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Many features of cell behavior are regulated by Rho family GTPases, but the most profound effects of these proteins are on the actin cytoskeleton and it was these that first drew attention to this family of signaling proteins. Focusing on Rho and Rac, we will discuss how their effectors regulate the actin cytoskeleton. We will describe how the activity of Rho proteins is regulated downstream from growth factor receptors and cell adhesion molecules by guanine nucleotide exchange factors and GTPase activating proteins. Additionally, we will discuss how there is signaling crosstalk between family members and how various bacterial pathogens have developed strategies to manipulate Rho protein activity so as to enhance their own survival.

All research has its antecedents, but occasionally papers are published that erupt on the scientific landscape seemingly without warning. So it was with the two papers by Ridley and Hall and their coworkers, published in *Cell* in August 1992 (Ridley and Hall, 1992; Ridley et al., 1992). This work brought the members of the Rho family of GTPases to center stage and out from under the shadow of their cousins in the Ras family. In reality, there had been several papers on Rho GTPases that laid the groundwork for these two seminal contributions, but most of us in the field date our interest in, if not our awareness of, Rho GTPases back to these two publications. This work caught the eyes (literally) of researchers in the cytoskeletal field, because two of the most prominent cytoskeletal structures in cells in culture are stress fibers and membrane ruffles. At the time, however, little was known about what governs the assembly of these structures. Ridley and Hall established that, not only are they regulated by Rho and Rac respectively, but that these GTPases act in signaling pathways downstream from growth factors and other agents that act on receptors at the cell surface. This work was important because it revealed that there are signaling pathways beyond those that regulate intracellular calcium, PIP_2 and cyclic AMP, which were then the focus of those studying cytoskeletal regulation. In addition, these papers caught the attention of those working on cell adhesion. The most prominent cell-matrix adhesions in tissue culture are focal adhesions, and the first of the Ridley and Hall papers revealed that assembly of these structures depends on Rho activity.

Rho proteins have been found to regulate many cellular activities besides the cytoskeleton and cell adhesion, such as cell polarity, endocytosis, vesicle trafficking, progression through the cell cycle, differentiation, oncogenesis, and gene transcription (Etienne-Manneville and Hall, 2002). Indeed, it now seems that there are few cellular activities that are not directly or indirectly affected by Rho family proteins. The two *Cell* papers triggered a search for downstream effectors of Rho, Rac, and other family members. This work also initiated the exploration of upstream signaling pathways that regulate the activity of these proteins. In this review, we will concentrate primarily on Rho and Rac, discussing both their upstream regulation and their downstream effectors. We will confine our discussion to the mammalian proteins, although many lessons have been learned from family members expressed in other organisms. In their original papers, Ridley and Hall revealed for the first time that there is crosstalk between Rac and Rho, as well as between Ras and Rho family GTPases. At the time, most signaling pathways were portrayed as strictly linear, but today crosstalk and feedback loops have become an expectation among those who study signal transduction. We will discuss several of the pathways by which Rho family proteins interact with each other, as well as with other members of the Ras superfamily.

The Rho Family of Proteins

The Ridley and Hall articles in *Cell* deal with two proteins, Rho and Rac. To date, 20 genes encoding proteins with a small GTPase domain of the Rho consensus type have been described in humans (Figure 1). With the human genome being almost completely characterized, this is likely to be the final number (Wherlock and Mellor, 2002). It is interesting to note that already in 1992, 8 mammalian Rho proteins had been described. Today, based on primary sequence and known functions, the 20 Rho proteins can roughly be divided into 5 groups, the Rho-like, Rac-like, Cdc42-like, Rnd, and RhoBTB subfamilies. Three of the Rho proteins, RhoD, Rif, and RhoH/TTF do not fall into any obvious grouping and, because little is known about them, they will not be discussed further. Recently, two additional GTPases, MIRO-1 and MIRO-2, were described as belonging to the Rho family (Fransson et al., 2003). However, due to their very low homology to the other Rho GTPases and their lack of the Rho-specific insert loop in their GTPase domains, discussion of these will not be included in this review.

The Rho-like subfamily are all very similar in sequence and when overexpressed as activated proteins, they each contribute to contractility and formation of stress fibers and focal adhesions. Few differences in binding to either effectors or upstream regulatory proteins are known, although RhoC has been suggested to bind more efficiently to Rho kinase (Sahai and Marshall, 2002) and certain Rho guanine nucleotide exchange factors (GEFs) that exchange nucleotide well on RhoA and RhoB, exchange ineffectively on RhoC (Arthur et al., 2002). However, functional differences are suggested by several observations. While RhoA and RhoC are growth-promoting, RhoB is in many cases growth-inhibiting (Du et al., 1999; Chen et al., 2000) and has been shown to

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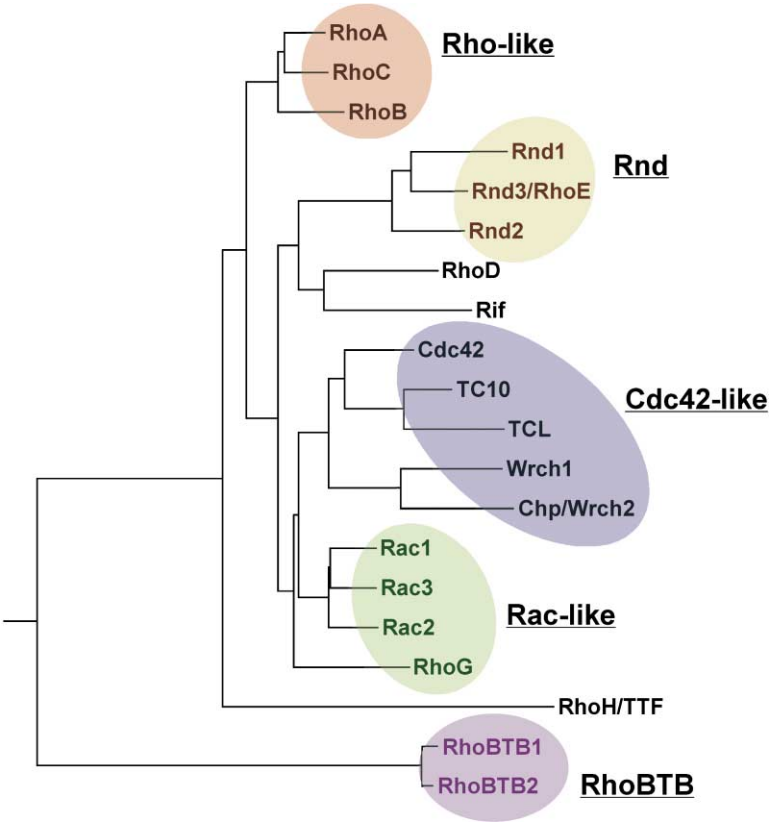

Figure 1. The Rho Family of Proteins

A dendrogram representing the relationships between the 20 Rho family members. The Rho domains of the family members were aligned and a dendrogram was calculated by the Bonsai application (http://calliope.gs.washington.edu/software/_). The five subfamilies, Rho-like, Rnd, Cdc42-like, Rac-like, and RhoBTB, are highlighted by circles.

be downregulated in cancer cells (Adnane et al., 2002). RhoB functions in vesicle trafficking and is localized both on the plasma membrane and on the outer membrane of multivesicular bodies, a late endosomal compartment. Internalized EGF receptor activates RhoB at this site (Gampel and Mellor, 2002) and activated RhoB inhibits transport of the EGF receptor to lysosomes (Gampel et al., 1999). RhoB, in addition, is a short-lived protein that is highly transcriptionally regulated (Zalcman et al., 1995). It is upregulated during the G1/S transition of the cell cycle and is also known to be upregulated during apoptosis (Zalcman et al., 1995; Liu et al., 2000). RhoC expression, on the other hand, has been shown to promote metastatic behavior in a way that the two other subfamily members do not and its expression is elevated in many invasive carcinomas (Suwa et al., 1998; Clark et al., 2000). The region that is most unique for these proteins is in their most C-terminal sequence residues. The interactions of these regions with distinct proteins probably is responsible for many of their different properties. In support of this idea, Zheng and colleagues have recently been able to suppress selectively the RhoA, B, or C phenotypes by expressing chimeric constructs in which the C termini of the respective Rho proteins have been fused to the GTPase activating protein (GAP) domain of p190RhoGAP (Wang et al., 2003). For simplicity, from here on, we will not distinguish between RhoA, B, and C, but refer to them collectively as "Rho", because in most experiments the distinction has not been made.

The Rac-like subfamily all stimulate the formation of lamellipodia and membrane ruffles, as described first for Rac1 (Ridley et al., 1992). While Rac1 and RhoG are widespread, Rac2 and 3 are largely restricted to hematopoietic and neural tissues respectively. (As for the Rho-like subfamily, we will not distinguish between Rac1, 2, and 3 in subsequent discussions.) Expression of RhoG is elevated during the G1 phase of the cell cycle, and it has been implicated in cell growth (Vincent et al., 1992). RhoG interacts with some but not all of the same effectors as Rac1 (Wennerberg et al., 2002; Prieto-Sanchez and Bustelo, 2003), but it has also been shown to activate Rac1 and Cdc42 (Gauthier-Rouviere et al., 1998). How important this is for RhoG's function is a matter of controversy (see below).

