IQGAP1: a key regulator of adhesion and migration

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Summary

The dynamic rearrangement of cell-cell adhesion is one of the major physiological events in tissue development and tumor metastasis. Polarized cell migration, another key event, is a tightly regulated process that occurs during tissue development, chemotaxis and wound healing. Rho-family small GTPases, especially Rac1 and Cdc42, play pivotal roles in these processes through one of their effectors, IQGAP1. Recent studies reveal that IQGAP1 regulates cadherin-mediated cell-cell adhesion both positively and negatively. It captures and stabilizes microtubules through the microtubule-binding protein CLIP-170 near the cell cortex, leading to establishment of polarized cell morphology and directional cell migration. Furthermore, Rac1 and Cdc42 link the adenomatous polyposis coli (APC) protein to actin filaments through IQGAP1 at the leading edge and thereby regulate polarization and directional migration.

Key words: IQGAP1, Rho-family GTPases, Rac1, Cdc42, Cadherin, CLIP-170, APC, Cell adhesion, Cell polarization

Introduction

Rho-family GTPases, such as Rho, Rac and Cdc42, cycle between a GTP-bound active state and a GDP-bound inactive state. The nucleotide state of Rho GTPases is controlled by three classes of regulator: guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP; Rho GDP dissociation inhibitors (Rho GDIs), which interact with GDP-bound Rho GTPases and inhibit the exchange of GDP for GTP; and GTPase-activating proteins (GAPs), which enhance the intrinsic GTPase activities of Rho GTPases. These regulators ensure that the activation and inactivation of Rho GTPases are tightly regulated spatio-temporally to generate specific and localized responses (Mackay and Hall, 1998; Kaibuchi et al., 1999; Gulli and Peter, 2001) through the numerous effectors of these GTPases that have been characterized (Van Aelst and D'Souza-Schorey, 1997; Hall, 1998; Kaibuchi et al., 1999). The effectors interact with Rho GTPases in the GTP-bound state and include molecules such as Rho kinase, members of the WASP family, PAK and IQGAP1 (Schwartz, 2004) (Table 1).

IQGAP1 is an effector for Rac and Cdc42 that contains binding sites for actin (Fukata et al., 1997), extracellular signal-regulated kinase 2 (ERK2) (Roy et al., 2004), calmodulin (Hart et al., 1996; Joyal et al., 1997; Ho et al., 1999), myosin essential light chain (Weissbach et al., 1998), S100B (Mbele et al., 2002), Rac/Cdc42 (Hart et al., 1996; Kuroda et al., 1996; Swart-Mataraza et al., 2002; Mataraza et al., 2003a), β-catenin (Fukata et al., 1999; Briggs et al., 2002), E-cadherin (Kuroda et al., 1998; Li et al., 1999), CLIP-170 (Fukata et al., 2002) and adenomatous polyposis coli (APC) (Watanabe et al., 2004) (Fig. 1). It appears to play a pivotal role in the control of cell adhesion, polarization and migration. Here, we review recent work that has provided insight into how IQGAP1 functions in these processes.

Cadherin-mediated cell-cell adhesion

Cadherins comprise a major group of cell-cell adhesion molecules that mediate intercellular adhesion by engaging in Ca^{2+}-dependent, homophilic, trans interactions (Takeichi, 1995; Adams and Nelson, 1998; Gumbiner, 2000; Tepass et al., 2000). By forming cis homodimers, cadherins can cluster through a zipper-like mechanism, with their intracellular domains anchored to the actin cytoskeleton through α-catenin and β-catenin. Anchorage of cadherins to the actin cytoskeleton and their clustering are indispensable for the development of strong and rigid adhesion (Tsukita et al., 1992).

More than 80 members of the cadherin superfamily have been identified in the human genome, including classical cadherins (e.g. E-cadherin, VE-cadherin and N-cadherin), Fat-like cadherins, and seven-pass transmembrane cadherins (Tepass et al., 2000; Yagi and Takeichi, 2000). All cadherins possess extracellular cadherin (EC) domains (also known as cadherin repeats), typically organized as tandem repeats, which mediate their homophilic interactions. Their adhesive activity can be regulated by extracellular Ca^{2+} and cytoplasmic signaling. Binding of Ca^{2+} to the linker region between the EC domains allows cadherin molecules to form a rigid and organized structure that is resistant to proteolysis (Steinberg and McNutt, 1999). Under these conditions, they can form cis dimers and trans dimers. Removal of Ca^{2+} by EGTA leads to a disordered structure and loss of cadherin-mediated cell-cell adhesion. The cytoplasmic regions of classical cadherins comprise two domains: the C-terminal distal β-catenin-binding domain (DβD); and the juxtamembrane domain (JMD), which is the p120-catenin-binding site and is thought to regulate clustering, transport and endocytosis of cadherins (Takeichi, 1995; Adams and Nelson, 1998; Gumbiner, 2000; Tepass et al., 2000).

Table 1. Effectors for Rho family GTPases

Rho	
Rho-kinase (also known as ROK or ROCK) and the myosin-binding subunit (MBS) of myosin phosphatase	smooth muscle contraction, stress fiber formation, focal adhesion formation, neurite retraction, and cell motility
PKN/PRK1	gene expression and membrane trafficking
PRK2	actin cytoskeleton organization
mDia and PI5-kinase	actin polymerization and stabilization of microtubule
Citron and BORGs	cytokinesis and cell cycle progression
Rac	
IQGAP1	cell-cell adhesion and actin reorganization
WAVE complex	actin polymerization
PI3-kinase	cell migration
PAK	gene expression and actin reorganization
Por1 and p140Sra-1	membrane ruffling
POSH and MLK	gene expression
NADPH oxidase	proliferation
Cdc42	
WASP/N-WASP	actin polymerization and filopodia formation
Ack	vesicle transport
PAR-6	scaffolding protein and cell polarization
Myotonic-dystrophy-kinase-related Cdc42-binding kinase (MRCK)	filopodia formation
Coatamer	membrane trafficking

