Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II–dependent manner

Quint le Duc,1 Quanming Shi,2 Iris Blonk,1 Arnoud Sonnenberg,5 Ning Wang,3 Deborah Leckband,2,4 and Johan de Rooij1

1Hubrecht Institute, University Medical Centre Utrecht, 3584 CT Utrecht, Netherlands
2Department of Chemical and Biomolecular Engineering, 3Department of Mechanical Science and Engineering, and 4Department of Chemistry, University of Illinois at Urbana-Champaign, Champaign, IL 61801
5Division of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, Netherlands

Cell surface receptors integrate chemical and mechanical cues to regulate a wide range of biological processes. Integrin complexes are the mechanotransducers between the extracellular matrix and the actomyosin cytoskeleton. By analogy, cadherin complexes also function as mechanosensors at cell–cell junctions, but this capacity of cadherins has not been directly demonstrated. Furthermore, the molecular composition of the link between E-cadherin and actin, which is needed to sustain such a function, is unresolved. In this study, we describe nanomechanical measurements demonstrating that E-cadherin complexes are functional mechanosensors that transmit force between F-actin and E-cadherin. Imaging experiments reveal that intercellular forces coincide with vinculin accumulation at actin-anchored cadherin adhesions, and nanomechanical measurements show that vinculin potentiates the E-cadherin mechanosensory response. These investigations directly demonstrate the mechanosensory capacity of the E-cadherin complex and identify a novel function for vinculin at cell–cell junctions. These findings have implications for barrier function, morphogenesis, cell migration, and invasion and may extend to all soft tissues in which classical cadherins regulate cell–cell adhesion.

Introduction

Cell surface receptors integrate both chemical and mechanical cues to regulate biological processes as diverse as differentiation, vascular development, tumor growth, and malignancy (Bershadsky et al., 2003; Discher et al., 2005; Vogel and Sheetz, 2006; Lecuit and Lenne, 2007; Kumar and Weaver, 2009). Integrin-based adhesion complexes are sensors of force between the ECM and the contractile actomyosin cytoskeleton (Bershadsky et al., 2003). Direct assays for integrin mechanosensing apply external force to ligand-coated beads bound to the cell surface and determine force-dependent reinforcement (or force-induced cell stiffening) from the reduction in bead displacement upon prolonged application of force (Wang et al., 1993; Choquet et al., 1997). Talin and vinculin are strongly implicated in this mechanoresponse (Giannone et al., 2003). In vitro force-dependent unfolding of talin opens up binding sites for vinculin (del Rio et al., 2009). In intact cells, recruitment of vinculin to tensile focal adhesions (FAs) is mediated by paxillin rather than direct talin binding (Pasapera et al., 2010). Overexpressing vinculin deletion mutants uncouples integrin-mediated adhesion from its regulation by cytoskeletal force (Humphries et al., 2007), and the absence of vinculin reduces cell stiffness (Mierke et al., 2008).

During morphogenesis, contractile forces at intercellular junctions direct cell patterning, drive convergence and extension.
E-cadherin–mediated mechanosensing and localizes to tension-bearing sites in cell–cell junctions to mediate mechanoregulation of cell–cell adhesion.

Results and discussion

The E-cadherin complex is a mechanosensor. We used magnetic twisting cytometry (MTC; Fig. 1 A; Wang and Ingber, 1995) to test whether mechanical stimulation affects the viscoelastic properties of junctions between F9 cells and Fc–E-cadherin–coated beads. All measurements were performed after 20 min of bead–cell contact to resolve force-dependent from force-independent changes in stiffening, which plateaus after \( \sim 15 \) min (see Fig. 4 B). During continuous shear modulation, bead displacement amplitude decreased with the forcing time (Fig. 1 A), amounting to a force-actuated 70% increase in stiffness, which is relative to previously unperturbed cells (Fig. 1 B). Latrunculin B, cytochalasin D, and blebbistatin strongly affected this response, demonstrating that cadherin force transmission requires a direct mechanical link to an organized and contractile actin cytoskeleton.

In this study, we present direct evidence that the E-cadherin complex is a mechanosensor that probes the mechanical environment to elicit a proportional change in the mechanics of the junctions. Furthermore, we show that vinculin potentiates E-cadherin–mediated mechanosensing and localizes to tension-bearing sites in cell–cell junctions to mediate mechanoregulation of cell–cell adhesion.

