes a GAP called YopE, which has an anti-phagocytic function by stimulating Rho inactivation (Black and Bliska, 2000).

The virulence of many pathogens also depends on upregulation of Rho activity. For example, entry of *Shigella flexneri*, the agent of bacillary dysentery in humans, into epithelial cells requires Rho function (Adam et al., 1996; Mounier et al., 1999). Other pathogens that require Rho for entry include *Bartonella bacilliformis*, which causes a severe and often lethal form of anemia (Verma et al., 2000) ; (P-) *Neisseria gonorrhoeae*, the leading cause of bacterial meningitis and sepsis in children and young adults in the United States (Kallstrom et al., 2000); Respiratory Syncytial Virus, which causes severe and life-threatening respiratory infections in infants and immuno-compromised adults (Gower et al., 2001); and the intracellular mature virus (IMV) of the poxvirus family (Locker et al., 2000). Uropathogenic *Escherichia coli* often associated with urinary tract infections, also secrete a virulence factor, CNF1, which modulates the Rho GTPase by placing it in a constitutively active, GTP bound state through deamidation of glutamine 63 (Flatau et al., 1997; Schmidt et al., 1997).

Viral pathogenesis

The Rho pathway is potentially implicated in modulating infection by the HIV-1 AIDS virus based on the finding that the HIV-1 transmembrane protein, gp41, interacts with the p115 Rho GEF, leading to apparent inhibition of p115-mediated Rho activation (Zhang et al., 1999; Wang et al., 2000). The physiologic role of these

findings remains to be determined.

Hepatic disease

Fibrosis is one of the main characteristics of liver cirrhosis; and activation of hepatic stellate cells, leading to actin-based changes in cell morphology, plays a key role in the development of fibrosis (Yee, 1998). Several studies implicate Rho in actin cytoskeletal and morphology changes which occur during stellate cell activation (Kato et al., 1999; Kawada et al., 1999). Moreover, portal vein constriction is inhibited by the Y27632 ROK inhibitor (Yee, 1998), indicating a possible therapeutic role for this drug in treating the portal hypertension associated with liver cirrhosis. In terms of liver cancer, the DLC-1 Rho GAP is reported to be frequently deleted in human liver cancer (Yuan et al., 1998), indicating that it may be a tumor suppressor gene.

Developmental disorders

The Rho pathway is linked to one of the leading causes of deafness resulting from inner ear hair cell defects (reviewed by Muller and Littlewood-Evans, 2001). The Rho effector, p140Dia (discussed above), is the locus for a genetically inherited form of human deafness, non-syndromic deafness (DFNA1), due to its being a major component of the cytoskeleton of inner ear hair cells (Lynch et al., 1997). In addition, ARHGAP6 has been identified as a gene in the critical region for the X-linked dominant, male-lethal disorder, Microphthalmia with linear skin defects syndrome (MLS). Since cell morphology and motility, two processes requiring Rho-mediated actin organization, are severely disrupted in MLS, mutations in ARHGAP6 are proposed to play a role in MLS (Schaefer et al., 1997; Prakash et al., 2000). In addition, the Trio Rho/Rac GEF is implicated in skeletal muscle deformity and neuronal disorders (O'Brien et al., 2000), based on a murine genetic knock-out model.

Conclusions

Rho GTPase is required for multiple cellular processes based on its regulation of actin filament assembly and SRE-mediated gene transcription. Rho is stimulated by certain GPCR agonists and by cell adhesion, although the mechanisms are partially understood. While multiple Rho regulators and effectors have been found, questions remain regarding their role, their own control, and mechanisms of action. The ROK effector plays a key role in Rho-induced acto-myosin contractility. Differences in the normal function and disease link between RhoA, B, and C have begun to emerge. The Rho pathway is implicated in cancer, metastasis, cardiovascular hypertension and hypertrophy, liver fibrosis, developmental defects, and infection by pathogens. Further elucidation of the function and role of Rho pathway components in the aforementioned disease processes should potentiate development of novel therapeutic agents for intervention in defects involving cell contractility, adhesion, migration and growth.

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