INTRODUCTION

Dynamic rearrangement of cell adhesion contacts enables cells to perform a variety of functions, for example: induction of morphological changes during early development, cell migration, establishment of epithelial-cell polarity and wound healing. It also allows tumors to metastasise (Takeichi, 1995; Gumbiner, 1996; Adams and Nelson, 1998). Cell adhesion can be classified into two types: (1) cell-substratum adhesion mediated by molecules such as integrins and (2) cell-cell adhesion mediated by molecules such as cadherins.

Cell-substratum adhesion

Cell-substratum adhesion is mediated by adhesion molecules such as integrins, which are receptors for the extracellular matrix (ECM) components (e.g. fibronectin). Integrins are heterodimers composed of two transmembrane glycoprotein subunits, α and β. Frequently, they are clustered to form specialized adhesive structures, focal adhesions and focal complexes, in which many signaling molecules are concentrated (Clark and Brugge, 1995). Focal adhesions are large adhesion contacts found at the ends of stress fibers. Focal complexes are smaller adhesion contacts found at the tips of filopodia or lamellipodia. The clustering of integrins into focal adhesions and focal complexes enables cells to adhere to ECM components, such as fibronectin. The cytoplasmic domains of integrins directly or indirectly bind to talin, α-actinin, vinculin, paxillin and p125 focal-adhesion kinase (FAK), which link integrins to the actin cytoskeleton. This link between integrins and the actin cytoskeleton is essential for strong adhesion and integrin clustering (Clark and Brugge, 1995; Hughes and Pfaff, 1998). Cell-substratum adhesion contacts are dynamically rearranged during various cellular processes, such as chemoattractant-activated leukocyte adhesion (Laudanna et al., 1996), and cell migration (Gumbiner, 1996). However, little was known about the regulatory mechanism underlying the initiation, maturation and turnover of focal adhesions and focal complexes until recently.

Cell-cell adhesion

Adhesion molecules such as cadherins mediate cell-cell adhesion through homophilic interactions (Takeichi, 1995; Gumbiner, 1996; Adams and Nelson, 1998). The cytoplasmic domain of E-cadherin directly interacts with β-catenin or plakoglobin (also known as γ-catenin) (Ozawa et al., 1989; Tsukita et al., 1992), which in turn associates with α-catenin. The latter appears to link the cadherin-β-catenin-α-catenin complex (cadherin-catenins complex) to the actin cytoskeleton directly, or indirectly through other cytoskeletal proteins such as α-actinin and vinculin. This link between cadherin and the actin cytoskeleton (via catenins) is essential for strong and rigid adhesion (Tsukita et al., 1992). Indeed, α-catenin-deficient mouse teratocarcinoma F9 cells display a scattered phenotype under the conditions in which parental or α-catenin-reexpressing cells form compact colonies (Maeno et al., 1999). Loss of α-catenin expression has also been observed in lung
carcinomas (Watabe et al., 1994) and gastric carcinomas (Ochiai et al., 1994) that show scattered cell growth.

Cell-cell adhesion seems to be a static process, but a dynamic rearrangement of cell-cell adhesion contacts accompanies various cellular processes, such as epithelial-cell scattering, dispersal of cancer cells and early embryonic cell migration (Adams and Nelson, 1998). Indeed, certain epithelial cell lines rapidly migrate even when confluent. This indicates that cell-cell adhesion is transiently perturbed in migrating areas (M. Fukata, M. Nakagawa, S. Kuroda and K. Kaibuchi, unpublished data). Recently, studies using optical tweezers and single-particle tracking have indicated that approximately 50% of the E-cadherin in the plasma membrane in epithelial cells is connected to the actin cytoskeleton, probably by α-catenin, but that the rest appears to be unattached (Sako et al., 1998). These observations suggest that the cell-cell adhesion contacts are constantly rearranged through remodeling of cadherin-catenin complexes. However, the mechanism underlying this dynamic rearrangement remains to be clarified.

