Chapter 7

CDC42 AND RAC CONTROL OF THE ACTIN CYTOSKELETON

CHENG-GEE KOH AND LOUIS LIM[1]
Institute of Molecular and Cell Biology, Singapore; [1] also at Institute of Neurology, University College, London

Abstract: Cell shape changes are critical to cell differentiation, movement and motility. Reorganization of the actin cytoskeleton is crucial to these changes, which are elicited in response to extracellular stimuli. This reorganization is achieved by the action of kinases, phosphatases and effectors of Rho GTPases on actin-containing filaments. In this review, we shall discuss how the different proteins such as WASP, WAVE, PAK and IQGAP, which are downstream of Cdc42 and Rac affect the actin cytoskeleton. Guanine nucleotide exchange factors that activate different Rho GTPases also contribute towards the regulation of the cytoskeleton. Many of the Rho GTPase targets are kinases or are regulated by phosphorylation. Thus phosphatases are essential in the control of cell adhesion and spreading as well. The integration and modulation of the different signalling pathways downstream of the Rho proteins are key to the final cellular responses. How the different downstream proteins are shuttled and shuffled is also of interest because the same molecules may participate in pathways that are regulated by Cdc42, Rac and the antagonistic RhoA.

1. INTRODUCTION

The small GTPases of the Rho family play key roles in transducing extracellular stimuli into distinct responses including cell motility, adhesion, cell division and phagocytosis. The GTPases cycle between GTP-bound and GDP-bound forms where activation requires the action of guanine nucleotide exchange factors (GEFs) to promote conversion of GDP to GTP states. Individual members of the Rho GTPases are known to cause specific changes to the actin cytoskeleton of the cells. Active RhoA promotes actin

E. Manser (ed.), RHO Family GTPases, 137-156.

stress fibre formation, while expression of dominant active Cdc42 and Rac1 induces the formation of filopodia and lamellipodia respectively. The rearrangement of the cytoskeletal structures is pivotal to the outcome of the signal transduction events downstream of the Rho GTPases (reviewed by Lim et al., 1996; Van Aelst and D'Souza-Schorey, 1997).

Some of the downstream effectors of the Rho GTPases and the pathways they regulate have been well studied. In particular, Rho-kinase/ROK (Amano et al., 1997; Leung et al., 1995) and mDia (Watanabe et al., 1997), which are downstream of RhoA have been shown to promote the formation of stress fibres. ROK can phosphorylate and inactivate the myosin binding subunit of the light chain (MLC) phosphatase (Kimura et al., 1996). This results in an increase in phosphorylated MLC, its enhancement of actin binding/bundling activity and consequently of stress fibre formation. The effector proteins downstream of Rac1 in lamellipodia formation were not as well characterized, until the recent discovery of the WAVE subfamily of the WASP proteins. WAVE promotes the formation of lamellipodia downstream of Rac1 (Miki et al., 1998b; Miki et al., 2000). POR1 may also be involved in this process (Van Aelst et al., 1996). N-WASP mediates the link between Cdc42 and the Arp2/3 proteins in actin polymerization, and participates in the formation of filopodia (Miki et al., 1998a; Rohatgi et al., 1999). The ROK-related target MRCK is involved in the formation of focal complexes and filopodia as a kinase-inactive mutant can block these processes downstream of Cdc42 (Leung et al., 1998).

In this review, we discuss the control of cytoskeleton by Rac and Cdc42 as well as their effectors, activators and other proteins that interact with the downstream targets of these GTPases. The effect of RhoA shall not be described extensively.

2. THE ACTIN CYTOSKELETON AND CELL SHAPE CHANGES

Cell shape changes are crucial to cellular movement and cell motility. The rearrangement of the cortical actin filaments is essential for the changes in cell shape, elicited in response to upstream signals. Hematopoietic cells need to migrate through the blood vessels walls and tissue spaces to reach the site of infection. Metastatic cancer cells move and invade other tissues to cause the spread of the disease. Neuronal cells send out neurites and axons and also migrate from the site of proliferation to other sites in response to developmental cues. Actin polymerization at the leading edge of the cell is responsible for driving the cell membrane forward, involving peripheral cell structures such as lamellipodia and filopodia. Lamellipodia are made up of

short branches of actin filaments forming a network. Each actin filament has branches at an angle of 70°, with the barbed end towards the cell membrane (Svitkina and Borisy, 1999). On the other hand, filopodia do not contain a meshwork of actin branches and are made up of long actin bundles (Figure 1). The spatial-temporal modulation of actin polymerization, branching and depolymerization is important to the maintenance of lamellipodia and filopodia at the leading edge of the cells. However, the formation of the trimeric nucleus for actin filament assembly is very slow although monomeric globular actin protein (G-actin) spontaneously forms trimeric nuclei. This nucleation process was shown to be initiated and promoted by the Arp 2/3 complex (Machesky et al., 1994) formed by the two actin-related proteins, Arp2 and Arp3, and five novel subunits. Increasing biochemical purification of the Arp2/3 complex led to a decline in its nucleation activity. This is because the Arp 2/3 complex needs to be activated first. One of the earliest activators found was ActA (Welch et al., 1998), a protein from the

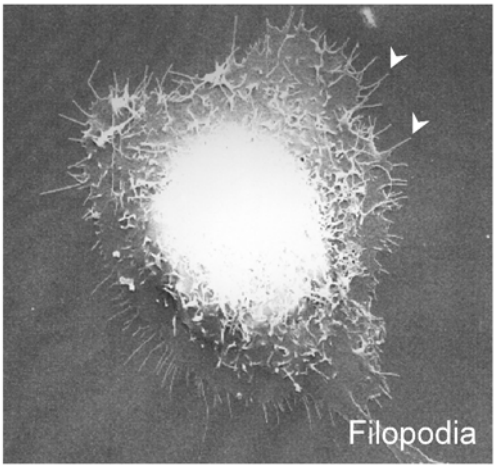

Figure 1. Electron micrographs of filopodia, ruffles and lamellipodia.

bacterium Listeria. Others have found WASP, myosin-I and cortactin to bind and activate the Arp2/3 complex (Lechler et al., 2000; Lee et al., 2000; Machesky et al., 1999; Rohatgi et al., 1999; Weaver et al., 2001).

