Correction

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Correction for "E-cadherin is under constitutive actomyosin-generated tension that is increased at cell–cell contacts upon externally applied stretch," by Nicolas Borghi, Maria Sorokina, Olga G. Shcherbakova, William I. Weis, Beth L. Pruitt, W. James Nelson, and Alexander R. Dunn, which appeared in issue 31, July 31, 2012, of *Proc Natl Acad Sci USA* (109:12568–12573; first published July 16, 2012; 10.1073/pnas.1204390109).

The authors note that that in the constructs EcadTSMod, EcadTSModΔcyto, and EcadTSModΔmTFP, the Förster resonance energy transfer (FRET) acceptor fluorophore was monomeric enhanced yellow fluorescent protein (mEYFP)—not Venus as was originally reported. The photophysical properties of mEYFP and Venus, including absorption spectra, emission spectra, and fluorescence quantum yields are highly similar, leading to negligible changes in the Förster radius with the FRET donor monomeric Teal Fluorescent Protein (mTFP) (1). This substitution thus does not affect the FRET data reported in the original manuscript or their interpretation. The protein mEYFP is based on EYFP (Clontech) with the addition of the mutation A206K to suppress dimerization (2).

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E-cadherin is under constitutive actomyosin-generated tension that is increased at cell–cell contacts upon externally applied stretch

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Classical cadherins are transmembrane proteins at the core of intercellular adhesion complexes in cohesive metazoan tissues. The extracellular domain of classical cadherins forms intercellular bonds with cadherins on neighboring cells, whereas the cytoplasmic domain recruits catenins, which in turn associate with additional cytoskeleton binding and regulatory proteins. Cadherin/catenin complexes are hypothesized to play a role in the transduction of mechanical forces that shape cells and tissues during development, regeneration, and disease. Whether mechanical forces are transduced directly through cadherins is unknown. To address this question, we used a Förster resonance energy transfer (FRET)-based molecular tension sensor to test the origin and magnitude of tensile forces transmitted through the cytoplasmic domain of E-cadherin in epithelial cells. We show that the actomyosin cytoskeleton exerts pN-tensile force on E-cadherin, and that this tension requires the catenin-binding domain of E-cadherin and αE-catenin. Surprisingly, the actomyosin cytoskeleton constitutively exerts tension on E-cadherin at the plasma membrane regardless of whether or not E-cadherin is recruited to cell–cell contacts, although tension is further increased at cell–cell contacts when adhering cells are stretched. Our findings thus point to a constitutive role of E-cadherin in transducing mechanical forces between the actomyosin cytoskeleton and the plasma membrane, not only at cell–cell junctions but throughout the cell surface.

mechanotransduction | mechanobiology | signal transduction | mechanosensor | morphogenesis

Embryonic development is fundamentally a mechanical process (1), as are the morphogenetic events that occur during tissue regeneration and diseases such as cancer. As tissues change shape, they generate mechanical forces that are propagated throughout the organism (2, 3). Ultimately, these forces may regulate genetic programs by activating transcription factors (4), thereby providing a role for mechanotransduction in a feedback loop between phenotype and genotype (3).

Recent studies have focused on measuring forces transmitted between cells in cohesive tissues and identifying the causes of those forces in relation to cell–cell and cell–matrix contact shape and size, and to cell migration (5–9). In cohesive tissues, cells adhere directly to their neighbors through intercellular adhesion complexes, and intercellular adhesion is dependent on the activity of the actomyosin cytoskeleton (10, 11), which provides a significant source of mechanical force within cells.