The Cdc42-like GTPases all stimulate the formation of filopodia, as originally described for Cdc42 (Kozma et al., 1995; Nobes and Hall, 1995), and they all bind to WASP or N-WASP, which are primary effectors mediating filopodia formation. Cdc42 is also important in cell polarization, but it is unknown if the other subfamily members share this function. TC10 and TCL are both involved in insulin-mediated metabolic events (Chiang et al., 2002). Wrch1 and Chp/Wrch2 are closely related, with one clear difference. Chp/Wrch2 is the only conventional Rho protein that lacks a prenylation signal in the C terminus, but it still localizes to membranes through its C-terminal tail (Aronheim et al., 1998). Wrch1 has been shown to be involved in Wnt signaling (Tao et al., 2001), while the signaling role of Chp/Wrch2 remains uncertain.

The Rnd subfamily all have amino acid substitutions rendering them GTPase inactive, indicating that they exist in cells as constitutively active, GTP bound pro-

teins (Foster et al., 1996; Nobes et al., 1998). This suggests that these proteins are regulated in ways other than by nucleotide binding, and at least with Rnd3/RhoE it seems that this protein is regulated by expression (Hansen et al., 2000; Riento et al., 2003). Rnd1 and Rnd2 are highly expressed in brain and have both been implicated in neurite outgrowth and branching. In contrast, Rnd3/RhoE seems to be widely expressed at low levels and its expression is upregulated by Ras-Raf signaling (Hansen et al., 2000). Interestingly, even though they are most closely related to the Rho subfamily, they antagonize the signaling from Rho (Guasch et al., 1998; Nobes et al., 1998). Mechanisms for how this is accomplished are discussed below.

The most unconventional members of the Rho family are the RhoBTB proteins. These have a large C-terminal extension containing two BTB domains. In the case of RhoBTB1 and RhoBTB2, the GTPase domain is Rho-like, whereas the GTPase domain in RhoBTB3 is not and should therefore not be considered as a Rho protein. The function of the RhoBTB proteins and their signaling is completely unknown. Of note, however, RhoBTB2, also known as DBC2 (deleted in breast cancer 2), is downregulated or mutated in many breast cancers, and its reexpression in T47D breast cancer cells inhibits cell growth (Hamaguchi et al., 2002).

Downstream Effectors

Rho, Stress Fibers, and Tension

In their first paper, Ridley and Hall concluded that stress fibers formed as a result of actin polymerization that was nucleated in focal adhesions. However, previously an alternative had been proposed that stress fibers assemble as a result of myosin-based contractility. Exploring whether Rho might act to induce stress fibers and focal adhesions by stimulating contractility confirmed that this was indeed the case and revealed that active Rho elevated myosin light chain (MLC) phosphorylation (Chrzanowska-Wodnicka and Burridge, 1996). Simultaneously, it was shown that one of the downstream Rho effectors, Rho-kinase (ROCK, ROK) elevates MLC phosphorylation by inhibiting the MLC phosphatase (Kimura et al., 1996). Subsequent work established that Rho-kinase can also take the place of the MLC kinase (MLCK) directly phosphorylating the regulatory MLC and thereby enhancing myosin activation (Amano et al., 1996). Together these studies established that Rho promotes myosin contractility and that the resulting tension drives the formation of stress fibers and focal adhesions (Figure 2). Perhaps the best evidence that tension itself can promote assembly of these structures comes from studies in which mechanical force was applied to single cells in which myosin activity was inhibited. In such cells, extrinsically applied force could take the place of the intrinsic tension, promoting assembly of stress fibers and focal adhesions (Riveline et al., 2001).

Does Rho-induced actin polymerization contribute to stress fiber development? Somewhat surprisingly, when this was examined directly, the level of polymerization in response to Rho activation was found to be relatively small (Machesky and Hall, 1997). Nevertheless, some actin polymerization may be important. Several groups noted that constitutively active forms of Rho-kinase

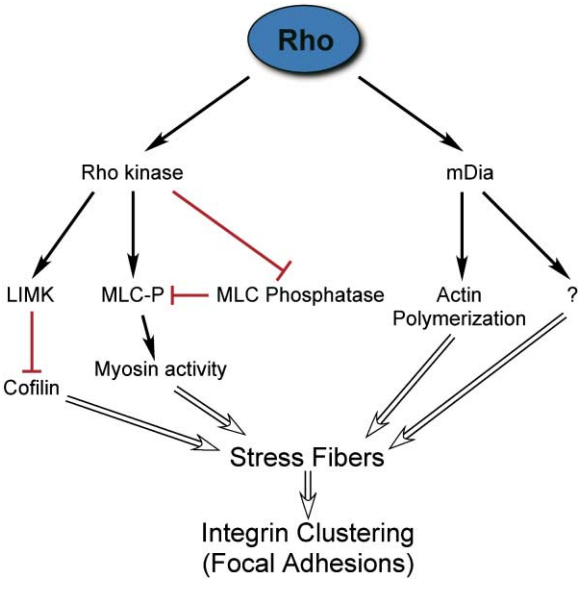

Figure 2. Signaling from Rho to the Cytoskeleton

The pathways from Rho to the formation of stress fibers are described in the text. Direct activating signals are presented by solid arrows. Inhibitory signals are depicted as red bars. Double-lined arrows and bars represent the net result of a signaling pathway. Abbreviations used: LIMK, LIM kinase; MLC-P, phosphorylated myosin II regulatory light chain; MLC phosphatase, myosin light chain phosphatase.

stimulated very prominent stress fibers, but their stellate arrangement was abnormal and they were much thicker than the stress fibers induced by active Rho. In contrast, more normal stress fibers could be induced by coexpression of activated forms of the mammalian homolog of diaphanous (mDia), another Rho effector (Watanabe et al., 1999). Expression of activated forms of mDia promote assembly of fine arrays of thin stress fibers that appear to be less bundled than the stress fibers induced by Rho activation or expression of constitutively active Rho-kinase. The density and appearance of stress fibers could be experimentally varied by titrating different levels of active Rho-kinase and mDia (Watanabe et al., 1999). Significantly, mDia, a member of the formin family of proteins, stimulates actin polymerization (Li and Higgs, 2003), but whether it is the actin polymerizing activity of mDia that contributes to stress fiber organization or some other activity has not been determined. In the study in which external force was applied to cells and promoted focal adhesion formation and growth, mDia was found to be a critical factor. External force did not induce focal adhesion formation if Rho activity was inhibited by C3 exotransferase, but could be rescued by expression of constitutively active mDia (Riveline et al., 2001). This study identified at least two components in stress fiber and focal adhesion assembly, the generation of tension, which could be either intrinsic, i.e., generated by myosin activity, or extrinsic, i.e., applied from the outside, and an activity supplied by activation of mDia (Figure 2).

Several other targets for Rho have been identified that are relevant in the context of the actin cytoskeleton (Bishop and Hall, 2000). These include citron kinase,

which, like Rho-kinase, stimulates MLC phosphorylation and myosin activity. However, citron kinase appears to function primarily following mitosis in the cleavage furrow of dividing cells (Madaule et al., 1998). Several cytoskeletal targets have also been identified downstream of Rho-kinase. These include LIM kinase and adducin. LIM kinase phosphorylates the actin depolymerizing protein, cofilin/ADF, inhibiting its function (Maekawa et al., 1999). The inhibition of cofilin stabilizes actin filament arrays such as stress fibers and the cell cortex. Phosphorylation of adducin by Rho-kinase increases its binding to F-actin (Kimura et al., 1998). Members of the ERM family were also reported to be substrates for Rho-kinase and to be activated by this phosphorylation. However, subsequent work has argued that although this phosphorylation is driven by Rho, it is mediated by PIP_2 and another kinase downstream from Rho that remains to be identified (Matsui et al., 1999).

When cells round up, either when entering mitosis or due to experimental manipulation, they lose stress fibers and focal adhesions. Intuitively, a decrease in Rho activity would be expected. Somewhat surprisingly, the reverse occurs (Ren et al., 1999; Maddox and Burridge, 2003). This indicates that there are mechanisms to disassemble stress fibers in the presence of high Rho activity. With mitotic cells, evidence was presented that high Rho activity contributed to rounding and a more rigid cell cortex. Arguments have been presented previously for the advantages to a mitotic cell being rounded (Maddox and Burridge, 2003), but why do cells rounded up in other situations have high Rho activity? We speculate that the elevation of Rho activity results in a rigid cortex, in part due to the suppression of cofilin activity by the Rho-kinase/LIM kinase pathway, in part through enhanced myosin activity, but possibly also due to other activities stimulated by Rho. The rigid cortex of a rounded cell provides resistance to mechanical forces and perhaps, therefore, protection against damage. This may be important for leukocytes within the circulation, but, other than in mitosis, the advantage for other cells is not clear. The decrease in Rho activity observed when suspended cells adhere to matrix (Ren et al., 1999), may be largely to facilitate the change from a rigid to a more dynamic actin cortex that allows spreading and cell migration (Arthur and Burridge, 2001).

Rho, Rac and Adhesion to the Matrix

Ever since Ridley and Hall demonstrated that Rho regulates focal adhesions, there has been much interest in Rho proteins and cell adhesion. Regarding adhesion to extracellular matrix (ECM), neither Rho nor Rac affect the affinity of integrins. However, in cells like fibroblasts, both induce integrin clustering and may thereby increase the strength of adhesion. With Rho, the clustering is very pronounced and results from tension aggregating dispersed integrins, such that they align through their attachment with the ends of stress fibers in focal adhesions (Chrzanowska-Wodnicka and Burridge, 1996). The inhibition of Rho in cells like fibroblasts decreases adhesion, causing a retraction of lamellae and rounding of the cell body. This cannot be due just to a loss of focal adhesions because many cells without these structures adhere well and have a spread morphology. The reason for decreased adhesion may be indirect and reflect a collapse of the cortical actin organization. Supporting this idea, a similar phenotype is generated by disrupting the actin cytoskeleton with drugs that promote actin depolymerization.

