JCS ePress online publication date 3 March 2009

Cell polarity triggered by cell-cell adhesion via E-cadherin

Ravi A. Desai[1], Lin Gao[1], Srivatsan Raghavan[1,2], Wendy F. Liu[1,2] and Christopher S. Chen[1,*]

[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
*Author for correspondence (e-mail: chrischen@seas.upenn.edu)

Accepted 5 December 2008
Journal of Cell Science 122, 905-911 Published by The Company of Biologists 2009
doi:10.1242/jcs.028183

Summary

Cell polarity is orchestrated by numerous extracellular cues, and guides events such as chemotaxis, mitosis and wound healing. In scrape-wound assays of cell monolayers, wound-edge cells orient their centrosomes towards the wound, a process that appears to depend on the formation of new cell–extracellular-matrix adhesions as cells spread into the wound. In direct contrast to scrape-wounded cells, isolated cells without cell-cell contacts failed to polarize, suggesting that asymmetry of cell-cell adhesions resulting from monolayer disruption might contribute to polarization. By using micropatterned substrates to engineer such asymmetries in kidney epithelial cells, we found that cell-cell contact induced displacement of the nucleus towards the contact, and also caused centrosomal reorientation and lamellipodial ruffling to the distal side of the nucleus. Upon release from micropatterned constraints, cells exhibited directed migration away from the cell-cell contact. Disrupting E-cadherin engagement randomized nuclear position and lamellipodial ruffling in patterned cultures, and abrogated scrape-wound-induced cell reorientation, but not migration rate. Polarity that was induced by cell-cell contact required an intact actin cytoskeleton and Cdc42 activity, but not RhoA or Rac signaling. Together, these findings demonstrate a novel role for cell-cell adhesion in polarization, and have implications for wound healing and developmental patterning.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/122/7/905/DC1

Key words: Polarity, Cell-cell adhesion, Migration

Introduction

Polarization of the cell-motility apparatus is a crucial first step for migration of cell populations in contexts such as morphogenesis and wound healing. During migratory polarization, many structures, including the centrosome, Golgi complex, lamellipodia and microtubules, organize towards the leading edge of the cell, whereas other structures, including the nucleus, stress fibers and mature focal adhesions, localize towards the cell rear (Ridley et al., 2003; Lauffenburger and Horwitz, 1996; Wittmann and Waterman-Storer, 2001). Scrape wounding of a monolayer of cells triggers reorientation of this migration machinery (Gotlieb et al., 1981; Kupfer et al., 1982; Etienne-Manneville and Hall, 2001; Gomes et al., 2005) and thus has been widely used to study cell polarization. In this model, Cdc42 is required for polarization in several cell types (Nobes and Hall, 1999; Palazzo et al., 2001; Watanabe et al., 2004; Tzima et al., 2003; Shen et al., 2008), and it has largely been assumed that adhesion to the extracellular matrix (ECM) in protrusions into the scrape wound is the source of the polarizing signal (Etienne-Manneville and Hall, 2001). Interestingly, fibroblast wound-edge cells polarize before significant cell protrusion takes place (Gomes et al., 2005) and intercellular communication appears to be required (Schlessinger et al., 2007), suggesting that cell-ECM adhesion is not the first or only polarizing signal. Asymmetries in cell-cell contact formed by monolayer wounding also might contribute to polarization of the cell-migration machinery.

In a variety of settings, cell-cell contact induces changes in the structural organization of cells. Cell-cell interaction at the immune synapse polarizes the nucleus and Golgi complex of the T cell to direct the transport of immune effectors (Kupfer et al., 1983). Epithelial cell-cell contact that is mediated by epithelial (E)-cadherin is required for proper establishment of apical-basal polarity (Wang et al., 1990; Nejsum and Nelson, 2007). Furthermore, E-cadherin engagement leads to significant rearrangements of actin and microtubule cytoskeletons, proximal (Chu et al., 2004; Drees et al., 2005; Yamada and Nelson, 2007; Scott et al., 2006; Kovacs et al., 2002) and distal (Ehrlich et al., 2002; Yamada and Nelson, 2007; Adams et al., 1998; Vaezi et al., 2002) to the cell-cell contact. These lines of evidence suggest that cell-cell adhesion can induce cells to segregate organelles and domains, and rearrange the cytoskeleton.

Here, we sought to investigate whether cell-cell contact might also guide polarization of the cell-migration machinery. Using a micropatterning approach, we found that E-cadherin-mediated cell-cell adhesion polarizes and directs the cell-migration machinery away from neighboring cells, and occurs prior to cell migration. Such polarity requires the actin cytoskeleton and Cdc42 activity. Polarity that is induced by cell-cell contact probably contributes to coordinated cell movements such as those occurring during morphogenesis and wound healing.

Results and Discussion

Cell-cell contact correlates with polarization of the centrosome, membrane ruffling and migration

We observed that, in the absence of serum, normal rat kidney epithelial cells (NRK-52E) failed to polarize and migrate in sparse culture, yet did so robustly at the edge of an experimental scrape wound (Fig. 1A). The dynamics of this process at the wound edges were such that cells were randomly oriented immediately after

wounding, but by 4 hours were oriented such that their centrosomes 'faced' the wound in both the presence or absence of serum (Fig. 1B-D; supplementary material Fig. S1). Such polarization was similar to that observed in other cell types (Kupfer et al., 1982; Nobes and Hall, 1999; Etienne-Manneville and Hall, 2001; Palazzo et al., 2001), and was accompanied by significant protrusive activity of the wounded edge.