2.1 The WASP family and Cdc42

The WASP (Wiskott-Aldrich syndrome) proteins are divided into two subfamilies: WASP and WAVE proteins. The WASP group consists of WASP and N-WASP, and the WAVE group has three very similar members (WAVE1-3). The expression of N-WASP is ubiquitous whereas WASP is expressed mainly in hematopoietic cells. These proteins contain many functional domains. The WH1 (WASP homology) domain binds phosphatidylinositol 4,5-bisphosphate (PIP2; Miki et al., 1996). It is thought that WASP/N-WASP anchors itself to the cell membrane by interacting with phospholipids. The GBD/CRIB domain is the binding site of Cdc42. It is this binding which regulates the activity of the WASP proteins. The proline-rich region binds several SH3-containing proteins. The VCA (Verpolin-Connecting/Cofilin-Acidic) domain at the C-terminus is important for binding to actin and the Arp2/3 complex. WASP proteins appear to adopt an auto-inhibitory conformation and need to be activated before they can bind to the Arp2/3 complex and actin. Co-expression of Cdc42 and N-WASP induces the formation of long filopodia (Miki et al., 1998a). A mutant N-WASP that cannot bind Cdc42 does not induce filopodia formation. The interaction of active Cdc42 and N-WASP causes a conformational change to expose the VCA domain and allow N-WASP to bind both actin and the Arp2/3 complex (Figure 2A).

However, N-WASP (-/-) knockout cells still form filopodia when microinjected with active Cdc42 (Snapper et al., 2001). There then must exist other proteins that can stimulate actin nucleation and polymerization, apart from the N-WASP-Arp2/3 complex. It has been argued that the Arp2/3 complex nucleates actin to form branches rather than long bundles of actin filament as in the case with filopodia. Perhaps the Arp2/3 complex does not regulate the extension of filopodia. On the other hand, IRSp53 could provide the link between Cdc42 and Ena/VASP (Mena) protein to direct actin nucleation and filament assembly of filopodia (Bear et al., 2002). IRSp53 was originally identified as a substrate of the insulin receptor kinase (Yeh et al., 1996) and there have been other reports that Cdc42 uses IRSp53 as an intermediary to form complexes which induce filopodia and neurite outgrowth (Govind et al., 2001; Krugmann et al., 2001). It has also been reported that Diaphanous-related formin, p134mDia2 (Drf3), could be the effector downstream of Cdc42 that facilitates filopodia formation (Peng et

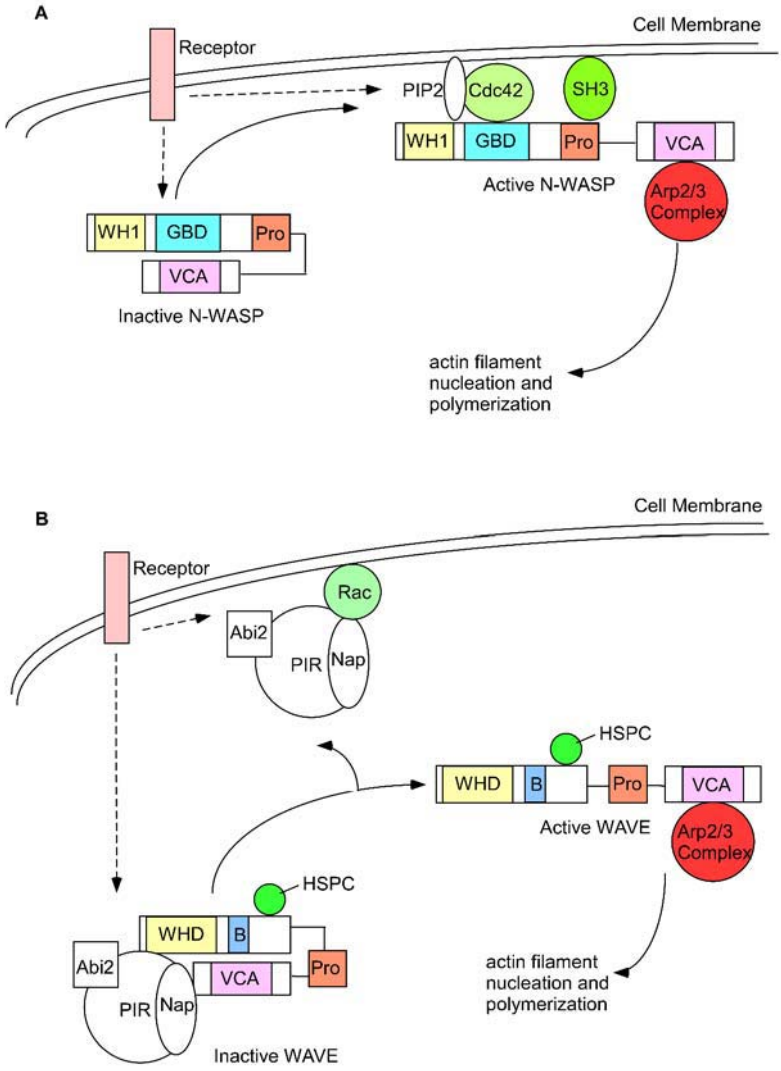

Figure 2. The activation of WASP and WAVE proteins. (A) When the cell receives activating signals from the receptor, WASP is targeted to the membrane. Active Cdc42 can then bind to WASP at the GTPase binding domain (GBD). Similarly, PIP2 and SH3- containing proteins such as Nck can also bind to their respective binding sites on WASP to activate WASP. Upon binding to Cdc42, PIP2, or Nck, the auto-inhibition of WASP is relieved and the VCA domain thus exposed is able to interact with and activate the Arp2/3 complex to stimulate actin nucleation. (B) WAVE1 is kept inactive by associating with four other proteins: Nap125, PIR121, Abi2 and HSPC300. When active Rac is present, the complex dissociates into NAP125-PIR121-Abi2 sub complex and WAVE1-HSPC300 sub complex. WAVE1 is now free to activate the Arp2/3 complex.

al., 2003). The formin homology domains, FH1-FH2 regions, of the yeast protein Bni1p have been proposed to nucleate actin filaments in vitro and promote the growth of actin filament at the barbed end. A similar and more potent actin nucleating activity was also observed in constructs containing the FH2 domain of mDia1 (Li and Higgs, 2003). mDia1 has also been reported to adopt an auto-inhibitory mode of regulation much like that of N-WASP. The N terminus of mDia1 inhibits the actin nucleation activity of the C-terminus. RhoA binding to the N terminus can only partially relieve the inhibition, implying the involvement of other regulatory molecules.