Focal complexes, the integrin clusters induced by Rac are smaller than focal adhesions, but little is known about how these assemble. Although it does not directly affect integrin affinity, Rac can recruit high-affinity integrins to regions where new membrane protrusion is occurring. This was demonstrated with the integrin $\alpha v\beta 3$ in migrating endothelial cells, but is likely to be important for many migrating cells where lamellipodia are stabilized by new adhesions (Kiosses et al., 2001).

In leukocytes, which lack both stress fibers and focal adhesions, the role of Rho in adhesion appears quite different. Indeed, inhibiting Rho elevates integrin-mediated adhesion (Worthylake et al., 2001). Here, it has been suggested that in the resting state, such as with suspended cells, the integrins are dispersed over the cell surface and prevented from being clustered by being tethered to the relatively rigid cortical actin network. Again, the stability of the cortical actin has been attributed to active Rho and the LIM kinase pathway maintaining the actin filament-severing protein, cofilin, in an inactive conformation. In this model, upon inhibition of Rho, cofilin becomes activated, disrupting the cortical actin network, thereby allowing integrins to be clustered either by binding multivalent extracellular ligands or by intracellular factors (Worthylake and Burridge, 2003). Interestingly, activated Rac promotes adhesion when introduced into lymphocytes. The increased adhesion was shown to be due to cell spreading, which was accompanied by a reorganization of the actin cytoskeleton and clustering of integrins adhering to ECM (D'Souza-Schorey et al., 1998). Again, we envisage a similar mechanism, but here one must invoke a mechanism to activate cofilin in the presence of active Rac, which via the p21-activated kinase (PAK)/LIM kinase pathway should be inhibiting cofilin activity (see below). Alternatively, there may be other ways to activate cofilin or other actin severing proteins. Severing proteins such as gelsolin are activated by elevated calcium and this should occur downstream from Rac's stimulation of phospholipase C (Snyder et al., 2003). As leukocytes spread, the initial engagement of integrins would be expected to lead to a positive feedback loop, arising from the integrins activating Rac and inhibiting Rho activity.

Migrating leukocytes are very responsive to chemotactic gradients. Recent work has shown that the polarity of these cells is regulated by different G protein-coupled receptors signaling to Rac and Rho (Xu et al., 2003). Within the cell, high Rac activity regulates behavior at the front, whereas Rho activity is critical at the rear. The antagonism between these signaling pathways determines the directional responsiveness to chemotactic signals. The activities of Rho and myosin at the rear inhibit protrusive activity in this region and confine protrusion to the front of the cell (Xu et al., 2003; Worthylake and Burridge, 2003).

Rac and Membrane Protrusion

In their second paper, Ridley, Hall, and coworkers observed that expression of constitutively activated Rac induced the formation of membrane ruffles and that ruffle formation induced by growth factors was suppressed by expression of a dominant-negative form of

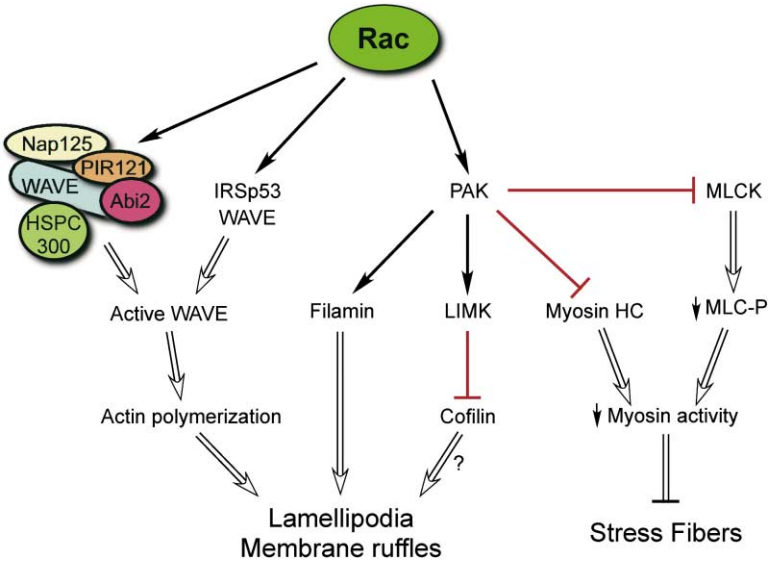

Figure 3. Signaling from Rac to the Cytoskeleton

The pathways leading from Rac activation to the formation of lamellipodia and membrane ruffles, and the loss of stress fibers, are described in the text. Direct activating signals are presented by solid arrows. Inhibitory signals are depicted as red bars. Double-lined arrows and bars represent the net result of a signaling pathway. Abbreviations used: PAK, p21-activated kinase; LIMK, LIM kinase; MLCK, myosin light chain kinase.

Rac (Ridley et al., 1992). Membrane ruffles are closely related to lamellipodia, and the terms are often used interchangeably. Lamellipodia are the thin protrusive structures generated at the leading edge of migrating cells. Frequently, lamellipodia evolve into ruffles, when these membrane protrusions fail to adhere and are swept backward on the dorsal surface. In other situations, however, ruffles can be induced de novo on the dorsal surfaces of cells. The significance of these structures is uncertain but may relate more to macropinocytosis than cell migration. In this regard, Ridley and coworkers observed that expression of activated Rac caused subconfluent cells to accumulate large vesicles containing material from the extracellular environment. Similar membrane ruffling and macropinocytosis had previously been observed in response to activated Ras (Bar-Sagi and Feramisco, 1986) and Ridley and colleagues showed that this Ras-induced ruffling was blocked by dominant-negative Rac (Ridley et al., 1992).

Whereas the role of Rho in actin polymerization has been controversial, Rac is a potent activator of polymerization. When Ridley et al. identified Rac as driving membrane protrusion at the leading edge, the dominant idea was that actin polymerization at such sites was driven by the uncapping of actin filaments, particularly by lipids such as PIP_2. The finding that Rac activates PI 5-kinase (Tolias et al., 1995) is consistent with earlier models and the generation of PIP_2 may, indeed, contribute to exposure of the barbed ends of actin filaments at the leading edge. However, with the discovery of the Arp2/3 complex and its role in nucleating actin polymerization, the focus turned to identifying how Rac can activate Arp2/3. First, a critical component between Rac and Arp2/3 was found to be the WAVE/Scar protein that belongs to the WASP family (Figure 3) (Miki et al., 1998; Machesky and Insall, 1998). Whereas Cdc42 binds WASP and N-WASP inducing a conformational change that activates these proteins such that they stimulate Arp2/3 to nucleate actin polymerization, the WAVE/Scar proteins do not bind Rac directly. Two different ways of activating WAVE/Scar downstream from Rac have been described. In the first, interactions between IRSp53 and both Rac and WAVE/Scar were identified (Miki et al., 2000), such that IRSp53 links Rac activity to WAVE/Scar activation. Others, however, have found that IRSp53 preferentially binds Cdc42 rather than Rac, raising the possibility that IRSp53 may be downstream from Cdc42 activation (Krugmann et al., 2001; Govind et al., 2001). An alternative pathway from Rac to WAVE activation of the Arp2/3 complex was suggested by the work of Eden et al. who found that WAVE existed in cells in an inactive complex, together with two Rac binding proteins, Nap125 and PIR121, along with HSPC300 and Abi2 (Eden et al., 2002). Activated Rac was found to cause dissociation of the Nap125, PIR121, and Abi2 complex from WAVE and HSPC300, leaving WAVE active with respect to stimulating Arp2/3-actin polymerization activity (Eden et al., 2002). Interestingly, the adaptor protein Nck also binds Nap125 and similarly promotes dissociation of the complex and WAVE activation. Both Rac and Nck are activated downstream from a variety of receptors and provide alternative or synergistic ways of coupling extracellular signals to actin polymerization.

Another downstream effector of Rac that has been implicated in cytoskeletal rearrangements and membrane ruffling is PAK. Here, however, the observations with PAK are complex and sometimes cell type specific (Bokoch, 2003). This kinase is activated by both Rac and Cdc42 and expression can promote formation of lamellipodia (Sells et al., 1997) and lead to the loss of stress fibers and focal adhesions (Manser et al., 1997). Some of these effects occur with expression of PAK mutants that cannot bind Rac or Cdc42, and, somewhat surprisingly, some of the effects are induced by expression of kinase-dead forms of the enzyme. Whereas loss of stress fibers and focal adhesions requires active PAK (Manser et al., 1997), the development of lamellipodia does not, although the morphology and polarized development of lamellipodia is affected by PAK activity (Sells et al., 1999). Several PAK substrates or binding partners have been implicated in the effects of PAK, including the actin binding protein filamin, LIM kinase, myosin, the paxillin/Pix/PKL complex, and the adaptor protein Nck.