To eliminate such protrusions as a potential cause of reorientation, we plated cells onto patterned substrates to form monolayers with defined edges. Here, edge cells polarized similarly to the scrape

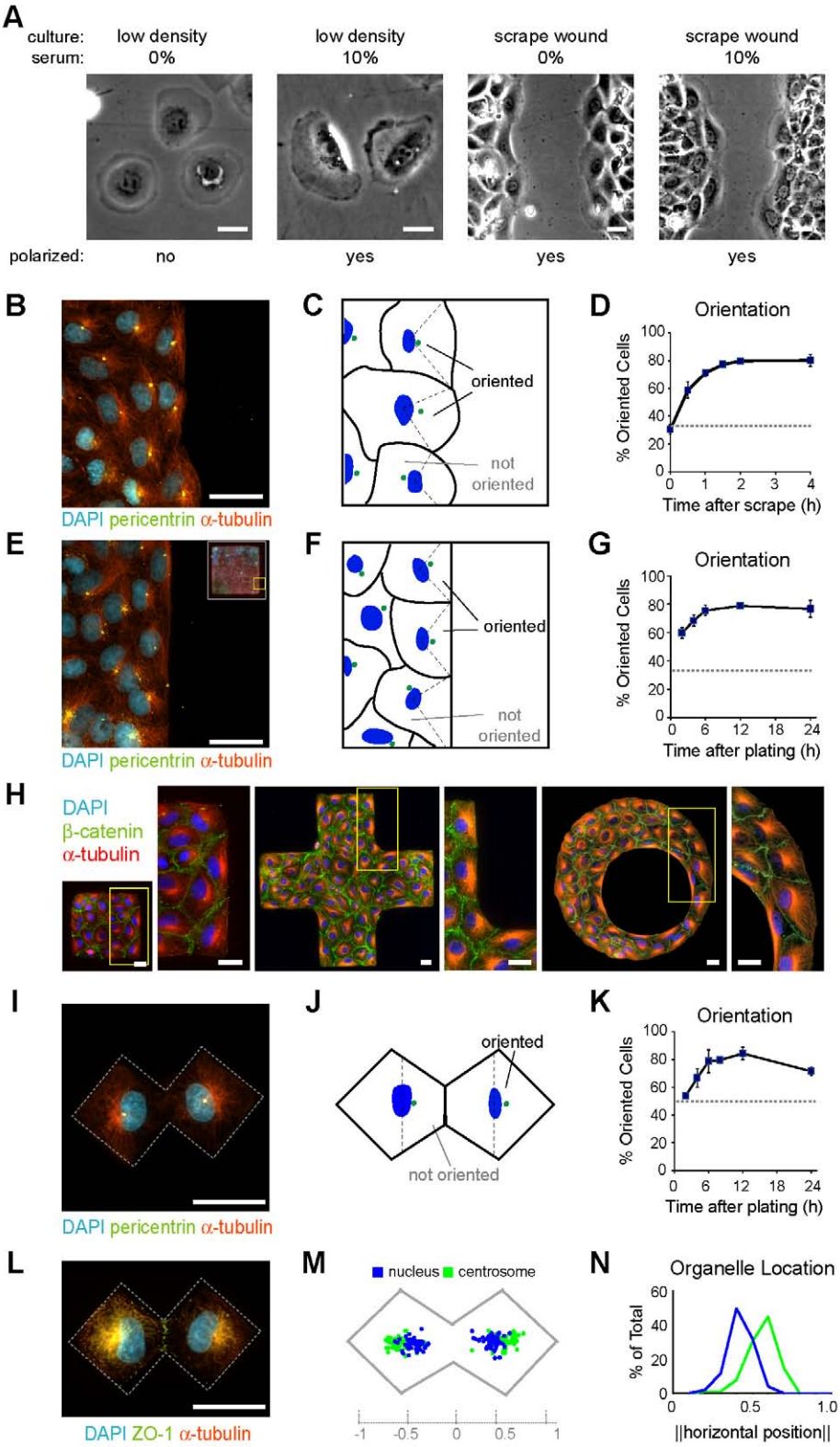

Fig. 1. Cell-cell contact polarizes the cell-motility apparatus. (A) Cells seeded in 10% serum at subconfluence or confluence were switched to 0% or 10% serum-containing media and imaged 33 hours later (5 hours after the scrape wound). (B) Cells 4 hours after scrape wounding. (C) Edge cells were scored as being oriented if their centrosome was distal from cell-cell contacts relative to the nucleus. (D) Kinetics of orientation. Broken line indicates random (33%) orientation; t=0 corresponds to time of scrape wounding. (E) Cells in a patterned monolayer 24 hours after plating. Inset is a 250,000 μm^2 patterned monolayer and shows the region of the main panel. (F) Edge orientation as in C. (G) Kinetics of orientation as in D, except that t=0 corresponds to cell plating. (H) Cells on patterns 24 hours after plating. Boxed areas are shown at higher magnification on the right of each panel. (I) Cells in a paired configuration 24 hours after plating. (J) Orientation as in C. (K) Kinetics of orientation as in D, except that t=0 corresponds to cell plating and the broken line indicates random (50%) orientation. (L) Cells in a paired configuration 8 hours after plating. (M) Pattern (gray), nuclear (blue) and centrosomal (green) positions were measured 12 hours after plating from micrographs of 50 patterns from three experiments. (N) Frequency histogram of organelle locations. In D, G and K, means ± s.e.m. are from at least three independent experiments. Scale bars: 25 μm.

wound (Fig. 1E-G). To explore preliminarily whether monolayer geometry might guide polarity, we examined multicellular patterns for which predicted forces vary along the edge (Fig. 1H) (Nelson et al., 2005). We observed similar high levels of orientation regardless of monolayer shape or size, suggesting that geometry did not contribute to edge-cell polarity.