Regulation of cadherin-mediated cell-cell adhesion by Rho GTPases

Several lines of evidence suggest that Rho-family GTPases are required for cadherin-mediated cell-cell adhesion. For example, microinjection of keratinocytes with dominant-negative Rac1 ($Rac1^{N17}$) or C3 botulinum toxin – which inactivates RhoA – inhibits the accumulation of cadherin at sites of cell-cell contact upon Ca^{2+}-induced intercellular adhesion (Braga et al., 1997). Similarly, overexpression of $Rac1^{N17}$ in MDCKII epithelial cells reduces the level of E-cadherin, β-catenin and actin filaments at sites of cell-cell contact, whereas overexpression of activated Rac ($Rac1^{V12}$) promotes it (Takaishi et al., 1997). Moreover, Cdc42 and Rac1 are required for E-cadherin-mediated cell-cell adhesion in MDCKII cells (Kuroda et al., 1997; Kodama et al., 1999). Tiam1, a Rac-GEF, localizes to cell-cell contact sites and inhibits hepatocyte growth factor (HGF)-induced cell scattering in MDCKII cells, probably by increasing E-cadherin-mediated cell-cell adhesion (Hordijk et al., 1997).

Studies using cell-dissociation assays to quantify E-cadherin activity in mouse L fibroblasts stably expressing E-cadherin mutants have examined the basis of these effects. Cells expressing wild-type E-cadherin (EL cells) and cells expressing an E-cadherin mutant in which the cytoplasmic domain has been replaced by the C-terminal domain of α-catenin (nEαCL cells) both exhibit E-cadherin-dependent cell-cell adhesion. However, in nEαCL cells, this clearly does not require the DβD of E-cadherin, β-catenin or the N-terminal region of α-catenin (Nagafuchi et al., 1994). Significantly, expression of $Rac1^{N17}$ or $Cdc42^{N17}$ markedly reduces E-cadherin-dependent adhesion in EL cells but not in nEαCL cells (Fukata et al., 1999). This observation indicates that Rac1 and Cdc42 regulate E-cadherin activity through the cadherin-catenin complex. By contrast, expression of dominant-negative Rho (Rho^{N19}) slightly reduces E-cadherin activity in both EL cells and nEαCL cells. This suggests that RhoA affects E-cadherin-mediated adhesive activity through another mechanism.

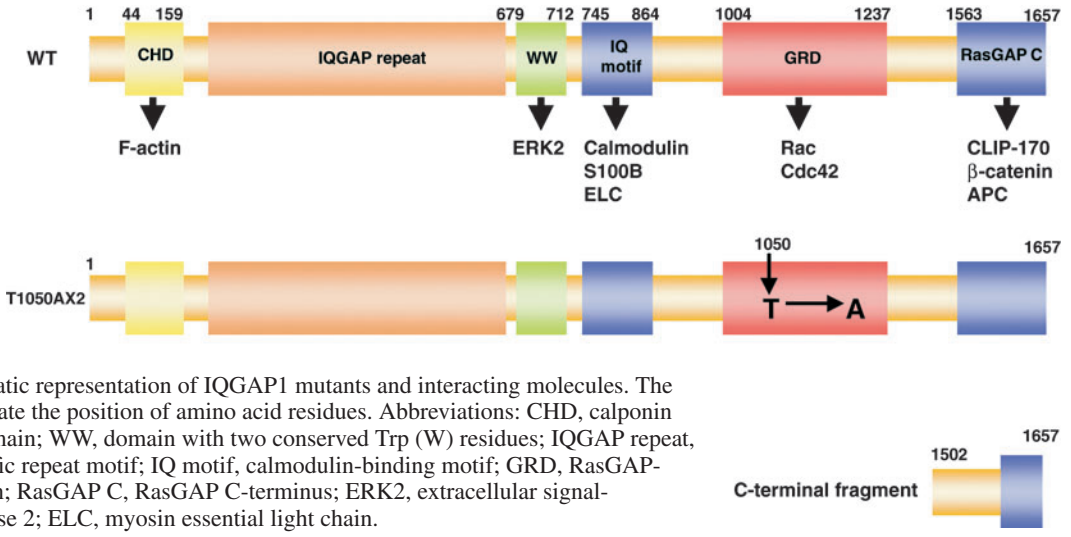

Fig. 1. Schematic representation of IQGAP1 mutants and interacting molecules. The numbers indicate the position of amino acid residues. Abbreviations: CHD, calponin homology domain; WW, domain with two conserved Trp (W) residues; IQGAP repeat, IQGAP-specific repeat motif; IQ motif, calmodulin-binding motif; GRD, RasGAP-related domain; RasGAP C, RasGAP C-terminus; ERK2, extracellular signal-regulated kinase 2; ELC, myosin essential light chain.

Regulation of cadherin-mediated cell-cell adhesion by IQGAP1

IQGAP1 localizes to sites of cell-cell contact (Kuroda et al., 1998) and, when overexpressed, it reduces E-cadherin-mediated cell-cell adhesion by interacting with β-catenin, causing the dissociation of α-catenin from the cadherin-catenin complex in EL cells (Kuroda et al., 1998). Activated Rac1 and Cdc42 positively regulate E-cadherin-mediated cell-cell adhesion by inhibiting the interaction of IQGAP1 with β-catenin (Fukata et al., 1999). We have therefore proposed that E-cadherin exists in a dynamic equilibrium between the E-cadherin–β-catenin–α-catenin complex and the E-cadherin–β-catenin–IQGAP1 complex at sites of cell-cell contact. The ratio between these two complexes could determine the strength of adhesion. However, until recently, the consequences of loss of function of IQGAP1 had been unclear.

Recent work, including the use of RNA interference (RNAi), has now examined this issue. We have shown that the inhibition of either IQGAP1 or Rac1 by RNAi reduces the accumulation of actin filaments, E-cadherin and β-catenin at sites of cell-cell contact in MDCKII cells (Noritake et al., 2004). In addition, we showed that expression of a putative constitutively active mutant of IQGAP1(T1050AX2) (Fig. 1) that cannot bind to Rac1/Cdc42 overcomes the effect of knocking down Rac1 (e.g. promoting actin accumulation). We also found that 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced cell scattering in cells in which IQGAP1 or Rac1 is knocked down is faster than in control cells (Noritake et al., 2004). IQGAP1 and Rac1 are thus both necessary for cell-cell adhesion.

