JCB Mini-Review

Direct cadherin-activated cell signaling: a view from the plasma membrane

Alpha S. Yap and Eva M. Kovacs

School for Biomedical Science and Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Brisbane, Australia 4072

Classical cadherin adhesion molecules are key determinants of cell recognition and tissue morphogenesis, with diverse effects on cell behavior. Recent developments indicate that classical cadherins are adhesion-activated signaling receptors. In particular, early–immediate Rac signaling is emerging as a mechanism to coordinate cadherin–actin integration at the plasma membrane.

Classical cadherin adhesion molecules exert profound and varied effects on cell behavior and tissue organization. It is commonly believed that cadherins support stable cell–cell contacts to maintain tissue cohesion, both during development and in post-embryonic life. But cadherins also participate in dynamic morphogenetic events: changes in cadherin repertoire influence cell sorting and tissue segregation (Godt and Tepass, 1998), whereas dynamic regulation of cadherin activity participates in synaptogenesis (Togashi et al., 2002) and in cell-on-cell locomotion during gastrulation (Brieher and Gumbiner, 1994). Conversely, in epithelial cancers, loss of E-cadherin activity is a major determinant of tumor progression and invasion (Semb and Christofori, 1998).

Given these diverse outcomes, a key issue for some time has been whether classical cadherins exert their biological effects solely through their undeniable contributions to cell surface adhesion, or whether cadherins also act as cell-signaling receptors. Classical cadherins are single-pass transmembrane glycoproteins that function as membrane-spanning macromolecular complexes (Adams and Nelson, 1998; Vleminckx and Kemler, 1999). The cadherin ectodomains mediate homophilic ligation and adhesive recognition, whereas the highly conserved cytoplasmic tails interact with proteins capable of linking cadherin adhesion to the actin cytoskeleton and cell-signaling pathways. In its simplest form, one might imagine that adhesive engagement of cadherin ectodomains would stimulate intracellular signaling. This classic paradigm of positive receptor–activated signaling characterizes many hormones, growth factors, and integrins. Such direct cadherin-activated signaling could provide an attractive mechanism for cell behavior to be altered in response to productive homophilic ligation.

Although often surmised, direct cadherin-activated signaling has been difficult to rigorously identify, although not for want of candidates. Indeed, many signaling molecules are reported to interact with classical cadherins, albeit under conditions that likely depend on cell type and context. The catalogue includes tyrosine kinases and phosphatases (Steinberg and McNutt, 1999), lipid kinases (Pece et al., 1999), heterotrimeric GTPases (Meigs et al., 2001), adaptor proteins (Xu et al., 1997), as well as β-catenin itself. Formally, these interactions might serve to regulate cadherin activity, reflect assembly of signaling complexes, or indeed represent mechanisms for cadherin-activated signaling. Although it is clear that cadherin binding acts as a tonic inhibitor, not a direct activator, of β-catenin signaling (Heasman et al., 1994), the function of the other interactions has been less forthcoming. Recently, however, new approaches to dissecting the specific cellular consequences of cadherin ligation have established that classical cadherins function as ligand-activated signaling receptors (Noren et al., 2001; Charrasse et al., 2002; Kovacs et al., 2002a). In particular, Rho family GTPases have emerged as part of a membrane-local signaling process capable of regulating cell shape and actin organization in response to cadherin adhesion. Without denying the likely existence of other modes of cadherin-dependent signaling, in this review we will concentrate on these new findings, and the light they may shed on how classical cadherins mediate cell–cell recognition.

Cadherin signaling by Rho family GTPases

Interest in the functional relationship between cadherin adhesion and Rho family GTPases was first prompted by the observation that several members of this family, including RhoA, Rac1, and Cdc42, localized to cadherin-based cell–cell contacts (for review see Braga, 2000). Indeed, cadherin accumulation in contacts, and the morphological integrity of those contacts, is often altered in cells when GTPase activity is manipulated by any of several approaches (Takaishi et al., 1997; Jou and Nelson, 1998; Braga et al., 1999). No simple functional pattern has emerged, at least in part because the consequences of GTPase signaling depend profoundly on

