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BREAKTHROUGHS AND VIEWS

Cdc42, Rac1, and Their Effector IQGAP1 as Molecular Switches for Cadherin-Mediated Cell–Cell Adhesion[1]

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Cell–cell adhesion is a dynamic process in various cellular and developmental situations. Cadherins, well-known Ca^{2+}-dependent adhesion molecules, are thought to play a major role in the regulation of cell–cell adhesion. However, the molecular mechanism underlying the rearrangement of cadherin-mediated cell–cell adhesion is largely unknown. Cdc42 and Rac1, belonging to the Rho small GTPase family, have recently been shown to be involved in the regulation of cell–cell adhesion. In addition, IQGAP1, an effector for Cdc42 and Rac1, has been shown to regulate the cadherin function through interaction with β-catenin, a molecule associated with cadherin. In this review, we will summarize the mode of action of Cdc42 and Rac1 as well as IQGAP1 as molecular switches for the cadherin function, and then discuss physiological processes in which the Cdc42/Rac1/IQGAP1 system may be involved.

I. E-CADHERIN AND ITS ASSOCIATED MOLECULES β-CATENIN AND α-CATENIN

E-cadherin, belonging to the classic cadherins, is a Ca^{2+}-dependent homophilic cell–cell adhesion molecule that is required for cell–cell adhesion and epithelial cell polarity (1–3). Strong adhesion of E-cadherin requires linkage of its cytoplasmic domain to the actin cytoskeleton (4–6). This linkage is mediated by direct interaction of β-catenin or plakoglobin/γ-catenin with the cytoplasmic domain of E-cadherin (4–6). β-Catenin and plakoglobin also interact with α-catenin. α-catenin has been thought to link the cadherin/β-catenin/α-catenin complex (cadherin/catenins complex) to the actin cytoskeleton directly or indirectly. There is evidence that the association of α-catenin with the E-cadherin/β-catenin complex is essential for the E-cadherin-mediated cell–cell adhesion (7, 8). For instance, PC9 cells, lacking the expression of α-catenin, do not form cell–cell adhesion, but the ectopic expression of α-catenin in PC9 cells restores E-cadherin-mediated cell–cell adhesion (7, 8). Thus, α-catenin is a key regulator of the cadherin/catenins complex.

Cell–cell adhesion seems to be a very static process and it is difficult to imagine that there is dynamic remodeling as long as fixed cells are used. However, it has been shown that, in confluent conditions, L cells stably expressing E-cadherin (EL cells), exhibit facilitated intercellular motility, while L cells stably expressing E-cadherin and α-catenin chimeric molecule (nEαL cells), exhibit suppressed intercellular motility, compared to parental L cells (9). Time-lapse video microscopic analysis has revealed that the cell–cell adhesion of EL cells is a dynamic process, and EL cells repeatedly attach to and detach from the adjacent EL cells, whereas the cell–cell adhesion of nEαL cells is very stable, and the cell–cell adhesion once established appears to be rarely disrupted in nEαL cells (9). In addition, dynamic rearrangement in the cell–cell adhesion can be seen in other cell lines including Madin-Darby canine kidney (MDCK) cells. MDCK cells rapidly migrate even in confluent conditions, indicating that the cell–cell adhesion process is transiently perturbed at migrating areas, enabling dynamic migration.[2] Therefore, the cell–cell adhesion is being constantly rearranged in real time, suggesting that the cadherin/catenins complex is dynamically remodeled.

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[2] A detailed analysis will be described elsewhere.

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However, the mechanism underlying the dynamic rearrangement in cell–cell adhesion remains unclear.

