Rac is a dominant regulator of cadherin-directed actin assembly that is activated by adhesive ligation independently of Tiam1

Astrid Kraemer,1 Marita Goodwin,1,2 Suzie Verma,1 Alpha S. Yap,1,2 and Radya G. Ali1,2
1Division of Molecular Cell Biology, Institute for Molecular Bioscience, and 2School of Biomedical Science, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia

Submitted 15 February 2006; accepted in final form 2 October 2006

Kraemer A, Goodwin M, Verma S, Yap AS, Ali RG. Rac is a dominant regulator of cadherin-directed actin assembly that is activated by adhesive ligation independently of Tiam1. Am J Physiol Cell Physiol 292: C1061–C1069, 2007. First published October 4, 2006; doi:10.1152/ajpcell.00073.2006.—Classic cadherins function as adhesion-activated cell signaling receptors. On adhesive ligation, cadherins induce signaling cascades leading to actin cytoskeletal reorganization that is imperative for cadherin function. In particular, cadherin ligation activates actin assembly by the actin-related protein (Arp)2/3 complex, a process that critically affects the ability of cells to form and extend cadherin-based contacts. However, the signaling pathway(s) that activate Arp2/3 downstream of cadherin adhesion remain poorly understood. In this report we focused on the Rac family GTPases Rac and Cdc42, which can signal to Arp2/3. We found that homophilic engagement of E-cadherin simultaneously activates both Rac1 and Cdc42. However, by comparing the impact of dominant-negative Rac1 and Cdc42 mutants, we show that Rac1 is the dominant regulator of cadherin-directed actin assembly and homophilic contact formation. To pursue upstream elements of the Rac1 signaling pathway, we focused on the potential contribution of Tiam1 to cadherin-activated Rac signaling. We found that Tiam1 or the closely-related Tiam2/STEF1 was recruited to cell-cell contacts in an E-cadherin-dependent fashion. Moreover, a dominant-negative Tiam1 mutant perturbed cell spreading on cadherin-coated substrata. However, disruption of Tiam1 activity with dominant-negative mutants or RNA interference did not affect the ability of E-cadherin ligation to activate Rac1. We conclude that Rac1 critically influences cadherin-directed actin assembly as part of a signaling pathway independent of Tiam1.

CLASSIC CADHERINS are a large family of cell surface adhesion receptors that function throughout development and postembryonic life to mediate events such as cell-cell adhesion, tissue patterning, and wound healing (40, 41). This is exemplified by E-cadherin, the principal cadherin found in epithelial tissues. E-cadherin supports epithelial morphogenesis and integrity, while its dysfunction promotes tumor progression to invasion and metastasis (3). At the cellular level, many of these morphogenetic effects of cadherin activity arise through close cooperation between the adhesion receptors and the actin cytoskeleton. Thus actin cytoskeletal integrity is necessary for cadherin function (2), while several different modes of actin organization, with the potential to serve distinct cellular functions, can be found at cadherin adhesive contacts (34, 46).

One important form of cytoskeletal activity is actin assembly itself: this can potentially generate force as cells form contacts with one another and produce filamemtous scaffolds for bundling and contractility (4, 19, 43). Of note, the actin-related complex (Arp)2/3 actin nucleator complex, a major determinant of actin assembly, is found at cadherin adhesions and influences the biogenesis of cell-cell contacts (19, 44). Arp2/3 can, indeed, be recruited to the cell cortex in response to adhesive ligation of the cadherin ectodomain itself. This suggests that Arp2/3 may provide local protrusive forces to drive the formation and efficient extension of cell-cell contacts (13, 19, 44).