Rac1 directly binds to IQGAP1 when the amount of its GTP-bound form increases. This tethers actin filaments, which are also linked to the cadherin–β-catenin complex through α-catenin. Under these conditions, IQGAP1 does not bind to β-catenin and cannot dissociate α-catenin from the cadherin-catenin complex, and the ratio of E-cadherin–β-catenin–α-catenin complex to E-cadherin–β-catenin–IQGAP1 complex is high. This state confers strong adhesive activity (Fig. 2). Since IQGAP1 has anti-GTPase activity (Hart et al., 1996), it might sustain the amount of GTP-bound Rac1 at sites of cell-cell contact, leading to stable adhesion. Izumi et al. recently reported that the inhibition of endocytosis of trans-interacting E-cadherin is mediated by reorganization of the actin cytoskeleton by the IQGAP1-Rac/Cdc42 complex (Izumi et al., 2004). Thus, IQGAP1 behaves as a positive regulator downstream of Rac1.

By contrast, IQGAP1 is freed from Rac1 and Cdc42, and interacts with β-catenin to dissociate α-catenin from the cadherin-catenin complex when the amounts of inactivated Rac1 and Cdc42 increase during the action of certain extracellular signals such as HGF or TPA (see below). In this case, the ratio of E-cadherin–β-catenin–IQGAP1 complex to E-cadherin–β-catenin–α-catenin complex is high, resulting in weak adhesion and cell-cell dissociation (Fig. 2). Thus, IQGAP1 negatively regulates E-cadherin-mediated cell-cell adhesion.

The physiological processes in which this Rac1/Cdc42/IQGAP1 system is involved are unclear. It functions in cell-cell dissociation during HGF- or TPA-induced cell scattering, which is thought to be a model for the epithelial-mesenchymal transition (EMT) and dispersal of cancer cells. Time-lapse analyses using green fluorescent protein (GFP)-tagged α-catenin showed that α-catenin disappears from cell-cell contacts before the cells dissociate during cell scattering. $Rac1^{V12}$, $Cdc42^{V12}$ and a dominant-

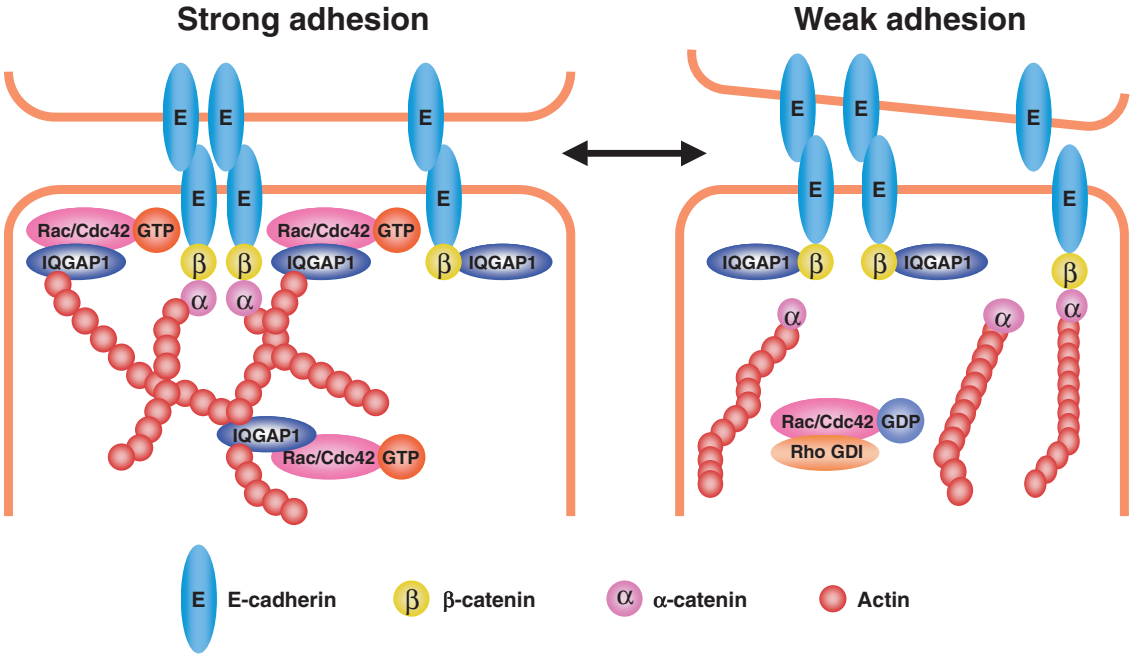

Fig. 2. Role of IQGAP1 in the regulation of E-cadherin-mediated cell-cell adhesion. When the amount of activated Rac1 increases, Rac1 interacts with IQGAP1, thereby crosslinking actin filaments. Under these conditions, IQGAP1 does not bind to β-catenin and cannot dissociate α-catenin from the cadherin-catenin complex, leading to strong adhesion. By contrast, when the amounts of inactivated Rac1 increases, IQGAP1 is freed from Rac1 and interacts with β-catenin to dissociate α-catenin from the cadherin-catenin complex. This results in weak adhesion.

negative mutant of IQGAP1 (a C-terminal fragment; see Fig. 1) that interacts with endogenous IQGAP1 and delocalizes it from sites of cell-cell contact inhibit the disappearance of α-catenin. Furthermore, on stimulation with HGF or TPA, the level of GTP-bound Rac1 and the proportion of Rac1 complexed with IQGAP1 decrease, and the proportion of IQGAP1 complexed with β-catenin increases (Fukata et al., 2001). α-catenin-deficient mouse teratocarcinoma F9 cells display a scattered phenotype under conditions in which parental or α-catenin-reexpressing cells form compact colonies (Li et al., 2000). This indicates that loss of α-catenin results in loss of cell-cell adhesion and a scattered phenotype. Since IQGAP1 dissociates α-catenin from the cadherin-catenin complex and dominant-negative IQGAP1 inhibits the disappearance of α-catenin from sites of cell-cell contact during cell scattering, these results indicate that the Rac1/Cdc42/IQGAP1 system is involved in cell-cell dissociation induced by HGF or TPA. Given that this represents a model for epithelial reorganization and the EMT, IQGAP1 might have a more general role in these processes. Indeed, the phenotypes of IQGAP1-knockout mice and its upregulation in certain cancer support this idea (see Perspectives section).

