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REGULATION OF THE CYTOSKELETON AND CELL ADHESION BY THE RHO FAMILY GTPASES IN MAMMALIAN CELLS

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■ **Abstract** Members of the Rho family of small Ras-like GTPases—including RhoA, -B, and -C, Rac1 and -2, and Cdc42—exhibit guanine nucleotide-binding activity and function as molecular switches, cycling between an inactive GDP-bound state and an active GTP-bound state. The Rho family GTPases participate in regulation of the actin cytoskeleton and cell adhesion through specific targets. Identification and characterization of these targets have begun to clarify how the Rho family GTPases act to regulate cytoskeletal structure and cell-cell and cell-substratum contacts in mammalian cells. The Rho family GTPases are also involved in regulation of smooth muscle contraction, cell morphology, cell motility, neurite retraction, and cytokinesis. However, the molecular mechanisms by which the Rho family GTPases participate in the regulation of such processes are not well established.

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INTRODUCTION

Dynamic rearrangements of the cytoskeleton and cell adhesion are required for various cellular processes, such as shape changes, migration, and cytokinesis (1–4). These temporal and spatial reorganizations of cell structure and cell contacts can be stimulated by extracellular signals, including growth factors, hormones, and other biologically active substances (1, 3, 4). The cytoskeleton consists mainly of three components: actin filaments, microtubules, and intermediate filaments (1, 3, 4). Cell-cell contacts are mediated by adhesion molecules, such as cadherins and their associated cytoplasmic proteins, α- and β-catenins (5–7). Cell-to-substratum contact is mediated by different types of adhesion molecules, such as integrins, and their interacting molecules, talin and vinculin (8, 9).

The Rho family GTPases (including Rho, Rac, and Cdc42) participate in regulation of the actin cytoskeleton and various cell adhesion events (10, 11). Rho has been implicated in the formation of stress fibers and focal adhesions (12), cell morphology (13), cell aggregation (14), cell motility (15), membrane ruffling (16), smooth muscle contraction (17, 18), neurite retraction in neuronal cells (19, 20), and cytokinesis (21, 22). Rac is involved in membrane ruffling (23), cell motility (24), actin polymerization (25), and cadherin-mediated cell-cell adhesion (26–28). Cdc42 participates in filopodia formation (29, 30) and cell-cell adhesions (28). The molecular mechanisms underlying the above processes have been largely unknown, until recently. Some of the molecular pathways that connect the Rho family GTPases to control of the cytoskeleton and cell adhesion have been established. The major emphasis in this review is on molecules that interact with the Rho family GTPases and their role in regulation of the cytoskeleton and cell adhesion.

MOLECULES THAT INTERACT WITH RHO FAMILY GTPASES

The Rho family GTPases belong to the Ras superfamily of small GTPases. At least 10 members of the Rho subfamily are known in mammals: RhoA-E, RhoG, Rac1 and -2, Cdc42, and TC10. These proteins share >50% sequence identity (31). RhoA, -B, and -C, Rac1 and -2, and Cdc42 are the best-studied members. RhoA, -B, and -C (collectively Rho) have the same amino acid sequence in their effector domains (approximately 32–41 amino acids), appear to be regulated in a similar manner, and seem to have similar functions. Like other GTP-binding proteins, the Rho family GTPases exhibit both GDP/GTP-binding and GTPase activ-

ities (31, 32). The GDP-bound inactive and GTP-bound active forms are interconvertible by GDP/GTP exchange and GTPase reactions (31, 32). There are several groups of proteins that interact with the Rho family GTPases, including regulators and effectors.

GDP/GTP Exchange Proteins

Guanine Nucleotide Exchange Factors There are guanine nucleotide exchange factors (GEFs), also known as GDP dissociation stimulators (GDSs), that facilitate the release of GDP from the Rho family GTPases, thereby promoting the binding of GTP (33) because the cytosolic concentration of GTP is more than fivefold higher than that of GTP. GTP binding activates the Rho family GTPases. GEFs for the Rho family GTPases share a common sequence motif, designated the Dbl-homology (DH) domain (33). The Dbl proto-oncogene was originally isolated as cDNA that induced foci when transfected into NIH3T3 cells (33). In 1991, Dbl was shown to release GDP from Cdc42 in vitro (34). Since then, a number of GEFs for various Rho family GTPases (in parentheses) have been identified, including the following: Dbl (Cdc42, Rho); Lbc (Rho) (35); Lfc (Rho) (36); Lsc (Rho) (36); Tiam (Rac) (37); p115 Rho GEF (Rho) (38); Vav (Rac, Cdc42, Rho) (39); Fgd1 (Cdc42) (40); Trio (Rac, Rho) (41); Ost (Rho, Cdc42) (42); Bcr (Rac, Cdc42, Rho) (43); Pix (Rac) (44); and Smg GDS (Rac, Cdc42, Rho) (45). In addition to the DH domain, GEFs for the Rho family GTPases also contain a nearby pleckstrin homology (PH) domain (except for Smg GDS) (45). Both the DH and PH domains are essential for GEF activity (33). As indicated, some Rho GEFs show activity toward several Rho family GTPases, whereas others have more restricted specificity. For example, p115 Rho GEF seems to be specific for Rho (38), whereas Fgd1 seems to be specific for Cdc42, at least in vitro (40). Vav is able to act on Rho, Rac, and Cdc42 in vitro (39, 46). Smg GDS was originally isolated as a GEF for Rap1, which is closely related to Ras (45, 47), but it was subsequently found to be active on both RhoA and K-Ras (48). Although a number of GEFs have been identified, the molecular mechanism by which their activity can be modulated by extracellular signals was unknown until recently (Figure 1).

Studies on heterotrimeric G proteins have clarified the mechanism by which p115 Rho GEF is regulated. Expression of constitutively active mutant forms of certain heterotrimeric G protein α subunits ($G\alpha_{12}$ or $G\alpha_{13}$) induce formation of stress fibers and focal adhesions in certain cells in a Rho-dependent manner (49). Mouse fibroblasts deficient in $G\alpha_{13}$ fail to display thrombin-induced cell migration, which is also thought to require Rho (50). Examination of the sequence of p115 Rho GEF revealed that its NH_2-terminal region has a regulator of G protein signaling domain (51). The regulator of G protein signaling domain of p115 Rho GEF specifically stimulates the intrinsic GTPase activity of $G\alpha_{12}$ and $G\alpha_{13}$ (51, 52). Conversely, activated $G\alpha_{13}$ binds to p115 Rho GEF and stimulates its ability to catalyze nucleotide exchange on Rho (52). Thus, on stimulation by extracellular signals such as thrombin, activation of $G\alpha_{13}$ is linked to activation of Rho via p115 Rho GEF (Figure 1).

Vav has DH, PH, and Src homology (SH2 and SH3) domains. Although Vav was originally thought to serve as a GEF for Ras (53), it actually functions as a GEF for Rac (39). Vav becomes phosphorylated on tyrosine residues when lymphoid cells are stimulated by antigens or mitogens. Tyrosine phosphorylation of Vav by a tyrosine kinase (Lck) enhances its GEF activity on Rac (39, 46). Rac is thought to be activated through processes that depend on phosphatidylinositol (PI) 3-kinase, because wortmannin (a relatively specific inhibitor of PI 3-kinase) or the expression of dominant-negative PI 3-kinase inhibits growth factor-induced Rac activation (54–58). Biochemical analysis showed that PI-3,4,5-triphosphate (a product of PI 3-kinase) enhances the phosphorylation and activation of Vav by Lck (59). Thus, activation of receptor-tyrosine kinases that recruit both Lck and PI 3-kinase may act synergistically to activate Vav, which in turn activates Rac. Further studies are required to identify the signaling molecules that couple other GEFs for the Rho family GTPases to upstream stimuli.