Importantly, the catalytic activity of Arp2/3 is tightly regulated in response to cell signals (21). The signaling pathways that promote Arp2/3-mediated actin assembly in response to cadherin adhesion, however, remain poorly understood. Small GTPases of the Rho subfamily, notably Rac and Cdc42, are well-known regulators of Arp2/3 activity (21). Cdc42 was previously reported to be activated when cells form contacts with one another (6, 16), although whether this occurs in response to cadherins themselves or reflects signaling by nectin adhesion receptors remains to be determined. Rac1 localizes to newly forming cadherin adhesions, both native intercellular contacts (1, 8, 27) and homophilic adhesions that cells make to immobilized cadherin ligands (11, 18). Moreover, Rac1 is activated when cells assemble contacts with one another or adhere to cadherin-coated substrata (18, 27, 29). Inhibition of Rac1 signaling either with dominant-negative (DN) mutants or by RNA interference (RNAi)-mediated protein depletion reduced the amount of actin filaments at cell-cell contacts and prevented the formation of cadherin-based contacts (4, 30, 39). These observations therefore suggest, but do not prove, that Rac1 may be a key determinant of actin assembly at cadherin contacts. To further understand the signaling pathways that regulate the cytoskeleton at cadherin contacts, we therefore sought to directly assess the contributions of Rac1 and Cdc42 to cadherin-activated actin assembly and to identify additional molecules that may regulate signaling by these GTPases in response to cadherin adhesion.

MATERIALS AND METHODS

Plasmids. Plasmids used in this study were 1) a p21-activated kinase (PAK)-Cdc42 and Rac interactive binding domain (CRIB) glutathione S-transferase (GST)-fusion construct (a kind gift from Dr. J. Chernoff, Fox Chase Cancer Center, Philadelphia, PA), 2) green fluorescent protein (GFP)-tagged DN Rac1 in pCDNA3.1(+) (a kind gift from Neil Hotchin, University of Birmingham, Birmingham, UK), 3) myc-tagged DN Rac1 in pCDNA3.1(+) (a kind gift from Alan...
Hall, UCL, London, UK), 4) GFP-tagged DN Cdc42 in enhanced GFP (EGFP)-C1 (a kind gift from Kozo Kaibuchi, Nagoya University, Japan), 5) EGFP-tagged IRSp53 (a generous gift of Drs. Taodami Takenawa and Hiroyuki Miki, Tokyo University, Japan), 6) pEGFP-N1 (Clontech), and 7) DN Tiam1 (amino acids 393–854), amplified via PCR using a 5′ primer containing a EcoRV site (indicated in bold) (GAT ATCC ATG AGC ACC ACC AAC AGC GAG AG) and a 3′ primer including a stop (indicated by underline) and NotI site (GG CGC CGC GTA TC ATC TGG ATC TTC TGC GTC G) and ligated into pCDNA3.1(+)(+) via EcoRV and NotI. GFP was excised from pEGFP-C1 via NheI/BglII and cloned in pCDNA3.1(+) via NheI/BamHI.

To generate a construct enabling expression of short hairpin RNA directed against Tiam1 under the control of the H1-RNA promoter a sense (GATCC CCAAG ATCGA CATGG ACGAG AAG) and an antisense (AGCTT TTCC AAAA AGATC GACAT GGAG AGAAG AGAAG GAGA AAG) oligo were annealed and subcloned into psUPER.retropuro (OligoEngine) digested with BglII and HindIII. The annealed oligos contain a BglII, a HindIII, a hairpin sequence, and a termination signal (indicated in bold). As negative control a scrambled sequence was used (sense oligo GATCC CCATC GTGGA GATAT GACTTC TCAAT CAGA GAGGT ATATC TCCAC GATT TTGG AAA, antisense oligo AGCTT TTCC AAAA TCGTGG GAGAT ATACCT TCTCT TTGAT GAGGAT GTGATA TCTCC ACGAT GGG). Cell culture. NMuMG and NM1F cells were cultured in DMEM containing 10% FCS and 10 μg/ml insulin. Generation and maintenance of Chinese hamster ovary (CHO) cells stably expressing human E-cadherin and production and purification of the recombinant protein consisting of the ectodomain of human E-cadherin fused to the Fc fragment of IgG (hE/Fc) were described previously (18, 19). Phoenix cells were maintained in DMEM supplemented with 10% FCS. CHO cells stably expressing human E-cadherin (hE-CHO) cells were transfected with Lipofectamine and PLUS reagent (Invitrogen) according to manufacturer’s instructions. NMuMG cells were transfected with Lipofectamine 2000 (Invitrogen). Phoenix cells were transfected by the calcium phosphate method.