Recently, the dissociation of α-catenin from the cadherin/catenins complex has been detected at the cultured cell-level (10, 11). The dissociation of α-catenin occurs during the passage of normal human breast epithelial cells (10). At early passage of the cells, α-catenin is colocalized with E-cadherin and β-catenin at the cell–cell contact sites. In contrast, at late passage of the cells, α-catenin appears in the entire cytoplasm, whereas E-cadherin and β-catenin are still localized at the cell–cell contact sites. Immunoprecipitation analysis has also revealed that α-catenin dissociates from the cadherin/catenins complex at late passage. In addition, treatment of leukemia cells expressing E-cadherin with pervanadate, a potent tyrosine phosphatase inhibitor, results in the reduction of E-cadherin activity, and in the dissociation of α-catenin from the cadherin/catenins complex (11). The dissociation of α-catenin from the complex correlates with the augmentation of the phosphorylation of E-cadherin, β-catenin, and plakoglobin. The adhesive activity of the E-cadherin and α-catenin chimeric molecule is not inactivated following the pervanadate treatment, suggesting that pervanadate acts on the cadherin/catenins complex and dissociates α-catenin from the complex, leading to the inactivation of E-cadherin activity. These studies suggest that the dissociation of α-catenin from the cadherin/catenins complex affects the process of cell–cell adhesion. However, the molecular mechanism underlying the remodeling of the cadherin/catenins complex remains to be clarified.

II. Cdc42, Rac1, AND THEIR EFFECTOR IQGAP1

Cdc42 and Rac1, belonging to the Rho small GTPase family, have been shown to be involved in various cellular processes, such as actin cytoskeletal reorganization, cell polarity, and cell growth. Comprehensive reviews are described elsewhere (12, 13). In addition to these functions, the Rho small GTPase family has recently been shown to participate in the regulation of cell–cell adhesion (14–19).

Cdc42 and Rac1 have two interconvertible forms; GDP-bound inactive and GTP-bound active forms, the latter of which interact with their effectors to exert biological functions (20, 21). The conversion from the GDP-bound to GTP-bound form is regulated positively and negatively by GDP/GTP exchange factor (GEF) and GDP-dissociation inhibitor (GDI), respectively, as described (21). The conversion from the GTP-bound to GDP-bound form is catalyzed by GTPase-activating protein (GAP) (21).

The mode of action of Cdc42 and Rac1 has well studied, and many effector molecules for these small GTPases have been identified (12, 22). The effectors for Cdc42 and Rac1 have been shown to be PAK (23–25), WASP (26, 27), IQGAP1 (28–30), and IQGAP2 (31), those for Cdc42 to be ACK (32), N-WASP (33) and MRCK (34), and those for Rac1 to be Sra-1 (35), POR1 (36) and POSH (37). Among these effectors, IQGAP1 (17, 28, 30) and MRCK (34) have been shown to be localized at cell–cell contact sites. For example, in MDCK cells, IQGAP1 is accumulated at cell–cell contact sites where E-cadherin, β-catenin, and α-catenin are localized (Fig. 1). Also, we have previously shown that IQGAP1 is accumulated at cell–cell contact sites in EL cells (9), whereas IQGAP1 is not at the sites in L cells expressing E-cadherin and a carboxyl-terminal domain of α-catenin chimeric molecule (nEαCL cells) (9), indicating that IQGAP1 is accumulated at the cell–cell contact sites in an E-cadherin/β-catenin-dependent manner (17). Thus, it is possible that IQGAP1 and MRCK, acting downstream of Cdc42 and Rac1, regulate the cell–cell adhesion.