Address correspondence to Alpha S. Yap, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia 4072. Tel.: 61-7-33654906. Fax: 61-7-33651766. E-mail: a.yap@mailbox.uq.edu.au

Key words: cadherin; Rac; actin cytoskeleton; Rho GTPase; PI3 kinase

The Journal of Cell Biology, Volume 160, Number 1, January 6, 2003 11–16
http://www.jcb.org/cgi/doi/10.1083/jcb.200208156

The Journal of Cell Biology

cellular context (Braga et al., 1999). Nonetheless, the most consistent results were obtained upon manipulation of either Rac or Rho signaling (Takaishi et al., 1997; Jou and Nelson, 1998; Braga et al., 1999). Stable localization of cadherins at cell–cell contacts often appeared to require Rho activity, whereas perturbation of Rac signaling had more pleotropic effects. In contrast, manipulation of Cdc42 seldom produced gross disturbances in cadherin localization or junctional assembly.

These studies did not, however, establish whether the Rho family GTPases might serve to relay signals emanating from outside adhesive contacts, or if these signals were being activated by cadherin ligation itself (not mutually exclusive possibilities). Cadherin function is commonly studied by comparing the behavior of cultured cells as they grow to confluence, or where cell–cell contacts are abruptly broken and allowed to reform through manipulation of extracellular calcium. Combining these approaches with affinity precipitation for the active GTP-loaded GTPase, it was recently reported that changes in GTPase activity accompanied the formation of cadherin-dependent cell–cell contacts. Steady-state Rac activity was increased when monolayers of VE-cadherin–null cells were complemented with VE-cadherin (Lampugnani et al., 2002). Rac activity also rose rapidly (over a time course of minutes) as cells formed contacts with one another (Noren et al., 2001). The effect of cell contact on other GTPases was less consistent. Noren et al. (2001) reported that Rho activity was inhibited in E-cadherin–expressing MDCK cells, no change was seen in another study (Nakagawa et al., 2001), and Rho was stimulated in N-cadherin–containing myogenic C2C12 cells (Charrasse et al., 2002). Similarly, stimulation of Cdc42 was found in some (Kim et al., 2000) but not all (Nakagawa et al., 2001) studies—discrepancies that are likely due to differences in cell type and assay conditions.

Clearly then, changes in Rho family GTPase activity can accompany the cadherin-dependent formation of cell–cell contacts. However, in studying native cell–cell interactions it is difficult, if not impossible, to discriminate cell signals that arise as direct consequences of cadherin ligation (direct cadherin signaling), from those due to juxtacrine signaling (i.e., surface-dependent signals that require cadherin adhesion to appose cells, but which are not themselves directly activated by the cadherin). For example, although inhibition of cadherin activity (e.g., using blocking antibodies) identifies some specific requirement for cadherin adhesion, this alone cannot distinguish secondary from primary signaling events. This distinction is fundamental for any rigorous mechanistic analysis of cadherin signaling.

An important advance was the recent development of recombinant cadherin-specific adhesive ligands. Several such proteins have now been described that utilize the complete ectodomains of C-cadherin (Brieher et al., 1996), N-cadherin (Lambert et al., 2002), or E-cadherin (Kovacs et al., 2002a; Niessen and Gumbiner, 2002). When presented on planar substrata or coated on beads, these ligands support cadherin-specific adhesion (Brieher et al., 1996) and lateral clustering (Yap et al., 1997), recruiting catenins (Lambert et al., 2002) as well as regulating the actin cytoskeleton (Kovacs et al., 2002a; Lambert et al., 2002). These reagents therefore present a powerful opportunity to isolate cellular consequences of homophilic adhesive binding, independent of secondary effects that occur when native cell surfaces come into contact with one another.