**Figure 1.** E-cadherin is a mechanosensor. (A) Continuous driving field modulation at 0.3 Hz for 60 s (2.4 Pa stress) and representative time course of the displacement of two E-cadherin– and poly-lysine (PL)–coated beads. (B) The force-induced stiffening of Fc–E-cadherin–coated beads relative to unperturbed bead–cell contacts in the absence (E-cad) or presence of latrunculin B (Lat B), cytochalasin D (Cyto D), or blebbistatin. (C) Fc–E-cadherin–coated beads versus beads coated with monoclonal E-cadherin antibody. (D) Fc–E-cadherin–coated beads in the absence or presence of 3 mM EGTA added just before MTC or blocking anti–E-cadherin antibody versus beads coated with poly-lysine. (E) The percent change in E-cadherin junction stiffness relative to unperturbed cells as a function of applied shear stress is shown. After 20 min of bead–cell contact, the beads were subjected to a modulated 0.3-Hz magnetic field for 60 s. The elastic shear modulus was determined at 50 s as a function of the amplitude of the applied shear stress. [B–E] Each data point represents >300 beads. Error bars represent SD.
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Cell–cell junctions (see Fig. 5 A; de Rooij et al., 2005). As shown in Fig. 2 A and Fig. S1, E-cadherin, α-, β-, p120-catenin, and EPLIN (Abe and Takeichi, 2008) are localized at similar levels in steady-state and HGF-stimulated cell–cell adhesions. Interestingly, vinculin scarcely localizes to steady-state cell–cell adhesions, whereas its colocalization with α-catenin is much more evident in junctions after HGF (Fig. 2 A). Treatment with blebbistatin for 10 min largely abolished vinculin localization to cell–cell junctions, suggesting that this localization is indeed tension dependent. We conclude that vinculin is recruited to cell–cell junctions in a myosin II–dependent manner.

Closer analysis of Fig. 2 B shows that vinculin does not colocalize with the E-cadherin complex all over cell–cell junctions but is restricted to those sites in which junctions contact F-actin bundles and, thus, likely experience increased tension. Moreover, dual-color imaging of GFP-vinculin and mCherry–p120-catenin shows that cell spreading induced by HGF is followed by an increase in contraction, which marks the onset of vinculin accumulation at discrete sites within cell–cell junctions (Video 1). Finally, Fig. 2 C and Video 2 show that EGFP-vinculin, but not mCherry–p120-catenin, rapidly disappears from cell–cell junctions when tension is relieved by inhibitors. Upon inhibitor washout, tension is restored concomitant with a relocalization of vinculin to cell–cell junctions (Fig. 2 C and Video 2). Quantification

Final, the relative stiffness increase at cadherin junctions increases with the applied bond shear up to a limiting plateau at stresses >2 Pa (Fig. 1 E). This increase in junction stiffness in proportion to the applied stress ultimately confirms that E-cadherin complexes are bona fide mechanosensors. Although regulation of E-cadherin complexes by contractile force is suggested by the myosin II requirement, direct evidence for force-dependent reinforcement of E-cadherin adhesions had not been presented to our knowledge. Thus, our data show for the first time that the E-cadherin complex is a mechanosensor.

Myosin-dependent recruitment of vinculin to active cell–cell junctions

We investigated the localization of putative cadherin–actin linker proteins after HGF stimulation, which increases tension on cell–cell junctions (see Fig. 5 A; de Rooij et al., 2005). As shown in Fig. 2 A and Fig. S1, E-cadherin, α-, β-, p120-catenin, and EPLIN (Abe and Takeichi, 2008) are localized at similar levels in steady-state and HGF-stimulated cell–cell adhesions. Interestingly, vinculin scarcely localizes to steady-state cell–cell adhesions, whereas its colocalization with α-catenin is much more evident in junctions after HGF (Fig. 2 A). Treatment with blebbistatin for 10 min largely abolished vinculin localization to cell–cell junctions, suggesting that this localization is indeed tension dependent. We conclude that vinculin is recruited to cell–cell junctions in a myosin II–dependent manner.