Antibodies. Primary antibodies were 1) rabbit antiserum raised against Tiam1/STIF (a kind gift from Dr. Mikio Hoshino, Kyoto University), 2) mouse MAb against Rac1 (Upstate Biotech), 3) mouse MAb against Cdc42 (BD Biosciences), 4) rabbit pAb against Tiam1 (Santa Cruz Biotechnology), 5) rabbit polyclonal antibody (PAb) against p34 (44), 6) mouse MAb against β-tubulin (Sigma), 7) mouse MAb against E-cadherin tail region (Transduction Laboratories), 8) rabbit PAb against human E-cadherin, raised against hE/Fc (13, 9) mouse MAb against E-cadherin, clone SHE 787 (Zymed), and 10) rabbit PAb against GFP (Roche). Actin was visualized with tetramethylrhodamine isothiocyanate (TRITC)-phalloidin (Sigma), A488- and A594-labeled secondary antibodies were obtained from Molecular Probes.

Bead and planar spreading assays. Bead recruitment assays and planar spreading assays were performed as described previously (12, 18).

Immunofluorescence and microscopy. Immunofluorescence was performed as described previously (18, 19). To visualize Tiam1/ Tiam2, cells were fixed with methanol for 5 min on ice. Images were acquired with a DX51 Olympus microscope equipped with an Hamamatsu Orca-ER camera. Quantification of fluorescence intensity around beads (recruitment index) was performed as previously described (44). Cell spreading was quantified in ImageJ by tracing the perimeter of the cell used as indicator for cell spreading.

Rac activation assay. Expression and purification of the GST-PAK-CRIB fusion protein was performed as described previously (18). Cells were serum starved for 3 h in serum-free medium before commencement of the planar spreading assay. At various times cells were scraped off the plates, pelleted by centrifugation, and lysed in 200 μl of lysis buffer [10 mM HEPES-KOH, pH 7.4, 10 mM MgCl2, 1% Triton X-100, 10% glycerol, 1 mM Na-vanadate, and EDTA-free protease inhibitors (Roche)]. Cleared lysates were mixed with 200 μl of wash buffer (10 mM HEPES-KOH, pH 7.4, 10 mM MgCl2, 1% glycerol, 1 mM Na-vanadate, and EDTA-free protease inhibitors) and incubated with 15 μg of GST-PAK-CRIB beads at 4°C for 45 min. Beads were washed and prepared for SDS-PAGE and immunoblotting. Quantification of Rac or Cdc42 activation was performed in ImageJ with the rectangular selection tool. After background correction, the mean band intensity was normalized to the intensity at time 0 min.

RESULTS

E-cadherin homophilic ligation activates Rac1 and Cdc42 signaling. As previously reported (18, 29), Rac1 is activated by cadherin homophilic ligating when cells adhere to substrata coated with recombinant cadherin ligands. To extend this analysis, we compared the response of both Cdc42 and Rac1 signaling to cadherin homophilic ligating. CHO cells stably expressing human E-cadherin (hE-CHO) cells and NMuMG cells were seeded onto plates coated with hE/Fc. At various times after attachment, GTP-loaded Rac1 and Cdc42 were precipitated from lysates by incubation with a GST fusion protein containing the CRIB domain of the Rac/Cdc42 effector domain. In both cell lines E-cadherin ligating, but not adhesion to the nonspecific adhesive ligand poly-L-lysine (PLL), induced a transient activation of Rac1 that peaked at ~30 min (Fig. 1, A, B, and E). Both cell lines also displayed an increase in GTP-loaded Cdc42 on adhesion to hE/Fc, but not to PLL, with a time course comparable to that for Rac activation (Fig. 1, C, D, and F). Thus E-cadherin homophilic ligating was capable of activating both Rac1 and Cdc42 signaling in these experiments.