Using this reductionist strategy, rapid stimulation of GTP.Rac levels was observed as cadherin-containing cells adhered to substrata coated with ligands for either *Xenopus* C-cadherin (Noren et al., 2001) or human E-cadherin (Kovacs et al., 2002a). Importantly, Rac activation was not seen when cells adhered to poly-L-lysine (Kovacs et al., 2002a), indicating that the rapid change in Rac signaling was a specific consequence of cadherin ligation, and not due to changes in cell shape concomitant upon spreading on planar substrata. Notably, Rac signaling increased within minutes of cadherin ligation (Kovacs et al., 2002a; Noren et al., 2001), a time course comparable to those associated with direct pathways activated by growth factors and integrins. In contrast, Rho or Cdc42 activity did not change as acute responses to cadherin ligation (Noren et al., 2001). This suggests that Rac may be principally activated as an early–immediate response to cadherin adhesion. Consistent with this, within individual cells Rac appeared to preferentially recruit to cadherins engaged in forming new adhesive contacts, but to dissipate as contacts aged (Ehrlich et al., 2002; Kovacs et al., 2002a). This implies that Rac is not activated continuously by all cadherins engaged in adhesion, but principally by those in the process of forming new contacts.

Over longer time frames (hours), however, homophilic ligation appears to have more diverse effects on GTPase signaling. Homophilic binding of C-cadherin inhibited Rho GTPase activity (Noren et al., 2001), whereas adhesion of mouse C2C12 cells to chick N-cadherin activated Rho signaling, with concomitant decreases in GTP.Rac and GTP.Cdc42 levels (Charrasse et al., 2002). Sustained engagement of cadherins is therefore likely to have complex consequences for GTPase signaling that are influenced by cell type, cross-talk between Rho and Rac signals (Rottner et al., 1999), cellular context, and perhaps even cadherin type.

Despite these complexities, these data established that classical cadherins function as ligand-activated receptors that modulate Rac and Rho GTPase activity upon adhesive ligation. In our view, the current data most clearly implicate Rac activation as a direct early–immediate response to cadherin ligation, and the remainder of this review will focus on cadherin-activated Rac signaling. In contrast, Cdc42 was stimulated when native cell contacts assembled (Kim et al., 2000), but not in cells bound to purified C-cadherin ligands (Noren et al., 2001). The jury remains out, but it is plausible that the changes in Cdc42 signaling seen when native cell contacts form are due to secondary signaling events associated with cell–cell contact. Certainly, this discrepancy highlights the notion that not all cadherin-dependent signaling events that arise as cells come into contact with one another are necessarily direct consequences of the cadherin receptor itself.

The role of cadherin-activated signaling in early cell–cell recognition

How then might early–immediate activation of Rac contribute to cadherin function? Although essential for the cohesion of mature tissues, classical cadherins also participate in the