**Rho family small GTPases**

Rho family GTPases cycle between GDP-bound inactive and GTP-bound active forms, formation of the latter being stimulated by extracellular signals, such as growth factors and hormones (Bourne et al., 1991; Takai et al., 1995). Members of this family, which includes Cdc42, Rac1 and Rho, are involved in regulation of the cytoskeleton and cell adhesion (Van Aelst and D’Souza-Schorey, 1997; Hall, 1998; Kaibuchi et al., 1999). Cdc42 participates in the formation of filopodia and focal complexes (Kozma et al., 1995; Nobes and Hall, 1995) and cell-cell adhesion (Kuroda et al., 1997; Kodama et al., 1999). Rac1 is involved in the formation of membrane ruffling and focal complexes (Ridley et al., 1992), cell motility (Ridley et al., 1995) and cell-cell adhesion (Braga et al., 1997; Takaishi et al., 1997; Kuroda et al., 1997). Rho has been implicated in the formation of actin stress fibers and focal adhesions (Ridley and Hall, 1992), cell-cell adhesion (Braga et al., 1997; Takaishi et al., 1997), cell motility (Takaishi et al., 1994), membrane ruffling (Nishiyama et al., 1994), smooth muscle contraction (Hirata et al., 1992; Gong et al., 1996), neurite retraction in neuronal cells (Nishiki et al., 1990; Jalink et al., 1994) and cytokinesis (Kishi et al., 1993; Mabuchi et al., 1993).

The molecular mechanisms underlying the above processes are largely unknown. However, some of the pathways that connect Rho family GTPases to control of the cytoskeleton and cell adhesion have been established through identification and characterization of specific effectors of these GTPases. Researchers have identified many effectors of the Rho family GTPases, using affinity chromatography, ligand-overlay assays or the yeast two-hybrid system (Van Aelst and D’Souza-Schorey, 1997; Kaibuchi et al., 1999). Rho effectors include Rho-kinase (MRCK). Some of these proteins, such as PAK and IQGAP1, interact with both Rac1 and Cdc42 (Van Aelst and D’Souza-Schorey, 1997; Kaibuchi et al., 1999). Here, we focus on how the Rho family GTPases regulate cell-substratum and cell-cell adhesion through their effectors. Regulation of the cytoskeleton by these GTPases has been reviewed elsewhere (Van Aelst and D’Souza-Schorey, 1997; Hall, 1998; Kaibuchi et al., 1999).

**REGULATION OF CELL-SUBSTRATUM ADHESION BY RHO GTPASES**

Several observations suggest that Rho regulates the formation of focal adhesions associated with actin stress fibers. In serum-starved Swiss 3T3 cells, few focal adhesions and actin stress fibers are observed. However, when these cells are stimulated by lysophosphatidic acid (LPA), new stress fibers and new focal adhesions at the ends of stress fibers appear. Prior treatment of the cells with C. botulinum C3 toxin, an inhibitor of Rho, inhibits the LPA-induced appearance of focal adhesions and stress fibers. Microinjection of dominant active RhoA (RhoA\(^{V14}\)) into the cells induces the formation of focal adhesions and stress fibers (Paterson et al., 1990; Ridley and Hall, 1992).

Cdc42 and Rac1 regulate the formation of focal complexes. In serum-starved Swiss 3T3 cells, bradykinin and platelet-derived growth factor (PDGF) promote the formation of focal complexes associated with filopodia and lamellipodia, respectively. Dominant negative Cdc42 (Cdc42\(^{N17}\)), which preferentially binds GDP rather than GTP, and thereby inhibits the activation of endogenous Cdc42 by titrating out its GEF, and dominant negative Rac1 (Rac1\(^{N17}\)), inhibit bradykinin-induced formation of focal complexes and PDGF-induced formation of focal complexes, respectively. Dominant active Cdc42 (Cdc42\(^{V12}\)) and dominant active Rac1 (Rac1\(^{V12}\)) induce formation of focal complexes associated with filopodia (Kozma et al., 1995; Nobes and Hall, 1995) and lamellipodia (Ridley et al., 1992), respectively.