Filamin is the major actin binding protein of the cell cortex and is enriched in membrane ruffles and found in many other sites where F actin is concentrated. It was identified in a screen for PAK binding proteins and was shown to be a substrate for PAK (Vadlamudi et al., 2002). Using cells lacking filamin or in which filamin had been reexpressed revealed that filamin was essential for membrane ruffling induced by growth factors or by PAK expression (Figure 3). Membrane ruffling induced by growth factors was blocked by expression of an autoinhibitory domain of PAK, although this did not block ruffling induced by PMA, indicating the existence of more than one pathway to ruffle formation (Vadlamudi et al., 2002). PAK and filamin interact via the C-terminal repeat domain of filamin, which is also involved in dimerization, and by the domain of PAK that binds Rac and Cdc42. Interestingly, the binding of filamin to PAK was shown to activate PAK. Filamin is likely to have multiple roles in lamellipodia and ruffles, serving both to crosslink F actin, thereby stabilizing the actin networks that support these membrane protrusions, but also serving as a scaffold for PAK as well as many other signaling proteins (Stossel et al., 2001). As a footnote on filamin, it also binds Rho, Rac, and Cdc42, but does not discriminate between the GTP and GDP loaded forms (Ohta et al., 1999). Given filamin's prominent role in the actin cortex, we speculate that this binding may restrict diffusion of Rac and Rho and confine their activity to local regions of the plasma membrane.

LIM kinase is activated by phosphorylation by both PAK (Edwards et al., 1999) and by Rho-kinase (Maekawa et al., 1999). In turn, LIM kinase phosphorylates and inactivates cofilin, a protein that promotes depolymerization of F-actin. The net result of PAK activation of LIM kinase is to stabilize actin filaments and filament arrays. This is somewhat paradoxical, because although decreasing the turnover of actin filaments in lamellipodia or ruffles should increase their stability, the induction of actin polymerization at these sites has been associated with active cofilin and rapid subunit cycling between polymer and monomer states (Blanchoin et al., 2000; Zebda et al., 2000). A possible explanation for this paradox would be if there is a spatial or temporal separation of active and inactive cofilin, with active cofilin being found more distally in a lamellipodium or ruffle, but with cofilin activity being inhibited further back as a result of PAK/LIM kinase phosphorylation. Such a scheme would allow cofilin to promote polymerization in cooperation with the Arp2/3 complex at the front of a protrusion, but would allow stabilization of the resulting network of actin filaments further back in the body of the lamellipodium or ruffle.

The association of PAK with the Pkl/paxillin/Pix complex potentially provides multiple downstream signaling possibilities. Pkl is an Arf GAP, thereby suggesting a point of convergence between Rac and the Arf family of GTPases (see below), whereas Pix is itself a Rac and Cdc42 GEF (Turner et al., 2001). The association of a downstream Rac effector with an upstream regulator of Rac activity suggests signaling feedback loops. The pairing of downstream effectors with GEFs also raises the intriguing possibility that this provides a mechanism whereby a particular GEF activates preferentially one out of many possible downstream pathways.

Another interesting association occurs between Nck and PAK. Nck is an adaptor protein that recruits PAK to receptor tyrosine kinases (Bokoch, 2003), but it is also implicated in activation of both WASP and WAVE/Scar, thereby stimulating Arp2/3 complex nucleation of actin polymerization. Whether the interaction of Nck with PAK serves mainly to recruit PAK to receptor tyrosine kinases or whether it also provides a link between PAK and actin polymerization has not been determined.

Ridley and colleagues noted that expression of active Rac stimulated formation of stress fibers in serum-starved quiescent cells. They showed that this was due to a downstream activation of Rho and was slower and never so robust an effect as activation by serum or LPA. Subsequent work has more commonly observed that growth factor stimulation of Rac activity antagonizes Rho resulting in disassembly of stress fibers and focal adhesions (Sander et al., 1999). The potential explanation for these different findings and how Rac may antagonize Rho activity are discussed below. It is worth mentioning here, however, that active PAK may contribute to the loss of stress fibers and focal adhesions by phosphorylating and inhibiting MLCK activity, thereby leading to a decrease in MLC phosphorylation (Figure 3) (Sanders et al., 1999). The effect of PAK on MLC phosphorylation is particularly controversial, with some groups reporting enhanced phosphorylation but others reporting a decrease (Bokoch, 2003). Additionally, phosphorylation of the myosin heavy chain by PAK has also been reported as a mechanism to inhibit myosin function and cause the disassembly of actomyosin structures (van Leeuwen et al., 1999).

Regulating Rho and Rac Activity

At the time of the two Ridley and Hall papers in *Cell*, it was well known that these proteins existed in two states, an inactive state with GDP bound and an active state in which the GDP was replaced by GTP. Already, examples of the three major classes of regulators, GAPs, GEFs, and guanine nucleotide dissociation inhibitors (GDIs), had been identified, but there was little understanding of the signaling pathways that modulate their activity.

Several Rho GAPs were already known in 1992, but since then the number of known genes encoding a Rho GAP domain has increased to about 80 (Moon and Zheng, 2003). The function of most of these are still unknown, but the variations in additional domains found in these proteins indicate that they act in a wide variety of signaling pathways in different tissues. In addition, there is evidence that some of these Rho GAP domain-containing proteins act as Rho effectors and transmit signals downstream from Rho protein activation rather than just turning off the Rho signal. One example is the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase). It lacks Rho GAP activity, but uses this domain to bind Rho proteins and this binding regulates its activity (Zheng et al., 1994). Another example is N-chimaerin, where the actual binding to GTP-loaded Rac rather than its GAP activity is the determining factor for its biological activity (Kozma et al., 1996).

The only known GEF for Rac and Rho in 1992 was Smg-GDS, which is an unusual GEF in that, at least in

vitro, it exchanges nucleotides on a wide variety of small GTPases of different families (Hiraoka et al., 1992). At this time, Dbl, the prototype GEF containing the Dbl-homology (DH)/Pleckstrin homology (PH) domain tandem repeat, common to all Dbl family members, had been characterized as a Cdc42 GEF (Hart et al., 1991). Since then, the Dbl family of exchange factors has been established as primarily responsible for GEF activity on Rho proteins and around 60 members have been found in mammalian genomes (Schmidt and Hall, 2002). More recently, an additional family of Rho GEFs lacking the DH-PH tandem domains has been identified. The founding member of this family was DOCK180 and about 10 members have been found (Meller et al., 2002; Cote and Vuori, 2002). In the case of DOCK180, it requires a cofactor protein (ELMO) to exchange nucleotide on Rac (Brugnera et al., 2002). Other members of this family seem to be capable of nucleotide exchange without the help of additional proteins (Cote and Vuori, 2002).

At the time of the Ridley and Hall papers, RhoGDI had already been discovered. Today, three RhoGDIs are known, and a few other molecules have been suggested to have RhoGDI activity. RhoGDI binds to a subset of Rho proteins, inhibits nucleotide exchange and sequesters these proteins away from membranes, where normally they would be active. It has been established that the concentration of RhoGDIs is roughly equal to the concentration of the Rho proteins being bound by them (Michaelson et al., 2001), indicating that at a given time, a large fraction of the Rho proteins in the cell is likely to be bound to GDIs. With this in mind, it is clear that the regulation of the binding between GDIs and their target Rho proteins is an important factor in Rho signaling. Several of the RhoGDIs have been shown to bind to other molecules, which stimulate release of the Rho protein. In this way, a Rho protein can be delivered by the GDI to a particular site of action in the cell in response to a signal. Overall, however, these mechanisms are poorly understood but currently receiving increased attention.

Signaling from Growth Factor Receptors to Rho Proteins

The Ridley and Hall *Cell* papers were the first to describe signaling from the cell surface to Rho proteins. LPA was established as a Rho activator, whereas bombesin was shown to independently activate both Rho and Rac. Growth factors, like PDGF, EGF, and insulin, were observed to stimulate Rac, leading to a subsequent Rho activation, and PKC agonists such as PMA were found to activate Rac without activating Rho. At that time, the function of the Dbl family of proteins as Rho exchange factors was not known, so the involvement of these proteins was not implied. Since then, the molecular pathways for several of these signals have been elucidated and many additional pathways from the cell surface to Rho proteins have been described (Figure 4). The activation of Rho by LPA has been worked out. The receptors for LPA, Edg/LPA receptors are seven transmembrane receptors that couple to heterotrimeric G proteins. The signal to Rho is mediated by the activation of $G_{\alpha 12/13}$ subunits, which in turn bind to the RGS domain of particular Rho GEFs, bringing them to the membrane and activating them (Hart et al., 1998; Kozasa et al., 1998). Rho GEFs containing an RGS domain are p115RhoGEF, PDZ-RhoGEF, and LARG, and they have all been shown to be activated by heterotrimeric G proteins (Fukuhara et al., 2001). In addition, LPA signals to Rac, even though this was not discovered in the original papers. Rho activation is followed by a Rac activation, which seems to be dependent on phospholipase activation, calcium/calmodulin-dependent kinase and Tiam1 (Fleming et al., 1998). Bombesin is likely to signal to Rho in a similar way as LPA, but has also been reported to activate Arf6. In turn, Arf6 can stimulate Rac and inhibit Rho signaling (Boshans et al., 2000). PMA stimulates Rac through a PKC-dependent phosphorylation and activation of Tiam1 (Mertens et al., 2003). In their original study, Ridley and coworkers observed that PMA was unique in not subsequently leading to Rho activation. They suggested that this might be due to other pathways activated by PKC that antagonized Rho or the assembly of focal adhesions and stress fibers. Recently, the inactivation of Rho by PKC has been ascribed to stimulation of p190RhoGAP activity by c-Src (Brandt et al., 2002).