We reasoned that asymmetric cell-cell contacts experienced by cells at the edges of scrape wounds and patterns might underlie their orientation. To test this idea, we plated cells into agarose microwells (Nelson et al., 2007), such that two cells land within a microwell, spread against the substrate and form a cell-cell contact between them (Fig. 1I,L). In this setting, protrusion and adhesion beyond the pattern are prevented. Soon (2 hours) after plating, few cells had made contact, but those that had were randomly oriented (Fig. 1J,K). However, 6 hours after plating, 80% of cells were oriented, similar to the level observed in scrape-wounded and patterned monolayers, both in the presence or absence of serum (supplementary material Fig. S2). Orientation was reminiscent of what occurs following mitosis, in which daughter cells polarize and respread away from the cytokinetic furrow (Robbins and Gonatas, 1964). However, cells that were inhibited from proliferating with mitomycin C still polarized (supplementary material Fig. S3), indicating that mitosis did not drive orientation here.

Measurements revealed that 12 hours after plating, the nucleus was located near the cell-cell contact, whereas the centrosome was oriented to the distal side of the nucleus (Fig. 1M,N), accounting for polarity. The disruption of microtubules with nocodozole (10 μM) led to mislocalization of the centrosome (supplementary material Fig. S4), which is consistent with the results from studies that demonstrated the importance of microtubules in centrosome centration (Vallee and Stehman, 2005; Gomes et al., 2005). Together, these data suggest that cell-cell contact is important in directing cell polarization.

Although nuclear and centrosomal positions are associated with directional ruffling and migration (Ridley et al., 2003; Gotlieb et al., 1981), such ruffling is prevented in agarose microwells. To examine ruffling activity when the nucleus and centrosome were polarized, we patterned cells on a flat surface that enabled membrane protrusion (Fig. 2A; supplementary material Movie 1). Kymographs from time-lapse recordings suggested that membrane ruffling was present distal, but not proximal, to the cell-cell contact. To report the extent and location of ruffling, we summed the absolute difference images between all successive frames of time-lapse recordings. The resulting 'activity map' revealed higher activity distal compared with proximal to the cell-cell contact (Fig. 2B), indicating that membrane ruffling was also polarized.

Together with the organelle analysis, these data suggested that cells were 'primed' for directed movement away from cell-cell contacts. To test this directly, we used electrodesorption (Jiang et al., 2003) to release cell patterns, allowing migration. Oriented cells that were released from patterns migrated away from each other in a directed fashion (Fig. 2C; supplementary material Movie 2). Collectively, these data implicate a role for cell-cell contact in initiating polarized cell structure, ruffling and migration.

E-cadherin at cell-cell contacts mediates polarity

Because cell-cell contact correlated with polarization of the migration apparatus, we turned to identifying the adhesion receptor(s) that are responsible. Removing Ca^{2+} from the media after cells had polarized abolished orientation (Fig. 3A), suggesting that Ca^{2+}-dependent cell-cell adhesion was required for polarity. E-cadherin is a crucial receptor in organizing epithelial cell-cell adhesions and associated cytoskeletal structures (Scott et al., 2006; Yamada and Nelson, 2007; Adams et al., 1998), and its homophilic binding is Ca^{2+}-dependent. To directly modulate E-cadherin binding, we infected cells with adenovirus harboring either GFP (Ad-GFP) or a truncated, dominant-negative mutant of E-cadherin (Ad-EΔ) prior to plating in patterns. Ad-GFP- but not Ad-EΔ-infected cells displayed typical E-cadherin localization to the cell-cell contact, and polarized in both the presence or absence of serum (Fig. 3B,C; supplementary material Fig. S5), indicating that E-cadherin engagement is required for the establishment of polarity. To determine whether E-cadherin engagement was required to also maintain polarity, we waited until cells polarized in the patterns (6 hours), and then infected them with either Ad-EΔ or Ad-GFP. Oriented cells that were infected with Ad-EΔ subsequently randomized their orientation (supplementary material Fig. S6), demonstrating that E-cadherin is required for both the establishment