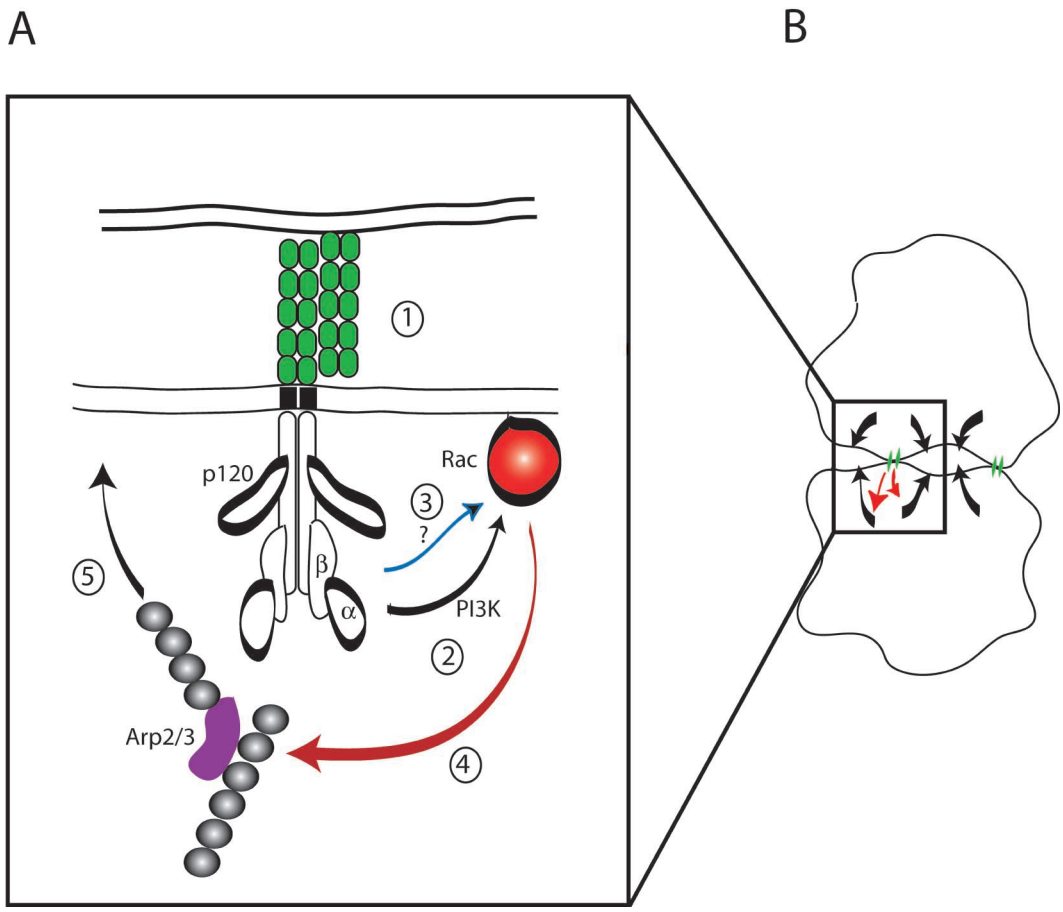

Figure 1. **A model for cadherin-activated Rac signaling participation in early cell–cell recognition.** (A) Productive cadherin ligation in newly forming contacts (1) activates Rac signaling at the plasma membrane via a PI3 kinase–dependent intermediary step (2) and possibly also a pathway independent of PI3 kinase (3). One key consequence of Rac activation is the stimulation of cadherin-directed actin assembly by Arp2/3 (4), thereby leading to protrusion of the cell surface (5). (B) Cadherin-directed actin assembly, coordinated by Rac activation, is predicted to direct the surface-protrusive activity of the actin cytoskeleton toward such nascent contacts, to extend the regions of contact and ultimately stabilize cell–cell adhesion.

early adhesive events involved in cell–cell recognition during development, cell migration, and wound-healing. The morphological details vary between experimental models (Adams et al., 1998; Raich et al., 1999; Vasioukhin et al., 2000), but in all cases productive cell–cell recognition entails the conversion of limited, nascent cadherin contacts into broader zones of adhesion. Rac appears to be critical for this process of contact-zone extension. When studied in native cell–cell contacts (Ehrlich et al., 2002), as well as when cells adhere to cadherin-coated substrata (Kovacs et al., 2002a), Rac is recruited to newly forming contacts and, indeed, principally localizes at the margins where contacts are being actively extended. Moreover, contact zone extension itself was significantly retarded by inhibiting Rac (Ehrlich et al., 2002; Kovacs et al., 2002a), and potentiated when Rac was stimulated (Kovacs et al., 2002a). By implication, it is attractive to postulate that the early activation of Rac by cadherin ligation couples homophilic recognition to contact zone extension.

One likely link between Rac signaling and contact zone extension is through regulation of the actin cytoskeleton. It has long been recognized that classical cadherins function in cooperation with actin filaments (Adams and Nelson, 1998). Originally, cortical actin was envisioned to stabilize adhesion by scaffolding cadherin–catenin complexes. Recent cellular and genetic studies now make it clear that cadherins also interact with more dynamic states of the actin cytoskeleton (Adams et al., 1998; Grevengoed et al., 2001; Vasioukhin et al., 2000; Ehrlich et al., 2002). Of relevance for understanding the early events in cell–cell recognition, surface-directed actin assembly is a protrusive mechanism that not only brings cells into contact with one another (Vasioukhin et al., 2000), but also participates in productively extending those nascent cadherin contacts. Thus, reorganization of the actin cytoskeleton occurs as contacts extend (Adams et al., 1998) and E-cadherin can interact biochemically with the Arp 2/3 actin nucleator complex (Kovacs et al., 2002b), a key determinant of actin assembly. Notably, homophilic ligation of E-cadherin alone could recruit Arp2/3 to nascent adhesive contacts, indicating that cadherin adhesion was sufficient to mark sites for actin assembly to occur at the cell surface (Kovacs et al., 2002b).