Analyses using dominant active and dominant negative mutants of small GTPases suggest that activation of Cdc42 leads to activation of Rac1, which in turn activates Rho (Ridley et al., 1992; Nobes and Hall, 1995). Recent experiments, however, show that Rac1/Cdc42 and Rho can be antagonistic in some situations, such as during neurite extension in neuronal cells (Kozma et al., 1997; Leeuwen et al., 1997; Hirose et al., 1999; Amano et al., 1998). In N1E-115 neuroblastoma cells, for example, Rho is implicated in serum- and LPA-induced neurite retraction and cell rounding, whereas Cdc42 and Rac1 are implicated in neurite extension. Downregulation of Rho or Rho-kinase induces neurite extension (Kozma et al., 1997; Hirose et al., 1999; Amano et al., 1998), whereas upregulation of Rac1 inhibits LPA-induced neurite retraction (Leeuwen et al., 1997). In addition, a more recent study suggests that the antagonistic relationship between Rac1/Cdc42 and Rho applies to the regulation of focal complexes and focal adhesions. The expression of Cdc42\(^{V12}\) or Rac1\(^{V12}\) causes loss of focal adhesions and stress fibers (Manser et al., 1997). The expression of Rac1\(^{N17}\) leads to a prominent increase in the size of focal adhesions. The expression of dominant active Rho (RhoA\(^{L63}\)) causes the transformation of pre-existing focal complexes, which have been induced by dominant active Rac1 (Rac1\(^{L61}\)), into focal adhesions. Treatment of the cells with Y27632, an inhibitor of Rho-kinase, causes a shift in the contact pattern from focal adhesions to peripheral focal complexes, which is accompanied by the induction of membrane ruffling (Rottner et
These observations suggest that Rac1/Cdc42 and Rho exert mutually antagonistic effects at some point in the process of contact initiation, maturation and turnover, possibly through their effectors, PAK and Rho-kinase (see below).

Rnd proteins (Rnd1, Rnd2 and Rnd3/RhoE), a new subfamily of Rho-related proteins, also appear to regulate integrin-mediated cell adhesion (Nobes et al., 1998; Guasch et al., 1998). Rnd1 has a much higher affinity for GTP than for GDP and lacks intrinsic GTPase activity, which suggests that it exists as the GTP-bound form in vivo. Prior injection of wild-type Rnd1 inhibits LPA-induced formation of stress fibers and focal adhesions, leading to cell rounding. Note that Rnd1 might also regulate cadherin-mediated cell adhesion: like Cdc42 and Rac1 (Kuroda et al., 1997), it colocalizes with E-cadherin at sites of cell-cell contact through its unique N-terminal region (Nobes et al., 1998). Further studies must address how Rnd proteins are regulated and how they exert their functions.

**The mechanism of regulation of cell-substratum adhesion by Rho GTPases**

The molecular mechanisms underlying Rho-induced formation of focal adhesions have been elucidated through the identification and characterization of two of its effectors: Rho-kinase and MBS. The expression of dominant active Rho-kinase induces the formation of stress fibers and focal adhesions in Swiss 3T3 and Madin-Darby canine kidney (MDCK) cells, whereas the expression of dominant negative Rho-kinase inhibits LPA- or RhoAV14-induced formation of stress fibers and focal adhesions (Leung et al., 1996; Amano et al., 1997; Ishizaki et al., 1997).

Rho-kinase regulates the phosphorylation of the myosin light chain (MLC) of myosin II by directly phosphorylating MLC at Ser19 (Amano et al., 1996) and by inactivating myosin phosphatase through phosphorylation of MBS (Kimura et al., 1996). MLC phosphorylation plays a pivotal role in the actin-myosin interaction for stress fibers in non-muscle cells (Huttenlocher et al., 1995) and in smooth-muscle contraction (Kamm et al., 1985). The expression of the MLC mutant MLCT18D,S19D, which mimics the phosphorylated form of MLC, inhibits RhoAV14-induced formation of stress fibers and focal adhesions (Leung et al., 1996; Amano et al., 1997; Ishizaki et al., 1997).