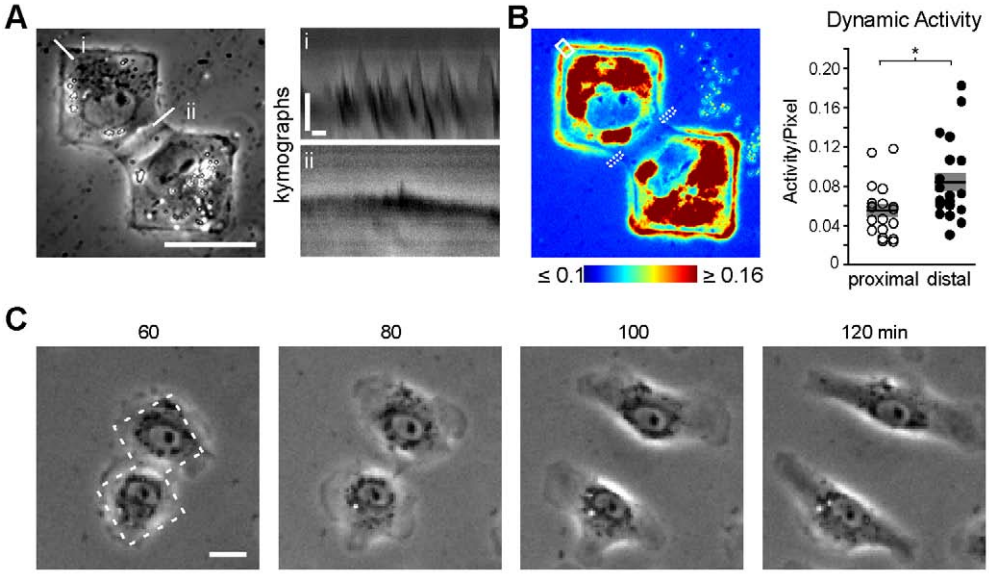

Fig. 2. Cell-cell contact polarizes cell dynamics. (A) Representative image and kymographs of cell membranes that are distal (i) or proximal (ii) to the cell-cell contact from a 15-minute time-series. In the kymographs, vertical and horizontal bars are 10 μm and 60 seconds, respectively. (B, left) Activity map of the cell pair in A. (B, right) Dynamic activity per pixel of membrane that is proximal (dashed boxes in the left image) or distal (solid box in the left image) to the cell-cell contact for 20 cells. Means (horizontal bars) ± s.e.m. (gray boxes) are indicated (*P=0.0112; Student's t-test). (C) Representative time series of a pair of cells after release (time indicated) from patterned constraints (broken lines) 20 hours after plating. Scale bars: 25 μm unless noted.

and maintenance of polarity. Antagonizing E-cadherin also contributed to loss of polarized membrane ruffling on patterned surfaces (Fig. 3D,E; supplementary material Movies 3 and 4). These findings suggest that E-cadherin engagement at the cell-cell contact is required to trigger polarization of the cell-migration machinery on patterned substrates.

The contribution of E-cadherin engagement to polarization and migration induced by scrape wounding remained unclear. Cells lacking E-cadherin engagement (supplementary material Fig. S7) were still able to develop lamellipodial extensions and migrate directionally to close an experimental scrape wound at a rate that was comparable to their control counterparts (Fig. 3F). Surprisingly, even in the presence of this directional extension, new adhesion and motility, Ad-EΔ-infected cells exhibited random centrosomal-nuclear polarity during wound closure (Fig. 3G). These data suggested that cells might principally use cell-cell contact to guide polarization of the nuclear-centrosomal axis.

Polarity that is triggered by cell-cell contact requires the actin cytoskeleton and Cdc42 activity

The actin cytoskeleton is required for scrape-wound-induced reorientation (Gomes et al., 2005). To test whether the actin