2.2 The WAVE Proteins and Rac

The WAVE proteins act down stream of Rac to induce actin polymerization responsible for lamellipodia formation. As with WASP, all three members of the WAVE proteins contain multiple functional domains. They have a proline-rich domain followed by the VCA domain at the C-terminal. However, the N terminal WAVE/SCAR homology domain (WHD/SHD) is different from that of WASP and its function is not known (Bear et al., 1998). There is no GTPase-binding domain present in WAVE. How the WAVE proteins are regulated by Rac remained a puzzle until recently, although it has been reported that Rac binds to IRSp53, which in turn binds to WAVE2 to activate it (Miki et al., 2000). However, WAVE1 and 3 do not bind IRSp53. Purified WAVE1 also appears to be constitutively active. There must exist other mechanisms to activate Arp2/3 through WAVE. The puzzle was apparently solved with the discovery of an inhibitory complex that renders WAVE1 inactive (Eden et al., 2002). WAVE1 is kept inactive by associating with four other proteins: Nap125, PIR121, Abi2 and HSPC300. When active Rac is present, the complex dissociates into sub complexes of Nap125-PIR121-Abi2 and of WAVE1-HSPC300. WAVE1 is now free to active the Arp2/3 complex (see Figure 2B).

There is a further twist to the story. More recently, there have been findings which are contradictory to the inhibitory function of the Wave-Abi complex (Innocenti et al., 2004). It was reported that the WAVE2-Abi1-Nap1-PIR121 complex binds to Rac and was targeted to the lamellipodia. Contrary to the earlier report, the binding to Rac did not result in the dissociation of the complex and that the recruitment of the WAVE2-Abi1-Nap1-PIR121 complex to the lamellipodia actually resulted in site-directed nucleation of actin filaments. It was also shown that the undissociated complex was active in stimulating Arp2/3. Further work is required to reconcile the differences in the findings.

Cdc42 and Rac are not the only regulators of WASP and WAVE. Acidic lipids such as phosphatidylinositol 4,5-bisphosphate can also bind to and

activate N-WASP (Miki et al., 1996; Rohatgi et al., 2000). The adaptor protein Nck also participates through one or more of its SH3 domains in binding N-WASP and the WAVE1-complex (Eden et al., 2002; Rohatgi et al., 2001). Other SH3-containing proteins such as WISH (Fukuoka et al., 2001), Ash/Grb2 (Carlier et al., 2000) and Profilin (Suetsugu et al., 1998; Yang et al., 2000) bind to the proline-rich domain of N-WASP to relieve its auto-inhibition. Tyrosine phosphorylation of N-WASP at residue 291 has also been shown to activate N-WASP independently of Cdc42 (Cory et al., 2002).

2.3 IQGAP and Cdc42/Rac

IQGAP1 is another multi-domain protein that interacts with Cdc42 and Rac. Starting from the N-terminal, it has a Calponin homology domain that binds actin, a WW domain and four tandem repeats of the IQ motif which binds calmodulin, myosin light chain and S100B, a Zn2+- and Ca^{2+}-binding protein (Briggs and Sacks, 2003). There is also a GAP-related domain (GRD), which interacts with Cdc42 and Rac but not RhoA and Ras. This is followed by a RasGAP-like domain at the C-terminal. However, this domain does not have any GAP activity and instead it interacts with microtubule-binding protein CLIP170 and is necessary for binding E-cadherin. Mammalian IQGAP1 colocalizes with actin in lamellipodia (Hart et al., 1996) and induces filopodia and microspike formation when over-expressed (Swart-Mataraza et al., 2002). The precise mechanism of how IQGAP induces the formation of filopodia is not known but it has been shown that IQGAP inhibits the intrinsic GTPase activity of Cdc42 in vitro (Hart et al., 1996). This will stabilize GTP-bound Cdc42 and thus increase the pool of active Cdc42. There are also reports on the actin cross-linking activities of IQGAP1 (Bashour et al., 1997; Fukata et al., 1997).

2.4 PAK and Cdc42/Rac

PAK (p21-activated kinase, Manser et al., 1994) was one of the first effectors identified for Cdc42 and Rac. Thus far, three members of the conventional group one PAK have been identified (reviewed by Jaffer and Chernoff, 2002). The N-terminal regulatory region of PAK contains three proline-rich sequences, a GTPase binding (PBD/CRIB) and a kinase-inhibitory domain. The C-terminal kinase domain is similar to that of the Ste20 protein of the budding yeast. PAK normally exists as a dimer with the inhibitory domain of one binding to the kinase domain of another PAK molecule. Upon binding to active Cdc42 or Rac, the inhibition is relieved and PAK becomes activated and self-phosphorylates (Lei et al., 2000). PAK

is an important regulator of cytoskeleton dynamics and cell motility. It has been shown that activated PAK caused the disassembly of stress fibres and the dissolution of focal adhesion complexes (Manser et al., 1997). Others have also reported that PAK is recruited to the leading edge of cells (Dharmawardhane et al., 1999; Harden et al., 1996; Li et al., 2003) and that it is involved in axon guidance(Newsome et al., 2000). A recent and fuller account of PAK and its function can be found in the review of Bokoch (2003).

2.5 PAK, its substrates and the regulation of cytoskeleton

2.5.1 LIM kinases

The numbers of protein found to be phosphorylated by PAK have increased steadily following its discovery. Many of these substrates participate in the regulation of the cytoskeleton and cell motility. LIM kinases 1 and 2 are such substrates that have been implicated in actin cytoskeletal regulation (Edwards et al., 1999). They are serine/threonine kinases which phosphorylate and down-regulate the cofilin/actin depolymerising factor (ADF) family of proteins under the control of Rac GTPases (Arber et al., 1998; Yang et al., 1998). One of the main functions of cofilin/ADF in the cell is to depolymerize actin filaments. Cofilin/ADF increases the off-rate at the pointed ends of filaments without changing the off-rate at the barbed ends (Carlier et al., 1997). Inhibition of cofilin/ADF will result in increased cellular levels of F-actin. In short, increased Rac activity will lead to the activation of PAK and its phosphorylation of LIM kinase. The activated LIM kinase in turn will phosphorylate and inactivate cofilin/ADF. The final outcome is more actin polymerization. Interestingly, the critical Thr508 regulatory site in LIM kinase-1 can also be phosphorylated by the myotonic dystrophy-related Cdc42-binding kinase (MRCK) and by Rho-kinase (Maekawa et al., 1999; Sumi et al., 2001). This implies that the LIM kinases could be regulated by all members of the Rho GTPase family. Cross talk between the different Rho GTPases is likely to determine the outcome and the response.