III. THE ROLE OF Cdc42/Rac1 IN THE REGULATION OF E-CADHERIN ACTIVITY

Recent studies have revealed that the Rho small GTPases including Cdc42, Rac1, and Rho, are involved in the regulation of cadherin-mediated cell–cell adhesion (14–19). Braga's group has firstly demonstrated that blocking endogenous Rac1 or Rho by microinjection of dominant negative $Rac1^{Asn17}$, which binds preferentially GDP rather than GTP and thereby inhibits the activation of endogenous Rac1 by titrating out its GEF (38), or botulinum toxin C3 which inactivates Rho by ADP-ribosylation (39), respectively, removes cadherin from cell–cell contact sites when keratinocytes, cultured in low calcium media, are transferred to standard media to induce calcium-dependent cell–cell adhesion (14). They have additionally shown that the effects of Rac1 and Rho on localization of cadherin depend on the cell types (18); Rac1 and Rho are required for the localization of E-cadherin at cell–cell contact sites in keratinocytes, whereas they do not affect the VE-cadherin-localization in human umbilical cord endothelial cells. Rac1 is not required for the localization of E-cadherin in EL cells or Swiss 3T3 cells. It has also been reported that in MDCK cells stably expressing dominant active $Rac1^{Val12}$, which is thought to be the constitutively GTP-bound active form in the cells (38), immunofluorescent intensities of E-cadherin, β-catenin, and actin filament at cell–cell contact sites become more intense compared to the parental cells (15). Conversely, in the MDCK cells expressing $Rac1^{Asn17}$, those of E-cadherin, β-catenin and actin filament become less intense. In addition to Rac1, we have found that Cdc42 as well as Rac1 are required for the regulation of cell–cell adhesion in MDCK cells (16). Microinjection of Rho GDI, a negative regulator

for Cdc42, Rac1, and Rho, results in the perturbation of cell–cell adhesion in MDCK cells. Coinjection of either $Cdc42^{Val12}$ or $Rac1^{Val12}$ with Rho GDI reverses the inhibitory action of Rho GDI, whereas that of Rho^{Val14} with Rho GDI does not. Besides the Rho small GTPases, it has also been shown that Tiam1, one of the GEFs for Rac1, is involved in the regulation of cell–cell adhesion in MDCK cells (40). Ectopic expression of Tiam1 as well as $Rac1^{Val12}$ inhibits hepatocyte growth factor (HGF)-induced scattering of MDCK cells possibly by increasing E-cadherin-mediated cell–cell adhesion. Thus, it is likely that Cdc42, Rac1, and Rho as well as Tiam1 regulate cadherin-mediated cell–cell adhesion directly or indirectly.

However, direct evidence has yet to be presented that these small GTPases regulate cadherin activity. For our studies, we have chosen EL cells since they adhere to each other in an E-cadherin-dependent manner (9). We have recently shown that, using the dissociation assay, a quantitative assay for E-cadherin activity (9), expression of $Cdc42^{Asn17}$ or $Rac1^{Asn17}$ in EL cells markedly reduces the E-cadherin activity, while that of Rho^{Asn19} slightly reduces it (19). In contrast, expression of either $Cdc42^{Asn17}$, $Rac1^{Asn17}$, or $RhoA^{Asn19}$, into nEαCL cells slightly reduces the mutant E-cadherin activity. This result strongly suggests that Cdc42 and Rac1 directly regulate the E-cadherin activity, whereas RhoA indirectly regulates it possibly through the rearrangement of actin cytoskeleton. We have also confirmed that microinjection or transfection of either $Cdc42^{Asn17}$ or $Rac1^{Asn17}$ into EL cells does not affect the localization of E-cadherin or the immunofluorescent intensity of E-cadherin at cell–cell contact sites[2], indicating that the immunofluorescent intensity of E-cadherin at the sites does not always correlate with E-cadherin activity. Thus, due to the discrepancy between cadherin activity and its immunofluorescent intensity, the activity should be quantified by an adhesion assay such as the dissociation assay.