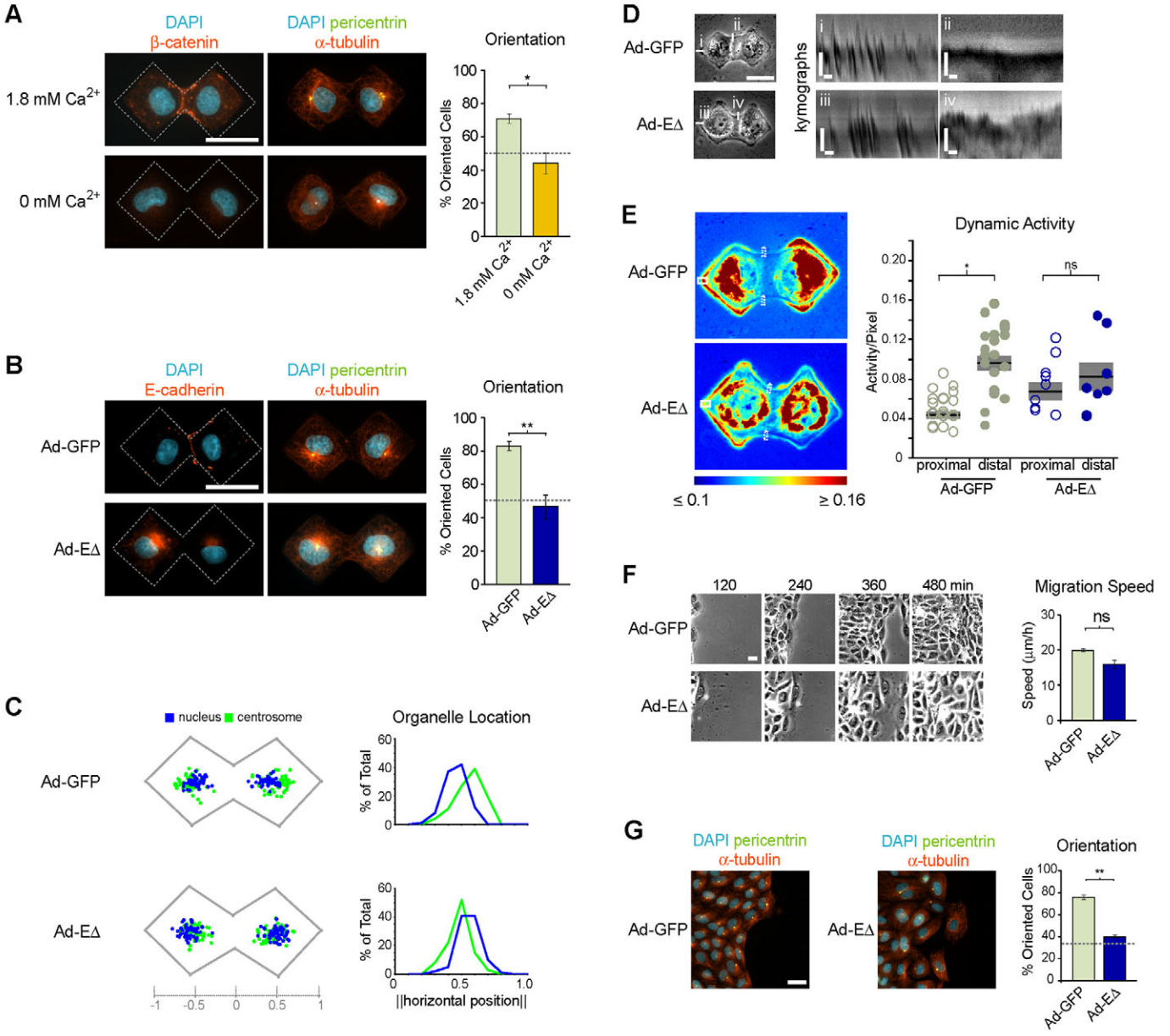

Fig. 3. E-cadherin is required for cell-cell-contact-induced polarity. (A) Cells were switched to minimum essential medium modified for suspension cultures (SMEM) containing 1.8 or 0 mM Ca^{2+} at 4 hours, and fixed at 24 hours, after plating; processed as in Fig. 1I-K (*P=0.018; Student's t-test). (B,C) Cells infected with Ad-GFP or Ad-EΔ at least 6 hours prior to, and fixed 24 after, plating; processed as in Fig. 1I-N (**P=0.0032; Student's t-test). (D) Representative images and kymographs from a 15-minute time series. In the kymographs, vertical and horizontal bars are 10 μm and 60 seconds, respectively. (E) Activity maps of the cell pairs in D, and dynamic activity per pixel of membrane that is proximal (dotted boxes) or distal (solid box) to the cell-cell contact for 20 cells (Ad-GFP, green) and nine cells (Ad-EΔ, blue). Means ± s.e.m. are indicated (*P<0.05; ns, P>0.05; ANOVA and Tukey's HSD). (F,G) Cells were infected at least 24 hours prior to wounding and imaged subsequently (F) or immunolabeled and scored 4 hours after wounding (G) (ns, P=0.45; **P=0.0031; Student's t-test). The broken line indicates random orientation, and means ± s.e.m. are from at least three independent experiments. Scale bars: 25 μm unless noted.