Rac1 signaling is a dominant regulator of cadherin-activated actin assembly. Rac1 and Cdc42 are known to have profound effects on the regulation of the actin cytoskeleton, especially on Arp2/3 activity (21). Accordingly, we then turned to examination of the contribution that activated Rac1 and Cdc42 might have on actin regulation by E-cadherin. In these experiments, we used latex beads coated with hE/Fc to present spatially defined cadherin adhesion signals to cells. As reported previously (19), adhesion of hE/Fc beads, but not of concanavalin A (ConA)-coated beads, caused cellular E-cadherin to accumulate at the sites of adhesion. This was accompanied by intense accumulation of F-actin at the leading edges of lamellipodia (Fig. 3, A and B), a process that requires Arp2/3 activity (13, 44). Transient expression of DN Rac/N1 (DN Rac1) reduced substantially the amount of F-actin found at cadherin contacts (Fig. 2, C and E). In contrast, DN Cdc42/N1 (DN Cdc42) had only a minor effect on F-actin accumulation (Fig. 2, D and E). This suggested that, of these two potential Arp2/3-regulatory signals, Rac1 had the preponderant effect on cadherin-directed actin assembly.

When cells adhere to hE/Fc-coated substrata they spread and extend prominent cadherin-based lamellipodia (11, 18, 19, 36), a process that requires Arp2/3 activity (44). To further assess the relative contributions of Rac1 and Cdc42 to cadherin regulation of the actin cytoskeleton, we next tested their impact on cell spreading in this assay (Fig. 3). As previously described (19), phalloidin staining of control cells revealed strong accumulation of F-actin at the leading edges of lamellipodia (Fig. 3,
A and B). Cells expressing DN Rac1 were able to attach to the hE/Fc substrate but failed to form lamellipodia and extend contact zones (Fig. 3, C and D). In contrast, expression of DN Cdc42 did not affect the ability of cells to spread on hE/Fc (Fig. 3, E and F). Integrin-dependent cell spreading on fibronectin, however, was inhibited by Cdc42N17 (not shown), as also observed by other investigators (31), confirming that the DN Cdc42 transgene was bioactive. Together these findings suggested that Rac1, but not Cdc42, was needed for the Arp2/3-dependent spreading of cells on cadherin-coated substrata.

To then assess whether Rac1 signaling might influence Arp2/3, we tested its ability to affect Arp2/3 recruitment to cadherin homophilic adhesions. As shown in Fig. 4, A, B, and E, the p34 subunit of Arp2/3 was recruited to hE/Fc beads but showed little accumulation around control beads coated with ConA. Transient expression of DN Rac1 significantly reduced the amount of p34 recruited to hE/Fc beads (Fig. 4, C and E). In contrast, DN Cdc42 did not affect p34 recruitment (Fig. 4, D and E). This suggests that Rac1 signaling is necessary for E-cadherin adhesion to recruit Arp2/3 to the cell cortex.

Tiam1 localizes at cell-cell contacts in an E-cadherin-dependent fashion. Together these data indicated that Rac was the dominant regulator of Arp2/3 activity at cadherin contacts. We then sought to pursue regulatory proteins that might specifically determine the activation of Rac signaling by E-cadherin adhesion. Several classes of proteins control expression of Rho family GTPase signaling, notably GTPase activating proteins, as shown in Fig. 1.
guanine-nucleotide dissociation inhibitors, and guanine nucleotide exchange factors (GEFs). Of these, GEFs transform the inactive, GDP-bound conformation of a GTPase into the active, GTP-bound conformation and are the best-understood activators of signaling (33). Although there are more than 80 known or predicted Rho family GEFs (32), only two Rac1-specific GEFs, Vav2 and Tiam1, have been implicated in cadherin signaling to date (10, 14, 22).