2.5.2 Regulatory myosin light chain and myosin light chain kinase

It has long been known that Cdc42 and Rac induce protrusions at the leading edge of cells whereas RhoA promotes retraction. Myosin II contractility is

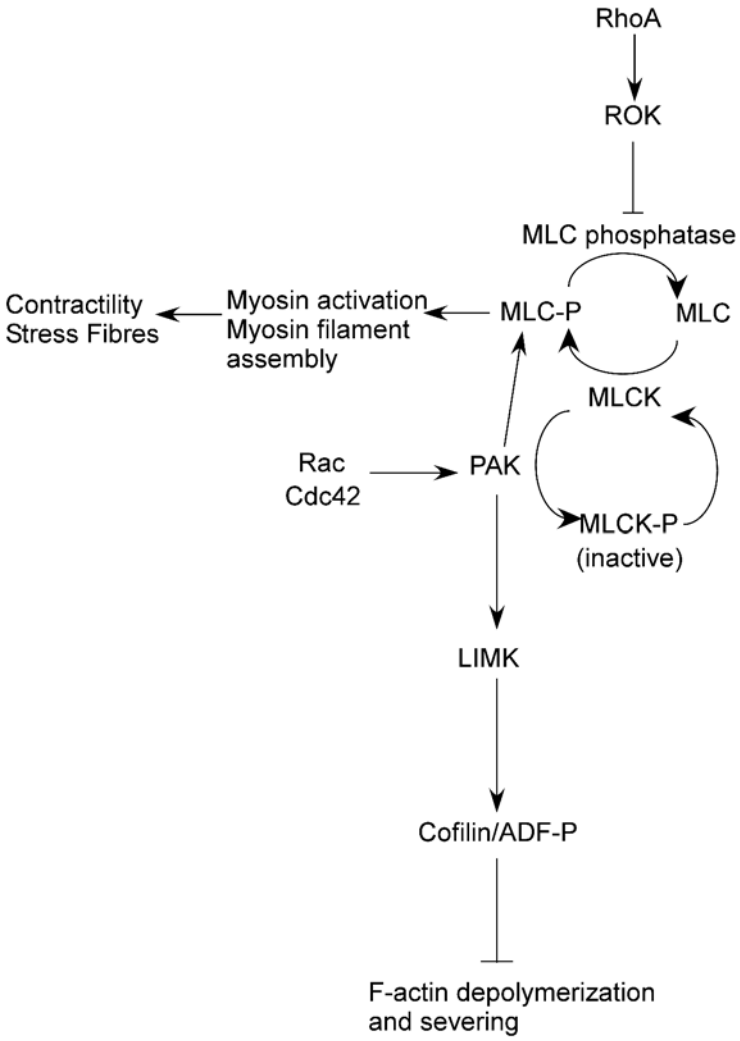

Figure 3. The regulation of stress fibres and myosin/actin contractility in the cell. Members of the Rho GTPase family regulate the actin cytoskeleton through activities of their effectors. ROK/ROCK is activated by binding to RhoA and PAK is activated by active Cdc42 and Rac. ROK/ROCK phosphorylates myosin light chain (MLC) phosphatase and inhibits the phosphatase activity. Thus MLC remains phosphorylated and can promote myosin and myosin filament assembly, which leads to contractility of stress fibres. MLC is phosphorylated by MLC Kinase (MLCK) which itself can be phosphorylated by PAK and becomes inactive. However, PAK can also phosphorylate MLC directly, resulting in myosin activation. Another substrate of PAK is LIM kinase (LIM-K). Active LIM-K phosphorylates and inactivates cofilin/ADF, thus inhibiting the F-actin depolymerization and severing activity of cofilin. The balance of the two activities of PAK is crucial in the maintenance of stress fibres.

crucial to such cell shape changes. Myosin forms filaments that consist of two heavy chains and two light chains. Myosin filaments interact with actin to generate the contractile forces required for cell retraction or spreading. The phosphorylation of the regulatory myosin light chain (R-MLC) at Thr18 and Ser19 by Ca^{2+}-dependent myosin light chain kinase (MLCK) has been reported to be responsible for the modulation of myosin contractility. Phosphorylation at these two residues induces conformational changes to allow the myosin molecules to form filaments. PAK has been shown to phosphorylate and inhibit MLCK (Goeckeler et al., 2000; Sanders et al., 1999). Phosphorylated/inhibited MLCK can no longer phosphorylate the regulatory myosin light chain (R-MLC), which results in a decrease in actin-myosin filament assembly. RhoA has the opposite effect on R-MLC. RhoA acting via Rho- kinase, ROK (Kimura et al., 1996) promotes the phosphorylation and subsequent inactivation of myosin light chain phosphatase. The resultant increase in phosphorylated R-MLC enhances myosin filament assembly and thus generates tension on the actin filaments and bundles them into stress fibres. Interestingly, ROK can also directly phosphorylate R-MLC, bypassing the MLCK pathway (Amano et al., 1996). On the other hand, there are also reports of PAK phosphorylation of the R-MLC at the critical Ser19 that could result in increasing contractility (Chew et al., 1998; Zeng et al., 2000). Here again, cross talk between Cdc42, Rac and Rho is clearly shown to be important in coordinating the complex events regulating the actin-myosin contractility affecting cell protrusion or retraction (see Figure 3).

Another Cdc42 effector, MRCK, can also phosphorylate MLC (Leung et al., 1998). In addition, over-expression of MRCKα promoted filopodia formation while that of a MRCK kinase-deficient mutant inhibited the induction of filopodia by Cdc42. The mechanisms are not known.

3. GUANINE NUCLEOTIDE EXCHANGE FACTORS AND THE ACTIN CYTOSKELETON

Cycling of Rho GTPases between the GTP-bound and GDP-bound states serves as a molecular switch to turn on and turn off the downstream pathways. Rac, Cdc42 and Rho are the best studied of the Rho GTPases. Although the Rho family proteins have intrinsic GTPase activities, the rate of hydrolysis of the GTP to GDP is slow. This rate and thus down regulation of the Rho proteins are enhanced by GAPs. The GDP-bound GTPase is kept in the cytosol by RhoGDI (Guanine nucleotide dissociation inhibitor). Rho GTPases are prenylated at their C-terminal, enabling them to anchor to the membrane. The binding of RhoGDI leads to the masking of the prenylated

tail so that the complex remains in the cytosol and interaction with the membrane is inhibited (Olofsson, 1999). The GTPase becomes active again when a GEF (Guanine nucleotide exchange factor) comes along and catalyses the exchange of GDP for GTP and also dissociates the GTPase from its complex with RhoGDI. The active GTP-bound GTPase can now translocate to the membrane where its activity is required.