Rho GDP Dissociation Inhibitor GDP dissociation inhibitor (GDI) was originally isolated as a molecule that interacts specifically with GDP·Rho and inhibits the dissociation of GDP from Rho (32, 60, 61). In addition to Rho GDI itself, at

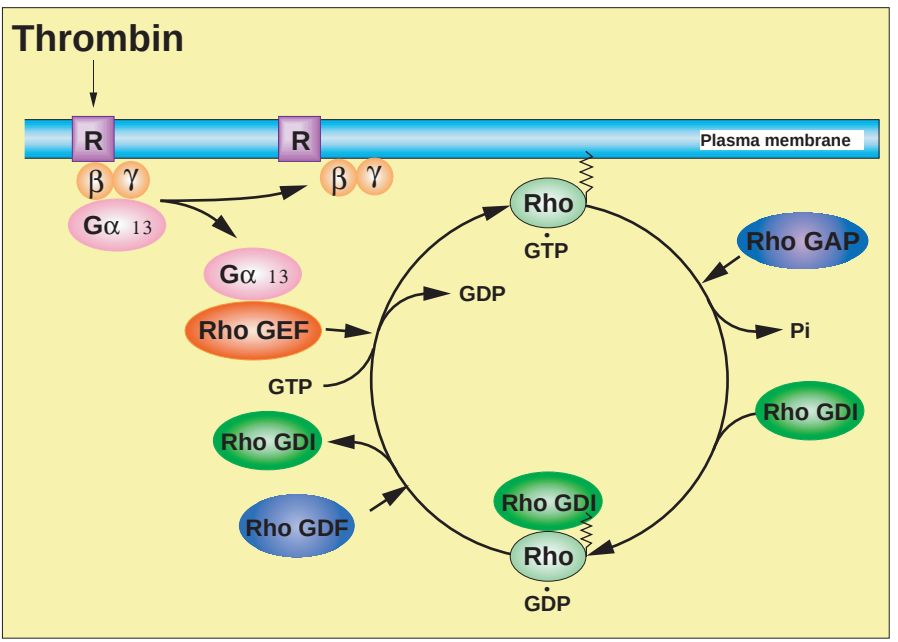

Figure 1 Mode of activation of the Rho family GTPases. GEF, Guanine nucleotide exchange factors; GAP, GTPase-activating proteins; GDI, GDP dissociation inhibitor; GDF, GDI dissociation factor.

least two other isoforms, named D4-GDI and Rho GDI3, have been identified (62, 63). Rho GDI was subsequently found to also be active on Cdc42 and Rac (64). Rho GDI prevents the binding of GDP·Rho, but not GTP·Rho, to cell membranes and can extract GDP·Rho from membranes (65). Thus, in a sense, Rho GDI acts as a chaperone to regulate the translocation of the Rho family GTPases between membranes and the cytosol. In resting cells, the Rho family GTPases, including Rho, Rac, and Cdc42, exist mostly in the GDP-bound form and in complexes with Rho GDI in the cytosol (32). Because Rho GDI counteracts the action of GEFs, such as Dbl, it is thought that release of Rho GDI from Rho is necessary before Rho becomes susceptible to a GEF (66, 67). The molecular mechanism underlying dissociation of Rho GDI from Rho was unknown until recently. Biochemical analysis suggests a tentative model by which Rho GDI is released from Rho (68) (Figure 1). Rho GDI can be coimmunoprecipitated with moesin, a member of the ERM (ezrin, radixin, and moesin) family of proteins (69). In vitro, the NH_2-terminal region of radixin binds to a GDP·Rho/Rho GDI complex, dissociates Rho GDI from GDP·Rho, and thereby enhances the ability of GEF to induce GDP/GTP exchange (68). Thus, Rho GDI dissociation factors (GDFs), such as the ERM family proteins, which stimulate dissociation of Rho GDI from GDP·Rho, may play a critical role in regulating the GDP/GTP exchange reaction.

GTPase–Activating Proteins

Rho GTPase-activating proteins (GAPs) were identified as molecules that stimulate the intrinsic GTPase activity of the Rho GTPases, leading to their conversion to the inactive GDP-bound state (32, 70). The first GAP identified for the Rho family GTPases was purified from cell extracts and designated p50 Rho-GAP (71). P50 Rho-GAP was subsequently found to be more active on Cdc42 than on Rho or Rac (72). Since the discovery of p50 Rho-GAP, a number of GAPs for various Rho family GTPases (in parentheses) have been identified, including the following: Bcr (Rac > Cdc42) (73); Abr (Rac, Cdc42) (74); β-chimerin (Rac) (75); p190GAP (Rho > Rac, Cdc42) (76); 3BP-1 (Rac, Cdc42) (77); p122 Rho GAP (Rho) (78); Myr5 (Rho > Cdc42) (79); and RalBP1 (Cdc42 > Rac) (80). GAPs shares a related GAP homology domain that encompasses about 140 amino acids of the protein. The substrate specificity in vivo may be more restricted than in vitro, however.

Based on the preceding sections, the activity of the Rho family GTPases appears to be regulated cyclically, as follows (Figure 1). In the cytosol of the resting cells, the Rho family GTPases are present in the GDP-bound form complexed with Rho GDI. When cells are stimulated by certain extracellular signals, Rho GDI is dissociated via the action of GDFs and specific GEFs for the Rho family GTPases are activated; then the GDP-bound form of the Rho family GTPase is converted to the GTP-bound form. The GTP-bound form of Rho family GTPases is targeted to cell membranes by its COOH-terminal prenyl group and interacts with its specific targets (32, 65, 81). GAPs act as negative regulators by enhancing the GTPase

activity of the Rho family GTPases and reconverting them to the inactive GDP-bound form. Rho GDI can then form a complex with the GDP-bound form and extract it from the membrane back into the cytosol.

Targets/Effectors

Rho Targets A number of proteins have been identified as targets of Rho by means of affinity chromatography, ligand overlay assay, or the yeast two-hybrid system. These targets include Rho-kinase/ROK/ROCK, the myosin-binding subunit (MBS) of myosin phosphatase, protein kinase N (PKN)/PRK1, rhophilin, rhotekin, citron, and p140 mDia (10) (Figure 2).

Rho-kinase was identified as a GTP·Rho-binding protein of extracts from bovine brain by affinity column chromatography on matrix-bound GTP·Rho (82). Rho-kinase was also identified as ROKα (83) and ROCK2 (84). ROKβ (85)/ROCK1 (86) is an isoform of Rho-kinase/ROKα/ROCK2. The kinase domain of Rho-kinase is situated at the NH_2-terminal end and has 72% sequence homology with the catalytic domain of myotonic dystrophy kinase (82). Rho-kinase has a putative coiled-coil domain in its middle portion and a PH domain at its COOH-terminal end. GTP-bound Rho interacts with the COOH-terminal portion of the coiled-coil domain and activates the phosphotransferase activity of Rho-kinase (82). Rho-kinase is involved in regulation of smooth muscle contrac-

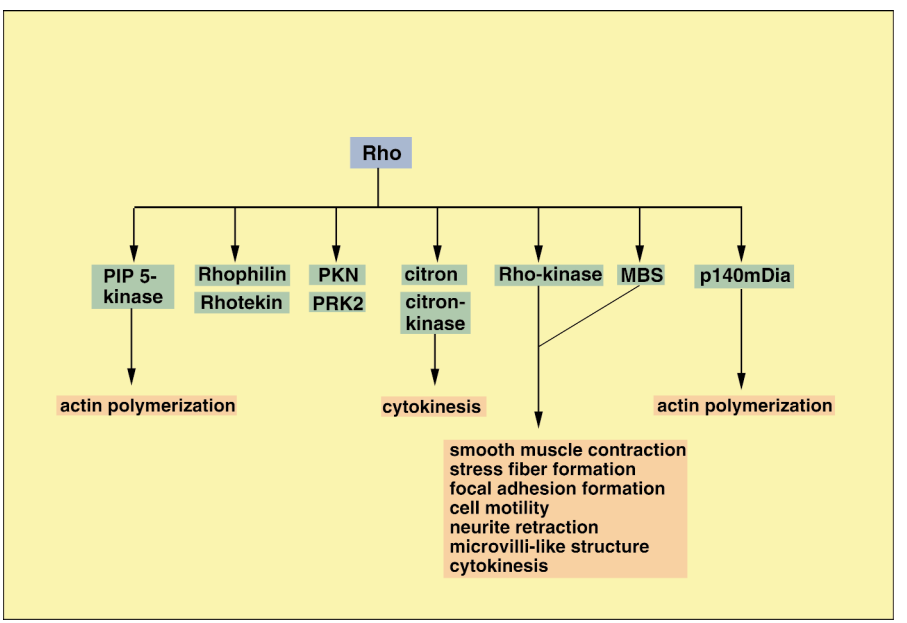

Figure 2 Mammalian targets of Rho. PIP, Phosphatidylinositol 4,5-bisphosphate; PKN, protein kinase N.

tion (87–89), the formation of stress fibers and focal adhesions (85, 90, 91), neurite retraction (92–94), and cytokinesis (95).