We chose to focus on Tiam1, because it has been identified at cell-cell contacts (20) and implicated in epithelial organization (14). Tiam1 was readily identifiable by immunoblotting in NMuMG cell lysates; hE-CHO cells did not express Tiam1 but did express Tiam2/STEF (Fig. 5A), the close relative of Tiam1, which appears to share a degree of functional redundancy with Tiam1 (23, 24, 28). Moreover, endogenous Tiam1 (Fig. 5B) or Tiam2 (Fig. 5C) was clearly localized at intercellular contacts in NMuMG and hE-CHO cells, respectively. However, NMF cells, a cadherin-deficient subclone of NMuMG cells, showed only diffuse cytoplasmic localization of Tiam1 (Fig. 5B), and cadherin-negative parental CHO cells showed a similarly cytoplasmic localization of Tiam2 (Fig. 5C). Furthermore, inhibition of cadherin function in hE-CHO cells with a blocking antibody delocalized Tiam2 from intercellular contacts (Fig. 5D). Thus both Tiam1 and Tiam2 appeared to localize to cell-cell contacts in an E-cadherin-dependent fashion.

As a first test of the potential contribution of Tiam1 to cadherin signaling, we investigated whether a DN mutant could affect the ability of hE-CHO cells to spread on hE/Fc-coated substrata, our earlier data having shown that this process was highly sensitive to Rac signaling (Fig. 3; Ref. 18). We used a DN Tiam1 construct comprising the region responsible for membrane localization (PHn-CC-Ex); this construct, as well as the homologous region from Tiam2, has commonly been used to disrupt Tiam1/Tiam2 signaling (15, 23, 24, 26, 29).

Fig. 3. Rac but not Cdc42 signaling is required for cadherin-based cell spreading. hE-CHO cells were allowed to adhere and spread on hE/Fc-coated substrata for 90 min. Cells were transiently transfected with enhanced GFP (EGFP) alone (control) or with either GFP-tagged DN Rac1 or GFP-DN Cdc42. Cell margins were identified by TRITC-phalloidin staining (A, C, E), and spreading was quantitated by expressing cell area as a ratio of the cell perimeter (area/perimeter, G); data are means ± SD. **P < 0.01, 2-tailed Student’s t-test. Control cells extended broad lamellipodia on adhesion to hE/Fc (A, B), and this was substantially reduced by expression of DN Rac1 but not by DN Cdc42 (E, F, G).

Fig. 4. Rac signaling is required for the recruitment of Arp2/3 to cadherin homophilic adhesions. Beads coated with either hE/Fc or ConA were allowed to adhere to the dorsal surfaces of hE-CHO cells for 90 min and then fixed and stained with anti-p34 antibodies to detect the Arp2/3 complex. Prominent p34 was detected at sites of adhesion with hE/Fc-coated beads (A) but not with the ConA controls (B) and was substantially reduced by transient expression of myc-tagged DN Rac1 (C) but not DN Cdc42 (D). E: fluorescence intensity of p34 recruitment was quantified with the recruitment index as described in MATERIALS AND METHODS; data are means ± SD. **P < 0.01, 2-tailed Student’s t-test compared with control hE/Fc beads.

Fig. 5. Tiam1 and Tiam2 localize to cell-cell contacts in an E-cadherin-dependent fashion.
37). We found that transient expression of this mutant dramatically reduced the ability of cells to spread on hE/Fc-coated substrata (Fig. 6). In contrast, cadherin-based spreading was not altered in control cells expressing GFP alone (Fig. 6). This indicated that DN Tiam1 could readily perturb the Rac-dependent spreading of cells on cadherin substrata.