Over-expression of many GEFs can lead to diverse changes in cell morphology such as increased formation of lamellipodia (Tiam1, Vav) and filopodia (hPem2) as well as of stress fibres (Dbl) (Ava and Aaronson, 1985; Bustelo, 2000; Bustelo, 2001; Habets et al., 1994; Hart et al., 1991; Reid et al., 1999). These changes can be attributed to the global activation of the relevant Rho family proteins. However, under physiological conditions, activation of the Rho GTPases is likely to be restricted to different cellular compartments. In migrating cells, the establishment and maintenance of cell polarity is fundamental to cell movement. Thus spatial and temporal activation of the different Rho GTPases is important. This could be achieved by specific localization of GEF or GAP to activate or inactive Rho GTPases at different sub-cellular locales. For example, Cdc42 is thought to control cell polarity through its interaction with the Par6-Par3-PKC□ complex (Etienne-Manneville and Hall, 2003). The final outcome of this interaction is the localization and reorganization of the microtubule-organizing centre (MTOC). The Cdc42-induced MTOC orientation appears to facilitate microtubule growth into the lamella and the microtubule-mediated delivery of membrane proteins via the Golgi vesicles to the leading edge. Active Cdc42 at the leading edge also leads to the exclusion of the Pten lipid phosphatase at the front, and Pten accumulation at the rear end of the cell. Phosphotidylinositol-3 kinase (PI3K) is also found to accumulate at the leading edge of the cell. The products of PI3K are PIP3 and PIP2, which have been reported to activate a number of Rac GEFs such as Vav and Tiam1 (Bustelo, 2000; Bustelo, 2001; Mertens et al., 2003) besides recruiting these GEFs through their PH domains. Once Rac is activated, several positive feedback loops are available to maintain continual activation of Rac and its downstream pathways to generate lamellipodia and other cell protrusions.

The localization of GEF is important to its activity. In the case of Tiam1, translocation to the membrane is crucial for its activation of Rac to induce membrane ruffles. The mechanism responsible for the translocation and activation of Tiam1 is still not clear. However, it has been observed that the N-terminal of Tiam 1 is important for membrane localization, presumably through the interaction of the N-terminal PH domain with phosphoinositides (Stam et al., 1997; Michiels et al., 1997). There are also reports that Tiam 1 is phosphorylated, possibly activated and translocated by Ca^{2+}/calmodulin

kinase II (CaMK-II) and protein kinase C (PKC) when Swiss 3T3 cells are treated with lysophosphatidic acid or PDGF (Fleming et al., 1998; Fleming et al., 2000). Other GEFs may contain domains that are responsible for their cellular localization. Peripheral localization of the Rac/Cdc42 GEF, β1PIX, is crucial for its activation of Cdc42 and Rac to induce formation of microvilli and ruffles, respectively (Koh et al., 2001). Deletion of the membrane targeting sequences in β1PIX prevented such localized activation of the GTPases. From these various reports, it is clear that the sub-cellular location of the GEFs is critical for its downstream activities. A number of GEFs were found to form dimers or multimers. β1PIX exists as dimers and Dbl can form oligomers (Kim et al., 2001; Koh et al., 2001; Zhu et al., 2001). Dimerization of PIX provides the prospect of forming multimeric complexes with PAK, GIT1/p95PKL and other associated proteins (Bagrodia et al., 1999; Turner et al., 1999; Zhao et al., 2000). This prospect allows for the generation of distinct functional outcomes, depending on the composition of the complexes. With Dbl, oligomerization resulted in the formation of a protein complex that could co-ordinate and further augment its GEF activities (Zhu et al., 2001).

In summary, the localization and activation of these RhoGEFs play crucial roles in the organization of the cytoskeleton and cell morphology though their activation of the Rho GTPases dictated by different spatial and temporal cues.

4. PHOSPHATASES AND THE ACTIN CYTOSKELETON

Cell adhesion and migration are dynamic processes that depend on cellular systems, whose protein components can be rapidly and precisely regulated. An example of this regulation is the modification of the proteins by phosphorylation and dephosphorylation. The balance between the activities of the kinases and phosphatases is crucial to achieve the desirable outcome. Many of the effectors of Rho GTPases are kinases. Thus regulation of the phosphorylation status of the substrates and even of the kinases themselves becomes critical for the modulation of signaling pathways. Earlier studies have indicated that phosphatases participate in the regulation of cell attachment and migration. It has been shown that the spreading of BHK21 cells on fibronectin could be inhibited by vanadate, which is a tyrosine phosphatase inhibitor (Edwards et al., 1991). Similarly Okadaic acid, which is a PP2A inhibitor, has been used to inhibit the migration of macrophages (Wilson et al., 1991).

Protein phosphatases can be classified as protein tyrosine phosphatases (PTPs) or serine/threonine phosphatases (PPs). As the name implies, PTPs dephosphorylate the phospho-tyrosine residues. Members of this family include the soluble PTPs, the receptor PTPs (RPTPs), dual specificity PTPs and low-molecular weight PTPs. The catalytic domains of all the PTPs are very conserved. The dual specificity PTPs can dephosphorylate phosphorylated tyrosine, serine and threonine residues. Pten which is a phosphatidylinositol 3,4,5-triphosphate lipid phosphatase can also be considered as a dual specificity phosphatase because it has tyrosine phosphatase activity as well.

The PPs dephosphorylate phospho-serine and phospho-threonine residues. Most of the PPs are holoenzymes and require one or two other regulatory subunits for activity. The PPs can further classified into phosphoprotein phosphatase (PPP) and metal ion-dependent protein phosphatase (PPM) subfamilies. PPMs require Mg2+ or Mn2+ ions for activities. PP2C phosphatases belong to this class. Members of the PPP subfamilies include PP1, PP2A, PP2B (calcineurin), PP4 and PP5.

Phosphatases such as Pten and PP2A directly act on proteins that are known to participate in cell adhesion or migration such as FAK, Src, and Paxillin. The activities of Rho GTPases can also be regulated through the inactivation or activation of their GAPs and GEFs via dephosphorylation. The Rac GEF Vav is activated by tyrosine phosphorylation (Crespo et al., 1997). It has been speculated that tyrosine phosphatase PTP-PEST (PTP-proline, glutamate, serine and threonine-rich domain) could inhibit Rac activation by dephosphorylating and down-regulating Vav (Sastry et al., 2002). There are also other reports showing that PTP-PEST can suppress Rac activity by acting through p130Cas (Garton and Tonks, 1999) and Paxillin (Shen et al., 1998).

Another PTP which could affect the activity of Rho GTPases is SHP-2 (Src homology region 2 containing PTP-2). It has been reported that SHP-2 levels and activity affect cytoskeleton organization, cell adhesion and migration (Inagaki et al., 2000; Oh et al., 1999; Saxton et al., 1997). The exact mechanisms are not known, with groups reporting SHP-2 to activate or to inhibit the activity of RhoA. It could well be that SHP-2 can function as both a positive and negative regulator of RhoA (Lacalle et al., 2002). SHP-2 could act as an activator of RhoA via the dephosphorylation of p190BRhoGAP, thereby decreasing the GAP activity. At the same time, SHP-2 might also reduce the activities of GEFs of the Vav family.