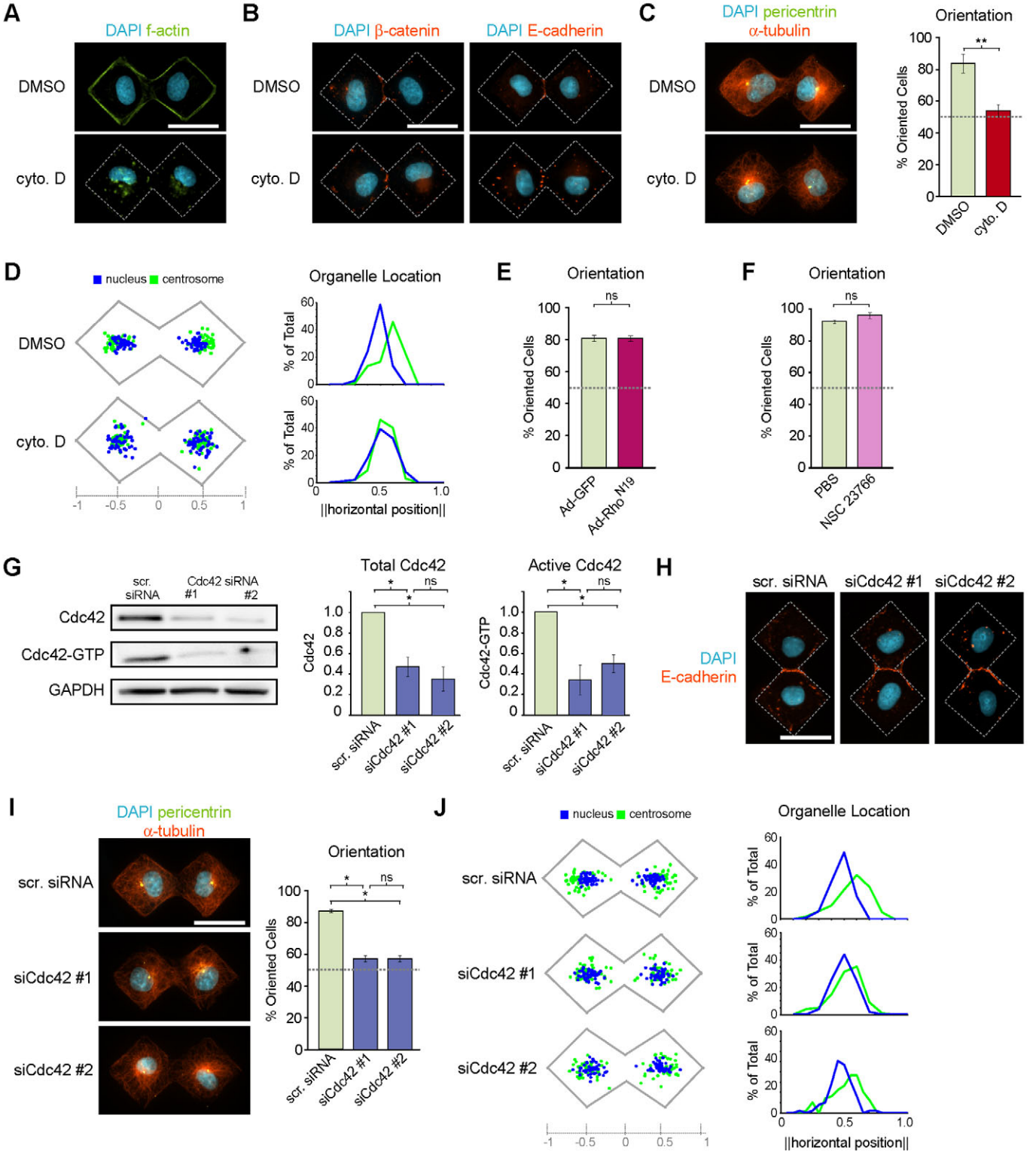

Fig. 4. Polarity triggered by cell-cell contact requires the actin cytoskeleton and Cdc42. (A-D) G0-synchronized cells were treated with 1% DMSO or 1 μM cytochalasin D (cyto. D) at 3 hours, and fixed at 8 hours, after plating; processed as in Fig. 1I-N (**P=0.0072; Student's t-test). (E) Cells were infected with Ad-GFP or Ad-Rho^{N19} at least 12 hours prior to, and fixed 24 hours after, plating; processed as in Fig. 1I-K (ns, P=1; Student's t-test). (F) Cells were handled as in A, except that they were treated with PBS or 50 μM NSC23766 (ns, P=0.17; Student's t-test). (G) Representative immunoblot and quantification of total and GTP-bound Cdc42, measured via pull-down assay from cells transfected with 10 nM siRNA (*P<0.05; ns, P>0.05; ANOVA and Tukey's HSD). (H-J) siRNA-transfected cells fixed 8 hours after plating and processed as in Fig. 1I-N (*P<0.05; ns, P>0.05; ANOVA and Tukey's HSD). Broken lines in C, E, F and I indicate random orientation, and means ± s.e.m. are from at least three independent experiments. Scale bars: 25 μm.

cytoskeleton was involved also in cell-cell-contact-induced polarity, we disrupted actin filaments with cytochalasin D (1 μM) (Fig. 4A). Although this manipulation did not prevent the accumulation of β-catenin and E-cadherin at the cell-cell contact (Fig. 4B), it substantially disrupted polarity in both the presence or absence of serum (Fig. 4C; supplementary material Fig. S8). In particular, shifts in both nuclear and centrosomal position accounted for randomized orientation in cells that had a compromised actin cytoskeleton (Fig. 4D).

The Rho family of GTPases – RhoA, Rac1 and Cdc42 – are central mediators of actin-cytoskeleton reorganization (Ridley and Hall, 1992; Ridley et al., 1992; Nobes and Hall, 1999), and have been linked to cadherin-mediated cell-cell adhesion (Braga and Yap, 2005). Inhibiting RhoA signaling via adenoviral-based delivery of the dominant-negative mutant Rho^{N19}, the ROCK inhibitor Y27632 (50 μM) or the myosin-II inhibitor blebbistatin (25-100 μM) all attenuated actomyosin-based stress fibers (data not shown), but had no impact on cell-cell contact-induced polarity (Fig. 4E; supplementary material Fig. S9). Likewise, antagonizing Rac1 signaling by treating cells with NSC23766 (50 μM) abrogated lamellipodial formation (data not shown), but had no impact on polarity (Fig. 4F). Taken together, these results demonstrate that RhoA and Rac1 signaling are dispensable for polarity induced by cell-cell contact.

Cdc42 is known to be essential for polarity in numerous settings (Etienne-Manneville, 2004) and can be activated by E-cadherin-mediated cell-cell adhesion (Kim et al., 2000; Noren et al., 2001). To test whether Cdc42 is involved also in polarity that is induced by cell-cell contact, we antagonized Cdc42 signaling via short interfering RNA (siRNA). We observed significant inhibition of Cdc42 expression and activity, but no attenuation of the cell-cell contact, relative to scrambled siRNA (Fig. 4G,H). Cells with antagonized Cdc42 signaling in microwells failed to orient in the presence or absence of serum (Fig. 4I; supplementary material Fig. S10). Similar to disruption of the actin cytoskeleton, shifts in both nuclear and centrosomal position accounted for this loss in polarity (Fig. 4J). Taken together, these data suggest that the polarizing signal that is triggered by cell-cell adhesion involves E-cadherin, the actin cytoskeleton and Cdc42.

It has been thought that the primary orientation signal at the edges of scrape-wounded monolayers arises from new cell-matrix adhesions as cells extend into the woundbed (Etienne-Manneville and Hall, 2001). Here, we show that asymmetric, E-cadherin-mediated cell-cell contact at wound edges is required to orient cells, even on patterned substrates that prevent cell-matrix protrusions beyond the pattern. These findings are consistent with prior reports in which scrape-induced polarization occurs prior to significant protrusion formation (Gomes et al., 2005). Interestingly, our studies show that cells with compromised cell-cell contacts have disrupted polarization axes, but nonetheless extend lamellipodia and close wounds with similar efficiency as their control counterparts. These data suggest that cell-cell contact is required for polarization of the nuclear-centrosomal axis, but additional signals such as new cell-ECM adhesion are required for fully directed migration. This decoupling of polarization of various intracellular structures highlights the complexity by which cells probe asymmetries in their extracellular environment to produce coordinated responses, and motivates a more detailed examination of the separate effects of cell-cell and cell-matrix cues.