Importantly, actin assembly by the Arp2/3 complex is quite strictly activated by cell signals, including Cdc 42 and Rac (Pollard et al., 2000). One function of cadherin-activated Rac signaling may therefore be to stimulate catalytic activity of the Arp2/3 complex when it is recruited to the

cell surface by cadherin ligation. Consistent with this notion, both Rac and Arp2/3 localized in newly forming cadherin contacts (Kovacs et al., 2002a,b), whereas inhibition of Rac signaling blocked actin assembly at sites of adhesion between cells and N-cadherin–coated beads (Lambert et al., 2002). As a working model, we therefore propose that the membrane-local activation of Rac plays a key role in early adhesive cell recognition by recruiting and/or activating the actin assembly apparatus in response to E-cadherin ligation (Fig. 1), thereby directing Arp2/3-based surface protrusiveness to efficiently expand zones of cell contact. Later effects of cadherin signaling may further remodel the actin cytoskeleton, for example through regulation of myosin-based contractility by Rho (Charrasse et al., 2002; Vaezi et al., 2002).

Local GTPase activation may also influence cadherin function by affecting the activity and/or composition of the cadherin–catenin complex. Notably, IQGAP, a multidomain protein that can act as a downstream effector of both Cdc42 and Rac, is found at cadherin-based cell–cell contacts and has the potential to regulate cadherin adhesiveness (Kuroda et al., 1997). Overexpression of IQGAP reduced cadherin-based cell adhesiveness, perhaps by displacing α-catenin from the cadherin–catenin complex. However, the physiological significance of these observations remains to be determined. IQGAP is commonly viewed to simply antagonize cadherin adhesion (Kuroda et al., 1997), but it is worth remembering that regulated decreases in adhesion are also important for the biological contribution of cadherins during morphogenesis (Brieher and Gumbiner, 1994). Nor is it yet clear whether IQGAP might act in response to cadherin-activated signaling, or mediate the effects of other signaling pathways on cadherin function. Nonetheless, this highlights the potential for membrane-local cadherin-activated signals to mediate cooperation between surface adhesion and actin cytoskeletal activity.

How classical cadherins activate Rho family GTPases: control of GTPase competance and membrane localization

Rho family GTPases, like other GTP-binding proteins, function as molecular switches (Hall, 1998; Braga, 2000). Their participation in cell signaling depends on both their intrinsic capacity to interact with downstream effector molecules and their correct subcellular localization (Symons and Settleman, 2000). Emerging evidence suggests that classical cadherins can, directly or indirectly, contribute to both these processes.

Like other GTPases, nucleotide status determines whether Rho family proteins can interact with, and activate, downstream effector molecules (Hall, 1998). To date, guanine nucleotide exchange factors (GEFs)* appear to be principally responsible for promoting exchange of GDP for GTP, thereby rendering Rho family GTPases competant to signal (Schmidt and Hall, 2002). Several potential candidate GEFs exist that might mediate the early activation of Rac by classical cadherins. VAV2, which activates Rho, Rac, and Cdc42 (Schmidt and Hall, 2002), can interact with p120-ctn, which binds the cytoplasmic tail of classical cadherins (Noren et al., 2000). Alternatively, Tiam-1 is a Rac-specific GEF capable of affecting E-cadherin expression and the stability of epithelial adherens junctions (Hordijk et al., 1997). The precise role of these, or other GEFs, in cadherin-activated signaling is an urgent question for investigation.

One important clue to the upstream components of the cadherin-activated Rac pathway comes from the lipid kinase, PI3 kinase. PI3 kinase is capable of activating Rac, probably by recruiting to the membrane GEF(s) containing PH domains that recognize PI-(3,4,5)-P_3 (PIP_3) (Hawkins et al., 1995; Coniglio et al., 2001). Indeed, several Rac-specific GEFs, including Tiam-1, contain PH domains that recognize PIP_3. Importantly, cadherin ligation can recruit Type 1A PI3 kinase to the cadherin complex and stimulate PI3 kinase activity (Pece et al., 1999; Kovacs et al., 2002a). Moreover, inhibition of PI3 kinase activity prevented full stimulation of Rac by E-cadherin (Nakagawa et al., 2001; Kovacs et al., 2002a). Together, these data suggest a role for PI3 kinase as an upstream activator of Rac in cadherin-activated signaling. It should be noted that although PI3 kinase inhibition very potently blocked cadherin contact formation and adhesion, it did not fully abolish Rac activation by E-cadherin (Kovacs et al., 2002a). Therefore, PI3 kinase is unlikely to be the sole mechanism for E-cadherin to activate Rac.