Pten can also suppress the activity of Rac1 and Cdc42. The inhibition is dependent on the lipid phosphatase activity of Pten (Liliental et al., 2000). Fibroblast cell lines from Pten (-/-) knockout mouse show increased cell motility and also significant increase in Rac1 and Cdc42 activities.

Reintroduction of wild type Pten, but not the catalytically inactive or lipid phosphatase-deficient mutants, reduced the enhanced cell motility of these Pten (-/-) cells.

Phosphatases may also have an effect on the actin cytoskeleton by regulating the targets of Rho GTPases. Serine/threonine phosphatases of the PP2C family, POPX1 and POPX2 (Partner of PIX, the PAK interacting exchange factor), have been found to dephosphorylate and down-regulate PAK (Koh et al., 2002). Co-expression with POPX prevents activated PAK from promoting the loss of stress fibres. PP2A has also been found in the same complex as PAK (Westphal et al., 1999) and to dephosphorylate PAK. Interestingly, PP2A can also dephosphorylate ADF/cofilin resulting in an increase in cell motility.

5. CONCLUSION

Although many proteins have been identified to participate in the organization of actin cytoskeleton downstream of Cdc42 and Rac, how the different complexes and pathways are integrated is still unclear. It is apparent that the same molecule can lie downstream of antagonistic signals such as RhoA versus Rac and Cdc42. Much work is needed to determine the mechanisms for achieving the right balancing of different signals through cross talk.

ACKNOWLEDGEMENTS

We thank Dr Carol Heckman for providing the SEM photo of cell ruffles and filopodia. This work is supported by the GSK (Singapore) Research Fund.

REFERENCES

Amano, M., K.Chihara, K.Kimura, Y.Fukata, N.Nakamura, Y.Matsuura, and K.Kaibuchi. 1997. Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. Science 275:1308-1311.

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

Arber, S., F.A.Barbayannis, H.Hanser, C.Schneider, C.A.Stanyon, O.Bernard, and P.Caroni. 1998. Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. Nature 393:805-809.

Ava, A. and S.A.Aaronson. 1985. Isolation of a new human oncogene from a diffused B-cell lymphoma. Nautre 316:273-275.

Bagrodia, S., D.Bailey, Z.Lenard, M.Hart, J.L.Guan, R.T.Premont, S.J.Taylor, and R.A.Cerione. 1999. A tyrosine-phosphorylated protein that binds to an important regulatory region on the cool family of p21-activated kinase-binding proteins. *J Biol Chem* 274:22393-22400.

Bashour, A.M., A.T.Fullerton, M.J.Hart, and G.S.Bloom. 1997. IQGAP1, a Rac- and Cdc42-binding protein, directly binds and cross-links microfilaments. J Cell Biol 137:1555-1566.

Bear, J.E., J.F.Rawls, and C.L.Saxe, III. 1998. SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late Dictyostelium development. *J Cell Biol* 142:1325-1335.

Bear, J.E., T.M.Svitkina, M.Krause, D.A.Schafer, J.J.Loureiro, G.A.Strasser, I.V.Maly, O.Y.Chaga, J.A.Cooper, G.G.Borisy, and F.B.Gertler. 2002. Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* 109:509-521.

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

Briggs, M.W. and D.B.Sacks. 2003. IQGAP proteins are integral components of cytoskeletal regulation. *EMBO Rep.* 4:571-574.

Bustelo, X.R. 2000. Regulatory and signaling properties of the Vav family. *Mol Cell Biol* 20:1461-1477.

Bustelo, X.R. 2001. Vav proteins, adaptors and cell signaling. *Oncogene* 20:6372-6381.

Carlier, M.F., V.Laurent, J.Santolini, R.Melki, D.Didry, G.X.Xia, Y.Hong, N.H.Chua, and D.Pantaloni. 1997. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *J Cell Biol* 136:1307-1322.

Carlier, M.F., P.Nioche, I.Broutin-L'Hermite, R.Boujemaa, C.Le Clainche, C.Egile, C.Garbay, A.Ducruix, P.Sansonetti, and D.Pantaloni. 2000. GRB2 links signaling to actin assembly by enhancing interaction of neural Wiskott-Aldrich syndrome protein (N-WASp) with actin-related protein (ARP2/3) complex. *J Biol Chem* 275:21946-21952.

Chew, T.L., R.A.Masaracchia, Z.M.Goeckeler, and R.B.Wysolmerski. 1998. Phosphorylation of non-muscle myosin II regulatory light chain by p21-activated kinase (gamma-PAK). *J Muscle Res Cell Motil.* 19:839-854.

Cory, G.O., R.Garg, R.Cramer, and A.J.Ridley. 2002. Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. *J Biol Chem* 277:45115-45121.

Crespo, P., K.E.Schuebel, A.A.Ostrom, J.S.Gutkind, and X.R.Bustelo. 1997. Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* 385:169-172.

Dharmawardhane, S., D.Brownson, M.Lennartz, and G.M.Bokoch. 1999. Localization of p21-activated kinase 1 (PAK1) to pseudopodia, membrane ruffles, and phagocytic cups in activated human neutrophils. *J Leukoc. Biol* 66:521-527.

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

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

Edwards, J.G., G.Campbell, A.W.Grierson, and S.R.Kinn. 1991. Vanadate inhibits both intracellular adhesion and spreading on fibronectin of BHK21 cells and transformed derivatives. *J Cell Sci* 98:363-368.

Etienne-Manneville, S. and A.Hall. 2003. Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature* 421:753-756.

Fleming, I.N., C.M.Elliott, and J.H.Exton. 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.

Fleming, I.N., A.Gray, and C.P.Downes. 2000. Regulation of the Rac1-specific exchange factor Tiam1 involves both phosphoinositide 3-kinase-dependent and -independent components. *Biochem J* 351:173-182.

Fukata, M., S.Kuroda, K.Fujii, T.Nakamura, I.Shoji, Y.Matsuura, K.Okawa, A.Iwamatsu, A.Kikuchi, and K.Kaibuchi. 1997. Regulation of cross-linking of actin filament by IQGAP1, a target for Cdc42. *J Biol Chem* 272:29579-29583.

Fukuoka, M., S.Suetsugu, H.Miki, K.Fukami, T.Endo, and T.Takenawa. 2001. A novel neural Wiskott-Aldrich syndrome protein (N-WASP) binding protein, WISH, induces Arp2/3 complex activation independent of Cdc42. *J Cell Biol* 152:471-482.

Garton, A.J. and N.K.Tonks. 1999. Regulation of fibroblast motility by the protein tyrosine phosphatase PTP-PEST. *J Biol Chem* 274:3811-3818.