**Tiam1 and Tiam2 are not essential for E-cadherin to activate Rac1.** We then tested the effect of DN Tiam1 on cadherin-activated Rac1 signaling. hE-CHO cells or NMuMG cells were transiently transfected with GFP-DN Tiam1 or with GFP alone; typically we obtained >70% transfection efficiency in these experiments. Cells were then allowed to adhere to hE/Fc-coated substrate, and GTP-Rac1 levels were measured with PAK-CRIB pull-down assays. To our surprise, DN Tiam1 had no effect on Rac1 activation in either cell line (Fig. 7, A–C), despite its potent impact on cadherin-based lamellipodial extension. To confirm that the DN Tiam1 mutant could, indeed, perturb Tiam1 signaling, we analyzed the response of Rac to lysophosphatidic acid (LPA) stimulation in NMuMG cells. The LPA1 receptor, a well-known Rho activator, also activates Rac via a pathway involving G_i, phosphatidylinositol 3-kinase (PI3-kinase), and Tiam1 (42). Consistent with this, LPA treatment of NMuMG cells induced a transient rise in GTP-Rac1 levels (Fig. 7D) and expression of DN Tiam1 significantly reduced the response of GTP-Rac1 to LPA compared with GFP alone (Fig. 7E).

To further test the role of Tiam1 in cadherin-activated Rac signaling, we then used RNAi to deplete Tiam1 levels in...
NMuMG cells. We restricted our efforts to NMuMG cells because the sequence of hamster Tiam2 is not yet known. Using a retroviral delivery system with high efficiency, combined with drug selection for the expressing population, we were able to reduce Tiam1 expression by \( \frac{90}{102} \) compared with control cells (Fig. 8A). Rac activation on binding to hE/Fc-coated substrata was measured with PAK-CRIB pull-down assays. Both Tiam1 knockdown cells [NMuMG(T−)] and control cells [NMuMG(T+)] showed a comparable transient rise in GTP.Rac with a maximum at 30 min (Fig. 8, B and C). Similarly, Tiam1 depletion did not affect the ability of cells to activate Rac1 when they reassembled contacts with one another after depletion of extracellular calcium (Fig. 8D). In contrast, the GTP.Rac response to LPA was significantly reduced in NMuMG(T−) cells compared with NMuMG(T+) cells (Fig. 8E). This, together with the fact that DN Tiam1 does not inhibit GTP-loading of Rac, indicates that Tiam1 and Tiam2 were not critical for E-cadherin ligation to activate Rac.

**DISCUSSION**

It is increasingly apparent that classic cadherins can act as adhesion-activated cell-surface receptors, capable of signaling through multiple downstream intracellular pathways (45). We have been exploring the concept that cadherins activate membrane-local signaling pathways that regulate cooperation between adhesion receptors and the actin cytoskeleton. This cooperation may involve multiple modes of actin cytoskeletal activity mediated by distinct actin regulatory proteins and signaling pathways (34). One important task is to characterize

Fig. 7. E-cadherin-activated Rac signaling is unaffected by DN Tiam1. A and B: hE-CHO cells were transiently transfected with either GFP alone or GFP-tagged DN Tiam1 and then allowed to adhere to hE/Fc-coated substrata for various times before being processed for PAK-CRIB pull-down assays. Samples were separated by SDS-PAGE and immunoprobed for Rac1. Rac activation was quantitated as described in MATERIALS AND METHODS and Fig. 1. C: Rac activation was measured in NMuMG cells transfected with GFP alone or with GFP-DN Tiam1 after adhesion to hE/Fc-coated substrata. D and E: DN Tiam1 inhibits lysophosphatidic acid (LPA)-induced Rac activation. LPA (5 μM) induced transient activation of Rac1 in serum-starved NMuMG cells (D). Expression of GFP-DN Tiam1, but not GFP alone, inhibited Rac1 activation by LPA (E). All immunoblots are representative of at least 2 independent experiments.