IV. THE ROLE OF IQGAP1 IN THE REGULATION OF E-CADHERIN ACTIVITY

How do Cdc42 and Rac1 regulate E-cadherin activity? Among the effectors for Cdc42 and Rac1, IQGAP1 (17, 28, 30) and MRCK (34) have been reported to be accumulated at the cell–cell contact sites, raising the possibility that these molecules regulate the E-cadherin activity downstream of Cdc42 and Rac1. Interestingly, the localization of IQGAP1 at cell–cell contact sites is not dependent on the ability of IQGAP1 to interact with Cdc42 or Rac1[2]. We have previously found that IQGAP1 is accumulated at the cell–cell contact sites in an E-cadherin and/or β-catenin-dependent manner (17). IQGAP1 directly interacts with β-catenin and the cytoplasmic domain of E-cadherin both *in vitro* and *in vivo* (17). IQGAP1 interacts with the amino-terminus of β-catenin (1–183 amino acids) (19), which contains the α-catenin-binding domain (120–151 aa) (41). IQGAP1 indeed inhibits the binding of α-catenin to β-catenin, and dissociates α-catenin from the β-catenin/α-catenin complex *in vitro* (19). In addition, overexpression of IQGAP1 in EL cells results in the dissociation of α-catenin from the cadherin/catenins complex and in concomitant reduction of E-cadherin activity (17). The overexpression of IQGAP1 in nEαCL cells does not affect the mutant E-cadherin activity, suggesting that IQGAP1 directly regulates E-cadherin activity *in vivo* at least through the dissociation of α-catenin from the cadherin/catenins complex (17). Thus, IQGAP1 is likely to serve as a negative regulator of E-cadherin function through the dissociation of α-catenin from the cadherin/catenins complex (Fig. 2).

Then, how do Cdc42 and Rac1 regulate IQGAP1 activity? We have found that GST-Cdc42 and GST-Rac1 bound to guanosine 5′-(3-*O*-thio)triphosphate (GTPγS), a non-hydrolyzable analogue of GTP, inhibit the interaction of IQGAP1 with β-catenin, whereas their GDP-bound forms or GTPγS · GST-RhoA do not *in vitro* (19). Coexpression of $Cdc42^{Val12}$ with IQGAP1 in EL cells inhibits the dissociation of α-catenin from the cadherin/catenins complex induced by IQGAP1 (19) and concomitantly restores the E-cadherin activity (17). Thus, this result indicates that activated Cdc42 suppresses the inhibitory action of IQGAP1 by preventing IQGAP1 from interacting with β-catenin, and thereby stabilizes the cadherin/catenins complex. Thus, it is likely that Cdc42 and Rac1 serve as positive regulators of E-cadherin function (Fig. 2).

In addition to IQGAP1, MRCK has been shown to be localized at cell–cell contact sites when MRCK is coexpressed with dominant active $Cdc42^{Val12}$ (34). MRCK, which structurally resembles one of the Rho effectors, ROK (42)/ROCK (43)/Rho-kinase (44), has been shown to be capable of phosphorylating the myosin light chain (34), suggesting that MRCK regulates the actomyosin-based contractility at cell–cell contact sites by phosphorylating the myosin light chain. Therefore, it is likely that IQGAP1 and MRCK, acting downstream of Cdc42 and Rac1, regulate the cell–cell adhesion.

V. PHYSIOLOGICAL PROCESSES IN WHICH THE Cdc42/Rac1/IQGAP1 SYSTEM OPERATES

It remains to be demonstrated in which physiological processes this Cdc42/Rac1/IQGAP1 system operates, however, we examined the role of Cdc42/Rac1/IQGAP1 in the physiological processes where dynamic cell–cell rearrangement takes place. Dynamic rearrangement can be seen in various situations such as developmental patterning (1–3).