Myosin phosphatase is composed of three subunits: MBS, a 37-kDa type 1 phosphatase catalytic subunit, and a 20-kDa regulatory subunit (96). Myosin phosphatase binds to phosphorylated myosin light chain (MLC) via MBS and dephosphorylates it. The COOH-terminal domain of MBS interacts with GTP-bound Rho (88). Rho-kinase phosphorylates both MLC (87) and MBS, the latter of which leads to inactivation of myosin phosphatase (88). Rho-kinase and MBS are therefore believed to regulate the level of MLC phosphorylation cooperatively, as described later.

PKN/PRK1 contains an NH_2-terminal regulatory domain and a COOH-terminal catalytic domain. The catalytic domain is highly homologous to that of protein kinase C, whereas the NH_2-terminal domain shares no obvious homology with protein kinase C or any other protein kinases (97). The regulatory domain contains three leucine zipper-like motifs. GTP-bound Rho interacts with the first leucine zipper-like motif in the NH_2-terminal portion (98, 99) and activates the catalytic activity of PKN (98). The physiological functions of PKN/PRK1 are unknown. PRK2 is an isoform of PKN/PRK1 and appears to associate with GTP·Rac. PRK2 also interacts with Rho, but in a GDP/GTP-independent manner (100, 101). The expression of a kinase-deficient form of PRK2 disrupts actin stress fibers (101), which implicates PRK2 in actin cytoskeleton reorganization.

Rhophilin and rhotekin have sequence similarity to the Rho-binding domains of both MBS and PKN/PRK1 and interact with GTP-bound Rho (99, 102). This region of homology represents one of the consensus motifs for a Rho-interacting surface. Rhophilin and rhotekin do not have any other diagnostic features in their primary structures, and their functions are unknown.

Citron was originally isolated as a Rho-binding protein in a yeast two-hybrid system (103). The overall domain structure of citron is similar in sequence to Rho-kinase, but it lacks a kinase domain. A splice variant of citron with an NH_2-terminal kinase domain homologous to that of the Rho-kinase family (citron-kinase) was recently identified (104). Citron-kinase is localized at the cleavage furrow and mid-body during cytokinesis and this is implicated in cytokinesis (104).

p140mDia is a mammalian homologue of *Drosophila melanogaster* diaphanous, which is required for cytokinesis, and belongs to the formin-related family of proteins (105). p140mDia also has sequence similarity to yeast Bni1 (106). The NH_2-terminal portion of p140mDia interacts with GTP-bound Rho. p140mDia also contains repetitive polyproline stretches that can bind profilin (105). p140mDia appears to be involved in actin polymerization (105).

Kv1.2, the delayed rectifier potassium channel, can associate with GTP-bound Rho (107). The overexpression of Rho reduces the basal current generated by exogenously expressed Kv1.2 in *Xenopus* oocytes. Clostridial C3 toxin, which ADP ribosylates and inactivates Rho (108, 148, 149), blocks the suppression of Kv1.2 current by one type (m1) of muscarinic acetylcholine receptors in human 293 embryonic kidney cell line. Thus, G protein-coupled receptors may activate Rho, which in turn regulates Kv1.2 activity.

Phospholipase D (PLD) is thought to be activated by Rho (109), although the molecular mechanism is not known. PLD hydrolyzes phospholipids to yield phosphatidic acid and their head groups. Phosphatidic acid is claimed to be a direct second messenger (109, 110), but it can also be metabolized to other demonstrated second messengers, including diacylglycerol and lysophosphatidic acid (LPA). The activity of PLD is stimulated by a variety of extracellular signals, such as growth factors and hormones. Biological and biochemical studies have indicated that Rho regulates PLD activity cooperatively with Arf, another member of the Ras-related superfamily of small GTPases (111–114).

PI 5-kinase has been implicated in Rho signaling (115, 116). Previous work has shown that PI 4,5-bisphosphate (PIP_2), the product of PI 5-kinase, can regulate the interactions of a number of actin-binding proteins, including profilin, α-actinin, gelsolin, and p39CapZ in vitro (117). Thus, it is possible that alterations in PIP_2 synthesis affect actin polymerization and the association of actin filaments with focal adhesion zones. However, whether effects on PIP_2 are part of a mechanism by which Rho controls the cytoskeleton in intact cells remains controversial.

Rac/Cdc42 Targets A number of proteins have been identified as targets for Rac and/or Cdc42, including p21-activated kinases (PAKs), WASP/N-WASP, IQGAP1, MRCK, Por1, p140Sra-1, and Posh. It is interesting that several of these proteins interact with both Rac and Cdc42 (10) (Figure 3).

To date, at least three isoforms of PAK (PAK1–3) have been identified (118–122). PAKs are serine/threonine kinases, which comprise an NH_2-terminal regulatory domain and a COOH-terminal catalytic domain (118). In the NH_2-terminal region, there is a conserved domain of approximately 20 residues that is responsible for interaction with Cdc42/Rac; this motif is called the CRIB (Cdc42/Rac-interactive binding) site (123). Several other potential targets for Cdc42 and Rac share this motif (see below). The GTP-bound forms of Cdc42 and Rac1 interact with the CRIB site and activate all PAK isoforms (118–122). PAKs are components of pathways that regulate cell morphology, including formation of lamellipodia and disassembly of stress fibers and focal adhesions (124, 125). PAKs are also upstream elements in the JNK and p38 kinase pathways that control gene expression (126, 127). PAK interacts with Pix, a GEF for Rac (44). This interaction is necessary for PAK-induced lamellipodia formation (128). Moreover, the PAK-induced lamellipodia formation is inhibited by dominant-negative Rac (Rac^{N17}). Hence, Cdc42-dependent activation of PAK leads to Pix-mediated activation of Rac.

The WASP gene was originally identified as the locus mutated in patients with Wiskott-Aldrich syndrome (129). Its neural isoform, N-WASP, was identified as a molecule interacting with the SH3 domain of Grb2/Ash (130). The WASP and N-WASP molecules consist of PH, CRIB, proline-rich, verprolin-homologous, and cofilin-like domains (129, 130). N-WASP, but not WASP, also contains an IQ motif, which is able to bind calmodulin. WASP is expressed exclusively in hematopoietic cells, whereas N-WASP is ubiquitously expressed, although especially abundant in brain (131). WASP and N-WASP interact with GTP-bound

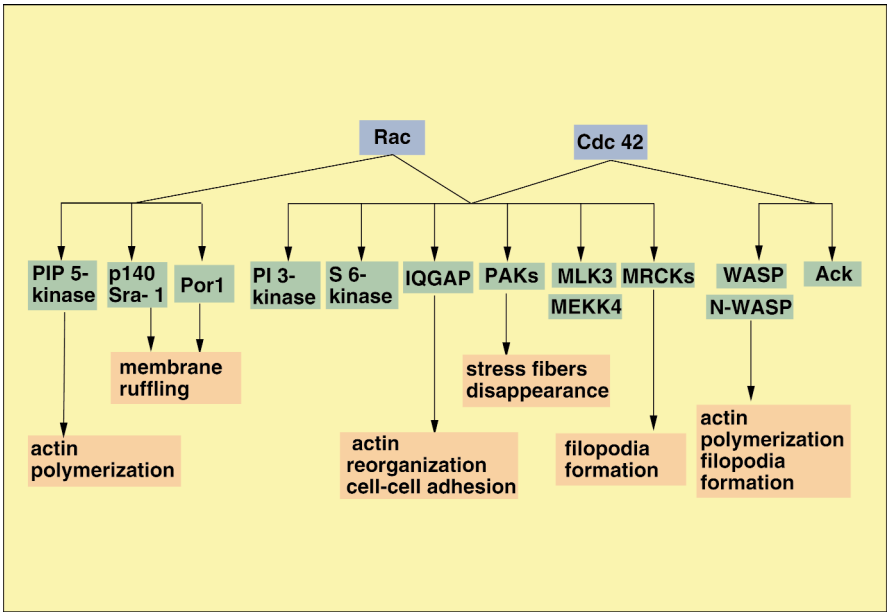

Figure 3 Mammalian targets of Rac and Cdc42. PIP, Phosphatidylinositol 4,5-bisphosphate; PI, phosphatidylinositol; PAK, p21-activated kinases.