Exploring the pathways from growth factor receptors to Rac activation revealed that the induction of membrane ruffles by various growth factors was blocked by inhibitors of PI 3-kinase activity (Nobes et al., 1995). These inhibitors, however, did not block ruffling induced by expression of activated Rac, indicating that the products of PI 3-kinase were acting upstream of Rac and suggesting that either directly or indirectly they were stimulating Rac GEFs. Subsequent studies have confirmed that many Rac GEFs are activated by PIP_2 or PIP_3 (Schmidt and Hall, 2002; Mertens et al., 2003). For example, the Rac GEF Tiam1 is recruited to membranes and thereby activated by PIP_3 binding to its amino-terminal PH domain (Mertens et al., 2003). Another mechanism involves the activation of a Rac GEF complex containing Sos1, Eps8, and Abi1/E3B1 (Nimnual et al., 1998; Scita et al., 1999), which localizes to membranes and the sites of PI-3 kinase activation by direct binding to the p85 subunit of PI 3-kinase (Innocenti et al., 2003). Several other GEFs are also activated by interactions with PI 3-kinase or its products (Schmidt and Hall, 2002). There are also PI-3 kinase independent pathways by which Rac GEFs can be activated. Tiam1 contains a Ras binding domain, similar to the one in Raf. GTP bound Ras binds to this domain thereby stimulating Tiam1's Rac GEF activity (Lambert et al., 2002). Furthermore, PDGF stimulation of Swiss 3T3 fibroblasts causes a Phospholipase C- and calcium/calmodulin-dependent protein kinase II-dependent threonine phosphorylation of Tiam1, leading to its localization to membranes and activation (Mertens et al., 2003).

The relationship between Rac activation and phosphoinositides is complex, because not only are many Rac GEFs stimulated by PIP_2 and PIP_3, but Rac (and Cdc42) bind to and activate both PI 4,5-kinase and PI 3-kinase (Tolias et al., 1995). This has led to the idea of positive feedback loops between Rac activity and PIP_2 and PIP_3 synthesis, which may be important for a rapid response to extracellular signals, such as chemotactic factors. Consistent with this idea, in migrating cells, activated Rac as well as PIP_2 and PIP_3 are concentrated in the leading lamellipodium or in membrane ruffles (Kraynov et al., 2000). Phosphoinositides have also been implicated in the downstream pathways from Rac, promoting Arp2/3 driven actin polymerization (Rohatgi et al.,

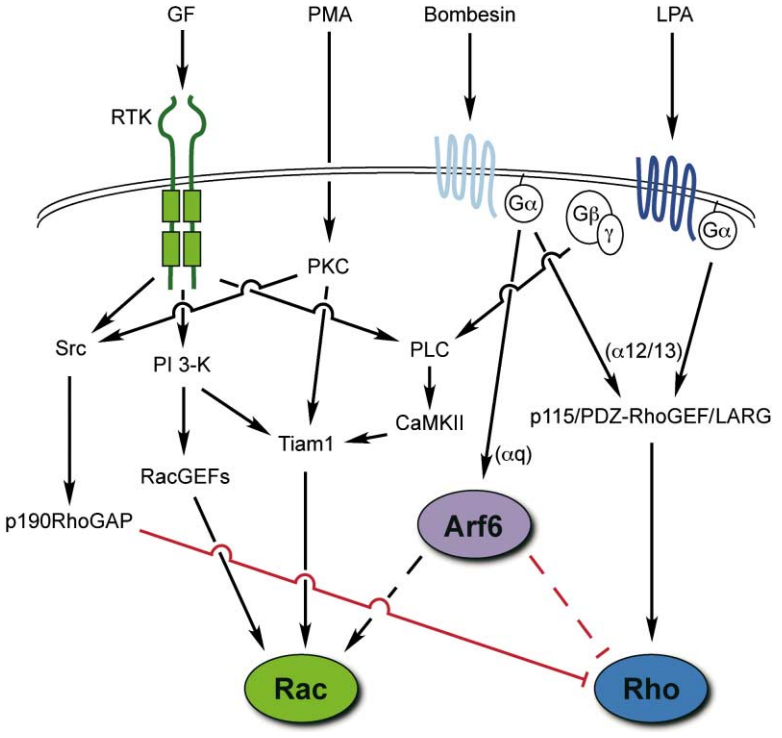

Figure 4. Regulation of Rac and Rho by Soluble Factors

Depiction of signaling pathways from serum components leading to regulation of Rho and Rac, as decribed in the text. Abbreviations: GF, growth factor such as PDGF, EGF, or insulin; RTK, receptor tyrosine kinase; PLC, phospholipase C; PKC, protein kinase C; PI 3-K, phosphatidylinositol 3-kinase.

2001). It is interesting that Rac also binds and activates phospholipase C-β, which suggests a way that Rac may terminate these positive feedback loops (Snyder et al., 2003).

Signaling from Adhesion Receptors to Rho Proteins

During the past several years, increasingly it has been recognized that not only are Rho proteins regulated downstream from G protein-coupled receptors and receptor tyrosine kinases, but that many cell adhesion molecules also affect Rho protein activity. This is not surprising, given the pronounced effect that many adhesion molecules have on cytoskeletal organization. Several of the classes of cell adhesion molecules have been shown to affect Rho, Rac, or Cdc42 activity, including integrins (DeMali et al., 2003), cadherins (Braga, 2002) and Ig superfamily members (Thompson et al., 2002). Upon cadherin engagement, there is an elevation in the activities of Rac and Cdc42, which may contribute to enhanced cadherin function in a positive feedback pathway. Cadherin engagement, however, drastically suppresses Rho activity, in part by increased p190RhoGAP activity (Noren et al., 2003). Identifying the GEFs and GAPs downstream from various adhesion molecules and the pathways involved has become a major pursuit.

Crosstalk

Currently, one of the exciting aspects of this field concerns the interactions between Rho family proteins. The foundation for this area was also laid in the Ridley and Hall *Cell* papers. In their second paper, they demonstrated that the induction of membrane ruffling by Ras was due to activation of Rac, and that this led subsequently to formation of stress fibers, in a Rho-dependent fashion (Ridley et al., 1992). Together, these observations delineated a signaling pathway whereby Ras activated Rac, which in its turn activated Rho. Later on, it was found that Cdc42 could also feed in to the same pathway by activating Rac (Kozma et al., 1995; Nobes and Hall, 1995). More recently, many examples of crosstalk between members of the Rho family, as well as with other members of the Ras superfamily have been identified. There are examples where one family member depresses the activity of another by stimulating a GAP, or elevates activity by stimulating a GEF. In addition, there are proteins, like Bcr and Abr, that contain both GAP and GEF domains for different Rho members (Schmidt and Hall, 2002). The interplay between family members is also mediated by interactions between their respective downstream signaling pathways.

With respect to the activation of Rho by Rac, more recently, the dogma has turned toward an inverse relationship, where activation of Rac leads to the inactivation of Rho and vice versa (Sander et al., 1999). In fact, Ridley and Hall pointed out in their first paper that the effect of growth factors or Rac in stimulating stress fibers was something seen only in serum-starved cells (Ridley and Hall, 1992). This suggests that there is a serum component that is modulating the response of cells to Rac activation. One mechanism for how Rac can inhibit Rho was identified recently (Figure 5). Rac-mediated production of oxygen radicals causes an inhibition of the low molecular weight protein tyrosine phosphatase (LMW-PTP), leading to increased phosphorylation and activation of p190RhoGAP. In turn, this results in the inactivation of Rho (Nimnual et al., 2003). Signaling events downstream from Rac may also inhibit Rho signaling pathways. For example, as mentioned earlier, constitutively active PAK promotes loss of stress fibers and focal adhesions. This may occur via inhibition

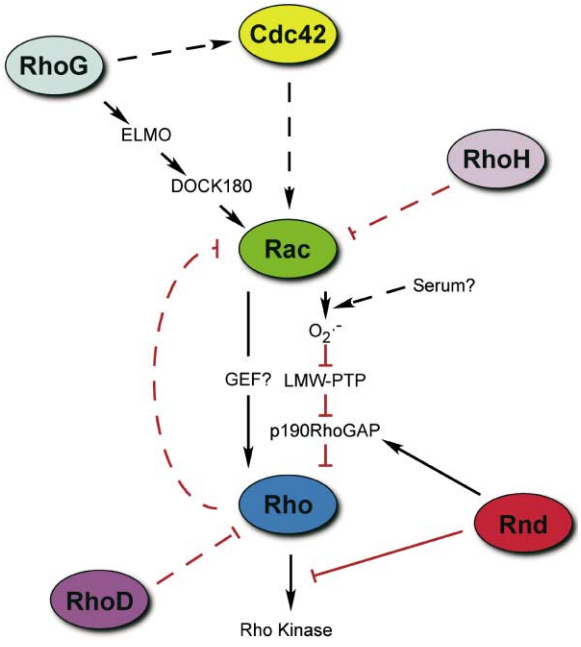

Figure 5. Crosstalk between Rho GTPases

Activating signals are shown as black arrows, inactivating as red. Direct signals are drawn as solid lines, whereas signals with unknown signaling intermediates are drawn as dashed lines. Abbreviations used: $O_2^{\bullet-}$, Superoxide anion; LMW-PTP, low molecular weight protein tyrosine phosphatase.