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**Upstream regulation of Rho GTPases in cell-substratum adhesion**

Recently, two groups reported that integrin-mediated cell-substratum adhesion, like stimulation by growth factors, induces the activation of the Rho family GTPases (Price et al., 1998; Ren et al., 1999). The engagement of integrins with fibronectin activates Cdc42, which subsequently activates Rac1 during cell spreading 5 minutes after the attachment of cells to the substratum (Price et al., 1998). Moreover, activation of Rho can occur later independently of the activation of Cdc42 (Price et al., 1998).

How does integrin-mediated cell adhesion activate Rho family GTPases? Regarding Rac1, one potential candidate is Vav, which is a GEF for Rac1. The GEF activity of Vav toward Rac1 is enhanced by tyrosine phosphorylation (Crespo et al., 1997; Han et al., 1997). Yron et al. (1999) have presented evidence to suggest that integrin-mediated cell adhesion induces tyrosine phosphorylation of Vav. Thus, integrin-
mediated cell adhesion probably activates Rac1 via phosphorylation of Vav. Further work will be required if we are to elucidate how integrin-mediated cell adhesion activates the Rho family GTPases.

REGULATION OF CELL-CELL ADHESION BY RHO GTPASES

Recently, several investigations have suggested that the Rho family GTPases are required for cadherin-mediated cell-cell adhesion. Microinjection of Rac1N17 or C3 inhibits the accumulation of cadherin at sites of cell-cell contact when keratinocytes are transferred from low-calcium medium to standard medium (which restores calcium-dependent cell-cell adhesion) (Braga et al., 1997). The effects of Rac1 and Rho on the localization of cadherin probably depend on the maturation status of the junction (i.e. on the time after induction of contacts and on confluence) and the cell types (Braga et al., 1999); for example, Rac1 and Rho are required for localization of E-cadherin to sites of cell-cell contact in keratinocytes, whereas they are not required for the VE-cadherin localization in human umbilical cord endothelial cells.

Takaishi et al. (1997) have shown that, in MDCK cells stably expressing Rac1V12, the immunofluorescent intensities of E-cadherin, β-catenin and actin filaments at sites of cell-cell contact are increased relative to those in the parental cells. By contrast, in cells expressing Rac1N17, the immunofluorescent intensities of E-cadherin, β-catenin and actin filaments are reduced (Takaishi et al., 1997). Microinjection of C3 inhibits cadherin-mediated cell-cell adhesion in wild-type MDCK cells, but not in MDCK cells expressing Rac1V12. This suggests that Rho activity is required for cadherin-mediated cell-cell adhesion but not for a Rac1-regulated cadherin-mediated cell-cell adhesion pathway (Takaishi et al., 1997).

In addition, we have shown that Cdc42, Rac1 and Rho are required for E-cadherin-mediated cell-cell adhesion in MDCK cells (Kuroda et al., 1997). Microinjection of Rho GDI, a negative regulator of Cdc42, Rac1 and Rho, results in perturbation of cell-cell adhesion in MDCK cells. Co-microinjection of either Cdc42V12 or Rac1V12, but not RhoAV14, with Rho GDI reverses the inhibitory effect (Kuroda et al., 1997). In addition, Tiam1, which colocalizes with E-cadherin at sites of cell-cell contact and has GEF activity for Rac1, is implicated in the regulation of cell-cell adhesion in MDCK cells (Hordijk et al., 1997). Expression of Tiam1 or Rac1V12 inhibits scatter factor/hepatocyte growth factor (HGF)-induced cell scattering of MDCK cells, probably by increasing E-cadherin activity (Hordijk et al., 1997). Thus, Cdc42, Rac1 and Rho, as well as Tiam1, appear to regulate cadherin-mediated cell-cell adhesion.