Cdc42 and induce actin polymerization when overexpressed (129, 131). N-WASP appears to play a crucial role in filopodia formation (131).

The IQGAP1 gene was originally isolated as a member of the Ras GAP family (132, 133). However, no GAP activity toward any small GTPases has been demonstrated for IQGAP1. At least two isoforms of IQGAP have been identified, IQGAP1 and IQGAP2. Both isoforms directly interact with GTP-bound Cdc42 and Rac, but not with the GDP-bound forms (134–137). IQGAP1 consists of a calponin-homologous domain, IQGAP-repeat sequences that are the hallmarks of IQGAP1 and IQGAP2, a WW domain, an IQ motif, and a GRD sequence (Ras-GAP related domain). IQGAPs, unlike PAKs and WASP, do not contain a CRIB site; the COOH-terminal GRD domain is responsible for the interaction with Cdc42. IQGAP1 (138, 139) and IQGAP2 (140) directly interact with actin filaments and cross-link the actin filaments, and GTP-bound Cdc42 enhances the cross-linking activity (138). In addition to its role in actin filament organization, IQGAP1, acting downstream of Cdc42 and Rac1, appears to regulate cell-cell adhesion through the cadherin-catenins pathway, as described below (141).

MRCKα and -β interact with GTP-bound Cdc42 through a CRIB site (142). MRCKs are composed of an NH_2-terminal kinase domain, a coiled-coil region, a cysteine-rich segment, a PH motif, and a CRIB site. The kinase domains of MRCKs show sequence similarity with those of myotonic dystrophy kinase and Rho-kinase. MRCKα is colocalized with Cdc42 at the cell periphery (142).

MRCKs appear to regulate the formation of focal adhesive complexes and filopodia in a Cdc42-dependent manner. Gek, a *D. melanogaster* ortholog of MRCK, binds to Cdc42 in a GTP-dependent manner (143). Homozygous gek mutants die as larvae, and egg chambers homozygous for gek mutations exhibit an abnormal accumulation of F-actin and are defective in producing fertilized eggs (143).

Por1, p140Sra-1, and Posh have been identified as Rac-specific targets (144–146). Por1 and p140Sra-1 may participate in regulation of lamellipodia formation (144, 145), whereas Posh may regulate JNK activity (146).

Bacterial Toxins

The Rho family GTPases are targets for at least three groups of bacterial toxins that either inactivate or activate the GTPases (147). The first group is the C3 exoenzyme family. Members of this toxin family include *Clostridium botulinum* C3 ADP-ribosyltransferase, *Clostridium limosum* transferase, *Bacillus cereus* transferase, and epidermal differentiation inhibitor (EDIN). *C. botulinum* C3 toxin is a useful tool for identifying specific functions of Rho. *C. botulinum* C3 toxin specifically ADP ribosylates Rho at Asn^{41} using NAD as the donor substrate (148, 149). ADP ribosylation of Rho is thought to interfere with the ability of Rho to interact with its putative targets, thereby inhibiting the functions of Rho. *C. botulinum* C3 toxin ADP ribosylates only poorly other Rho family members, such as Rac and Cdc42.

Bacterial toxins in the second group are large clostridial cytotoxins. Members of this toxin family include *Clostridium difficile* toxins A and B and *Clostridium sordellii* HT and LT (147). *C. difficile* toxins specifically glycosylate Rho at Thr^{37}, Rac at Thr^{35}, and Cdc42 at Thr^{35} by using UDP-glucose as the donor substrate (150). The biological effects of *C. difficile* toxins indicate that glycosylation inactivates the Rho family GTPases (151).

The third group is cytotoxic necrotizing factors (CNFs). Members of this toxin family include *Escherichia coli* CNF1 and CNF2 and *Bordetella* spp. DNTs (147). CNFs induce a deamidation of Gln^{63} of Rho, resulting in its conversion to Glu^{63} (152). Loss of Gln^{63} inhibits both the intrinsic and the GAP-stimulated GTPase activity of Rho, thereby sustaining Rho in its active GTP-bound form (152). CNF acts on Cdc42 as well as on Rho. Thus, CNFs may activate several Rho family GTPases in vivo.

FUNCTIONS OF THE RHO FAMILY GTPASES

Smooth Muscle Contraction and Contractility

Specific agonists, such as carbacol and endothelin, cause an increase in cytosolic Ca^{2+} and subsequent activation of Ca^{2+}/calmodulin-dependent MLC kinase in smooth muscle. MLC kinase phosphorylates MLC and activates myosin ATPase, thereby inducing contraction of smooth muscles (153–155). However, because

the levels of MLC phosphorylation and degree of contraction are not always proportional to the cytosolic Ca^{2+} levels, an additional mechanism that regulates MLC phosphorylation and contraction has been proposed (156). Because the agonist-induced MLC phosphorylation and contraction of permeabilized smooth muscles occur at fixed suboptimal concentrations of Ca^{2+} and are GTP dependent, a GTP-binding protein is thought to be involved in receptor-mediated sensitization of MLC phosphorylation (157, 158).

Evidence that Rho is the GTP-dependent factor involved in sensitizing smooth muscle contraction at suboptimal Ca^{2+} levels has been obtained in several laboratories (159). GTP-bound Rho causes contraction of permeabilized smooth muscles at fixed suboptimal concentrations of Ca^{2+} (17), and *C. botulinum* C3 toxin and EDIN inhibit contraction induced by the guanosine 5′-(3-O-thio)-triphosphate (GTPγS) (a nonhydrolyzable GTP analog) (17). *C. botulinum* C3 toxin inhibits the GTPγS-induced MLC phosphorylation in permeabilized smooth muscle cells (160). GTP-bound Rho stimulates MLC phosphorylation in permeabilized smooth muscle, and EDIN inhibits the ability of carbacol-induced Ca^{2+} elevation to stimulate MLC phosphorylation (18).

Until recently, the molecular mechanism by which Rho regulates MLC phosphorylation was largely unknown. Recent analyses suggest the model shown in Figure 4, in which Rho regulates MLC phosphorylation through its effectors, Rho-kinase and MBS (87, 88). GTP-bound Rho interacts with both Rho-kinase and MBS of myosin phosphatase, resulting in activation of Rho-kinase and translocation of MBS. Activated Rho-kinase phosphorylates MBS, thereby inactivating myosin phosphatase (88). Concomitantly, Rho-kinase phosphorylates MLC at the same site that is phosphorylated by MLC kinase and activates myosin ATPase (87). Both events appear to be necessary for an increase in MLC phosphorylation. Consistent with this view, addition of dominant-active Rho-kinase to permeabilized vascular smooth muscle induces contraction through MLC phosphorylation (89). A specific chemical inhibitor for Rho-kinase (Y27632) has recently been developed (161). Y27632 selectively inhibits smooth muscle contraction by inhibiting GTP-dependent sensitization to suboptimal Ca^{2+} and suppresses hypertension in several hypertensive rat models (161) as well as vasospasm of porcine coronary artery (H Shimokawa, unpublished observations). Thus, Rho-kinase–mediated sensitization of smooth muscle contraction may be involved in the pathophysiology of hypertension and vasospasm.