Previous studies have identified a role for Cdc42 in mediating cell-ECM-induced polarization of actin (Etienne-Manneville and Hall, 2001; Etienne-Manneville, 2004; Thery et al., 2006). Our data indicate that cell-cell contact also requires Cdc42 activity and the actin cytoskeleton to polarize cells. Interestingly, whereas some components of the polarizing machinery are shared (actin, Cdc42), others are not; serum and myosin II are required in some scrape-induced polarity models (Palazzo et al., 2001; Gomes et al., 2005) but not here in cell-cell contact-induced polarity. It is therefore not unreasonable to suggest that motile cells might integrate directional signals from multiple sources, including soluble factors, cell-ECM adhesion and cell-cell adhesion, in order to direct migration. Although additional studies will be required to elucidate the interaction of these and other signals in mediating polarity, it is clear that cells have developed mechanisms to respond to asymmetries in cell-cell adhesions, such as those occurring during morphogenesis and wound healing.

Materials and Methods

Cell culture and reagents

NRK-52E cells (ATCC) were cultured in 10% fetal bovine serum (Gibco) in DMEM (Gibco) unless otherwise indicated. Cells were G0-synchronized as previously described (Liu et al., 2006). Reagents included: 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma), anti-pericentrin (Covance), anti-α-tubulin (Sigma), anti-β-catenin (Santa Cruz Biotechnology), anti-ZO-1 (Zymed), anti-Cdc42 (BD), anti-E-cadherin (BD), phalloidin–Alexa-Fluor-488 (Invitrogen), mitomycin C (Sigma), blebbistatin (EMD), Y27632 (Tocris), NSC23766 (Calbiochem), scrambled (5′-UAACGACGCGACGACGUAAtt-3′) and Cdc42 (5′-GACUACGACCG-UUAAGUUAtt; CAACUGUUUUUGACAACUAtt-3′) siRNA (Ambion), OptiMEM (Gibco), and Lipofectamine 2000 (Invitrogen). Adenoviral vectors harboring mutant E-cadherin or Rho bicistronic to GFP were prepared as previously described (Liu et al., 2006; Nelson and Chen, 2003).

Micropatterned substrates

Surface and microwell patterns were generated via microcontact printing and agarose patterning as previously described (Tan et al., 2004; Nelson et al., 2007). Dynamically adhesive substrates were generated using self-assembled monolayers of $HS(CH_2)_{15}CH_3$ (Sigma) and $HS(CH_2)_{11}(OCH_2CH_2)_3OH$ (EG_3; ProChimia) on gold (Jiang et al., 2003). A potentiostat (Gamry Instruments) was used to desorb EG_3 and thereby permit cell migration.

Immunofluorescence

Cells were fixed in ice-cold 1:1 methanol:acetone for 10 minutes at –20°C (for E-cadherin), or pre-warmed 2% paraformaldehyde in microtubule-stabilizing buffer [1 mM EGTA, 1 mM $MgSO_4$, 4% (w/v) poly(ethylene glycol) 8000 and 1% (v/v) Triton X-100 in 0.1 M PIPES, pH 6.75] for 10 minutes at 37°C (for α-tubulin, β-catenin, pericentrin, ZO-1 and phalloidin), and labeled with antibodies in 10% goat serum.

Cdc42 activity assay

GTP-loaded Cdc42 was measured using a kit (Upstate) as previously described (Liu et al., 2006). Total and GTP-loaded Cdc42 were normalized to α-tubulin or GAPDH and quantified relative to control.

We thank D. M. Cohen and N. J. Sniadecki for helpful discussions, and M. W. Wozniak, C. J. Shen and J. D. Baranski for careful reading of the manuscript. This work was supported by NIH grants EB00262 and HL73305. R.A.D. and W.F.L. acknowledge the National Science Foundation, and S.R. acknowledges the Whitaker Foundation, for financial support. R.A.D. dedicates his contributions to the project to Anand Narain Mathur. Deposited in PMC for release after 12 months.

References

Adams, C. L., Chen, Y. T., Smith, S. J. and Nelson, W. J. (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.

Braga, V. M. and Yap, A. S. (2005). The challenges of abundance: epithelial junctions and small GTPase signalling. *Curr. Opin. Cell Biol.* **17**, 466-474.

Chu, Y. S., Thomas, W. A., Eder, O., Pincet, F., Perez, E., Thiery, J. P. and Dufour, S. (2004). Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. *J. Cell Biol.* **167**, 1183-1194.

Drees, F., Pokutta, S., Yamada, S., Nelson, W. J. and Weis, W. I. (2005). Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* **123**, 903-915.