The above experiments did not indicate whether the Rho family GTPases regulate E-cadherin-mediated cell-cell adhesion by acting directly on the cadherin-catenins complex or by acting indirectly on the actin cytoskeleton or other cytoskeleton components. This issue has been addressed by use of the cell dissociation assay (a quantitative assay for E-cadherin activity) in three stable cell lines derived from mouse L fibroblasts (which do not express endogenous cadherins): (1) EL cells that express E-cadherin and adhere to each other in an E-cadherin-dependent manner (Nagafuchi et al., 1994); (2) nEaCL cells expressing an E-cadherin mutant in which the cytoplasmic domain is deleted and replaced by the C-terminal domain of α-catenin, and the cadherin-catenins complex is not remodelled (Nagafuchi et al., 1994); (3) LAP86 cells that express neuropilin-1, which is a calcium-independent cell-adhesion molecule (Kawakami et al., 1996), and require neither an actin-based cytoskeleton (which EL and nEaCL cells require) nor catenins for adhesion (Fukata, M. et al., 1999).

Expression of Cdc42N17 or Rac1N17 in EL cells markedly reduces E-cadherin activity, whereas expression of dominant negative RhoA (RhoAN19) slightly reduces it (Fukata, M. et al., 1999). In contrast, expression of Cdc42N17, Rac1N17 or RhoAN19 in nEaCL cells slightly reduces the mutant E-

**Fig. 1.** Antagonistic effect of Rho and Rac1/Cdc42 on the formation of focal adhesions. Rho, through Rho-kinase, plays a critical role in the formation of focal adhesions and stress fibers by activating myosin II, whereas Rac1/Cdc42, through PAK, disassembles focal adhesions and stress fibers. α, integrin α; β, integrin β.
Cadherin activity. Furthermore, expression of Cdc42\textsuperscript{N17}, Rac1\textsuperscript{N17} or RhoA\textsuperscript{N19} in LAP86 cells does not affect adhesion. Since \( \beta \)-catenin, the E-cadherin cytoplasmic domain and the N-terminal region of \( \alpha \)-catenin are not required for the adhesion in nEaCL cells, and the effect of Cdc42\textsuperscript{N17} or Rac1\textsuperscript{N17} in nEaCL cells is less than that in EL cells, Cdc42 and Rac1 appear to regulate E-cadherin-mediated cell adhesion, acting directly on the cadherin-catenins complex. Since RhoA\textsuperscript{N19} has similar effects on E-cadherin activity in EL cells and nEaCL cells (whose adhesion requires the actin cytoskeleton) and no effect in LAP86 cells (whose adhesion does not require the actin cytoskeleton), Rho presumably regulates E-cadherin activity indirectly by acting on the actin cytoskeleton (Fukata, M. et al., 1999).

**The mechanism of regulation of cell-cell adhesion by Rho GTPases**

We have recently proposed a mechanism by which Cdc42 and Rac1 might regulate E-cadherin activity (Kuroda et al., 1998, 1999). We and others have shown that an effector of Cdc42 and Rac1, IQGAP1, colocalizes with cadherin-catenins at sites of cell-cell contact (Hart et al., 1996; Kuroda et al., 1996, 1998). IQGAP1 might therefore regulate E-cadherin-mediated cell-cell adhesion. IQGAP1 accumulates at sites of cell-cell contact.
in EL cells but not in nEtαCL cells, which indicates that this accumulation depends on the presence of the cytoplasmic domain of E-cadherin and β-catenin (Kuroda et al., 1998). IQGAP1 directly interacts with β-catenin and the cytoplasmic domain of E-cadherin both in vitro and in vivo, but not with α-catenin (Kuroda et al., 1998). Since the binding affinity of IQGAP1 for β-catenin is higher than that of E-cadherin in vitro (half-maximal binding is at 20 nM and 400 nM, respectively) and in vivo (Kuroda et al., 1998), it is likely that IQGAP1 functions mainly through β-catenin. IQGAP1 interacts with the N-terminal region of β-catenin (residues 1-183), which contains the α-catenin-binding domain (residues 120-151) (Fukata, M. et al., 1999).