Finally, classical cadherins may influence the precise sites at the plasma membrane where Rho family signaling occurs. As noted above, interest in Rho family signaling was first occasioned by evidence that these molecules localized to adherens junctions. At least for Rac, it is clear that this molecule is not found at all cadherin contacts: instead it appears to principally recruit to newly forming contacts. This may be an indirect consequence of cadherin signaling, especially local generation of PIP_3 by PI3 kinase (Hansen et al., 2002; Kovacs et al., 2002a). In addition, it is also possible that proteins of the cadherin–catenin complex can associate directly with Rho family proteins. Of note, Magie et al. (2002) demonstrated recently that purified α-catenin and p120-ctn could both bind to Rho, supporting earlier evidence for a direct biochemical association between p120-ctn and Rho (Anastasiadis et al., 2000). Moreover, p120-ctn was necessary for Rho to accumulate in *Drosophila* adherens junctions. Multiple mechanisms may therefore exist to ensure the spatial fidelity of cadherin signaling.

Signaling to and away from the membrane

To conclude, we believe that recent developments firmly establish the principle that classical cadherins function both as mediators of cell surface adhesion, and as adhesion-activated cell surface receptors. These functions are likely to be intimately interrelated. We have focused on positive signaling pathways responsible for cadherin–actin cooperation at the plasma membrane, processes relevant to contact formation, cell-upon-cell locomotion, and cellular recognition. However, there are already signs that cadherin signaling is even more complex. Both Rac and PI3 kinase signaling ramify beyond the plasma membrane to control processes such as cell proliferation and apoptosis. Interestingly, cadherin activated PI3 kinase signaling induced the recruitment to, and phos-

*Abbreviations used in this paper: GEF, guanine nucleotide exchange factor; PIP_3, PI-(3,4,5)-P_3.

phorylation of, Akt (PKB) (Watton and Downward, 1999; Kovacs et al., 2002a), an enzyme well implicated in controlling apoptosis. Moreover, it is possible that the numerous protein–protein interactions reported between classical cadherins and other signaling molecules reflect alternate modes of cadherin signaling. For example, VE-cadherin can condition VEGF signaling in endothelial cells (Carmeliet et al., 1999) and it has been suggested that E-cadherin may cocluster and activate EGF receptor signaling in a manner independent of EGF itself (Pece and Gutkind, 2000). Heterotypic cis-interactions between cadherins and receptor tyrosine kinases may therefore constitute a separate paradigm of cadherin-activated signaling. All told, it is likely to be an exciting and challenging time for those of us interested in understanding what happens when two cells touch.

We thank our laboratory colleagues, particularly Astrid Kraemer, Radiya Ali, and Marita Goodwin, for many stimulating discussions; further afield, Nicole Noren, Keith Burridge, Aki Kusumi, Marc Symons, and Al Reynolds for sharing data, ideas, and setting us straight as necessary; Rob Krauss, Cara Gottardi, and Carien Niessen for critically reading the manuscript; and the reviewers of this manuscript for their collegial and insightful comments.

Work in our laboratory was funded by grants from the National Health and Medical Research Council and the American Research Council. A.S. Yap is a Wellcome Trust Senior International Research Fellow.

Submitted: 27 August 2002
Revised: 20 November 2002
Accepted: 22 November 2002

References

Adams, C.L., Y.-T. Chen, S.J. Smith, and W.J. Nelson. 1998. Mechanisms of epithelial cell–cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein. *J. Cell Biol.* 142:1105–1119.