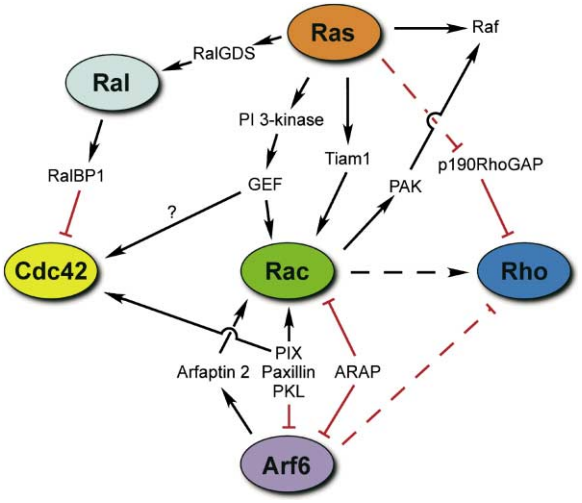

Figure 6. Crosstalk between the Rho Family and Other Ras Superfamily GTPases

Signals between Rho GTPases and other small GTPases are depicted, as well as signaling intermediates where these are known. Activating signals are shown as black arrows, inhibitory signals as red bars, direct signals as solid lines, and indirect as dashed lines. Abbreviations used: RalGDS, Ral guanine nucleotide dissociation stimulator; RalBP1, Ral-binding protein 1.

of the MLCK (Sanders et al., 1999), or by direct phosphorylation of myosin II heavy chain by PAK, both events leading to inhibition of myosin function (van Leeuwen et al., 1999).

Several studies indicate that Ras can also activate Rho, but here the signaling pathways appear to be complex. In MDCK cells, Ras transformation causes an activation of Rho, mediating the epithelial-mesenchymal transition (Sander et al., 1999). This activation, however, occurs independent of Rac activation, since Rac is inactivated at the same time. In some cells, the signaling from Ras to Rho has been reported to be positive, in others negative (Sahai et al., 2001).

A particularly interesting example of crosstalk occurs between the Rnd family and Rho (Figure 5) (Chardin, 2003). This was recently shown to be accomplished through several different signals. All three Rnd proteins bind and activate p190RhoGAP, leading to an inactivation of Rho (Wennerberg et al., 2003). In addition, Rnd3/RhoE, but not Rnd1 and Rnd2, was shown to bind and inactivate Rho Kinase, leading to loss of Rho signaling (Riento et al., 2003). This exemplifies a common theme in Rho signaling where an upstream-signaling protein can act at several levels downstream, thereby generating a more potent effect. It is significant that GEM, another small G protein of the Ras superfamily, also appears to use this strategy to inhibit Rho action by targeting both downstream effectors and a GAP. Like the Rnd proteins, GEM inhibits Rho-kinase and it interacts with another Rho GAP, GMIP (Chardin, 2003).

RhoG was reported to signal to and activate Rac and Cdc42 (Gauthier-Rouviere et al., 1998) and a signaling pathway for the signaling to Rac was recently described. Activated RhoG binds ELMO1, which in turn binds and activates the unconventional Rac GEF Dock180 (Katoh and Negishi, 2003). The importance for RhoG's signaling to Rac remains controversial, however. While some reports indicate that this pathway is needed for RhoG signaling and its biological effects (Gauthier-Rouviere et al., 1998), others argue against this (Wennerberg et al., 2002; Prieto-Sanchez and Bustelo, 2003).

Some of the examples of crosstalk between Rho proteins and other small GTPases are illustrated in Figure 6. The activation of a Rac GEF, Tiam1, by Ras binding was discussed earlier. Rac also feeds into the mitogenic signals of Ras by causing a coactivating phosphorylation of the Ras effector Raf (King et al., 1998). Rac, in addition, cooperates with Arf6 in regulating membrane protrusions (Radhakrishna et al., 1999) and there are several proteins and protein complexes that contain protein domains regulating both Rac and Arf6 activities. A particularly interesting link is the Arf and Rac binding protein Arfaptin 2/POR1 (Van Aelst et al., 1996; Kanoh et al., 1997). This protein binds Rac in either a nucleotide-free manner or in a GDP-dependent manner (Shin and Exton, 2001; Tarricone et al., 2001). Interestingly, the Rac binding site, which is made up of an Arfaptin 2 dimer, has structural similarities to a Dbl domain and binds Rac in a similar manner as Tiam1 binds Rac (Cherfils, 2001). In addition, Arfaptin 2 binds Arf proteins of all three classes in a GTP-dependent manner and this has been shown to release the Rac molecule (Tarricone et al., 2001). Therefore, this protein could create a link between Arf activation, Rac activity, and membrane ruffling, where it can release Rac at sites of Arf activation in a RacGDI-like manner. Truncated mutants of Arfaptin 2 lacking either the Arf or the Rac binding site block

membrane ruffling caused by Arf6 (D'Souza-Schorey et al., 1997). Several other intriguing links between Rac and Arf proteins include the ARAP family, which contain GAP domains for both proteins, and the complex between the Rac GEF PIX and the Arf GAP PKL/Git/Cat (Figure 6) (Turner et al., 2001).

Pathogens that Target Rho Proteins

A wide range of bacterial pathogens have developed strategies to subvert host cell function by regulating Rho proteins. Some pathogenic bacteria manipulate Rho proteins to prevent their uptake and death by phagocytosis, whereas others promote phagocytosis by host cells so as to occupy immunologically inaccessible intracellular locations. The bacterial proteins that modify Rho family members are powerful tools that have been used since the earliest studies on these GTPases. The prototypical agent is the C3 exotransferase produced by *Clostridium botulinum*. This protein ADP-ribosylates RhoA, B, and C on Asn41, inhibiting nucleotide exchange catalyzed by GEFs, and rapidly leading to inactive proteins (Barbieri et al., 2002). C3 has been a valuable reagent for identifying roles for Rho in many situations, although at high concentrations C3 has some activity toward Rac (Ridley et al., 1992). Many clostridial species produce toxins that glucosylate Rho proteins (Barbieri et al., 2002). Examples are the A and B toxins of *C. difficile*, which glucosylate Rho, Rac, and Cdc42, and the lethal toxin of *C. sordellii*, which not only glucosylates Rho, Rac, and Cdc42, but also Ras. With Rho, the glucose is transferred to Thr37. This blocks GTP exchange, GAP activity, as well as the interaction of Rho with effectors. Whereas the above toxins inhibit signaling by Rho proteins, cytotoxic-necrotizing factor (CNF) produced by several pathogenic strains of *E. coli* activates Rho by deamidation of Gln63 (Barbieri et al., 2002). This blocks the intrinsic and GAP-activated GTPase activity, rendering Rho constitutively active.

Salmonella promotes its own uptake by host epithelial cells lining the intestine by injecting two proteins, SopE and SopE2, into the host cells (Stebbins and Galan, 2001). These proteins are potent GEFs for Rac and Cdc42, that induce very active membrane ruffling at the sites of bacterial adhesion, thereby leading to phagocytosis of the bacterium. Having achieved entry into the host cell, the Salmonella secretes a second protein, SptP that is both a tyrosine phosphatase as well as a GAP for Rac and Cdc42 (Stebbins and Galan, 2001). Presumably, once entry into the host cell has been accomplished, it is advantageous for the bacterium to suppress Rac and Cdc42 activity and so restore a more quiescent cytoskeletal state. Enteropathic *Yersinia* also express several proteins that modulate Rho protein activity (Finlay and Cossart, 1997; Bliska, 2000). To get out of the intestine, these bacteria adhere to M cells in the intestinal epithelium, stimulating their own phagocytosis and subsequent passage across the epithelial barrier into the body (Finlay and Cossart, 1997). *Yersinia* then adhere strongly to immune cells within Peyer's patches but now inhibit their own phagocytosis and potential destruction by the immune cells by introducing into these cells several proteins (Yops) that inhibit Rho family GTPases (Bliska, 2000). YopE is a broad specificity GAP that inactivates Rho, Rac, and Cdc42. YopT is a protease that specifically cleaves the C terminus of Rho, Rac, and Cdc42, such that the resulting proteins are effectively deprenylated and dissociated from cell membranes, where their normal sites of action would occur. YopO is a serine-threonine protein kinase that binds both Rho and Rac, but its role has not been fully elucidated. Finally, YopH is a potent tyrosine phosphatase. Several tyrosine phosphorylated proteins in focal adhesions have been identified as substrates (Bliska, 2000), but this tyrosine phosphatase may also contribute to downregulation of Rho family GTPase activity by dephosphorylating GEFs that are normally stimulated by tyrosine phosphorylation, such as members of the Vav family.

Physiological Relevance

The induction of stress fibers and focal adhesions by Rho and the induction of membrane ruffles by Rac are striking observations, but these structures are to a degree artifacts of tissue culture. Stress fibers and focal adhesions are rarely seen in cells in organisms and appear to be a "wound" response to the tissue culture environment. Similarly, the ruffling phenotype induced by Rac is particularly pronounced due to the two-dimensional nature of tissue culture surfaces. Nevertheless, these cytoskeletal structures point to the roles of Rho and Rac in generating contractile force and membrane protrusion, respectively. Rho and Rac are often antagonistic in their effects but their coordinated activities are critical for effective cell migration (Nobes and Hall, 1999; Xu et al., 2003). In itself, migration is of fundamental importance to our own biology. Cell migration underlies much of our embryology and development, and it continues to play essential roles in the adult organism, ranging from normal physiological activities, such as wound healing and the recruitment of leukocytes to sites of inflammation, to pathological situations such as rheumatoid arthritis and tumor invasion. Additionally, many related activities, such as phagocytosis, smooth muscle contraction, cell shape determination, and the development of cell-cell junctions depend on the actions of Rho and Rac through their regulation of the actin cytoskeleton (Etienne-Manneville and Hall, 2002).

The Ridley and Hall papers in *Cell* in 1992 were not alone in the launch of this field, but their impact was unique in the momentum they gave to this area. Did the authors have any inkling that Rho and Rac would be found to regulate so many activities and would expand to touch almost every area of cell biology?