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adhesions contain E-cadherin (Fig. 3 B) and α-, β-, and p120-catenin (Fig. S2, A and F) but not proteins found at other types of cell–cell junctions (nectin and claudin; Fig. S2 F) or FAs (paxillin; Fig. 3 B). Furthermore, they depend on actomyosin activity and calcium (Fig. S2, B and C) and are insensitive to blocking anti-β1- and α6-integrins, which mediate basal ECM adhesion in MDCK cells (Fig. S2, D and E). The fact that vinculin strongly localizes to E-cadherin–COMP adhesions (Fig. 3 B) leads to the conclusion that vinculin is recruited to the E-cadherin complex that forms a direct and functional interaction with actomyosin in cells on E-cadherin–COMP.

In immunoprecipitation (IP) after reversible chemical cross-linking followed by boiling and trituration of insoluble material that includes the cell–cell junctions (Hinck et al., 1994), endogenous vinculin precipitates with GFP–E-cadherin, and endogenous E-cadherin precipitates with GFP-vinculin (Fig. 3 C). Compared with total protein levels, β-catenin more efficiently precipitates with E-cadherin, whereas paxillin more efficiently precipitates with vinculin. We could not reproduce the efficient co-IP of vinculin and E-cadherin observed in other cell lines (Hazan et al., 1997; Maddugoda et al., 2007; Peng et al., 2010). Nevertheless, the inefficient co-IP of vinculin and E-cadherin from MDCK cells correlates well with the low abundance of vinculin in their cell–cell adhesions observed by fluorescence (Figs. 2 and 3). Thus, E-cadherin and vinculin reside in one complex, which, in MDCK cells, is not very abundant compared with other complexes in which these proteins reside. The amount of coprecipitated E-cadherin and vinculin of the EGFP-vinculin intensity shows that the loss of vinculin from cell–cell junctions upon myosin inhibition follows the same time curve as the loss of vinculin from FAs. We conclude that during activation of cell–cell adhesions by HGF, a pool of vinculin accumulates at discrete, actin-anchored sites in cell–cell junctions, which is concurrent with increased myosin II-dependent tension.

Vinculin interacts with the E-cadherin complex at cell–cell junctions

Total internal reflection fluorescence (TIRF) microscopy (Fig. 2 C and Video 2) shows that the pool of vinculin in cell–cell junctions is distinct from the vinculin in FAs. Furthermore, the FA protein paxillin does not colocalize with vinculin at cell–cell contacts after HGF (Video 3). Finally, tyrosine-118–phosphorylated (pY118) paxillin, which mediates myosin II–dependent recruitment of vinculin to FAs (Pasapera et al., 2010), shows no colocalization with the cell–cell junction pool of vinculin (Fig. 3 A). Thus, we conclude that vinculin is recruited to cell–cell junction complexes, which do not contact the basal ECM, through intermediates that are distinct from those that mediate recruitment of vinculin to FAs.

To separate E-cadherin adhesions from other cell–cell adhesion complexes, we plated MDCK cells on coverslips coated with E-cadherin–cartilage oligomerizing protein (COMP), a pentamerizing fusion of the ectodomain of E-cadherin and COMP (Tomschy et al., 1996), to induce the formation of actin-anchored E-cadherin adhesions (Fig. S1 A). These E-cadherin–COMP adhesions contain E-cadherin (Fig. 3 B) and α-, β-, and p120-catenin (Fig. S2, A and F) but not proteins found at other types of cell–cell junctions (nectin and claudin; Fig. S2 F) or FAs (paxillin; Fig. 3 B). Furthermore, they depend on actomyosin activity and calcium (Fig. S2, B and C) and are insensitive to blocking antibodies to β1- and α6-integrins, which mediate basal ECM adhesion in MDCK cells (Fig. S2, D and E). The fact that vinculin strongly localizes to E-cadherin–COMP adhesions (Fig. 3 B) leads to the conclusion that vinculin is recruited to the E-cadherin complex that forms a direct and functional interaction with actomyosin in cells on E-cadherin–COMP.

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Vinculin mediates E-cadherin mechanoregulation through interactions in its head domain (Bakolitsa et al., 2004). Whether β-catenin is the main recruiter of vinculin, as previously described in a different cell type (Peng et al., 2010), remains to be investigated.