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

Anastasiadis, P.Z., S.Y. Moon, M.A. Thoreson, D.J. Mariner, H.C. Crawford, Y. Zheng, and A.B. Reynolds. 2000. Inhibition of RhoA by p120 catenin. *Nat. Cell Biol.* 2:637–644.

Braga, V. 2000. Epithelial cell shape: cadherins and small GTPases. *Exp. Cell Res.* 261:83–90.

Braga, V.M., A. Del Maschio, L. Machesky, and E. Dejana. 1999. Regulation of cadherin function by Rho and Rac: modulation of junction maturation and cellular context. *Mol. Biol. Cell.* 10:9–22.

Brieher, W.M., and B.M. Gumbiner. 1994. Regulation of cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J. Cell Biol.* 126: 519–527.

Brieher, W.M., A.S. Yap, and B.M. Gumbiner. 1996. Lateral dimerization is required for the homophilic binding activity of C-cadherin. *J. Cell Biol.* 135: 487–489.

Carmeliet, P., M.G. Lampugnani, L. Moons, F. Breviario, V. Compernolle, F. Bono, G. Balconi, R. Spagnuolo, B. Oostuyse, M. Dewerchin, et al. 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. *Cell.* 98: 147–157.

Charrasse, S., M. Meriane, F. Comunale, A. Blangy, and C. Gauthier-Rouviere. 2002. N-cadherin-dependent cell–cell contact regulates Rho GTPases and β-catenin localization in mouse C2C12 myoblasts. *J. Cell Biol.* 158:953–965.

Coniglio, S.J., T.-S. Jou, and M. Symons. 2001. Rac1 protects epithelial cells against anoikis. *J. Biol. Chem.* 276:28113–28120.

Ehrlich, J.S., M.D.H. Hansen, and W.J. Nelson. 2002. Spatio-temporal regulation of Rac1 localization and lamelliodia dynamics during epithelial cell-cell adhesion. *Dev. Cell.* 3:259–270.

Godt, D., and U. Tepass. 1998. *Drosophila* oocyte localization is mediated by differential cadherin-based adhesion. *Nature.* 395:387–391.

Grevengoed, E.E., J.J. Loureiro, T.L. Jesse, and M. Peifer. 2001. Abelson kinase regulates epithelial morphogenesis in *Drosophila*. *J. Cell Biol.* 155:1185–1198.

Hall, A. 1998. Rho GTPases and the actin cytoskeleton. *Science.* 279:509–514.

Hansen, M.D., J.S. Ehrlich, and W.J. Nelson. 2002. Molecular mechanism for orienting membrane and actin dynamics to nascent cell–cell contacts in epithelial cells. *J Biol Chem.* 277:45371–45376.

Hawkins, P.T., A. Eguinoa, R.-G. Qiu, D. Stokoe, F.T. Cooke, R. Walters, S. Wennstrom, L. Claesson-Welsh, T. Evans, M. Symons, and L. Stephens. 1995. PDGF stimulates an increase in GTP-Rac via activation of phosphoinositide 3-kinase. *Curr. Biol.* 5:393–403.

Heasman, J., A. Crawford, K. Goldstone, P. Garner-Hamrick, B. Gumbiner, P. McCrea, C. Kintner, C. Yoshida Noro, and C. Wylie. 1994. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell.* 79:791–803.

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

Jou, T.-S., and W.J. Nelson. 1998. Effects of regulated expression of mutant RhoA and Rac1 small GTPases on the development of epitehlial (MDCK) cell polarity. *J. Cell Biol.* 142:85–100.

Kim, S.H., Z. Li, and D.B. Sacks. 2000. E-cadherin-mediated cell-cell attachment activates Cdc42. *J. Biol. Chem.* 275:36999–37005.

Kovacs, E.M., R.G. Ali, A.J. McCormack, and A.S. Yap. 2002a. E-cadherin homophilic ligation directly signals through Rac and PI3-kinase to regulate adhesive contacts. *J. Biol. Chem.* 277:6708–6718.

Kovacs, E.M., M. Goodwin, R.G. Ali, A.D. Paterson, and A.S. Yap. 2002b. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr. Biol.* 12:379–382.

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

Lambert, M., D. Choquet, and R.M. Mege. 2002. Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J. Cell Biol.* 157:469–479.