IQGAP1 and α-catenin compete for binding to β-catenin, and IQGAP1 dissociates α-catenin from the β-catenin-α-catenin complex in vitro (Fukata, M. et al., 1999). In addition, overexpression of IQGAP1 in EL cells, but not in nEtαCL cells, results in the dissociation of α-catenin from the cadherin-catenin complex and in a decrease in the level of E-cadherin-mediated cell-cell adhesion (Kuroda et al., 1998). Thus, IQGAP1 appears to regulate E-cadherin-mediated adhesion in vivo by causing α-catenin to dissociate from the cadherin-catenin complex (Kuroda et al., 1998).

How do Cdc42 and Rac1 regulate the E-cadherin-mediated cell-cell adhesion through IQGAP1? Cdc42 and Rac1 bound to guanosine 5′-(3-O-thio)triphosphate (GTPγS), a non-hydrolyzable analog of GTP, inhibit the interaction between IQGAP1 and β-catenin in vitro, whereas their GDP-bound forms and GTPγS-RhoA do not (Fukata, M. et al., 1999). Coexpression of Cdc42V12 with IQGAP1 in EL cells inhibits the dissociation of α-catenin from the cadherin-catenin complex induced by IQGAP1 (Fukata, M. et al., 1999) and counteracts the inhibitory effect of IQGAP1 on the E-cadherin-mediated cell-cell adhesion (Kuroda et al., 1998). Activated Cdc42 must therefore suppress the inhibitory action of IQGAP1 by preventing IQGAP1 from interacting with β-catenin and thereby stabilize the cadherin-catenin complex.

These observations suggest a mechanism by which Cdc42 and Rac1, together with IQGAP1, regulate cadherin-mediated cell-cell adhesion (Fig. 2). In their GTP-bound, active forms, Cdc42 and Rac1 interact with IQGAP1, preventing it from interacting with β-catenin and thereby stabilizing the cadherin-catenin complex. This results in strong cell-cell adhesion. In contrast, in their GDP-bound, inactive forms, Cdc42 and Rac1 cannot interact with IQGAP1, and IQGAP1 interacts with β-catenin, dissociating α-catenin from the cadherin-catenin complex. This results in weak cell-cell adhesion. Such a model can explain the dynamic rearrangement of cell-cell adhesion. A mixture of E-cadherin-β-catenin-α-catenin and E-cadherin-β-catenin-IQGAP1 complexes should exist at sites of cell-cell contact. The ratio between the two complexes could determine the adhesive activity. The fact that approximately 50% of E-cadherin appears to be connected to the actin cytoskeleton, probably via α-catenin in EL cells, but that the rest appears to be free, is consistent with this (Sako et al., 1998).

Most GTPases known thus far exert their functions by binding to effectors and changing the activities of effectors. For example, GTP-bound Gsα binds to adenylyl cyclase, causing its activation, and GTP-bound Ras binds to Raf, causing its activation. The interaction between GTP-bound Cdc42/Rac1 and IQGAP1, which inhibits IQGAP1 function by preventing it from interacting with β-catenin, represents a different case. This sequestration of IQGAP1 by Cdc42 and Rac1 might be a novel mechanism for GTPase action. Gsα acts similarly on adenylyl cyclase (type V), which is inhibited by GTP-bound Gsα but not by GDP-bound Gsα, through direct interaction (Tausig et al., 1994; Dessauer et al., 1998; Wittbrodt et al., 1999).

Analyses by electron microscope have recently revealed that the morphology of the cell-cell adhesion site in MDCK cells stably expressing RacV12 is different from that of those expressing Cdc42V12. The lateral membranes in the former intermingle more markedly than those in cells expressing Cdc42V12 (Takaishi et al., 1997; Kodama et al., 1999), which suggests that IQGAP1, downstream of Cdc42 and Rac1, regulates cadherin activity cooperatively with Cdc42- or Rac1-specific effectors, such as MRCK.

MRCK is another effector of Cdc42 and localizes to sites of cell-cell contact when coexpressed with Cdc42V12 in HeLa cells (Leung et al., 1998). It can phosphorylate MLC (Leung et al., 1998), which suggests that MRCK regulates actomyosin-based contractility at sites of cell-cell contact by such a mechanism. Cadherin activity could therefore be dually regulated through direct regulation of the cadherin-catenin complex by IQGAP1 and indirect regulation of actin filaments by MRCK downstream of Cdc42 and Rac1.