Cell polarization and Rho family GTPases

Cells dynamically polarize during directed cell migration (e.g. chemotaxis and wound healing), responses of T cells to antigen, and the establishment of epithelial apico-basolateral polarity and axon specification in neuronal cells. This requires asymmetric distribution of signaling molecules, cell-adhesion molecules and the cytoskeleton, as well as directed membrane trafficking. During wound healing, for example, migrating fibroblasts develop a polarized morphology characterized by membrane ruffling and filopodia at the leading edge, capture of microtubule plus-ends near the leading edge (which enables motor proteins such as dynein and kinesin to perform directed membrane trafficking), and reorientation of the microtubule-organizing center (MTOC) and the Golgi apparatus towards the direction of migration. Each process must be coordinated, particularly the reorganization of the actin cytoskeleton and microtubules (Goode et al., 2000).

Time-lapse imaging has begun to reveal when and where signaling molecules, cytoskeletal components, cell adhesion molecules and vesicles are asymmetrically distributed during cell polarization. The molecular mechanisms involved and how the overall series of events are integrated remain mysterious. However, it is clear that Rho-family GTPases play an important role through regulation of the cytoskeleton and assembly of matrix adhesion complexes (Hall, 1998; Fukata et al., 2003; Nelson, 2003; Ridley et al., 2003; Raftopoulou and Hall, 2004) in various cell types, including T cells (Stowers et al., 1995), fibroblasts (Nobes and Hall, 1999), macrophages (Allen et al., 1998), astrocytes (Etienne-Manneville and Hall, 2001; Etienne-Manneville and Hall, 2003), epithelial cells (Kroschewski et al., 1999) and neuronal cells (Luo, 2000; Schwamborn and Püschel, 2004). Rac1 and Cdc42 participate in polarization of T cells towards antigen-presenting cells, in the directed movement of fibroblasts, macrophages and astrocytes, and in the apico-basolateral polarization of epithelial cells (Etienne-Manneville and Hall, 2002). Cdc42 is also known to participate in cell polarization in *Caenorhabditis elegans* embryos by interacting with the PAR-3–PAR-6–PKC-3 complex (Gotta et al., 2001; Kay and Hunter, 2001). Recently, the regulatory mechanisms by which Rac1 and Cdc42 control the actin cytoskeleton have been revealed through the identification and characterization of effectors such as N-WASP, PAK and IQGAP1 (Kaibuchi et al., 1999; Raftopoulou and Hall, 2004). Rho-family GTPases also seem to regulate microtubule organization and dynamics (Fukata et al., 2003; Gundersen et al., 2004). Again IQGAP1 is implicated, and intensive analyses are beginning to clarify how Rho-family GTPases use this protein to regulate cell polarity through reorganization of the actin cytoskeleton and microtubules.

Cell polarization by IQGAP1 and CLIP-170

Links between the plus-ends of microtubules and cortical regions are essential for the establishment of cell polarity and directional migration. Studies using GFP fusion proteins have revealed that plus-end-tracking proteins (+Tips) (Schuyler and Pellman, 2001), including the CLIP-170 family (Perez et al., 1999), the EB1 family (Mimori-Kiyosue et al., 2000a) and the dynein-dynactin complex (Vaughan et al., 1999), accumulate at the plus-ends of growing microtubules and play crucial roles in sensing cortical capture sites. CLIP-170 links microtubules to endosomes (Pierre et al., 1992), and CLIP-170 and EB1 are among the components needed for capture of microtubules at specific cortical regions (Schuyler and Pellman, 2001; Gundersen, 2002).

CLIP-170 interacts with IQGAP1 (Fukata et al., 2002), which can associate with microtubules through CLIP-170 in vitro. In fibroblasts, IQGAP1 colocalizes with actin filaments at the polarized leading edge, to which CLIP-170 and microtubules are targeted. Activated Rac1/Cdc42 forms a tripartite complex with IQGAP1 and CLIP-170, enhances the interaction of IQGAP1 with CLIP-170, and captures GFP–CLIP-170 at the leading edge and the base of filopodia. Expression of the constitutively active mutant IQGAP1(T1050AX2) bypasses the stimulatory effect of Rac1/Cdc42 on the IQGAP1–CLIP-170 interaction and induces formation of multiple leading edges (Fukata et al., 2002). We therefore propose that Rac1/Cdc42 marks cortical spots to which the IQGAP1–CLIP-170 complex is targeted, leading to formation of polarized microtubule arrays and cell polarization. Knocking down IQGAP1 by RNAi decreases the number of immobilized GFP–CLIP-170 complexes at the cell periphery facing a wound, which indicates that stabilization of the plus-ends of microtubules at these sites requires IQGAP1 (Watanabe et al., 2004).

Microtubules frequently follow similar tracks during polymerization. They might therefore be guided along common cytoskeletal elements to specific sites in cells. How is this accomplished during polarization? The most likely candidates for such tracks are actin filaments (Fig. 3). The actin-based motor myosin VI interacts with CLIP-190 (Lantz and Miller, 1998), the *Drosophila* homolog of CLIP-170, and some actin-binding proteins can interact with +Tips, such as IQGAP1. ACF7, a member of the spectraplakin family of cytoskeletal crosslinking proteins, interacts with both actin and microtubules, and seems to play a key role. In ACF7-null endodermal cells, EB1 and CLIP-170 localize to the tips of

microtubules, but microtubules do not grow along actin filaments (Kodama et al., 2003). Furthermore, in the absence of ACF7, the microtubules do not pause and become tethered to actin-rich cortical sites. Instead, they are less-stable, longer structures that have skewed cytoplasmic trajectories (Kodama et al., 2003).