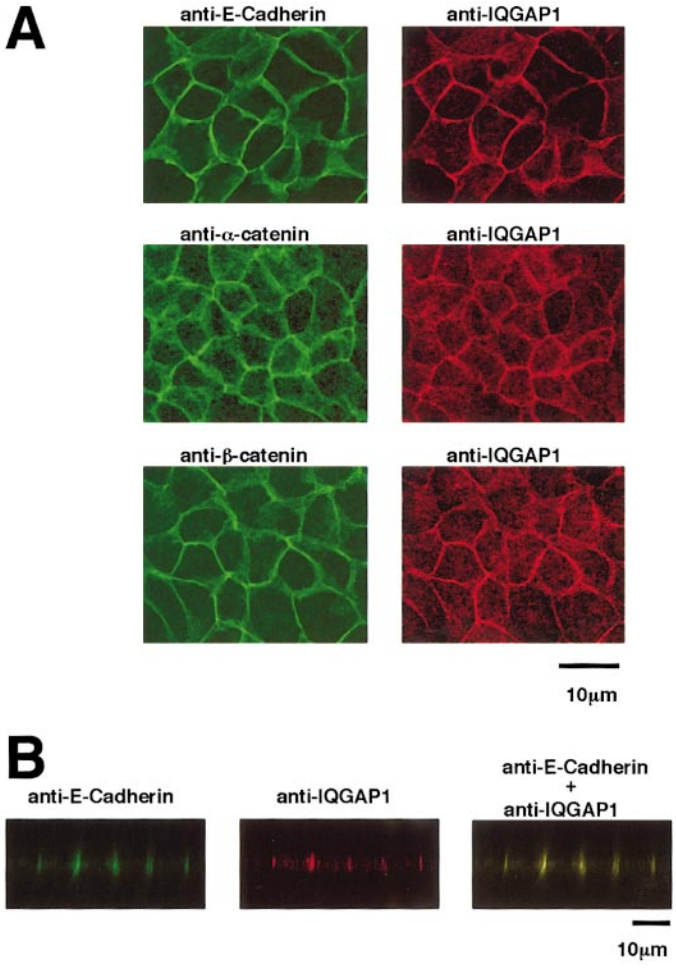

FIG. 1. Colocalization of IQGAP1 with E-cadherin, α-catenin, and β-catenin at cell–cell contact sites in MDCK cells. (A) Colocalization of IQGAP1 with E-cadherin, α-catenin, or β-catenin at cell–cell contact sites of MDCK cells. Confluent MDCK cells were fixed and doubly stained with antibodies against respective proteins and their distributions were analyzed by confocal microscopy. (B) Cross-sectional images of IQGAP1 and E-cadherin in MDCK cells. Cross-sectional images of these proteins were analyzed by confocal microscopy under the same conditions as described for A.

In mammalian early embryogenesis, it has been shown that the dynamic rearrangement in E-cadherin-mediated cell–cell adhesion underlies the compaction of the eight-cell embryo during which the embryo develops from a collection of loosely adherent blastomeres into a tightly packed epithelium called a blastcyst (45). This morphogenic process entails the rapid activation of E-cadherin at the cell surface without marked change of its expression (46–48). Although the mechanism regulating E-cadherin activity in embryos remains unknown, it is possible that remodeling of the cadherin/catenins complex is involved. Kemler's group precisely analyzed the expression of cadherin and catenins, and their assembly in mouse early embryogenesis (49). Although a certain amount of the cadherin/catenins complex is already present before the compaction, considerable amounts of free β-catenin and α-catenin also exist. As the compaction proceeds, β-catenin and α-catenin associate with E-cadherin, suggesting that the total amount of cadherin/catenins complex increases. Thus, it is tempting to speculate that Cdc42 and Rac1 are inactivated before the compaction and IQGAP1 thereby acts to inactivate the E-cadherin activity. As the compaction proceeds, Cdc42 and Rac1 are activated to some extent, resulting in the inhibition of IQGAP1 activity, and thereby formation of a stable E-cadherin/catenins complex, enabling the compaction. Very recently, it has been shown that microinjection of $Cdc42^{Val12}$ into the mouse four-cell embryo results in abnormal polarization such as nuclear displacement and polarized microfilament organization, but does not induce premature compaction (50). This suggests that activation of Cdc42 is not sufficient for the compaction, or the Cdc42/Rac1/IQGAP1 system is not involved in this process. Further study will tell whether the Cdc42/Rac1/IQGAP1 system is involved in the regulation of compaction.