Acknowledgments

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References

Adnane, J., Seijo, E., Chen, Z., Bizouarn, F., Leal, M., Sebti, S.M., and Munoz-Antonia, T. (2002). RhoB, not RhoA, represses the transcription of the transforming growth factor beta type II receptor by a mechanism involving activator protein 1. J. Biol. Chem. *277*, 8500–8507.

Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y., and Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). J. Biol. Chem. *271*, 20246–20249.

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

Arthur, W.T., and Burridge, K. (2001). RhoA inactivation by p190RhoGAP regulates cell spreading and migration by promoting membrane protrusion and polarity. Mol. Biol. Cell *12*, 2711–2720.

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

Barbieri, J.T., Riese, M.J., and Aktories, K. (2002). Bacterial toxins that modify the actin cytoskeleton. Annu. Rev. Cell Dev. Biol. *18*, 315–344.

Bar-Sagi, D., and Feramisco, J.R. (1986). Induction of membrane ruffling and fluid-phase pinocytosis in quiescent fibroblasts by ras proteins. Science *233*, 1061–1068.

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

Blanchoin, L., Pollard, T.D., and Mullins, R.D. (2000). Interactions of ADF/cofilin, Arp2/3 complex, capping protein and profilin in remodeling of branched actin filament networks. Curr. Biol. *10*, 1273–1282.

Bliska, J.B. (2000). Yop effectors of Yersinia spp., and actin rearrangements. Trends Microbiol. *8*, 205–208.

Bokoch, G.M. (2003). Biology of the p21-activated kinases. Annu. Rev. Biochem. *72*, 743–781.

Boshans, R.L., Szanto, S., Van Aelst, L., and D'Souza-Schorey, C. (2000). ADP-ribosylation factor 6 regulates actin cytoskeleton remodeling in coordination with Rac1 and RhoA. Mol. Cell. Biol. *20*, 3685–3694.

Braga, V.M. (2002). Cell-cell adhesion and signalling. Curr. Opin. Cell Biol. *14*, 546–556.

Brandt, D., Gimona, M., Hillmann, M., Haller, H., and Mischak, H. (2002). Protein kinase C induces actin reorganization via a Src- and Rho-dependent pathway. J. Biol. Chem. *277*, 20903–20910.

Brugnera, E., Haney, L., Grimsley, C., Lu, M., Walk, S.F., Tosello-Trampont, A.C., Macara, I.G., Madhani, H., Fink, G.R., and Ravichandran, K.S. (2002). Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. Nat. Cell Biol. *4*, 574–582.

Chardin, P. (2003). GTPase regulation: getting aRnd Rock and Rho inhibition. Curr. Biol. *13*, R702–R704.

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

Cherfils, J. (2001). Structural mimicry of DH domains by Arfaptin suggests a model for the recognition of Rac-GDP by its guanine nucleotide exchange factors. FEBS Lett. *507*, 280–284.

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

Chrzanowska-Wodnicka, M., and Burridge, K. (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol. *133*, 1403–1415.

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

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

D'Souza-Schorey, C., Boettner, B., and Van Aelst, L. (1998). Rac regulates integrin-mediated spreading and increased adhesion of T lymphocytes. Mol. Cell. Biol. *18*, 3936–3946.

D'Souza-Schorey, C., Boshans, R.L., McDonough, M., Stahl, P.D., and Van Aelst, L. (1997). A role for POR1, a Rac1-interacting protein, in ARF6-mediated cytoskeletal rearrangements. EMBO J. *16*, 5445–5454.

DeMali, K.A., Wennerberg, K., and Burridge, K. (2003). Integrin signaling to the actin cytoskeleton. Curr. Opin. Cell Biol. *15*, 572–582.

Du, W., Lebowitz, P.F., and Prendergast, G.C. (1999). Cell growth inhibition by farnesyltransferase inhibitors is mediated by gain of geranylgeranylated RhoB. Mol. Cell. Biol. *19*, 1831–1840.

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

Edwards, D.C., Sanders, L.C., Bokoch, G.M., and Gill, G.N. (1999). Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat. Cell Biol. *1*, 253–259.

Etienne-Manneville, S., and Hall, A. (2002). Rho GTPases in cell biology. Nature *420*, 629–635.

Finlay, B.B., and Cossart, P. (1997). Exploitation of mammalian host cell functions by bacterial pathogens. Science *276*, 718–725.

Fleming, I.N., Elliott, C.M., and Exton, J.H. (1998). Phospholipase C-gamma, protein kinase C and Ca2+/calmodulin-dependent protein kinase II are involved in platelet-derived growth factor-induced phosphorylation of Tiam1. FEBS Lett. *429*, 229–233.

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

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

Fukuhara, S., Chikumi, H., and Gutkind, J.S. (2001). RGS-containing RhoGEFs: the missing link between transforming G proteins and Rho? Oncogene *20*, 1661–1668.

Gampel, A., and Mellor, H. (2002). Small interfering RNAs as a tool to assign Rho GTPase exchange-factor function in vivo. Biochem. J. *366*, 393–398.

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

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

Govind, S., Kozma, R., Monfries, C., Lim, L., and Ahmed, S. (2001). Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin. J. Cell Biol. *152*, 579–594.

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

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

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

Hart, M.J., Eva, A., Evans, T., Aaronson, S.A., and Cerione, R.A. (1991). Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the dbl oncogene product. Nature *354*, 311–314.

Hart, M.J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W.D., Gilman, A.G., Sternweis, P.C., and Bollag, G. (1998). Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. Science *280*, 2112–2114.

Hiraoka, K., Kaibuchi, K., Ando, S., Musha, T., Takaishi, K., Mizuno, T., Asada, M., Menard, L., Tomhave, E., Didsbury, J., et al. (1992). Both stimulatory and inhibitory GDP/GTP exchange proteins, Smg

GDS and Rho GDI, are active on multiple small GTP-binding proteins. Biochem. Biophys. Res. Commun. *182*, 921–930.

Innocenti, M., Frittoli, E., Ponzanelli, I., Falck, J.R., Brachmann, S.M., Difiore, P.P., and Scita, G. (2003). Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. J. Cell Biol. *160*, 17–23.

Kanoh, H., Williger, B.T., and Exton, J.H. (1997). Arfaptin *1*, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. J. Biol. Chem. *272*, 5421–5429.

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

Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J.H., Nakano, T., Okawa, K., et al. (1996). Regulation of myosin phosphatase by Rho and Rho-Associated kinase (Rho-kinase). Science *273*, 245–248.

Kimura, K., Fukata, Y., Matsuoka, Y., Bennett, V., Matsuura, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1998). Regulation of the association of adducin with actin filaments by Rho-associated kinase (Rho-kinase) and myosin phosphatase. J. Biol. Chem. *273*, 5542–5548.

King, A.J., Sun, H., Diaz, B., Barnard, D., Miao, W., Bagrodia, S., and Marshall, M.S. (1998). The protein kinase Pak3 positively regulates Raf-1 activity through phosphorylation of serine 338. Nature *396*, 180–183.

Kiosses, W.B., Shattil, S.J., Pampori, N., and Schwartz, M.A. (2001). Rac recruits high-affinity integrin $\alpha_v \beta 3$ to lamellipodia in endothelial cell migration. Nat. Cell Biol. *3*, 316–320.

Kozasa, T., Jiang, X., Hart, M.J., Sternweis, P.M., Singer, W.D., Gilman, A.G., Bollag, G., and Sternweis, P.C. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. Science *280*, 2109–2111.

Kozma, R., Ahmed, S., Best, A., and Lim, L. (1995). The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. Mol. Cell. Biol. *15*, 1942–1952.

Kozma, R., Ahmed, S., Best, A., and Lim, L. (1996). The GTPase-activating protein n-chimaerin cooperates with Rac1 and Cdc42Hs to induce the formation of lamellipodia and filopodia. Mol. Cell. Biol. *16*, 5069–5080.

Kraynov, V.S., Chamberlain, C., Bokoch, G.M., Schwartz, M.A., Slabaugh, S., and Hahn, K.M. (2000). Localized Rac activation dynamics visualized in living cells. Science *290*, 333–337.

Krugmann, S., Jordens, I., Gevaert, K., Driessens, M., Vandekerckhove, J., and Hall, A. (2001). Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. Curr. Biol. *11*, 1645–1655.

Lambert, J.M., Lambert, Q.T., Reuther, G.W., Malliri, A., Siderovski, D.P., Sondek, J., Collard, J.G., and Der, C.J. (2002). Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. Nat. Cell Biol. *4*, 621–625.

Li, F., and Higgs, H.N. (2003). The mouse formin mDia1 is a potent actin nucleation factor regulated by autoinhibition. Curr. Biol. *13*, 1335–1340.

Liu, A., Du, W., Liu, J.P., Jessell, T.M., and Prendergast, G.C. (2000). RhoB alteration is necessary for apoptotic and antineoplastic responses to farnesyltransferase inhibitors. Mol. Cell. Biol. *20*, 6105–6113.

Machesky, L.M., and Hall, A. (1997). Role of actin polymerization and adhesion to extracellular matrix in Rac- and Rho-induced cytoskeletal reorganization. J. Cell Biol. *138*, 913–926.

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

Madaule, P., Eda, M., Watanabe, N., Fujisawa, K., Matsuoka, T., Bito, H., Ishizaki, T., and Narumiya, S. (1998). Role of citron kinase as a target of the small GTPase Rho in cytokinesis. Nature *394*, 491–494.

Maddox, A.S., and Burridge, K. (2003). RhoA is required for cortical retraction and rigidity during mitotic cell rounding. J. Cell Biol. *160*, 255–265.