Migration mechanisms involving IQGAP1 and APC

The dynein-dynactin motor complex is thought to pull on microtubules stabilized at the leading edge to induce the reorientation of the MTOC and Golgi apparatus towards the decision of migration. This facilitates growth of microtubules into the lamellipodium and microtubule-mediated delivery of vesicles to the leading edge, providing the membrane and associated proteins needed (Gundersen, 2002; Ridley et al., 2003). In astrocytes, the activation of Cdc42 is necessary for the reorientation of the MTOC, but that of Rac1 and RhoA is not (Etienne-Manneville and Hall, 2001). Cdc42 is thought to regulate the reorientation of the MTOC by stabilizing APC at the cell periphery, acting through the PAR-6–aPKC complex and glycogen synthase kinase (GSK)-3β (Etienne-Manneville and Hall, 2003). APC, an important tumor suppressor in the human colon, destabilizes β-catenin in the Wnt signaling pathway, and several intracellular locations for APC have been described, including clusters at the cortical regions where microtubules are targeted (Näthke, 2004). It also accumulates at the ends of microtubules that extend into actively migrating regions (Barth et al., 2002; Mimori-Kiyosue et al., 2000b). It could stabilize the microtubule array; subsequently, dynein-dynactin at the cell periphery could pull the stabilized microtubules to reorient the MTOC (Palazzo et al., 2001).

In directionally migrating cells, IQGAP1 accumulates at the leading edge (Hart et al., 1996; Kuroda et al., 1996; Mataraza et al., 2003b) and crosslinks actin filaments (Briggs and Sacks, 2003). Furthermore knocking down IQGAP1 by RNAi or transfection of a dominant-negative IQGAP1 mutant markedly reduces cell motility (Mataraza et al., 2003b). More recent experiments have shown that IQGAP1 directly interacts with APC and that they both colocalize at the leading edge with Rac1 and Cdc42. Activated Rac1 and Cdc42 form a tripartite complex with IQGAP1 and APC (Watanabe et al., 2004). The depletion of IQGAP1 or APC by RNAi inhibits formation of an actin meshwork at the leading edge, as well as cell migration, immobilization of the plus-ends of microtubules and polarization of the MTOC. This often induces formation of protrusions in the direction of movement instead of a typical leading edge. These atypical structures might be caused by lack of an actin meshwork in the space beneath the leading edge, which longer microtubules have invaded (Bradke and Dotti, 1999). Constitutively active IQGAP1, which can induce formation of multiple leading edges (Fukata et al., 2002), provides anomalous accumulation sites for APC; this depends on actin filaments and inhibits proper cell migration. Thus, IQGAP1 appears to anchor APC to actin filaments at specific cortical sites.

On the basis of the study discussed above, we can propose the following model for migration (Fig. 4). Rac1 and Cdc42 are activated by extracellular signals through receptors and GEFs at the leading edge. We have recently shown that the PAR-6–PAR-3 complex mediates the Cdc42-induced Rac1 activator through the direct interaction with Rac-specific GEFs, STEF and Tiam1 (Nishimura et al., 2005). Rac1 and Cdc42 then induce the polymerization of actin filaments through various effectors. They also mark spots where IQGAP1 tethers actin filaments. IQGAP1 links APC to actin filaments and captures the plus-ends of microtubules through CLIP-170. Then APC directly and/or indirectly stabilizes microtubules, which is necessary for generation of a stable actin meshwork at the leading edge. APC binds to APC-stimulated GEF (Asef), activating its Rac1 GEF activity (Kawasaki et al., 2000), and Rac1 activated in this way may affect not only microtubules but also actin filaments, through other effectors. This would provide a local positive-feedback loop that sustains cell migration.

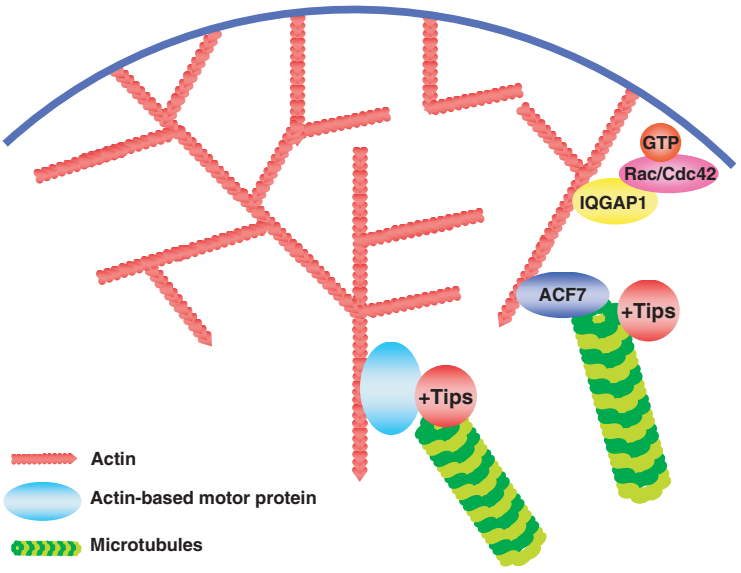

Fig. 3. Schematic representation of the movement of microtubules along actin filaments. The coordinated reorganization of the cytoskeleton is essential for cell polarization and migration. Actin filaments are one candidate for guidance tracks for microtubules. Actin-binding proteins such as myosin VI interact with CLIP-190, the *Drosophila* homolog of CLIP-170, to slide microtubules along actin filaments. In higher eukaryotes, scaffold proteins such as ACF7 link actin filaments and microtubules. These actin-binding proteins and +Tips seem to stabilize mictotubule-actin interactions. They also stabilize cell migration or cell adhesion.

Perspectives

The work discussed in this Commentary indicates that IQGAP1 plays a crucial role in cell-cell adhesion, cell polarization and directional cell migration by linking Rho-family GTPases with the actin cytoskeleton and microtubules. Recent knockout work and clinical studies have further revealed the physiological importance of IQGAP1. IQGAP1-knockout mice exhibit gastric hyperplasia and dysplasia (Li et al., 2000), which suggests that IQGAP1 plays an important role in reorganization of the gastric epithelium. The mice do not display any developmental defects, which might be the result of the functional redundancy with IQGAP2 (Brill