In nonmuscle cells, MLC phosphorylation is thought to modulate interaction of myosin with actin to regulate stress fiber contraction and thereby affect cell motility (162). Several studies have established roles for Rho and Rho-kinase in MLC phosphorylation in nonmuscle cells. MBS is phosphorylated, and myosin phosphatase activity is inactivated, during the action of thromboxane A2 in platelets, and both responses are blocked by prior treatment of platelets with *C. botulinum* C3 toxin (163). Similar observations have been made in endothelial cells responding to thrombin (164). Expression of dominant-active Rho (Rho^{V14}) or constitutively active Rho-kinase induces MLC phosphorylation in COS7 cells

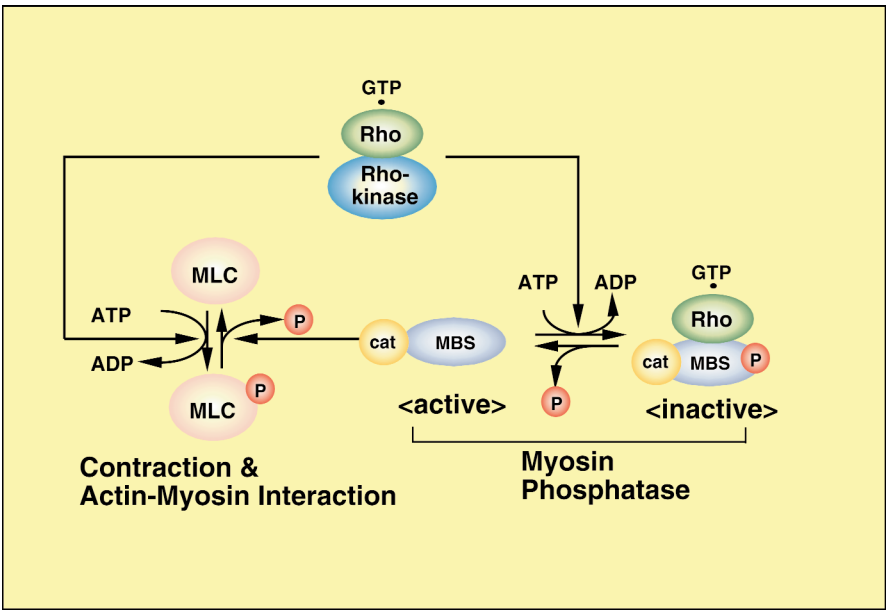

Figure 4 Model for regulation of MLC (myosin light chain) phosphorylation by Rho, Rho-kinase, and myosin phosphatase. cat, Catalytic subunit; MBS, myosin-binding subunit.

(165) and NIH3T3 cells (92), whereas the expression of a dominant-negative Rho-kinase variant inhibits serum-induced MLC phosphorylation (92). Thus, Rho-kinase appears to regulate MLC phosphorylation downstream of Rho in nonmuscle cells as well as in smooth muscle.

Stress Fibers and Focal Adhesions

Rho is believed to regulate the formation of actin stress fibers and focal adhesions, based on the following observations. Confluent, serum-starved Swiss 3T3 cells have few actin stress fibers, which are composed of actin filaments and associated myosin filaments that confer contractility to the fibers on MLC phosphorylation (162). When these cells are stimulated by LPA, new stress fibers, which increase in number and diameter, appear (12). Prior treatment of the cells with *C. botulinum* C3 toxin inhibits LPA-induced stress fiber formation. Microinjection of $RhoA^{V14}$ into the cells induces stress fiber formation (12, 13). Rho is also required for the LPA-induced formation of focal adhesions (12), the sites at which stress fibers are linked via integrins to extracellular matrix components, such as fibronectin (8, 9). Certain molecules, including vinculin, α-actinin, and talin, assemble with integrin that form the focal adhesions and serve as the

anchors for stress fibers. Expression of dominant-active Rho-kinase induces formation of stress fibers in Swiss 3T3 and MDCK cells, whereas the expression of dominant-negative Rho-kinase inhibits the LPA- or $RhoA^{V14}$-induced formation of stress fibers (85, 90, 91). The expression of MLC mutant, $MLC^{T18D,S19D}$ (substitution of Thr and Ser by Asp), which is known to cause activation of myosin ATPase and a conformational change of myosin II when reconstituted with myosin heavy chain in vitro (166–168), also enhances the formation of stress fibers and focal adhesions (92). Pharmacological analysis suggests that contractility of stress fibers driven by MLC phosphorylation governs focal adhesion formation (169). Taken together, these observations indicate that Rho through Rho-kinase plays a critical role in the formation of stress fibers and focal adhesions via myosin II activation (Figure 5).

Lamellipodia and Filopodia

Membrane ruffling, which is characterized by dynamically fluctuating movement of membrane protrusions consisting of lamellipodia and filopodia, is rapidly induced in cells in response to certain extracellular signals (170). The molecular mechanism of membrane ruffling is not known, but it has been suggested that polymerization and depolymerization of actin beneath the plasma membrane drives the

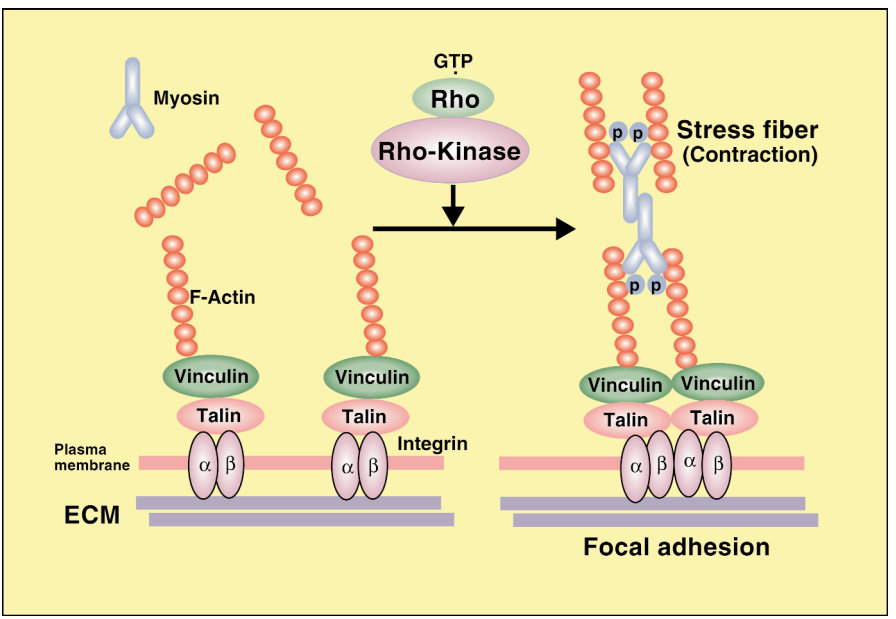

Figure 5 Model for formation of focal adhesions induced by the Rho/Rho-kinase pathway.

process (171). Accumulating evidence indicates that Rac serves as a key control element in lamellipodia formation and membrane ruffling induced by certain growth factors, such as platelet-derived growth factor, and by activated Ras in fibroblasts (23), whereas Cdc42 appears to regulate filopodia formation (29, 30). Injection of dominant-active Rac (Rac^{V12}) triggers lamellipodia and membrane ruffling followed by stress fiber formation, whereas injection of dominant-negative Rac^{N17} inhibits these processes induced by the same growth factors (23). Injection of dominant-active Cdc42 ($Cdc42^{V12}$) rapidly induces filopodia formation followed by lamellipodia and membrane ruffling (29, 30). The cytoskeletal changes induced by Rac and Cdc42 are associated with integrin-based focal complexes distinct from the adhesion sites induced by Rho (23, 29, 30). Judging from the kinetics of the cytoskeletal changes induced by Rho, Rac, and Cdc42, cross talk appears to exist: Cdc42 can activate Rac, and Rac can activate Rho (11). It should be noted that Rho is implicated in the membrane ruffling (lamellipodia) induced by hepatocyte growth factor (HGF) and 12-*O*-tetradecanoylphorbol-13-acetate in certain cells, such as KB cells (16) and MDCK cells (172), whereas Rac is implicated in the insulin-induced membrane ruffling in KB cells (16). These observations indicate that Rho and Rac receive distinct signals but generate similar outputs in certain cells. This convergence could be partly explained by the fact that Rho and Rac/Cdc42 interact with different protein kinase targets; nevertheless, these enzymes share a high degree of similarity in their catalytic domains, for example Rho-kinase and MRCKα.