Maekawa, M., Ishizaki, T., Boku, S., Watanabe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohashi, K., Mizuno, K., and Narumiya, S. (1999). Signaling from rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science *285*, 895–898.

Manser, E., Huang, H.Y., Loo, T.H., Chen, X.Q., Dong, J.M., Leung, T., and Lim, L. (1997). Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. Mol. Cell. Biol. *17*, 1129–1143.

Matsui, T., Yonemura, S., Tsukita, S., and Tsukita, S. (1999). Activation of ERM proteins in vivo by Rho involves phosphatidyl-inositol 4-phosphate 5-kinase and not ROCK kinases. Curr. Biol. *9*, 1259–1262.

Meller, N., Irani-Tehrani, M., Kiosses, W.B., Del Pozo, M.A., and Schwartz, M.A. (2002). Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. Nat. Cell Biol. *4*, 639–647.

Mertens, A.E., Roovers, R.C., and Collard, J.G. (2003). Regulation of Tiam1-Rac signalling. FEBS Lett. *546*, 11–16.

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

Miki, H., Suetsugu, S., and Takenawa, T. (1998). WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. EMBO J. *17*, 6932–6941.

Miki, H., Yamaguchi, H., Suetsugu, S., and Takenawa, T. (2000). IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. Nature *408*, 732–735.

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

Nimnual, A.S., Yatsula, B.A., and Bar-Sagi, D. (1998). Coupling of Ras and Rac guanosine triphosphatases through the Ras exchanger Sos. Science *279*, 560–563.

Nimnual, A.S., Yatsula, B.A., and Bar-Sagi, D. (2003). Redox-dependent downregulation of Rho by Rac. Nat. Cell Biol. *5*, 236–241.

Nobes, C.D., and Hall, A. (1995). Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell *81*, 53–62.

Nobes, C.D., and Hall, A. (1999). Rho GTPases control polarity, protrusion, and adhesion during cell movement. J. Cell Biol. *144*, 1235–1244.

Nobes, C.D., Hawkins, P., Stephens, L., and Hall, A. (1995). Activation of the small GTP-binding proteins rho and rac by growth factor receptors. J. Cell Sci. *108*, 225–233.

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

Noren, N.K., Arthur, W.T., and Burridge, K. (2003). Cadherin engagement inhibits RhoA via p190RhoGAP. J. Biol. Chem. *278*, 13615–13618.

Ohta, Y., Suzuki, N., Nakamura, S., Hartwig, J.H., and Stossel, T.P. (1999). The small GTPase RalA targets filamin to induce filopodia. Proc. Natl. Acad. Sci. USA *96*, 2122–2128.

Prieto-Sanchez, R.M., and Bustelo, X.R. (2003). Structural basis for the signaling specificity of RhoG and Rac1 GTPases. J. Biol. Chem. *278*, 37916–37925 Published online June 12, 2003. 10.1074/jbc.M301437200.

Radhakrishna, H., AlAwar, O., Khachikian, Z., and Donaldson, J.G. (1999). ARF6 requirement for Rac ruffling suggests a role for membrane trafficking in cortical actin rearrangements. J. Cell Sci. *112*, 855–866.

Ren, X.D., Kiosses, W.B., and Schwartz, M.A. (1999). Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. *18*, 578–585.

Ridley, A.J., and Hall, A. (1992). The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth-factors. Cell *70*, 389–399.

Ridley, A.J., Paterson, H.F., Johnston, C.L., Diekmann, D., and Hall, A. (1992). The small GTP-binding protein Rac regulates growth-factor induced membrane ruffling. Cell *70*, 401–410.

Riento, K., Guasch, R.M., Garg, R., Jin, B., and Ridley, A.J. (2003). RhoE binds to ROCK 1 and inhibits downstream signaling. Mol. Cell. Biol. *23*, 4219–4229.

Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A.D. (2001). Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J. Cell Biol. *153*, 1175–1186.

Rohatgi, R., Nollau, P., Ho, H.Y., Kirschner, M.W., and Mayer, B.J. (2001). Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. J. Biol. Chem. *276*, 26448–26452.

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

Sahai, E., Olson, M.F., and Marshall, C.J. (2001). Cross-talk between Ras and Rho signalling pathways in transformation favours proliferation and increased motility. EMBO J. *20*, 755–766.

Sander, E.E., ten Klooster, J.P., van Delft, S., van der Kammen, R.A., and Collard, J.G. (1999). Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. J. Cell Biol. *147*, 1009–1022.

Sanders, L.C., Matsumura, F., Bokoch, G.M., and de Lanerolle, P. (1999). Inhibition of myosin light chain kinase by p21-activated kinase. Science *283*, 2083–2085.

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

Scita, G., Nordstrom, J., Carbone, R., Tenca, P., Giardina, G., Gutkind, S., Bjarnegard, M., Betsholtz, C., and Di Fiore, P.P. (1999). EPS8 and E3B1 transduce signals from Ras to Rac. Nature *401*, 290–293.

Sells, M.A., Knaus, U.G., Bagrodia, S., Ambrose, D.M., Bokoch, G.M., and Chernoff, J. (1997). Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. Curr. Biol. *7*, 202–210.

Sells, M.A., Boyd, J.T., and Chernoff, J. (1999). p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J. Cell Biol. *145*, 837–849.

Shin, O.H., and Exton, J.H. (2001). Differential binding of arfaptin 2/POR1 to ADP-ribosylation factors and Rac1. Biochem. Biophys. Res. Commun. *285*, 1267–1273.

Snyder, J.T., Singer, A.U., Wing, M.R., Harden, T.K., and Sondek, J. (2003). The pleckstrin homology domain of phospholipase C-beta2 as an effector site for Rac. J. Biol. Chem. *278*, 21099–21104.

Stebbins, C.E., and Galan, J.E. (2001). Structural mimicry in bacterial virulence. Nature *412*, 701–705.

Stossel, T.P., Condeelis, J., Cooley, L., Hartwig, J.H., Noegel, A., Schleicher, M., and Shapiro, S.S. (2001). Filamins as integrators of cell mechanics and signalling. Nat. Rev. Mol. Cell Biol. *2*, 138–145.

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

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

Tarricone, C., Xiao, B., Justin, N., Walker, P.A., Rittinger, K., Gamblin, S.J., and Smerdon, S.J. (2001). The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signalling pathways. Nature *411*, 215–219.

Thompson, P.W., Randi, A.M., and Ridley, A.J. (2002). Intercellular adhesion molecule (ICAM)-1, but not ICAM-2, activates RhoA and stimulates c-fos and rhoA transcription in endothelial cells. J. Immunol. *169*, 1007–1013.

Tolias, K.F., Cantley, L.C., and Carpenter, C.L. (1995). Rho family GTPases bind to phosphoinositide kinases. J. Biol. Chem. *270*, 17656–17659.

Turner, C.E., West, K.A., and Brown, M.C. (2001). Paxillin-ARF GAP signaling and the cytoskeleton. Curr. Opin. Cell Biol. *13*, 593–599.

Vadlamudi, R.K., Li, F., Adam, L., Nguyen, D., Ohta, Y., Stossel, T.P., and Kumar, R. (2002). Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1. Nat. Cell Biol. *4*, 681–690.

Van Aelst, L., Joneson, T., and Bar-Sagi, D. (1996). Identification of a novel Rac1-interacting protein involved in membrane ruffling. EMBO J. *15*, 3778–3786.

van Leeuwen, F.N., van Delft, S., Kain, H.E., van der Kammen, R.A., and Collard, J.G. (1999). Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. Nat. Cell Biol. *1*, 242–248.

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

Wang, Y., Lang, L., Luo, Y., and Zheng, Y. (2003). A novel strategy for specifically downregulating individual Rho GTPase activity in tumor cells. J. Biol. Chem. *278*, 44617–44625 Published online June 25, 2003. 10.1074/jbc.M308929200.

Watanabe, N., Kato, T., Fujita, A., Ishizaki, T., and Narumiya, S. (1999). Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat. Cell Biol. *1*, 136–143.

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

Wennerberg, K., Forget, M.A., Ellerbroek, S.M., Arthur, W.T., Burridge, K., Setoyama, M., Der, C.J., and Hansen, S.H. (2003). Rnd proteins function as RhoA antagonists by activating p190 RhoGAP. Curr. Biol. *13*, 1106–1115.

Wherlock, M., and Mellor, H. (2002). The Rho GTPase family: a Racs to Wrchs story. J. Cell Sci. *115*, 2–4.

Worthylake, R.A., and Burridge, K. (2003). RhoA and ROCK promote migration by limiting membrane protrusions. J. Biol. Chem. *278*, 13578–13584.

Worthylake, R.A., Lemoine, S., Watson, J.M., and Burridge, K. (2001). RhoA is required for monocyte tail retraction during transendothelial migration. J. Cell Biol. *154*, 147–160.

Xu, J., Wang, F., Van Keymeulen, A., Herzmark, P., Straight, A., Kelly, K., Takuwa, Y., Sugimoto, N., Mitchison, T., and Bourne, H.R. (2003). Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. Cell *114*, 201–214.

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

Zebda, N., Bernard, O., Bailly, M., Welti, S., Lawrence, D.S., and Condeelis, J.S. (2000). Phosphorylation of ADF/cofilin abolishes EGF-induced actin nucleation at the leading edge and subsequent lamellipod extension. J. Cell Biol. *151*, 1119–1127.

Zheng, Y., Bagrodia, S., and Cerione, R.A. (1994). Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85. J. Biol. Chem. *269*, 18727–18730.