Vinculin potentiates mechanosensing by the E-cadherin complex

Finally, we measured fluorescence resonance energy transfer (FRET) between vinculin and cell–cell junction complex members by means of acceptor photobleaching (see Materials and methods; Fig. S3). GFP-vinculin–expressing cells, stimulated for 1 h with HGF, were extracted in cytoskeleton-preserving (CSK) buffer, fixed, and stained for vinculin as donor (Alexa Fluor 488) and β-catenin or occludin as acceptor (rhodamine). Clear colocalization was found between vinculin and β-catenin at cell–cell junctions, whereas vinculin and occludin colocalized less often. FRET was measured in regions of colocalization. Higher FRET values were found for vinculin and β-catenin compared with vinculin and occludin (Fig. 3 D, left). This difference does not reflect a difference in acceptor concentration because regions were chosen with equal levels of acceptor as judged by rhodamine intensity (Fig. 3 D, right). The close proximity of vinculin to β-catenin suggests a specific association with the E-cadherin complex at cell–cell junctions.

Mutating alanine 50 to isoleucine resulted in a strong loss of vinculin’s localization to cell–cell junctions and to integrin–dependent FAs (Fig. 3 E). This indicates that vinculin associates with the E-cadherin complex through mechanistically conserved interactions in its head domain (Bakolitsa et al., 2004). Whether β-catenin is the main recruiter of vinculin, as previously described in a different cell type (Peng et al., 2010), remains to be investigated.
Vinculin mediates the recruitment of active myosin to cell–cell junctions

We have previously shown that HGF signaling increases the presence of active myosin II (phosphorylated myosin light chain [pMLC]) in areas of cell–cell adhesion (de Rooij et al., 2005). A closer inspection of Fig. 5B reveals that pMLC is present at F-actin structures that connect to p120-labeled cell–cell junctions. As we show in Fig. 2, inhibition of myosin activity results in a loss of these actin-connected, vinculin-containing junctions. Conversely, we now investigated whether a loss of vinculin would affect these contractile actomyosin structures connected to cell–cell junctions. An \( \approx 90\% \) knockdown of vinculin (Fig. 5F) resulted in a strong reduction in vinculin levels in the cytoplasm but did not completely deplete vinculin from FAs or intercellular junctions in MDCK cells. Although depletion of vinculin from cell–cell junctions was not complete, we observed a significant effect on the reorganization of junctions in response to HGF, which resulted in a strong loss of pMLC recruitment to cell–cell adhesions (Fig. 5C). Fig. 5D shows that this loss is specific for cells with a strongly reduced amount of vinculin. To quantify this, we used custom software that automatically divides the pixel intensity of the pMLC image in areas of cell–cell adhesion (Fig. 5E, \( I_{pMLC, junctions} \), green) by the pixel intensity of the pMLC signal in the cytoplasm (Fig. 5E, \( I_{pMLC, cytoplasm} \), red). As shown in Fig. 5E, there is a strong reduction of the HGF-induced recruitment of

ko cells displayed an \( \approx 50\% \) reduction in force-dependent reinforcement of cadherin junctions, which was fully restored by reconstitution with chicken vinculin (Fig. 4D). Thus, loss of vinculin significantly reduces the mechanoresponse by the E-cadherin complex.

By immunofluorescence (IF), we noticed that vinculin and F-actin are already recruited to unforced beads. For quantification, we measured the fluorescence intensity at an increasing radius from the bead center, which was normalized to the peak level for each bead so that background levels, instead of peak intensity levels, vary. As shown in Fig. 4E, the vinculin signal condenses around beads upon force. Furthermore, the intensity of vinculin (the curve area above background at 1-\( \mu \)m width around the intensity peak; Fig. 4E, gray) slightly increases upon force. Clearly, this situation is quite different from the situation at intercellular junctions, where we observed de novo recruitment of vinculin. It is possible that the vinculin levels around unforced beads represent the buildup of intrinsic contractile actomyosin around larger beads (Choquet et al., 1997). Indeed, there was no evidence of either actin or vinculin accumulation near smaller E-cadherin–coated beads (Perez et al., 2008). In conclusion, these data show that vinculin potentiates the E-cadherin mechanoresponse concurrent with its strong increase at intercellular junctions, which experience increased tension, and with a moderate increase around forced beads.
pMLC to cell–cell junctions in vinculin knockdown cells. Thus, we conclude that the recruitment of vinculin to active cell–cell junctions upon HGF is important for the remodeling of the cytoskeleton that connects to these junctions.