**Upstream regulation of Rho GTPases in cell-cell adhesion**

The above model and analogies with integrin-mediated cell adhesion lead us to speculate that E-cadherin-mediated cell-cell adhesion induces activation of the Rho family GTPases. Very recent observations support this idea. The engagement of E-cadherins in homophilic calcium-dependent cell-cell interactions results in a rapid phosphoinositide 3-kinase (PI 3-kinase)-dependent activation of Akt/PKB in MDCK cells (Pece et al., 1999). Activated PI 3-kinase activates Rac1 (Kotani et al., 1994, 1995; Hawkins et al., 1995; Reif et al., 1996). Therefore, it may prove worthwhile to examine whether cell-cell contact induces the activation of the Rho family GTPases. Indeed, by measuring the level of GTP-bound Rac1, we have already found that, during cadherin-mediated cell adhesion, the amount of activated Rac1 increases (M. Nakagawa, M. Fukata, S. Kuroda and K. Kaibuchi, unpublished data).

**The role of tyrosine phosphorylation**

Over the past ten years, a considerable number of studies on the regulation of cadherin function have been performed. The mechanisms underlying regulation, however, are not fully understood. Among them, tyrosine phosphorylation of β-catenin is the best-studied mechanism. Increased levels of tyrosine phosphorylation of β-catenin frequently characterize dysfunctional cadherin-mediated cell-cell adhesion induced by v-Src (Matsuyoshi et al., 1992; Behrens et al., 1993) or by treatment with growth factors such as epidermal growth factor (EGF) or HGF (Shibamoto et al., 1994). Treatment of cells with pervanadate, a potent inhibitor of tyrosine phosphatases, also induces perturbation of cadherin-mediated cell-cell adhesion, which is accompanied by tyrosine phosphorylation of β-catenin and dissociation of α-catenin from the cadherin-catenin complex (Ozawa and Kemler, 1998a). Therefore, the tyrosine phosphorylation of β-catenin probably plays a crucial role in regulation of cadherin function.
Given these findings, together with our model for IQGAP1-mediated regulation of cadherin, it is tempting to speculate that tyrosine-phosphorylated β-catenin recruits IQGAP1 or GTPase-activating proteins (GAPs) for Cdc42 and/or Rac1 to sites of cell-cell contact. This would cause a reduction in cadherin activity through inhibition of Cdc42 and/or Rac1 activity. Takeda et al. (1995), however, have reported that tyrosine phosphorylation of β-catenin is not required for the reduction in cadherin activity induced by v-Src, given that expression of v-Src reduces cadherin activity in nEtαC1 cells as well as in E1 cells. Thus, the physiological role of the tyrosine phosphorylation of β-catenin in cadherin function remains unresolved.

**Cadherin dimerization**

Analyses by NMR spectroscopy and X-ray crystallography suggest that the extracellular domain of cadherin forms a dimer. They show that the two monomers interact with monomers from different dimers on the opposite membrane (see Fig. 2), which leads to zipper-like adhesion (Overdun et al., 1995; Shapiro et al., 1995). Therefore, dimerization of cadherin is another crucial contributor to strong adhesion. The mechanism, however, underlying dimer formation remains to be elucidated. Recent studies suggest that the membrane-proximal region of the cadherin cytoplasmic domain, which is separate from the distal β-catenin-binding domain, regulates cadherin dimerization and activity. In the case of E-cadherin, the membrane-proximal region inhibits dimerization and adhesive activity (Ozawa and Kemler, 1998b), whereas in the case of C-cadherin, this region supports lateral clustering of C-cadherin (Yap et al., 1998). Thus, the role of the membrane-proximal region of cadherin remains to be determined.