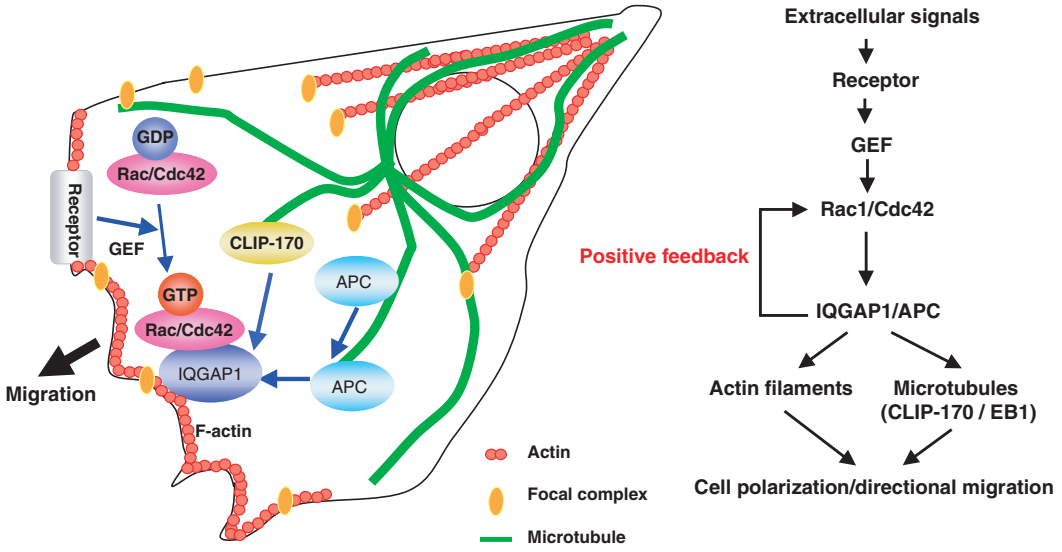

Fig. 4. Role of the IQGAP1-APC complex in cell polarization and migration. Directional cell migration is usually initiated in response to extracellular cues. Extracellular signals, including growth factors and chemokines, activate Rac1 and Cdc42 through their receptors and certain GEFs at leading edges. Activated Rac1 and Cdc42 induce the polymerization of actin filaments through their effectors. Activated Rac1 and Cdc42 also mark spots where IQGAP1 crosslinks actin filaments. There, APC is recruited through IQGAP1 to actin filaments. IQGAP1 captures the plus-ends of microtubules through CLIP-170. APC then directly and/or indirectly stabilizes microtubules, which are necessary for stable actin meshwork at leading edges.

et al., 1996) and IQGAP3. IQGAP1 is upregulated by gene amplification in some diffuse types of gastric cancer (Sugimoto et al., 2001) and is highly expressed in patients with moderate and/or severe atopic dermatitis (Matsumoto et al., 2002). Furthermore, there seems to be a correlation between dysfunction of E-cadherin-mediated adhesion in gastric tumors, increased membrane localization of IQGAP1 and decreased membrane localization of α-catenin (Takemoto et al., 2001). This indicates that the Rac1/Cdc42/IQGAP1 system might be involved in tumor progression. Presslauer et al. found that autoantibodies in patients with autoimmune bullous skin diseases recognize IQGAP1 as an antigen (Presslauer et al., 2003). Moreover, IQGAP1 might have a role in the organization of reactive oxygen species (ROS)-dependent vascular endothelial growth factor (VEGF) signaling, followed by the promotion of endothelial cell migration and proliferation, which may contribute to the regeneration of endothelial cells after vascular injury (Yamaoka-Tojo et al., 2004).

IQGAP1 thus seems to be involved in several human diseases, and it is conceivable that the dysregulation of cadherin-mediated cell-cell adhesion and the misregulation of cell polarization by IQGAP1 and Rho GTPases promote tumor metastasis and intractable inflammatory diseases that often lead to death. If so, modulation of the signaling pathways linking IQGAP1 and cadherins or cell polarization might provide the basis for therapies for these diseases.

References

Adams, C. L. and Nelson, W. J. (1998). Cytomechanics of cadherin-mediated cell-cell adhesion. *Curr. Opin. Cell Biol.* **10**, 572-577.

Allen, W. E., Zicha, D., Ridley, A. J. and Jones, G. E. (1998). A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* **141**, 1147-1157.

Barth, A. I., Siemers, K. A. and Nelson, W. J. (2002). Dissecting interactions between EB1, microtubules and APC in cortical clusters at the plasma membrane. *J. Cell Sci.* **115**, 1583-1590.

Bradke, F. and Dotti, C. G. (1999). The role of local actin instability in axon formation. *Science* **283**, 1931-1934.

Braga, V., Machesky, L. M., Hall, A. and Hotchin, N. A. (1997). The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *J. Cell Biol.* **137**, 1421-1431.

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

Briggs, M. W., Li, Z. and Sacks, D. B. (2002). IQGAP1-mediated stimulation of transcriptional co-activation by beta-catenin is modulated by calmodulin. *J. Biol. Chem.* **277**, 7453-7465.

Brill, S., Li, S., Lyman, C. W., Church, D. M., Wasmuth, J. J., Weissbach, L., Bernards, A. and Snijders, A. J. (1996). The Ras GTPase-activating-protein-related human protein IQGAP2 harbors a potential actin binding domain and interacts with calmodulin and Rho family GTPases. *Mol. Cell. Biol.* **16**, 4869-4878.

Etienne-Manneville, S. and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* **106**, 489-498.

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

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

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

Fukata, M., Kuroda, S., Nakagawa, M., Kawajiri, A., Itoh, N., Shoji, I., Matsuura, Y., Yonehara, S., Fujisawa, H., Kikuchi, A. et al. (1999). Cdc42 and Rac1 regulate the interaction of IQGAP1 with β-catenin. *J. Biol. Chem.* **274**, 26044-26050.

Fukata, M., Nakagawa, M., Itoh, N., Kawajiri, A., Yamaga, M., Kuroda, S. and Kaibuchi, K. (2001). Involvement of IQGAP1, an effector of Rac1 and Cdc42 GTPases, in cell-cell dissociation during cell scattering. *Mol. Cell. Biol.* **21**, 2165-2183.

Fukata, M., Watanabe, T., Noritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuura, Y., Iwamatsu, A., Perez, F. and Kaibuchi, K. (2002). Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. *Cell* **109**, 873-885.

Fukata, M., Nakagawa, M. and Kaibuchi, K. (2003). Roles of Rho-family GTPases in cell polarisation and directional migration. *Curr. Opin. Cell Biol.* **15**, 590-597.

Goode, B. L., Drubin, D. G. and Barnes, G. (2000). Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.* **12**, 63-71.

Gotta, M., Abraham, M. C. and Ahringer, J. (2001). CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. *Curr. Biol.* **11**, 482-488.