In conclusion, our nanomechanical experiments show that the E-cadherin complex is a bona fide mechanosensor. Furthermore, we uncover a novel role for vinculin in modulating E-cadherin–cytoskeleton mechanics and force-induced remodeling of cell–cell junctions. Because stiffness measured by MTC is a complex process, it is impossible to pinpoint the exact molecular mechanism that explains vinculin’s role in E-cadherin mechanoregulation from these experiments. It is tempting to speculate similarity to integrin adhesion, but the molecules involved in vinculin recruitment to integrin adhesions (talin and paxillin) are not present at cell–cell contacts. Moreover, the proposed integrin mechanism (vinculin recruitment to stretched talin) would predict full inhibition of mechanosensing in the absence of vinculin, whereas we measure only partial inhibition. Furthermore, our MTC measurements show a strong effect on the stress–strain relation for E-cadherin junctions (Fig. 4 C), which is not found for integrin junctions (Mierke et al., 2008). All of this indicates that the mechanism of integrin-dependent force sensing may differ from mechanosensing at E-cadherin junctions. Thus, our data uncover a novel role for vinculin in E-cadherin mechanosensing, but, as for integrins, the exact mechanism remains to be established. Our findings have broad implications because of the central role of E-cadherin in the development and maintenance of epithelial tissues. Given the similarities in structure and binding characteristics among classical cadherins, it is tempting to speculate that cadherins may constitute a new family of tension sensors. The involvement of vinculin in cadherin mechanosensing and its localization to subdomains in activated cell–cell junctions opens the door for further investigations of molecular mechanisms underlying E-cadherin mechanoregulation.

Materials and methods

Cell lines and culture

MDCK and F9 cells were routinely cultured in high glucose DME (Invitrogen) supplemented with 10% FCS (Sigma-Aldrich) and penicillin/streptomycin (Invitrogen) in standard tissue culture dishes coated with 0.1% gelatin (Sigma-Aldrich) in the case of F9. F9 ko cells were the y229 cells, and chicken vinculin-reconstituted R16 cells were described previously (Xu et al., 1998). MDCK cell lines stably expressing EGFP or mCherry-tagged constructs were generated by nucleofection (Lonza), G418 (Invitrogen) selection, and FACS sorting for intermediate levels of fluorescence. For imaging experiments, cells were grown on glass coverslips, glass-bottom dishes (WillCo Wells B.V.), or chamber coverslips (Thermo Fisher Scientific) coated with 10 µg/ml collagen type I (Sigma-Aldrich) or 20 µg/ml E-cadherin–COMP in medium supplemented with 0.5% FCS. 5 ng/ml HGF (Sigma-Aldrich) concentration was used.

Adhesion assays

Both MDCK cells and F9 cells were prepared by trypsinization from the culture dish, washed once in DME containing 10% FCS, incubated for 1 h under rotation in suspension in DME plus 10% FCS at 37°C, pelleted, and resuspended at the appropriate concentration in DME containing 0.5% FCS. MDCK adhesion assays were performed in 48-well plates coated with 20 µg/ml E-cadherin–COMP at a concentration of 100,000 cells per well. F9 cell adhesion was performed in a 96-well plates coated with 20 µg/ml E-cadherin–COMP at a concentration of 100,000 cells per well. Adhesion was allowed for 45 min followed by three steps of rigorous washing in PBS supplemented with 1 mM CaCl₂, and quantified by measuring acid phosphatase activity (de Rooij et al., 2005).

Immunocytochemistry and microscopy

For immunocytochemistry, cells were washed three times in PBS (containing 1 mM CaCl₂ in the case of growth on E-cadherin–COMP) and fixed using 4% paraformaldehyde in PBS. Alternatively, if indicated, cells were washed twice for 1 min in CSK buffer (300 mM sucrose, 0.5% TX-100, 10 mM Pipes, pH 7, 50 mM NaCl, 3 mM CaCl₂, and 2 mM MgCl₂) before fixation. Live cell imaging, E-cadherin–COMP IF, and the colocalization imaging of α-catenin and EPLIN in Fig. S2 were performed using a confocal microscope (TCS-SP2; Leica) with a 63× 1.32 NA objective lens. Acceptor photobleaching was achieved by scanning the central area of the image with a 20 times zoom using a 561 laser at full power. All widefield images, unless specifically indicated otherwise, and with the exception of the E-cadherin–COMP images in Fig. 3 B and Fig. 2 (A and F), were sharpened for display with an unsharp mask filter in ImageJ (National Institutes of Health; r = 3, weight = 0.6) and background subtracted by rolling ball (r = 40).