Lampugnani, M.G., A. Zanetti, F. Breviario, G. Balconi, F. Orsenigo, M. Corada, R. Spagnuolo, M. Betson, V. Braga, and E. Dejana. 2002. VE-cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam. *Mol. Biol. Cell.* 13:1175–1189.

Magie, C.R., D. Pinto-Santini, and S.M. Parkhurst. 2002. Rho1 interacts with p120ctn and α-catenin, and regulates cadherin-based adherens junctions in *Drosophila*. *Development.* 129:3771–3782.

Meigs, T.E., T.A. Fields, D.D. McKee, and P.J. Casey. 2001. Interaction of Galpha 12 and Galpha 13 with the cytoplasmic domain of cadherin provides a mechanism for beta-catenin release. *Proc. Natl. Acad. Sci. USA.* 98:519–524.

Nakagawa, M., M. Fukata, M. Yamagawa, M. Itoh, and K. Kaibuchi. 2001. Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. *J. Cell Sci.* 114:1829–1838.

Niessen, C.M., and B.M. Gumbiner. 2002. Cadherin-mediated cell sorting not determined by binding or adhesion specificity. *J. Cell Biol.* 156:389–400.

Noren, N.K., B.P. Liu, K. Burridge, and B. Kreft. 2000. p120 catenin regulates the actin cytoskeleton via rho family GTPases. *J. Cell Biol.* 150:567–579.

Noren, N.K., C.M. Niessen, B.M. Gumbiner, and K. Burridge. 2001. Cadherin engagement regulates Rho family GTPases. *J. Biol. Chem.* 276:33305–33308.

Pece, S., M. Chiariello, C. Murga, and J.S. Gutkind. 1999. Activation of the protein kinase Akt/PKB by the formation of E-cadherin-mediated cell-cell junctions. *J. Biol. Chem.* 274:19347–19351.

Pece, S., and J.S. Gutkind. 2000. Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell-cell contact formation. *J. Biol. Chem.* 275:41227–41233.

Pollard, T.D., L. Blanchoin, and R.D. Mullins. 2000. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu. Rev. Biophys. Biomol. Struct.* 29:545–576.

Raich, W.B., C. Agbunag, and J. Hardin. 1999. Rapid epithelial sheet sealing in the *Caenorhabditis elegans* embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* 9:1139–1146.

Rottner, K., A. Hall, and J.V. Small. 1999. Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* 9:640–648.

Schmidt, A., and A. Hall. 2002. Guanine nucleotide exchange factors for Rho GTP-

ases: turning on the switch. *Genes Dev.* 16:1587–1609.

Semb, H., and G. Christofori. 1998. The tumor-suppressor function of E-cadherin. *Am. J. Hum. Genet.* 63:1588–1593.

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

Symons, M., and J. Settleman. 2000. Rho family GTPases: more than simple switches. *Trends Cell Biol.* 10:415–419.

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

Togashi, H., K. Abe, A. Mizoguchi, K. Takaoka, O. Chisaka, and M. Takeichi. 2002. Cadherin regulates dendritic spine morphogenesis. *Neuron.* 35:77–89.

Vaezi, A., C. Bauer, V. Vasioukhin, and E. Fuchs. 2002. Actin cable dynamics and rho/rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev. Cell.* 3:367–381.

Vasioukhin, V., C. Bauer, M. Yin, and E. Fuchs. 2000. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell.* 100:209–219.

Vleminckx, K., and R. Kemler. 1999. Cadherins and tissue formation: integrating adhesion and signaling. *Bioessays.* 21:211–220.

Watton, S.J., and J. Downward. 1999. Akt/PKB localisation and 3′-phosphoinositide generation at sites of epithelial cell-matrix and cell-cell interaction. *Curr. Biol.* 9:433–436.

Xu, Y., D.-F. Guo, M. Davidson, T. Inagami, and G. Carpenter. 1997. Interaction of the adaptor protein Shc and the adhesion molecule cadherin. *J. Biol. Chem.* 272:13463–13466.

Yap, A.S., W.M. Brieher, M. Pruschy, and B.M. Gumbiner. 1997. Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function. *Curr. Biol.* 7:308–315.