Goeckeler, Z.M., R.A.Masaracchia, Q.Zeng, T.L.Chew, P.Gallagher, and R.B.Wysolmerski. 2000. Phosphorylation of myosin light chain kinase by p21-activated kinase PAK2. *J Biol Chem* 275:18366-18374.

Govind, S., R.Kozma, C.Monfries, L.Lim, and S.Ahmed. 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.

Habets, G.G., E.H.Scholtes, D.Zuydgeest, R.A.Van Der Kammen, J.C.Stam, A.Berns, and J.G.Collard. 1994. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. *Cell* 77:537-549.

Harden, N., J.Lee, H.Y.Loh, Y.M.Ong, I.Tan, T.Leung, E.Manser, and L.Lim. 1996. A Drosophila homolog of the Rac- and Cdc42-activated serine/threonine kinase PAK is a potential focal adhesion and focal complex protein that colocalizes with dynamic actin structures. *Mol Cell Biol* 16:1896-1908.

Hart, M., A.Ava, T.Evans, S.A.Aaronson, and R.A.Cerione. 1991. Catalysis of the guanine nucleotide exchange on the Cdc42Hs protein by the dbl oncogene product. *Nature* 354:311-314.

Hart, M.J., M.G.Callow, B.Souza, and P.Polakis. 1996. IQGAP1, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cdc42Hs. *EMBO J* 15:2997-3005.

Inagaki, K., T.Yamao, T.Noguchi, T.Matozaki, K.Fukunaga, T.Takada, T.Hosooka, S.Akira, and M.Kasuga. 2000. SHPS-1 regulates integrin-mediated cytoskeletal reorganization and cell motility. *EMBO J* 19:6721-6731.

Innocenti, M., A.Zucconi, A.Disanza, E.Frittoli, L.B.Areces, A.Steffen, T.E.Stradal, P.P.Di Fiore, M.F.Carlier, and G.Scita. 2004. Abi1 is essential for the formation and activation of a WAVE2 signalling complex. *Nat Cell Biol* 6:319-327.

Jaffer, Z.M. and J.Chernoff. 2002. p21-activated kinases: three more join the Pak. *Int. J Biochem Cell Biol* 34:713-717.

Kim, S., S.H.Lee, and D.Park. 2001. Leucine zipper-mediated homodimerization of the p21-activated kinase-interacting factor, beta Pix. Implication for a role in cytoskeletal reorganization. *J Biol Chem* 276:10581-10584.

Kimura, K., M.Ito, M.Amano, K.Chihara, Y.Fukata, M.Nakafuku, B.Yamamori, J.Feng, T.Nakano, K.Okawa, A.Iwamatsu, and K.Kaibuchi. 1996. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 273:245-248.

Koh, C.G., E.Manser, Z.S.Zhao, C.P.Ng, and L.Lim. 2001. Beta1PIX, the PAK-interacting exchange factor, requires localization via a coiled-coil region to promote microvillus-like structures and membrane ruffles. *J Cell Sci* 114:4239-4251.

Koh, C.G., E.J.Tan, E.Manser, and L.Lim. 2002. The p21-activated kinase PAK is negatively regulated by POPX1 and POPX2, a pair of serine/threonine phosphatases of the PP2C family. *Curr Biol* 12:317-321.

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

Lacalle, R.A., E.Mira, C.Gomez-Mouton, S.Jimenez-Baranda, A.Martinez, and S.Manes. 2002. Specific SHP-2 partitioning in raft domains triggers integrin-mediated signaling via Rho activation. *J Cell Biol* 157:277-289.

Lechler, T., A.Shevchenko, and R.Li. 2000. Direct involvement of yeast type I myosins in Cdc42-dependent actin polymerization. *J Cell Biol* 148:363-373.

Lee, W.L., M.Bezanilla, and T.D.Pollard. 2000. Fission yeast myosin-I, Myo1p, stimulates actin assembly by Arp2/3 complex and shares functions with WASp. *J Cell Biol* 151:789-800.

Lei, M., W.Lu, W.Meng, M.C.Parrini, M.J.Eck, B.J.Mayer, and S.C.Harrison. 2000. Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch. *Cell* 102:387-397.

Leung, T., X.Q.Chen, I.Tan, E.Manser, and L.Lim. 1998. Myotonic dystrophy kinase-related Cdc42-binding kinase acts as a Cdc42 effector in promoting cytoskeletal reorganization. *Mol Cell Biol* 18:130-140.

Leung, T., E.Manser, L.Tan, and L.Lim. 1995. A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. *J Biol Chem* 270:29051-29054.

Li, F. and H.N.Higgs. 2003. The mouse Formin mDia1 is a potent actin nucleation factor regulated by autoinhibition. *Curr Biol* 13:1335-1340.

Li, Z., M.Hannigan, Z.Mo, B.Liu, W.Lu, Y.Wu, A.V.Smrcka, G.Wu, L.Li, M.Liu, C.K.Huang, and D.Wu. 2003. Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. *Cell* 114:215-227.

Liliental, J., S.Y.Moon, R.Lesche, R.Mamillapalli, D.Li, Y.Zheng, H.Sun, and H.Wu. 2000. Genetic deletion of the Pten tumor suppressor gene promotes cell motility by activation of Rac1 and Cdc42 GTPases. *Curr Biol* 10:401-404.

Lim, L., E.Manser, T.Leung, and C.Hall. 1996. Regulation of phosphorylation pathways by p21 GTPases. The p21 Ras-related Rho subfamily and its role in phosphorylation signalling pathways. *Eur J Biochem* 242:171-185.

Machesky, L.M., S.J.Atkinson, C.Ampe, J.Vandekerckhove, and T.D.Pollard. 1994. Purification of a cortical complex containing two unconventional actins from Acanthamoeba by affinity chromatography on profilin-agarose. *J Cell Biol* 127:107-115.

Machesky, L.M., R.D.Mullins, H.N.Higgs, D.A.Kaiser, L.Blanchoin, R.C.May, M.E.Hall, and T.D.Pollard. 1999. Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proc Natl Acad Sci U S A* 96:3739-3744.

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

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

Manser, E., T.Leung, H.Salihuddin, Z.S.Zhao, and L.Lim. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* 367:40-46.

Mertens, A.E., R.C.Roovers, and J.G.Collard. 203. Regulation of Tiam-Rac signalling. *FEBS Lett.*11-16.

Michiels, F., J.C.Stam, P.L.Hordijk, R.A.Van Der Kammen, L.Ruuls-Van Stalle, C.A.Feltkamp, and J.G.Collard. 1997. Regulated membrane localization of Tiam1, mediated by the NH2-terminal pleckstrin homology domain, is required for Rac-dependent membrane ruffling and C-Jun NH2-terminal kinase activation. *J Cell Biol* 137:387-398.