Quantification of fluorescence imaging

To measure the decay of vinculin from cell–cell junctions and FAs after inhibition of actomyosin contractility, we cleaned up the time-lapse image series using the unsharp mask filter and background subtraction. Regions of interest (ROIs) were drawn that encompassed two to four closely grouped FAs or a vinculin-containing area of cell–cell adhesion. The decay in each of these ROIs was corrected for bleaching and fluctuations by neighboring background ROIs and normalized between 0 (background level at the end of the curve) and 1 (mean vinculin ROI intensity before addition of inhibitors). Normalized intensities from individual ROIs were averaged per frame and displayed in Fig. 2 C.

To quantify changes in pMLC intensity at cell–cell junctions, a custom function was written in MATLAB (MathWorks) that draws a grid of 9-pixel-wide horizontal and vertical lines on the images, which were first flattened by background subtraction. These lines are spaced 170 pixels apart from the edges of the image. Thresholding was used to determine the edges of cell islands, and lines were shortened to end at least 100 pixels from these edges (on the inside of the island) or from the edge of the image (Fig. 5 E, red). Peaks in pixel intensity in the p120-catenin image were used to automatically define the location of cell–cell junctions along these lines and mark 60-pixel fragments on the lines that span peaks (Fig. 5 E, green). Pixel intensities along these line fragments in the pMLC image were divided by pixel intensities along the rest of the red lines to calculate the relative pMLC levels near cell–cell junctions.

Acceptor photobleaching (FRET)

FRET efficiencies were measured by acceptor photobleaching. Donor (GFP-vinculin) and acceptor (indicated primary with rhodamine-labeled secondary antibodies) confocal images were collected before and after photobleaching of the acceptor in a defined region of the image (Fig. S3). Laser, microscope, and scanhead settings were identical throughout these experiments, and the images were corrected for background and nonspecific bleaching during scanning. The FRET values were calculated from the mean fluorescent values from ROIs. Three to five ROIs comprising cell–cell junctions outside the bleach area were used to calculate bleaching caused by imaging, and three to five ROIs inside the bleach area were used to calculate the percent loss in acceptor fluorescence and the gain in donor fluorescence upon acceptor bleaching. The postbleach donor values were corrected for acceptor photo conversion. Photo conversion of the acceptor was measured by imaging in 20 ROIs in five independent images of cells that were stained only with acceptor antibodies (β-catenin + anti–mouse rhodamine) and determined to be 1.12 ± 0.3%. The donor fluorescence loss in the prebleach donor image was calculated by subtracting the postbleach donor image from the corrected postbleach donor image and dividing this by the fraction of acceptor bleaching to correct for incomplete bleaching (in all experiments >80%). The FRET percent was calculated by relating the donor fluorescence loss in the prebleach donor image to the total donor fluorescence (prebleach donor image plus donor fluorescence loss in the prebleach donor image).

Antibodies and DNA constructs

Mouse monoclonal vinculin antibody (hVin-1) and rabbit polyclonal α-catenin antibody were obtained from Sigma-Aldrich. Mouse monoclonal Vinculin mediates E-cadherin mechanoregulation • le Duc et al. 1113
Fc–E-cadherin was purified from the serum-free conditioned medium on a 29-G insulin needle. The sample was put at 100°C for 10 min, diluted immediately before use) per 10-cm dish. Next, cells were incubated with beads and fixed without applying force or after force application. The cells were stained with either vinculin antibody or rhodamine-conjugated phalloidin. Imaging was performed on a microscope (200M; Carl Zeiss, Inc.) using a 100× 1.3 NA objective and a cooled charge-coupled device camera. Alexa Fluor 488 phalloidin. Imaging was performed on a microscope (200M; Carl Zeiss, Inc.) using a 100× 1.3 NA objective and a cooled charge-coupled device camera (AxioCam; Carl Zeiss, Inc.) using a 100× 1.3 NA objective and a cooled charge-coupled device camera (AxioCam; Carl Zeiss, Inc.). For each cell-attached bead, the fluorescent image was divided into concentric rings centered at the bead center, and the mean fluorescent intensity was calculated at a different radius position. This fluorescent intensity profile of each bead was subtracted by the background, normalized to its maximum, and averaged for > 300 beads at approximately bead per cell. The data follow a log normal distribution from which we obtained the mean and standard deviation. The Student’s t test was used to compare measurements, with P < 0.05 indicative of a statistically significant difference.