Fig. 9. IRSp53 localization is perturbed by DN Tiam1. A: GFP-EGFP-IRSp53 localizes to the leading edges of cadherin-based lamellipodia, characteristically being found continuously decorating the outer margins (Fig. 9A). In contrast, EGFP-IRSp53 was less apparent, being more discontinuously distributed at the residual lamellipodial margins of cells expressing DN Tiam1 (Fig. 9B).
Fig. 8. Rac activation in response to E-cadherin ligation is not affected by Tiam1 depletion by RNA interference (RNAi). A: Tiam1 RNAi successfully reduces Tiam1 expression in NMuMG cells. Immuno-blot of whole cell lysates from Tiam1 knockdown cells [NMuMG(T−)] and control cells [NMuMG(T+)] with a polyclonal anti-Tiam1 antibody. B and C: PAK-CRIB pull-down assays from NMuMG(T+) and NMuMG(T−) cells adhering to hE/Fc-coated substrata. D: Tiam1 RNAi does not affect Rac1 activation as cells assemble cell-cell contacts. Cell-cell contacts in monolayers of NMuMG(T+) and NMuMG(T−) cells were disrupted by chelating extracellular calcium. Rac1 activation was then measured with PAK-CRIB pull-down assays at 0–60 min after extracellular calcium was replaced to allow cells to reform contacts. E: Tiam1 RNAi successfully abolishes LPA-mediated Rac activation. PAK-CRIB pull-down assays from NMuMG(T+) and NMuMG(T−) cells exposed to LPA (5 μM, 2 min) are shown.

Fig. 9. DN Tiam1 mutant perturbs localization of IRSp53 at cadherin-based lamellipodia. hE-CHO cells were transiently cotransfected with EGFP-IRSp53 and either DN Tiam1 or its vector alone. The cells were then allowed to adhere to hE/Fc-coated substrata, and the localization of EGFP-IRSp53 was identified by fluorescence microscopy. A and B: cells transfected with vector alone showed prominent localization of EGFP-IRSp53 in continuous bands at the outer margins of cadherin-based lamellipodia. C and D: in cells coexpressing DN Tiam1 (not shown) EGFP-IRSp53 was discontinuous and less prominent. B and D represent detailed views of the areas enclosed by the boxes in A and C, respectively.
VE-cadherin is necessary for junctional localization of Tiam1 herin-null cells. This is consistent with earlier evidence that herin adhesion is necessary for this localization process, as Tiam1 (14), and additionally Tiam2, localized to cadherin-several reasons. First, we found that, as in earlier reports, date to participate in cadherin-activated Rac signaling for cell-cell interactions. Indeed, Tiam1 was an attractive candi-
which has been implicated in the regulation of cadherin-based
activation must also involve exchange factors that catalyze ever, remains to be fully elucidated.

The precise role that Cdc42 plays in cadherin function, how-
ticipate in signaling from E-cadherin to the Arp2/3 complex. Indeed, Tiam1 is an attractive candi-
that is recruited to cadherin adhesions and contributes to the biogenesis of those contacts (19, 44). Both Rac1 and Cdc42 possess a well-documented capacity to activate Arp2/3 in response to cell surface receptor signaling (21). Our present data indicate that while E-cadherin homophilic ligation can activate signaling by both these GTPases, Rac1 has the dom-
Figure 1. Rac1 and Cdc42 accumulate at cadherin contacts. A
Figure 1. Rac1 and Cdc42 accumulate at cadherin contacts. A key element of this process involves the Arp2/3 actin nucleator that is recruited to cadherin adhesions and contributes to the biogenesis of those contacts (19, 44). Both Rac1 and Cdc42 possess a well-documented capacity to activate Arp2/3 in response to cell surface receptor signaling (21). Our present data indicate that while E-cadherin homophilic ligation can activate signaling by both these GTPases, Rac1 has the dominant impact on cadherin-activated actin assembly.