Gulli, M. P. and Peter, M. (2001). Temporal and spatial regulation of Rho-type guanine-nucleotide exchange factors: the yeast perspective. *Genes Dev.* **15**, 365-379.

Gumbiner, B. M. (2000). Regulation of cadherin adhesive activity. *J. Cell Biol.* **148**, 399-404.

Gundersen, G. G. (2002). Evolutionary conservation of microtubule-capture mechanisms. *Nat. Rev. Mol. Cell. Biol*. **3**, 296-304.

Gundersen, G. G., Gomes, E. R. and Wen, Y. (2004). Cortical control of microtubule stability and polarization. *Curr. Opin. Cell Biol.* **16**, 106-112.

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

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

Ho, Y. D., Joyal, J. L., Li, Z. and Sacks, D. B. (1999). IQGAP1 integrates Ca^{2+}/calmodulin and CDC42 signaling. *J. Biol. Chem.* **274**, 464-470.

Hordijk, P. L., ten Klooster, J. P., van der Kammen, R. A., Michiels, F., Oomen, L. C., Collard, J. G. (1997). Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science* **278**, 1464-1466.

Izumi, G., Sakisaka, T., Baba, T., Tanaka, S., Morimoto, K. and Takai, Y. (2004). Endocytosis of E-cadherin regulated by Rac and Cdc42 small G proteins through IQGAP1 and actin filaments. *J. Cell Biol.* **166**, 237-248.

Joyal, J. L., Annan, R. S., Ho, Y. D., Huddleston, M. E., Carr, S. A., Hart, M. J. and Sacks, D. B. (1997). Calmodulin modulates the interaction between IQGAP1 and Cdc42. Identification of IQGAP1 by nanoelectrospray tandem mass spectrometry. *J. Biol. Chem*. **272**, 15419-15425.

Kaibuchi, K., Kuroda, S. and Amano, M. (1999). Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. *Annu. Rev. Biochem.* **68**, 459-486.

Kawasaki, Y., Senda, T., Ishidate, T., Koyama, R., Morishita, T., Iwayama, Y., Higuchi, O. and Akiyama, T. (2000). Asef, a link between the tumor suppressor APC and G-protein signaling. *Science* **289**, 1194-1197.

Kay, A. J. and Hunter, C. P. (2001). CDC-42 regulates PAR protein localization and function to control cellular and embryonic polarity in *C. elegans*. *Curr. Biol*. **11**, 474-481.

Kodama, A., Takaishi, K., Nakano, K., Nishioka, H. and Takai, Y. (1999). Involvement of Cdc42 small G protein in cell-cell adhesion, migration and morphology of MDCK cells. *Oncogene* **18**, 3996-4006.

Kodama, A., Karakesisoglou, I., Wong, E., Vaezi, A. and Fuchs, E. (2003). ACF7: an essential integrator of microtubule dynamics. *Cell* **115**, 343-354.

Kroschewski, R., Hall, A. and Mellman, I. (1999). Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nat. Cell Biol*. **1**, 8-13.

Kuroda, S., Fukata, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A. and Kaibuchi, K. (1996). Identification of IQGAP as a putative target for the small GTPases, CDC42 and Rac1. *J. Biol. Chem.* **271**, 23363-23367.

Kuroda, S., Fukata, M., Fujii, K., Nakamura, T., Izawa, I. and Kaibuchi, K. (1997). Regulation of cell-cell adhesion of MDCK cells by Cdc42 and Rac1 small GTPases. *Biochem. Biophys. Res. Commun.* **240**, 430-435.

Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H. et al. (1998). Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. *Science* **281**, 832-835.

Lantz, V. A. and Miller, K. G. (1998). A class VI unconventional myosin is associated with a homologue of a microtubule-binding protein, cytoplasmic linker protein-170, in neurons and at the posterior pole of *Drosophila* embryos. *J. Cell Biol.* **140**, 897-910.

Li, S., Wang, Q., Chakladar, A., Bronson, R. T. and Bernards, A. (2000). Gastric hyperplasia in mice lacking the putative Cdc42 effector IQGAP1. *Mol. Cell. Biol.* **20**, 697-701.

Li, Z., Kim, S. H., Higgins, J. M., Brenner, M. B. and Sacks, D. B. (1999). IQGAP1 and calmodulin modulate E-cadherin function. *J. Biol. Chem.* **274**, 37885-37892.

Luo, L. (2000). Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.* **1**, 173-180.

Mackay, D. J. and Hall, A. (1998). Rho GTPases. *J. Biol Chem.* **273**, 20685-20688.

Mataraza, J. M., Briggs, M. W., Li, Z., Frank, R. and Sacks, D. B. (2003a). Identification and characterization of the Cdc42-binding site of IQGAP1. *Biochem. Biophys. Res. Commun.* **305**, 315-321.

Mataraza, J. M., Briggs, M. W., Li, Z., Entwistle, A., Ridley, A. J. and Sacks, D. B. (2003b). IQGAP1 promotes cell motility and invasion. *J. Biol. Chem.* **278**, 41237-41245.

Matsumoto, Y., Oshida, T., Obayashi, I., Imai, Y., Matsui, K., Yoshida, N. L., Nagata, N., Ogawa, K., Obayashi, M., Kashiwabara, T. et al. (2002). Identification of highly expressed genes in peripheral blood T cells from patients with atopic dermatitis. *Int. Arch. Allergy Immunol.* **129**, 327-340.

Mbele, G. O., Deloulme, J. C., Gentil, B. J., Delphin, C., Ferro, M., Garin, J., Takahashi, M. and Baudier, J. (2002). The zinc- and calcium-binding S100B interacts and co-localizes with IQGAP1 during dynamic rearrangement of cell membranes. *J. Biol. Chem.* **277**, 49998-50007.

Mimori-Kiyosue, Y., Shiina, N. and Tsukita, S. (2000a). The dynamic behavior of the APC-binding protein EB1 on the distal ends of microtubules. *Curr. Biol.* **10**, 865-868.

Mimori-Kiyosue, Y., Shiina, N. and Tsukita, S. (2000b). Adenomatous polyposis coli (APC) protein moves along microtubules and concentrates at their growing ends in epithelial cells. *J. Cell Biol.* **148**, 505-518.