Quantification of vinculin around Fe–E-cadherin–coated beads

To investigate the effect of force on vinculin recruitment, the cells were incubated with beads and fixed without applying force or after force application. The cells were stained with either vinculin antibody or rhodamine-conjugated phalloidin. Imaging was performed on a microscope (200M; Carl Zeiss, Inc.) using a 100× 1.3 NA objective and a cooled charge-coupled device camera (AxioCam; Carl Zeiss, Inc.). For each cell-attached bead, the fluorescent image was divided into concentric rings centered at the bead center, and the mean fluorescent intensity was calculated at a different radius position. This fluorescent intensity profile of each bead was subtracted by the background, normalized to its maximum, and averaged for ~80 beads.

Online supplemental material

Fig. S1 shows localization of E-cadherin–actin linkers after HGF. Fig. S2 shows that E-cadherin–COMP adhesions are cadherin- and myosin II-dependent structures that contain the core E-cadherin complex. Fig. S3 further explains the FRET data shown in Fig. 3 D. Video 1 shows that vinculin is recruited to p120-catenin containing cell–cell junctions after HGF. Video 2 shows that the cell–cell junction pool of vinculin is distinct from the vinculin pool at the basal FAs and that they are both localized in an actomyosin-dense manner. Video 3 shows that vinculin at cell–cell contacts does not overlap with paxillin-positive FAs. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201001149/DC1.
References

Abe, K., and M. Takeuchi. 2008. EMLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. Proc. Natl. Acad. Sci. USA. 105:13–19. doi:10.1073/pnas.0710504105

Avizienyte, E., V.J. Fincham, V.G. Brunton, and M.C. Frame. 2004. Src SH3 domain-mediated peripheral accumulation of Src and phospho-myosin is linked to deregulation of E-cadherin and the epithelial-mesenchymal transition. Mol. Biol. Cell. 15:2794–2803. doi:10.1099/mcb.0.03-12-0879

Ayoilo, D.V., I.Y. Zhitnyak, J.M. Vasilei, and N.A. Gloushankova. 2009. Rearrangements of the actin cytoskeleton and E-cadherin-based adherens junctions caused by neoplastic transformation change cell-cell interactions. PLoS One. 4:e8027. doi:10.1371/journal.pone.0008027

Bakolitsa, C., D.M. Cohen, L.A. Bankston, A.A. Bobkov, G.W. Cadwell, L. Jennings, D.R. Critchley, S.W. Craig, and R.C. Lindington. 2004. Structural basis for vinculin activation at sites of cell adhesion. Nature. 430:583–586. doi:10.1038/nature02610

Bershadsky, A.D., N.Q. Balaban, and B. Geiger. 2003. Adhesion-dependent cell cytoskeleton modulates the spatial organization of cell-cell contacts in intercellular movement of epithelial cells. J. Cell Sci. 117:1247–1257. doi:10.1242/jcs.009972

Mierke, C.T., P. Kollmannsberger, D.P. Zitterbart, J. Smith, B. Fabry, and W.H. Goldmann. 2008. Mechnano-coupling and regulation of contractility by the vinculin tail domain. Biophys. J. 94:661–670. doi:10.1529/biophysj.107.074872

Miyake, Y., N. Inoue, K. Nishimura, N. Kinoshita, H. Hosoya, and S. Yonemura. 2006. Actomyosin tension is required for correct recruitment of adherens junction components and zona occludens formation. Exp. Cell Res. 312:1637–1650. doi:10.1016/j.yexcr.2006.01.031

Niessen, C.M., and B.M. Gumbiner. 2002. Cadherin-mediated cell sorting not determined by binding or adhesion specificity. J. Cell Biol. 156:389–399. doi:10.1083/jcb.200108040