The molecular mechanisms by which Rac and Cdc42 regulate lamellipodia and filopodia, respectively, are still largely unknown. Several targets of Rac and Cdc42 have been implicated in these processes.

Accumulating evidence suggests that cofilin plays a critical role in the rapid turnover of actin filaments in various cellular processes, including lamellipodia formation (171). Cofilin sequesters actin monomers and severs actin filaments, thereby inducing disassembly of actin filaments in vitro (171). Phosphorylation of cofilin at Ser3 reduces its actin-binding and -severing activities (173, 174). Recent studies indicate a role for LIM-kinase in Rac-induced lamellipodia formation, which appears to be mediated by the phosphorylation of cofilin (175, 176). Dominant active Rac (Rac^{V12}) activates LIM-kinase (175, 176), although the linkage between Rac and LIM-kinase is unknown. The expression of dominant-negative LIM-kinase inhibits Rac^{V12}-induced membrane ruffling. Activated LIM-kinase phosphorylates cofilin at Ser3, which may prevent cofilin from disassembling of actin filaments and inhibits the ability of cofilin to bind actin monomers, thereby promoting the formation of the proper actin meshwork for lamellipodia. Rac may also increase the production of PIP_2 through the action of PI 5-kinase (177). The change in PIP_2 levels may affect the functions of certain classes of actin-binding proteins and influence actin polymerization by uncapping or increased nucleation of actin filaments, perhaps in cooperation with cofilin (178).

Por1, a specific target of Rac, may participate in lamellipodia formation (144). A mutation in the effector-binding loop of Rac1 abolishes membrane ruffling and

also the interaction of Rac with Por1. Truncated versions of Por1 inhibit the induction of membrane ruffling by Rac^{V12} in fibroblasts. In addition, the overexpression of Por1 synergizes with dominant-active Ras (Ras^{V12}) in the induction of membrane ruffling, which suggests a potential role of Por1 in Rac1-mediated membrane ruffling. p140Sra-1, another Rac-specific target, is colocalized with activated Rac in the membrane ruffling area (145). p140Sra-1 directly interacts with actin filaments. IQGAP1, a target of Rac and Cdc42, specifically accumulates at the membrane ruffling area induced by insulin or Rac^{V12} (135). IQGAP1 (138) and IQGAP2 (139) directly interact with and cross-link actin filaments. A calponin-homologous domain mediates the interaction of IQGAP1 and IQGAP2 with actin filaments (138). GTP-bound Cdc42, but not its GDP-bound form, enhances the actin filament cross-linking activity of IQGAP1 in vitro (138). These proteins, including LIM-kinase, Por1, p140Sra-1, and IQGAPs, may contribute to formation of the actin meshwork required for membrane ruffling, although their relation is largely unknown.

The expression of N-WASP, but not WASP, enhances Cdc42-induced filopodia formation (130). Injection of anti-N-WASP antibody specifically blocks bradykinin-induced filopodia formation (131). Moreover, N-WASP severs actin filaments in vitro, and this activity is further enhanced by GTP-bound Cdc42, but not the GDP-bound form (131). Thus, N-WASP appears to play a crucial role in filopodia formation.

MRCKα is colocalized with Cdc42 at the cell periphery in HeLa cells (142). MRCKα preferentially phosphorylates MLC at Ser19 in vitro, as observed for Rho-kinase (142). The expression of $Cdc42^{V12}$ or constitutively active MRCKβ induces MLC phosphorylation in COS7 cells (T Nakamura, unpublished data). The expression of dominant-negative MRCKα blocks the $Cdc42^{V12}$-dependent formation of filopodia and focal complexes. The coexpression of MRCKα enhances the $Cdc42^{V12}$-induced formation of filopodia, which suggests that MRCKα regulates filopodia formation, presumably through reorganization of actin-myosin complexes.

Cell Motility and Migration

Reorganization of actin filaments and cell-substratum contacts is believed to be involved in cell motility. Membrane ruffling is observed at the leading edge of motile cells and is also thought to be essential for cell motility (179). A force arising from actin polymerization appears to drive lamellipodial protrusion (4), which is thought to be regulated by Rac (11, 23) (Figure 6). Actin at the area of membrane ruffling is thought to be continuously depolymerizing and repolymerizing during cell movement (4). A force derived from myosin II triggered by MLC phosphorylation, which appears to be regulated by Rho (87, 88), in the area of membrane ruffling and in the posterior region of motile cells may also contribute to cell movement (4, 180) (Figure 6). Indeed, injection of anti-MLC kinase diminishes the cell motility of macrophages (181) (Figure 6).

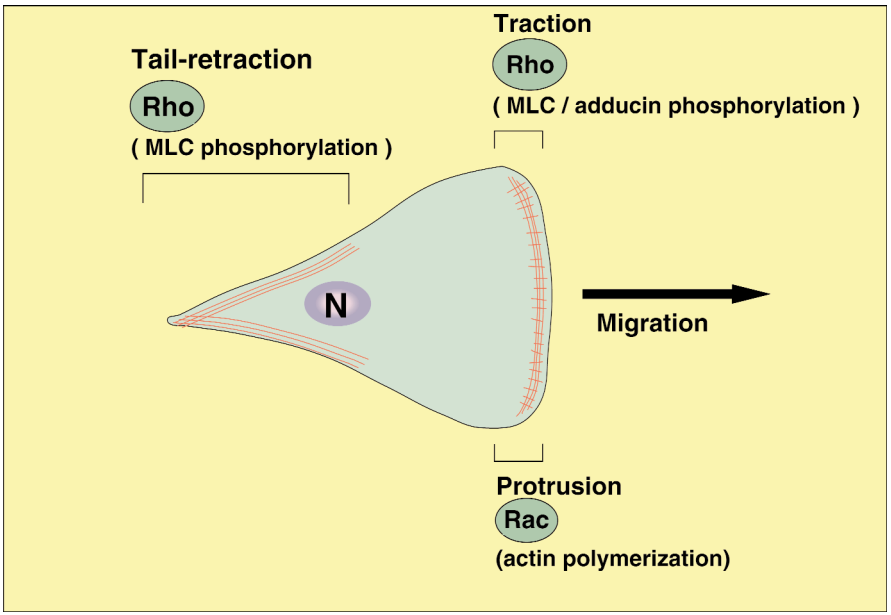

Figure 6 Roles of Rho and Rac in cell motility. MLC, Myosin light chain.

Accumulating evidence indicates that the Rho family GTPases regulate cell motility (15, 24, 182). Scatter factor/HGF stimulates the motility of epithelial cells, initially inducing a centrifugal spreading of cell colonies followed by a disruption of cell-cell adhesions and then cell scattering, accompanied by membrane ruffling. Roles of the Rho family GTPases in cell motility have been investigated mainly by two groups of investigators. Takaishi et al (15) have reported that HGF-induced cell motility of keratinocytes (308R cells) is inhibited by microinjection of either Rho GDI or *C. botulinum* C3 toxin, but not by microinjection of Rac^{N17}. The action Rho GDI is counteracted by comicroinjection of activated Rho, but not of activated Rac, indicating that Rho is necessary for the HGF-induced cell motility. Conversely, Ridley et al (24) have proposed that Rac, rather than Rho, is necessary for the HGF-induced cell motility of MDCK cells and that activated Rho inhibits the HGF-induced motility. These apparent contradictory results concerning the roles of Rho and Rac might be explained by the differences in cell types and reagents used. A more recent study has shown that the expression of Rho^{N19} or Rac^{N17} inhibits the HGF-induced membrane ruffling of MDCK cells (172). Thus, both Rho and Rac may play crucial roles in the motility of MDCK cells. The expression of dominant-negative Rho-kinase also inhibits the HGF-induced membrane ruffling and wound-induced cell migration, indicating that Rho-kinase is involved in cell motility (172).