Nagafuchi, A., Ishihara, S. and Tsukita, S. (1994). The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-α-catenin fusion molecules. *J. Cell Biol.* **127**, 235-245.

Näthke, I. (2004). APC at a glance. *J. Cell Sci.* **117**, 4873-4875.

Nelson, W. J. (2003). Adaptation of core mechanisms to generate cell polarity. *Nature* **422**, 766-774.

Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y. I., Ohno, S., Hoshino, M., Kaibuchi, K. (2005). PAR-6–PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. *Nat. Cell Biol.* **7**, 270-277.

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

Noritake, J., Fukata, M., Sato, K., Nakagawa, M., Watanabe, T., Izumi, N., Wang, S., Fukata, Y. and Kaibuchi, K. (2004). Positive role of IQGAP1, an effector of Rac1, in actin-meshwork formation at sites of cell-cell contact. *Mol. Biol. Cell* **15**, 1065-1076.

Palazzo, A. F., Joseph, H. L., Chen, Y., Dujardin, D. L., Alberts, A. S., Pfister, K. K., Vallee, R. B. and Gundersen, G. G. (2001). Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr. Biol.* **11**, 1536-1541.

Perez, F., Diamantopoulos, G. S., Stalder, R. and Kreis, T. E. (1999). CLIP-170 highlights growing microtubule ends in vivo. *Cell* **96**, 517-527.

Pierre, P., Scheel, J., Rickard, J. E., Kreis, T. E. (1992). CLIP-170 links endocytic vesicles to microtubules. *Cell* **70**, 887-900.

Presslauer, S., Hinterhuber, G., Cauza, K., Horvat, R., Rappersberger, K., Wolff, K. and Foedinger, D. (2003). RasGAP-like protein IQGAP1 is expressed by human keratinocytes and recognized by autoantibodies in association with bullous skin disease. *J. Invest. Dermatol.* **120**, 365-371.

Raftopoulou, M. and Hall, A. (2004). Cell migration: Rho GTPases lead the way. *Dev. Biol.* **265**, 23-32.

Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T. and Horwitz, A. R. (2003). Cell migration: integrating signals from front to back. *Science* **302**, 1704-1709.

Roy, M., Li, Z. and Sacks, D. B. (2004). IQGAP1 binds ERK2 and modulates its activity. *J. Biol. Chem*. **279**, 17329-17337.

Schuyler. S. C. and Pellman, D. (2001). Microtubule 'plus-end-tracking proteins': the end is just the beginning. *Cell* **105**, 421-424.

Schwamborn, J. C. and Püschel, A. W. (2004). The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. *Nat. Neurosci*. **7**, 923-929.

Schwartz, M. (2004). Rho signalling at a glance. *J. Cell Sci*. **117**, 5457-5458.

Steinberg, M. S. and McNutt, P. M. (1999). Cadherins and their connections: adhesion junctions have broader functions. *Curr. Opin. Cell Biol.* **11**, 554-560.

Stowers, L., Yelon, D., Berg, L. J. and Chant, J. (1995). Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. *Proc. Natl. Acad. Sci. USA* **92**, 5027-5031.

Sugimoto, N., Imoto, I., Fukuda, Y., Kurihara, N., Kuroda, S., Tanigami, A., Kaibuchi, K., Kamiyama, R. and Inazawa, J. (2001). IQGAP1, a negative regulator of cell-cell adhesion, is upregulated by gene amplification

at 15q26 in gastric cancer cell lines HSC39 and 40A. *J. Hum. Genet.* **46**, 21-25.

Swart-Mataraza, J. M., Li, Z. and Sacks, D. B. (2002). IQGAP1 is a component of CDC42 signaling to the cytoskeleton. *J. Biol. Chem.* **277**, 24753-24763.

Takaishi, K., Sasaki, T., Kotani, H., Nishioka, H. and Takai, Y. (1997). Regulation of cell-cell adhesion by Rac and Rho small G proteins in MDCK cells. *J. Cell Biol.* **139**, 1047-1059.

Takeichi, M. (1995). Morphogenetic roles of classic cadherins. *Curr. Opin. Cell Biol.* **7**, 619-627.

Takemoto, H., Doki, Y., Shiozaki, H., Imamura, H., Utsunomiya, T., Miyata, H., Yano, M., Inoue, M., Fujiwara, Y. and Monden, M. (2001). Localization of IQGAP1 is inversely correlated with intercellular adhesion mediated by E-cadherin in gastric cancers. *Int. J. Cancer* **91**, 783-788.

Tepass, U., Truong, K., Godt, D., Ikura, M. and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. *Nat. Rev. Mol. Cell. Biol.* **1**, 91-100.

Tsukita, S., Tsukita, S., Nagafuchi, A. and Yonemura, S. (1992). Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. *Curr. Opin. Cell Biol.* **4**, 834-839.

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

Vaughan, K. T., Tynan, S. H., Faulkner, N. E., Echeverri, C. J. and Vallee, R. B. (1999). Colocalization of cytoplasmic dynein with dynactin and CLIP-170 at microtubule distal ends. *J. Cell Sci*. **112**, 1437-1447.

Watanabe, T., Wang, S., Noritake, J., Sato, K., Fukata, M., Takefuji, M., Nakagawa, M., Izumi, N., Akiyama, T. and Kaibuchi, K. (2004). Interaction with IQGAP1 links APC to Rac1, Cdc42 and actin filaments during cell polarization and migration. *Dev. Cell.* **7**, 871-883.

Weissbach, L., Bernards, A. and Herion, D. W. (1998). Binding of myosin essential light chain to the cytoskeleton-associated protein IQGAP1. *Biochem. Biophys. Res. Commun.* **251**, 269-276.

Yagi, T. and Takeichi, M. (2000). Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes Dev.* **14**, 1169-1180.

Yamaoka-Tojo, M., Ushio-Fukai, M., Hilenski, L., Dikalov, S. I., Chen, Y. E., Tojo, T., Fukai, T., Fujimoto, M., Patrushev, N. A., Wang, N. et al. (2004). IQGAP1, a novel vascular endothelial growth factor receptor binding protein, is involved in reactive oxygen species-dependent endothelial migration and proliferation. *Circ. Res.* **95**, 276-283.