Gastrulation provides one of the best examples of dynamic rearrangement of the cadherin/catenins complex. In sea urchin embryo, cadherin is localized at the cell–cell contact sites throughout gastrulation, whereas α-catenin staining at the sites decreases markedly (51, 52). In *Drosophila*, *D*E-cadherin is degenerated or disrupted in mesoderm during gastrulation. This elimination step also accompanies the dissociation of *D*α-catenin from the cadherin/catenins complex (53). In addition, in mouse *rac1* (−/−) mutant embryos, Rac1 has recently been shown to be required for cell adhesion of embryonic mesoderm during the gastrulation (54). Thus, it is likely that the Cdc42/

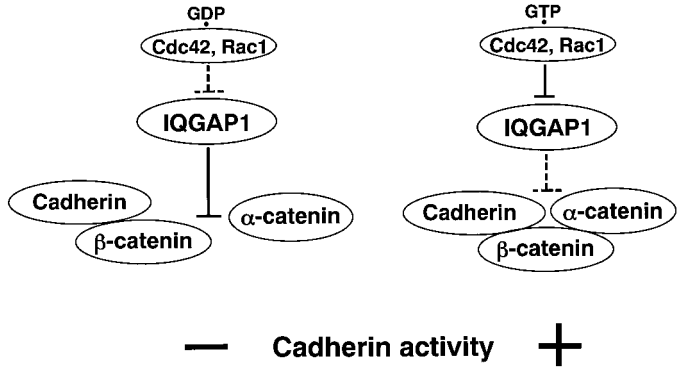

FIG. 2. Possible role of Cdc42, Rac1, and their effector IQGAP1 in the regulation of E-cadherin-mediated cell–cell adhesion. IQGAP1 dissociates α-catenin from the cadherin/catenins complex through direct interaction with β-catenin. Since the association of α-catenin with the complex is essential for E-cadherin activity, IQGAP1 is thought to act as a negative regulator of cadherin function. Activated Cdc42 and Rac1 interact with IQGAP1 and inhibit the interaction of IQGAP1 with β-catenin. This stabilizes the cadherin/catenins complex, resulting in the activation of E-cadherin activity. Therefore, Cdc42 and Rac1 are thought to act as positive regulators of cadherin function.

Rac1/IQGAP1 system is involved in the regulation of cell–cell adhesion during gastrulation.

Cell scattering provides one of the most prominent examples at the cultured cell–level of dynamic cell–cell adhesion and rearrangement (2, 3). HGF (55–58), *v*-Src (59), and phorbor ester (60) have been shown to induce scattering of epithelial cells including MDCK cells, with an increase in motility and reduction in cell–cell adhesion that results in the cells growing as a dispersed culture rather than in confluent islands. The ectopic expression of *v*-Src in MDCK cells suppresses the cadherin-mediated cell–cell adhesion with concomitant destruction of adherens junctions where cadherin/catenins complex acts as an adhesion molecule without affecting other junctions such as tight junctions and desmosomes (59). Therefore, cell scattering accompanies the dynamic rearrangement in cadherin-mediated cell–cell adhesion.

Rac1 and Rho have been implicated in the regulation of cell scattering induced by HGF (61–63) or phorbor ester (61). We have also found that microinjection of $Cdc42^{Val12}$ into MDCK cells blocks the disruption of cell–cell adhesion during the phorbor ester-induced cell scattering of MDCK cells[2]. Thus, it is likely that Cdc42 and Rac1 regulate cell–cell adhesion during the cell scattering. Although there is no evidence that IQGAP1 is involved in this process, it is intriguing to ask whether IQGAP1 plays a role in the suppression of cadherin activity possibly through the dissociation of α-catenin from the cadherin/catenins complex. We are currently trying to establish whether this is the case.

VI. CONCLUDING REMARKS

In addition to the well-known functions of Cdc42 and Rac1, such as in actin cytoskeletal control, cell polarity, and cell growth, recent evidence indicates the involvement of Cdc42 and Rac1 together with IQGAP1 in the regulation of cadherin-mediated cell–cell adhesion. Cdc42 and Rac1, and IQGAP1 appear to serve as positive regulators and a negative regulator of cadherin function, respectively. Although cadherin activity is regulated in various situations, the molecular mechanism controlling cadherin function has yet to be elucidated. Thus, Cdc42 and Rac1 as well as IQGAP1 are possible molecular linkages between the cadherin/catenins complex and various extracellular and intracellular signals. A study using Cdc42, Rac1, and IQGAP1 as molecular switches for cadherin function should shed light on the novel aspects of cadherin-mediated cell–cell adhesion.

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