Miki, H., K.Miura, and T.Takenawa. 1996. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. *EMBO J* 15:5326-5335.

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

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

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

Newsome, T.P., S.Schmidt, G.Dietzl, K.Keleman, B.Asling, A.Debant, and B.J.Dickson. 2000. Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in Drosophila. *Cell* 101:283-294.

Oh, E.S., H.Gu, T.M.Saxton, J.F.Timms, S.Hausdorff, E.U.Frevert, B.B.Kahn, T.Pawson, B.G.Neel, and S.M.Thomas. 1999. Regulation of early events in integrin signaling by protein tyrosine phosphatase SHP-2. *Mol Cell Biol* 19:3205-3215.

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

Peng, J., B.J.Wallar, A.Flanders, P.J.Swiatek, and A.S.Alberts. 2003. Disruption of the Diaphanous-related formin Drf1 gene encoding mDia1 reveals a role for Drf3 as an effector for Cdc42. *Curr Biol* 13:534-545.

Reid, T., A.Bathoorn, M.R.Ahmadian, and J.G.Collard. 1999. Identification and characterization of hPEM-2, a guanine nucleotide exchange factor specific for Cdc42. *J Biol Chem* 274:33587-33593.

Rohatgi, R., H.Y.Ho, and M.W.Kirschner. 2000. Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4, 5-bisphosphate. *J Cell Biol* 150:1299-1310.

Rohatgi, R., L.Ma, H.Miki, M.Lopez, T.Kirchhausen, T.Takenawa, and M.W.Kirschner. 1999. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* 97:221-231.

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

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

Sastry, S.K., P.D.Lyons, M.D.Schaller, and K.Burridge. 2002. PTP-PEST controls motility through regulation of Rac1. *J Cell Sci* 115:4305-4316.

Saxton, T.M., M.Henkemeyer, S.Gasca, R.Shen, D.J.Rossi, F.Shalaby, G.S.Feng, and T.Pawson. 1997. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. *EMBO J* 16:2352-2364.

Shen, Y., G.Schneider, J.F.Cloutier, A.Veillette, and M.D.Schaller. 1998. Direct association of protein-tyrosine phosphatase PTP-PEST with paxillin. *J Biol Chem* 273:6474-6481.

Snapper, S.B., F.Takeshima, I.Anton, C.H.Liu, S.M.Thomas, D.Nguyen, D.Dudley, H.Fraser, D.Purich, M.Lopez-Ilasaca, C.Klein, L.Davidson, R.Bronson, R.C.Mulligan, F.Southwick, R.Geha, M.B.Goldberg, F.S.Rosen, J.H.Hartwig, and F.W.Alt. 2001. N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. *Nat Cell Biol* 3:897-904.

Stam, J.C., E.E.Sander, F.Michiels, F.N.van Leeuwen, H.E.Kain, R.A.Van Der Kammen, and J.G.Collard. 1997. Targeting of Tiam1 to the plasma membrane requires the cooperative function of the N-terminal pleckstrin homology domain and an adjacent protein interaction domain. *J Biol Chem* 272:28447-28454.

Suetsugu, S., H.Miki, and T.Takenawa. 1998. The essential role of profilin in the assembly of actin for microspike formation. *EMBO J* 17:6516-6526.

Sumi, T., K.Matsumoto, A.Shibuya, and T.Nakamura. 2001. Activation of LIM kinases by myotonic dystrophy kinase-related Cdc42-binding kinase alpha. *J Biol Chem* 276:23092-23096.

Svitkina, T.M. and G.G.Borisy. 1999. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *J Cell Biol* 145:1009-1026.

Swart-Mataraza, J.M., Z.Li, and D.B.Sacks. 2002. IQGAP1 is a component of Cdc42 signaling to the cytoskeleton. *J Biol Chem* 277:24753-24763.

Turner, C.E., M.C.Brown, J.A.Perrotta, M.C.Riedy, S.N.Nikolopoulos, A.R.McDonald, S.Bagrodia, S.Thomas, and P.S.Leventhal. 1999. Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: A role in cytoskeletal remodeling. *J Cell Biol* 145:851-863.

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

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

Watanabe, N., P.Madaule, T.Reid, T.Ishizaki, G.Watanabe, A.Kakizuka, Y.Saito, K.Nakao, B.M.Jockusch, and S.Narumiya. 1997. p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J* 16:3044-3056.

Weaver, A.M., A.V.Karginov, A.W.Kinley, S.A.Weed, Y.Li, J.T.Parsons, and J.A.Cooper. 2001. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. *Curr Biol* 11:370-374.

Welch, M.D., J.Rosenblatt, J.Skoble, D.A.Portnoy, and T.J.Mitchison. 1998. Interaction of human Arp2/3 complex and the listeria monocytogenes ActA protein in actin filament nucleation. *Science*105-108.

Westphal, R.S., R.L.Coffee, Jr., A.Marotta, S.L.Pelech, and B.E.Wadzinski. 1999. Identification of kinase-phosphatase signaling modules composed of p70 S6 kinase-protein phosphatase 2A (PP2A) and p21-activated kinase-PP2A. *J Biol Chem* 274:687-92.

Wilson, A.K., A.Takai, J.C.Ruegg, and P.de Lanerolle. 1991. Okadaic acid, a phosphatase inhibitor, decreases macrophage motility. *Am J Physol* 260:L105-L112.

Yang, C., M.Huang, J.DeBiasio, M.Pring, M.Joyce, H.Miki, T.Takenawa, and S.H.Zigmond. 2000. Profilin enhances Cdc42-induced nucleation of actin polymerization. *J Cell Biol* 150:1001-1012.

Yang, N., O.Higuchi, K.Ohashi, K.Nagata, A.Wada, K.Kangawa, E.Nishida, and K.Mizuno. 1998. Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* 393:809-812.

Yeh, T.C., W.Ogawa, A.G.Danielsen, and R.A.Roth. 1996. Characterization and cloning of a 58/53-kDa substrate of the insulin receptor tyrosine kinase. *J Biol Chem* 271:2921-2928.

Zeng, Q., D.Lagunoff, R.Masaracchia, Z.Goeckeler, G.Cote, and R.Wysolmerski. 2000. Endothelial cell retraction is induced by PAK2 monophosphorylation of myosin II. *J Cell Sci* 113 (Pt 3):471-482.

Zhao, Z., E.Manser, T.H.Loo, and L.Lim. 2000. Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. *Mol Cell Biol* 20:6354-63.

Zhu, K., B.Debreceni, F.Bi, and Y.Zheng. 2001. Oligomerization of DH domain is essential for Dbl-induced transformation. *Mol Cell Biol* 21:425-437.