In contrast to the reasonably well-documented capacity for classic cadherins to activate Rac1, whether cadherins also signal through Cdc42 has been less clear. Cdc42 was reported to be activated when cells made contact with one another in some studies (6, 16) but not in others (10, 27). Moreover, those studies could not readily determine whether changes in GTPase signaling were immediate downstream responses to cadherin ligation or might reflect juxtacrine signaling [through, e.g., nectins (9)] that is activated when cells surfaces are brought together. Our data indicate that homophilic E-cadherin ligation, induced by allowing cells to adhere to recombinant cadherin ligands, is sufficient to activate both Cdc42 and Rac1 with similar time profiles. In contrast, neither GTPase was activated when cells adhered to nonspecific adhesive substrata. These observations thus demonstrate for the first time that E-cadherin homophilic ligation has the capacity to activate both Rac1 and Cdc42 simultaneously.

However, although both of these GTPases can activate the Arp2/3 complex, we found that Rac1 had the dominant impact on cadherin-directed actin assembly. Thus inhibition of Rac1 clearly reduced the accumulation of F-actin at sites of adhesion to cadherin-coated beads, a process that depends on Arp2/3 activity. In contrast, the minor effect of DN Cdc42 on F-actin accumulation was much less than the effect of DN Rac1. Supporting this, DN Rac1, but not DN Cdc42, profoundly inhibited the ability of cells to extend lamellipodia on cadherin-coated substrata, a functional response to homophilic ligation that requires Arp2/3 (44). Finally, expression of DN Rac1, but not DN Cdc42, inhibited the ability of E-cadherin homophilic adhesion to recruit the Arp2/3 complex itself to the cell cortex. This implies that Rac1 is the dominant GTPase signal that couples E-cadherin homophilic ligation to Arp2/3 activity. As both GTPases signal indirectly to Arp2/3, Rac-specific intermediary proteins (such as cortactin and/or WAVE) may participate in signaling from E-cadherin to the Arp2/3 complex. The precise role that Cdc42 plays in cadherin function, how-
ever, remains to be fully elucidated.

The signaling pathway that links E-cadherin ligation to Rac activation must also involve exchange factors that catalyze GTP-loading of Rac. We focused our attention on Tiam1, which has been implicated in the regulation of cadherin-based cell-cell interactions. Indeed, Tiam1 was an attractive candidate to participate in cadherin-activated Rac signaling for several reasons. First, we found that, as in earlier reports, Tiam1 (14), and additionally Tiam2, localized to cadherin-based cell-cell contacts. Moreover, our data suggest that cadherin adhesion is necessary for this localization process, as Tiam1 or -2 was lost from cell-cell contacts on treatment with function-blocking anti-cadherin antibodies, as well as in cadherin-null cells. This is consistent with earlier evidence that VE-cadherin is necessary for junctional localization of Tiam1 in endothelial cells (20). Second, Tiam1 is a PI3-kinase-responsive GEF, and cadherin-activated Rac signaling depends at least partially on PI3-kinase signaling (18, 27). Finally, we found that a DN Tiam1 mutant potently inhibited lamellipodial extension on cadherin-coated substrata, a process that requires Rac signaling.

It was therefore surprising that our subsequent studies failed to identify any role for Tiam1 in cadherin-activated Rac sig-
ning. Neither expression of mutant Tiam1 nor depletion of Tiam1 by RNAi altered the acute rise in GTP.Rac levels that occurred when cells adhered to cadherin-coated substrata, nor did Tiam1 knockdown affect the Rac activation that occurred when cells reassembled native cell-cell contacts after depletion of extracellular calcium. Taken together, these data indicate that, although Tiam1 may be recruited to cadherin adhesions, it does not appear to be necessary for homophilic ligation to activate Rac1 signaling. Instead, it is possible that Tiam1 is recruited to cadherin contacts to regulate other aspects of
juncti...