Taken together, the above observations suggest that MLC phosphorylation regulated by Rho and Rho-kinase is necessary for cell motility. In addition to

using MLC as a substrate, Rho-kinase also phosphorylates adducin and moesin directly and inactivates myosin phosphatase which dephosphorylates adduction and moesin, thereby increasing phosphorylation levels of adducin and moesin (172, 183–185). Adducin binds to F-actin and to the spectrin-F-actin complex (186, 187). Adducin subsequently promotes the binding of spectrin to F-actin or the spectrin-F-actin complex to form a spectrin-F-actin meshwork (186, 187). Adducin accumulates within the spectrin-F-actin meshwork at cell-cell contact sites and membrane ruffling areas (188). The phosphorylation of α-adducin by Rho-kinase enhances the interaction of α-adducin with F-actin (185). Rho-kinase phosphorylates α-adducin at Thr^{445} in the membrane ruffling area of MDCK cells during the action of HGF (172). The expression of α-$adducin^{T445A,T480A}$ (substitution of Thr residues by Ala), which is not phosphorylated by Rho-kinase, inhibits HGF-induced membrane ruffling and wound-induced cell migration. The expression of α-adducin ^{T445D,T480D} (substitution of Thr residues by Asp), which may mimic α-adducin phosphorylated by Rho-kinase, counteracts the inhibitory effect of dominant-negative Rho-kinase on HGF-induced membrane ruffling and wound-induced cell migration. Thus, phosphorylation of adducin by Rho-kinase appears to be necessary for membrane ruffling and cell motility.

Neurite Retraction and Extension

Chemoattractants and chemorepellants can exert significant effects on neural architecture, ranging from the stimulation of neurite outgrowth to the induction of growth cone collapse and neurite retraction (189, 190). The growth cone is a dynamic structure at a tip of a neurite and consists of filopodia and lamellipodia. Neurite extension is thought to be driven by actin polymerization in growth cones. Neurite retraction and the extension of developing neurites evoked by extracellular molecules are fundamental to nervous system development and neural plasticity. Rho is implicated in thrombin-, serum-, and LPA-induced neurite retraction and cell rounding in N1E-115 neuroblastoma and PC12 cells (19, 191), whereas Rac and/or Cdc42 is implicated in the promotion of filopodia and lamellipodia formation in growth cones and therefore in neurite extension (192). Competition appears to exist between chemoattractant- and chemorepellant-induced morphological pathways mediated by Rac and/or Cdc42 and by Rho, leading to either neurite development or collapse (192). The Rho-induced neurite retraction is presumed to be driven by a contraction of the cortical actin-myosin system (20), which is known to be regulated by Rho. Consistent with this conclusion, the expression of dominant-active Rho-kinase induces neurite retraction (92–94), whereas the expression of dominant-negative Rho-kinase inhibits the LPA-induced neurite retraction. The expression of mutant $MLC^{T18D,S19D}$ also enhances neurite retraction (92). Thus, myosin II activation appears to promote neurite retraction downstream of Rho and Rho-kinase.

Cell-Cell Adhesion

Cell-cell adhesions are categorized into several types, including tight junctions and adherens junctions (5–7, 193). Tight junctions, the most apical component of the junctional complex in columnar epithelium, form a diffusion barrier that regulates the flux of ions and hydrophilic molecules. Among the proteins that comprise tight junctions are claudin and occludin. Rho is thought to be required to maintain the "fence" function of the tight junction in epithelial cells (194, 195).

Adherens junctions are characterized by a well-developed plaque structure in which actin filaments are densely associated. Adherens junctions are composed of adhesion molecules such as cadherin (5). α- and β-Catenins are cadherin-associated cytoplasmic proteins that are required for cadherin-mediated cell-cell adhesion (5, 194). β-Catenin serves as a bridge between cadherin and α-catenin (Figure 7). α-Catenin is thought to link cadherin and catenins with actin filaments. Recent work has suggested that Rho, Rac, and Cdc42 are required for maintaining cadherin-mediated cell-cell adhesion (26–28). The expression of either $Cdc42^{N17}$ or Rac^{N17} in L cells expressing E-cadherin (EL cells), but not in L cells expressing E-cadherin–α-catenin chimeric protein (nEαCL cells), results in a reduction of E-cadherin–mediated cell-cell adhesive activity (M Fukata, unpublished observation). Because β-catenin is not required for the adhesion of nEαCL cell, Cdc42 and Rac appear to regulate E-cadherin–mediated cell-cell adhesion presumably through β-catenin.

Until recently, the mechanism underlying the regulation of E-cadherin-mediated cell-cell adhesion by the Rho family GTPases had been unknown. Studies of IQGAP, a target of Cdc42/Rac, have shed light on the molecular mechanism as follows (141). IQGAP1 is localized with E-cadherin and β-catenin at cell-cell contact sites in MDCK cells and EL cells, but not in nEαCL cells, indicating that IQGAP1 accumulates at cell-cell contact sites in a manner dependent on E-cadherin, β-catenin, or the NH_2-terminal domain of α-catenin (141). IQGAP1 interacts with E-cadherin and β-catenin both in vivo and in vitro (141). IQGAP1 promotes the dissociation of α-catenin from a cadherin-catenin complex in vitro and in vivo (141). These IQGAP1 effects are reversed by activated Cdc42. The overexpression of IQGAP1 in EL cells, but not in nEαCL, results in reduction of E-cadherin–mediated cell-cell adhesive activity. The inhibitory effect of IQGAP1 is counteracted by the coexpression of $Cdc42^{V12}$ (141). Thus, activated Cdc42 blocks the ability of IQGAP1 to inhibit assembly of a cadherin-catenins complex and thereby promotes formation of adherens junctions (Figure 7).

Microvilli

The ERM family proteins are essential components of microvilli-like structures (apical membrane protrusions) on polarized epithelial cells, fibroblasts, and lymphocytes (196–200) and are localized at the specific regions where actin filaments associate with plasma membranes, such as at the brush border of the intestinal epithelial cells, in cleavage furrows, at membrane ruffles, in filopodia,

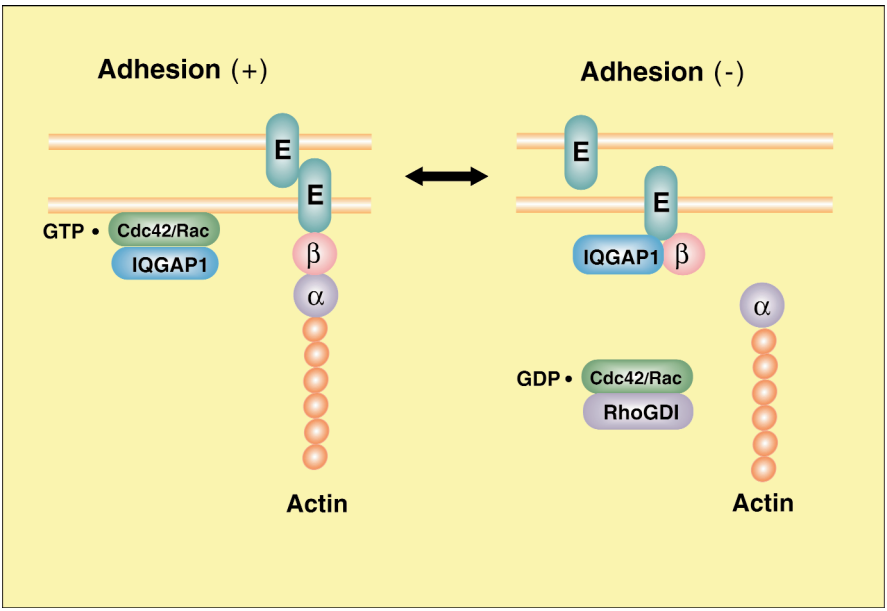

Figure 7 Role of IGQAP1 in regulation of E-cadherin–mediated cell-cell adhesion. E, E-cadherin; α, α-catenin; β, β-catenin.

at cell-cell adhesion sites, and at other microvilli-like structures (193, 198, 201–206). The ERM family proteins function as bridges between the plasma membrane and actin filaments (198–200). The NH_2-terminal domains of the ERM family proteins are thought to bind directly to some integral membrane proteins, such as CD44 (207), and the COOH-terminal domains of ERM proteins bind to actin filaments (208, 209). The NH_2- and COOH-terminal domains appear to mask each other, presumably through the intramolecular head-to-tail association between the NH_2- and COOH-terminal domains, thereby preventing the interaction of the ERM family proteins with their partners (210, 211). Once this intramolecular occlusion is relieved, the ERM family proteins are activated and translocate from the cytosol to the plasma membranes, where they serve to anchor actin filaments (212, 213). Formation of the complex between CD44 and the ERM family members appears to be regulated dynamically. Recent evidence suggests that Rho regulates formation of the complex between CD44 and the ERM family proteins (69). A permeable cell reconstitution assay showed that the ERM family proteins are essential for Rho- and Rac-induced cytoskeltal reorganization (214).