Ehrlich, J. S., Hansen, M. D. and Nelson, W. J. (2002). Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. *Dev. Cell* **3**, 259-270.
Etienne-Manneville, S. (2004). Cdc42-the centre of polarity. *J. Cell Sci.* **117**, 1291-1300.
Etienne-Manneville, S. and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* **106**, 489-498.
Gomes, E. R., Jani, S. and Gundersen, G. G. (2005). Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* **121**, 451-463.
Gotlieb, A. I., May, L. M., Subrahmanyan, L. and Kalnins, V. I. (1981). Distribution of microtubule organizing centers in migrating sheets of endothelial cells. *J. Cell Biol.* **91**, 589-594.
Jiang, X., Ferrigno, R., Mrksich, M. and Whitesides, G. M. (2003). Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements. *J. Am. Chem. Soc.* **125**, 2366-2367.
Kim, S. H., Li, Z. and Sacks, D. B. (2000). E-cadherin-mediated cell-cell attachment activates Cdc42. *J. Biol. Chem.* **275**, 36999-37005.
Kovacs, E. M., Goodwin, M., Ali, R. G., Paterson, A. D. and Yap, A. S. (2002). 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.
Kupfer, A., Louvard, D. and Singer, S. J. (1982). Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. *Proc. Natl. Acad. Sci. USA* **79**, 2603-2607.
Kupfer, A., Dennert, G. and Singer, S. J. (1983). Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc. Natl. Acad. Sci. USA* **80**, 7224-7228.
Lauffenburger, D. A. and Horwitz, A. F. (1996). Cell migration: a physically integrated molecular process. *Cell* **84**, 359-369.
Liu, W. F., Nelson, C. M., Pirone, D. M. and Chen, C. S. (2006). E-cadherin engagement stimulates proliferation via Rac1. *J. Cell Biol.* **173**, 431-441.
Nejsum, L. N. and Nelson, W. J. (2007). A molecular mechanism directly linking E-cadherin adhesion to initiation of epithelial cell surface polarity. *J. Cell Biol.* **178**, 323-335.
Nelson, C. M. and Chen, C. S. (2003). VE-cadherin simultaneously stimulates and inhibits cell proliferation by altering cytoskeletal structure and tension. *J. Cell Sci.* **116**, 3571-3581.
Nelson, C. M., Jean, R. P., Tan, J. L., Liu, W. F., Sniadecki, N. J., Spector, A. A. and Chen, C. S. (2005). Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl. Acad. Sci. USA* **102**, 11594-11599.
Nelson, C. M., Liu, W. F. and Chen, C. S. (2007). Manipulation of cell-cell adhesion using bowtie-shaped microwells. *Methods Mol. Biol.* **370**, 1-10.
Nobes, C. D. and Hall, A. (1999). Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J. Cell Biol.* **144**, 1235-1244.
Noren, N. K., Niessen, C. M., Gumbiner, B. M. and Burridge, K. (2001). Cadherin engagement regulates Rho family GTPases. *J. Biol. Chem.* **276**, 33305-33308.
Palazzo, A. F., Joseph, H. L., Chen, Y. J., Dujardin, D. L., Alberts, A. S., Pfister, K. K., Vallee, R. B. and Gundersen, G. G. (2001). Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr. Biol.* **11**, 1536-1541.
Ridley, A. J. and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70**, 389-399.
Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401-410.
Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T. and Horwitz, A. R. (2003). Cell migration: integrating signals from front to back. *Science* **302**, 1704-1709.
Robbins, E. and Gonatas, N. K. (1964). The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**, 429-463.
Schlessinger, K., McManus, E. J. and Hall, A. (2007). Cdc42 and noncanonical Wnt signal transduction pathways cooperate to promote cell polarity. *J. Cell Biol.* **178**, 355-361.
Scott, J. A., Shewan, A. M., den Elzen, N. R., Loureiro, J. J., Gertler, F. B. and Yap, A. S. (2006). Ena/VASP proteins can regulate distinct modes of actin organization at cadherin-adhesive contacts. *Mol. Biol. Cell* **17**, 1085-1095.
Shen, Y., Li, N., Wu, S., Zhou, Y., Shan, Y., Zhang, Q., Ding, C., Yuan, Q., Zhao, F., Zeng, R. et al. (2008). Nudel binds Cdc42GAP to modulate Cdc42 activity at the leading edge of migrating cells. *Dev. Cell* **14**, 342-353.
Tan, J. L., Liu, W., Nelson, C. M., Raghavan, S. and Chen, C. S. (2004). Simple approach to micropattern cells on common culture substrates by tuning substrate wettability. *Tissue Eng.* **10**, 865-872.
Thery, M., Racine, V., Piel, M., Pepin, A., Dimitrov, A., Chen, Y., Sibarita, J. B. and Bornens, M. (2006). Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. USA* **103**, 19771-19776.
Tzima, E., Kiosses, W. B., del Pozo, M. A. and Schwartz, M. A. (2003). Localized cdc42 activation, detected using a novel assay, mediates microtubule organizing center positioning in endothelial cells in response to fluid shear stress. *J. Biol. Chem.* **278**, 31020-31023.
Vaezi, A., Bauer, C., Vasioukhin, V. and Fuchs, E. (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.
Vallee, R. B. and Stehman, S. A. (2005). How dynein helps the cell find its center: a servomechanical model. *Trends Cell Biol.* **15**, 288-294.
Wang, A. Z., Ojakian, G. K. and Nelson, W. J. (1990). Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. *J. Cell Sci.* **95**, 137-151.
Watanabe, T., Wang, S., Noritake, J., Sato, K., Fukata, M., Takefuji, M., Nakagawa, M., Izumi, N., Akiyama, T. and Kaibuchi, K. (2004). Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration. *Dev. Cell* **7**, 871-883.
Wittmann, T. and Waterman-Storer, C. M. (2001). Cell motility: can Rho GTPases and microtubules point the way? *J. Cell Sci.* **114**, 3795-3803.
Yamada, S. and Nelson, W. J. (2007). Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. *J. Cell Biol.* **178**, 517-527.

Journal of Cell Science