Nishiyama, N., W.B. Kiooses, J. Han, and M.H. Ginsberg. 2005. An alpha-integrin-paxillin-Arf-GAP complex restricts Rac activation to the leading edge of migrating cells. Nat. Cell Biol. 7:343–352. doi:10.1038/ncb1234

Passaera, A.M., I.C. Schneider, E. Reirich, D.D. Schlaeger, and C.M. Waterman. 2010. Myosin II activity regulates vinculin recruitment to focal adhesions through F-actin-mediated phosphoinositol phosphorylation. J. Cell Biol. 188:877–890. doi:10.1083/jcb.200906012

Peng, X., L.E. Cuff, C.D. Lawton, and K.A. DeMali. 2010. Vinculin regulates cell-surface E-cadherin expression by binding to beta-catenin. J. Cell Sci. 123:567–577. doi:10.1242/jcs.056432

Perez, T.D., M. Tamada, M.P. Sheetz, and W.J. Nelson. 2008. Immediate-early signaling induced by E-cadherin engagement and adhesion. J. Biol. Chem. 283:5014–5022. doi:10.1074/jbc.M705209200

Pertz, O., D. Bozic, A.W. Koch, C. Fauser, A. Brancaccio, and J. Engel. 1999. A new crystal structure, Ca2+ dependence and mutational analysis reveal molecular details of E-cadherin homosassociation. EMBO J. 18:1738–1747. doi:10.1093/emboj/18.7.1738

Prakash, A., V.H. Chien, V. Maruthamuthu, and D.E. Leckband. 2006a. Calcium site mutations in cadherin: impact on adhesion and evidence of cooperativity. Biochemistry. 45:6930–6939. doi:10.1021/bi060213m

Prakash, A.K., V. Maruthamuthu, and D.E. Leckband. 2006b. Similarities between heterophilic and homophilic cadherin adhesion. Proc. Natl. Acad. Sci. USA. 103:15434–15439. doi:10.1073/pnas.0606701103

Schwartz, M.A., and D.W. DeSimone. 2008. Cell adhesion receptors in mechanotransduction. Curr. Opin. Cell Biol. 20:551–556. doi:10.1016/j.jcb.2008.05.005

Shewan, A.M., M. Maddugoda, A. Kraemer, S.J. Strehns, S. Verma, E.M. Kovacs, and A.S. Yap. 2005. Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. Mol. Biol. Cell. 16:4531–4542. doi:10.1091/mbc.E05-04-0330

Tomschy, A., C. Fauser, R. Landwehr, and J. Engel. 1996. Homophilic adhesion of E-cadherin occurs by a co-operative two-step interaction of N-terminal domains. EMBO J. 15:3507–3514.

Tzima, E., M. Irani-Tehrani, W.B. Kiooses, E. Dejana, D.A. Schultz, B. Engelhardt, G. Cao, H. DeLisser, and M.A. Schwartz. 2005. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature. 437:426–431. doi:10.1038/nature03952

Vogel, V., and M. Sheetz. 2006. Local force and geometry sensing regulate cell migration. J. Cell Sci. 119:3507–3512. doi:10.1242/jcs.056432

Wang, N., and D.E. Ingber. 1995. Probing transmembrane mechanical coupling and cytomembranes using magnetic twisting cytometry. Biochem. Cell Biol. 73:327–335. doi:10.1139/bcb-95-041

Wang, N., J.P. Butler, and D.E. Ingber. 1993. Mechanotransduction across the cell surface and through the cytoskeleton. Science. 260:1124–1127. doi:10.1126/science.7684161

Xu, W., J.L. Coll, and E.D. Adamson. 1998. Rescue of the mutant phenotype by reexpression of full-length vinculin in null F9 cells; effects on cell locomotion by domain deleted vinculin. J. Cell Sci. 111:1535–1544.

Yamada, S., S. Pokutta, F. Drees, W.I. Weis, and W.J. Nelson. 2005. Deconstructing the cadherin-catenin-actin complex. Cell. 123:889–901. doi:10.1016/j.cell.2005.09.020

Zhong, C., M.S. Kinch, and K. Burridge. 1997. Rho-stimulated contractility contributes to the fibroblastic phenotype of Ras-transformed epithelial cells. Mol. Biol. Cell. 8:2329–2344.