How does Rho regulate the activity of the ERM family proteins? In thrombin-activated platelets, moesin is phosphorylated at Thr^{558} (215). This phosphorylation is observed at filopodia in macrophages and is thought to be required for the

stable interaction of moesin with actin (216). Recent biochemical analysis has shown that Rho-kinase phosphorylates moesin at Thr^{558}, thereby inhibiting intramolecular association between the NH_2- and COOH-terminal domains of moesin (183, 185). Presumably Rho acts in vivo through Rho-kinase to phosphorylate and activate the ERM family proteins. Other studies have shown that LPA stimulation of serum-starved NIH3T3 cells results in relocalization of radixin into microvilli-like structures, which is blocked by *C. botulinum* C3 toxin (217). The expression of Rho^{V14} induces formation of microvilli-like structures and localizes radixin or moesin into these structures in both Rat1 and NIH3T3 cells. A more recent analysis has shown that the expression of Rho^{V14} in COS7 cells induces moesin phosphorylation and formation of microvilli-like structures at apical membranes where Thr^{558}-phosphorylated moesin accumulates, whereas the expression of dominant-negative Rho-kinase inhibits both these processes (218). The expression of dominant-active Rho-kinase also induces moesin phosphorylation and formation of microvilli-like structure. The expression of $moesin^{T558D}$ (substitution of Thr by Asp), which may mimic phosphorylated moesin, induces formation of microvilli-like structures, whereas the expression of $moesin^{T558A}$ (substitution of Thr by Ala), which is not phosphorylated by Rho-kinase, inhibits the Rho^{V14}-induced formation of microvilli-like structures (218). Thus, Rho-kinase does appear to regulate moesin phosphorylation downstream of Rho in vivo, and the phosphorylation of moesin by Rho-kinase seems to play a crucial role in the formation of microvilli-like structures.

Cytokinesis

Cells undergo cytokinesis at the end of M-phase of the cell cycle through the formation of a contractile ring beneath the plasma membrane. The contractile ring is composed mainly of actin filaments and myosin and contraction of this structure is thought to provide the force required to divide the cells into two daughter cells by pulling the membrane inward (cytoplasmic division) (2, 219). The cytoplasmic division of fertilized *Xenopus laevis* embryos is inhibited by microinjection of Rho GDI or *C. botulinum* C3 toxin, whereas nuclear division occurs normally (21, 22, 220). Coinjection of Rho GDI with activated RhoA prevents Rho GDI action. Thus, Rho appears to control cytoplasmic division through regulation of the contractility of the contractile ring.

Rho and Rho-kinase accumulate at the cleavage furrow (221, 222), where MLC phosphorylation occurs (180). The expression of dominant-negative Rho-kinase inhibits cytokinesis in *Xenopus* embryo and in mammalian cells, resulting in multinucleate cells (95). Thus, MLC phosphorylation by Rho-kinase may promote contraction of the contractile ring and play a critical role in cytokinesis. Rho-kinase also phosphorylates intermediate filament proteins, such as glial fibrillary acidic protein (GFAP) and vimentin (223, 224), MBS, and the ERM family proteins (Y Kawano, unpublished observations), exclusively at the cleavage furrow during cytokinesis. The expression of GFAP mutated at its Rho-kinase phosphorylation sites results in impaired glial filament segregation into postmi-

totic daughter cells (95). These results suggest that Rho-kinase is essential not only for cytokinesis but also for segregation of GFAP filaments into daughter cells, which in turn ensures efficient cellular separation.

Citron-kinase is localized at the cleavage furrow and midbody (104). The overexpression of kinase-deficient or constitutively active mutant of citron-kinase results in multinucleate cells, whereas that of a constitutively active kinase causes abnormal contraction during cytokinesis, which suggests that citron-kinase is involved in cytokinesis. The physiological substrates of citron-kinase remain to be identified.

Cell Transformation

Transformed cells show altered patterns of expression of cytoskeletal proteins and commonly have a disorganized actin cytoskeleton, which may be associated with the ability of transformed cells to grow in an anchorage-independent fashion (225). Ras-transformed fibroblasts typically show decreased numbers of actin stress fibers and possess sparse, less-prominent focal adhesions, which are localized only at the margins of cells, whereas Vav- and Dbl-transformed cells show well-developed stress fibers and focal adhesions (226). The critical involvement of the Rho family GTPases including Rho, Rac, and Cdc42, in Ras-induced transformation is supported by a number of experiments (227–230; see also 58, 231–233). Rac appears to participate in the Ras-induced low serum growth, whereas Cdc42 appears to participate in the Ras-induced anchorage independence (229). Although Rho activity is required for the Ras-induced foci formation, Rho activity may be down-regulated in stably transformed Rat1 cells because stress fibers and focal adhesions decrease in number at that stage (228). Consistently, Rat1 cells expressing Ras^{V12} show a severe disruption of actin stress fibers and cell adhesions, whereas the coexpression of Rho^{V14} restores not only the formation of stress fibers and focal adhesions, but also cell-cell adhesions (234). The coexpression of constitutively active Rho-kinase restores the assembly of stress fibers and focal adhesions (234). The treatment of Rat1 cells with LPA enhances stress fiber formation, whereas it fails to induce stress fiber formation in cells expressing Ras^{V12} (234). Thus, Rho-kinase may be inactivated in cells expressing Ras^{V12}, and this may contribute to oncogenic Ras-induced transformation. Because the expression of $Cdc42^{V12}$ or constitutively active PAK decreases the number of stress fibers and focal adhesions (124), Cdc42, acting through PAK, may inactivate the Rho and/or Rho-kinase. However, Ras-transformed MCF10A breast epithelial cells display a fibroblastic morphology with decreased cell-cell adhesions but increased focal adhesions and stress fibers (233, 235). Thus, the terminal morphologies of Ras-transformed cells may depend on cell types.

Other Functions of the Rho Family GTPases

The Rho family GTPases have been reported to regulate various cell functions, including transcription, cell growth, endo-exocytosis of vesicles, and development in mammals (10) and lower eukaryotes (236). Because this review focuses

on the regulation of cytoskeletons and cell adhesions in mammals, the above functions regulated by the Rho family GTPases are not described in detail [for such information, the reader is referred to two excellent reviews (10, 11)].

PERSPECTIVES

To understand the mechanism by which the Rho family GTPases regulate the cytoskeletons and cell adhesion, enormous effort has been made over the past several years to identify upstream regulators and downstream effectors of the Rho family GTPases. As a result, a number of the regulatory proteins and targets have been isolated. Intensive analyses of their functions have provided some insights into the modes of activation and action of the Rho family GTPases at the molecular level. For example, Rho-kinase has critical roles in divergent pathways downstream of Rho. Rho-kinase is involved in MLC phosphorylation, leading to the formation of stress fibers, focal adhesions, and neurite retraction, indicating a direct linkage between the Rho family GTPases and the cytoskeleton. Further studies will lead to a better understanding of how temporal and spatial signals are delivered to the cytoskeletons and cell contact sites through the Rho family GTPases and their targets.

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