Given that p120ctn (Ozawa and Kemler, 1998b; Yap et al., 1998) and δ-catenin (Lu et al., 1999), members of the Armadillo/β-catenin family, bind to the membrane-proximal region of the cadherin cytoplasmic domain, they might modulate E-cadherin-mediated cell-cell adhesion by regulating dimerization of cadherin. Recently, p120ctn (Yap et al., 1999; Ohkubo and Ozawa, 1999), and the expression of δ-catenin increases the cell scattering induced by HGF in MDCK cells (Lu et al., 1999). Further studies are necessary if we are to elucidate how p120ctn and δ-catenin regulate cadherin activity and which signalling pathway (e.g. tyrosine kinase or Rho family GTPases) is involved. Given that IQGAP1 binds to E-cadherin as well as β-catenin in vitro and in vivo (Kuroda et al., 1998), it might be also involved in the regulation of E-cadherin dimerization.

**Physiological processes in which the Rho family GTPases regulate cadherin activity**

Cadherin-mediated cell-cell adhesion is dynamically rearranged in various situations, such as during cell scattering, compaction of early embryogenesis, wound-induced cell migration and tumorigenesis (Takeichi, 1995; Gumbiner, 1996; Adams and Nelson, 1998). The physiological processes in which the Cdc42/Rac1/IQGAP1 system operates remain to be clarified, however.

Cell scattering provides one of the most prominent examples of dynamic rearrangement of cell-cell adhesion (Takeichi, 1995; Gumbiner, 1996). HGF or phorbol ester induces membrane ruffling and centrifugal spreading of MDCK cells in colonies and subsequently stimulates cell-cell dissociation followed by cell scattering (Hartmann et al., 1994). Dysfunction of E-cadherin-mediated cell-cell adhesion is believed to be essential for cell scattering. Rho family GTPases are implicated in the regulation of cell scattering induced by HGF or phorbol ester (Takaishi et al., 1994, 1995; Ridley et al., 1995). Microinjection of RhoAN19 or Rac1N17 inhibits HGF-induced membrane ruffling and subsequent cell scattering of MDCK cells (Fukata, Y. et al., 1999). Moreover, microinjection of Cdc42V12 or Rac1V12 into MDCK cells blocks the disruption of cell-cell adhesion during the HGF- and phorbol ester-induced cell scattering (Kodama et al., 1999; M. Fukata, M. Nakagawa, S. Kuroda and K. Kaibuchi, unpublished data). Thus, activation of Rho and Rac1 is probably necessary for membrane ruffling, and the inactivation of Cdc42 and Rac1 appears to be essential for disruption of cell-cell adhesion during the cell scattering (Fig. 3). In addition, α-catenin-deficient mouse teratocarcinoma F9 cells display the scattered phenotype, under the conditions in which parental or α-catenin-reexpressing cells form compact colonies (Maeno et al., 1999). Taken together, these results suggest that we should examine whether IQGAP1 plays a pivotal role in the suppression of cadherin activity during the cell scattering.

Dynamic rearrangement in E-cadherin-mediated cell-cell adhesion underlies the compaction of the eight-cell embryo, in which the embryo develops from a collection of loosely adherent blastomeres into a tightly packed epithelium called a blastocyst (Fleming and Johnson, 1988). Gastrulation provides another example of dynamic rearrangement of the cadherin-catenins complex. In sea urchin embryos, cadherin is localized to sites of cell-cell contact throughout gastrulation, whereas α-catenin staining at the sites decreases markedly (Miller and McClay, 1997a,b). Further studies must determine whether the Cdc42/Rac1/IQGAP1 system is involved in the regulation of cell-cell adhesion during compaction and gastrulation.

**CONCLUSION**

Over the past several years, researchers have made considerable effort to elucidate the mechanisms by which Rho family GTPases regulate the cytoskeleton and cell adhesions. Many effectors have been identified and characterized. As a result, some of the pathways that connect these GTPases to control of the cytoskeleton and cell adhesion have been established. For example, we now know that Rho-kinase is essential for integrin-mediated cell adhesion and is involved in MLC phosphorylation, which leads to the formation of focal adhesions. In addition, Cdc42 and Rac1, together with IQGAP1, are involved in regulation of cadherin-mediated cell-cell adhesion. The next step is to elucidate the physiological phenomena taking advantage of these mechanisms.

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