The classical cadherin/catenin complex is the best understood intercellular adhesion complex and is found in most metazoan cohesive tissues (12). Classical cadherins are transmembrane proteins that form intercellular bonds through interactions between their extracellular domains on apposed cells. The classical cadherin cytoplasmic domain binds β-catenin, which in turn binds α-catenin. Although it is generally thought that cadherins are physically connected to the cytoskeleton through catenins (13), the cadherin/catenin complex appears to bind poorly to actin in vitro (14, 15). Therefore, the cadherin/catenin/actin linkage may be highly dynamic, or its components may require a specific conformation not recapitulated in vitro to bind actin. This linkage may also involve additional actin-binding proteins such as activated vinculin or epithelial protein lost in neoplasm (EPLIN), which bind to α-catenin (16–21). In addition to actin, the classical cadherin/γ-catenin (plakoglobin) complex can also associate with intermediate filaments in some instances (22, 23).

Mechanical coupling between the plasma membrane and the cortical cytoskeleton involves the cadherin cytoplasmic domain (24). In addition, mechanical stimulation of cells through the extracellular domain of cadherins results in vinculin-dependent cell stiffening (25), and membrane protrusion reorientation via a mechanism involving γ-catenin and intermediate filaments (26). Conversely, actomyosin activity is proposed to induce conformational changes in α-catenin and the recruitment of vinculin to cell–cell contacts (27), which provides resistance to cell–cell contact disruption (28, 29). Moreover, cadherins can transmit cell-generated forces to the extracellular environment in a stiffness-dependent manner (30–33).

Taken together, these studies suggest that tension exerted across cell–cell contacts is transduced between the cadherin extracellular domain and the cytoskeleton through the cadherin/catenin complex, thus positioning this protein complex upstream of mechanotransduction pathways that remain to be elucidated. A condition for this model is that the cytoplasmic domain of cadherin directly transmits force between the cytoskeleton and the cell environment and therefore experiences variable tension depending on the activity of the cytoskeleton and/or external mechanical stimuli. However, current data equally support an alternate model by which the cytoplasmic domain of cadherin may simply recruit cytoskeletal regulatory proteins, while other membrane–cytoskeleton linker proteins mediate mechanotransduction. Therefore, we sought to directly test these possibilities by using a Förster resonance energy transfer (FRET)-based molecular tension sensor strategy (34). We inserted a pN-sensitive force sensor derived from spider silk (35, 36) in the cytoplasmic domain of E-cadherin to measure the tension experienced by the E-cadherin cytoplasmic

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domain at cell–cell contacts and at contact-free regions of the plasma membrane in Madin–Darby canine kidney (MDCK) epithelial cells. We then tested whether disruption of actomyosin activity, αE-catenin depletion, and externally applied mechanical stimuli affected the tension experienced by the cytoplasmic domain of E-cadherin. Our results provide direct evidence that mechanical forces are transduced through the cytoplasmic domain of E-cadherin and support a mechanosensory role of the cadherin/catenin complex not only at cell–cell contacts but also at contact-free plasma membrane throughout the cell.

Results

We hypothesized that if tension is exerted by the actomyosin cytoskeleton on E-cadherin, then it should be transmitted through the cytoplasmic domain (Fig. 1*A*). To directly measure tension, we generated a variant E-cadherin (EcadTSMod) that contained a tension sensor module (TSMod) (36) inserted in the cytoplasmic domain between the transmembrane domain and the catenin-binding domain (Fig. 1*B*; see *SI Materials and Methods*). This sensor allowed us to measure pN-range forces transmitted between the transmembrane domain and the catenin-binding domain by FRET microscopy (see *SI Materials and Methods*).

EcadTSMod localized prominently to the plasma membrane of MDCK cells and was recruited to cell–cell contacts, similar to the endogenous protein (Fig. 1*C*); EcadTSMod also labeled some endo-/exocytic vesicles and intracellular compartments, as already shown for E-cadherin-GFP (37). Importantly, EcadTSMod rescued cell–cell adhesion and plasma membrane recruitment of β-catenin and α-catenin in L fibroblasts (Fig. S1*A*) that normally lack cadherin expression (38). EcadTSModΔcyto, a control construct in which the β-catenin–binding domain was deleted, was also recruited to cell–cell contacts but did not recruit the catenins, as expected (Fig. S1*B*). Therefore, EcadTSMod recapitulated wild-type E-cadherin association with catenins and cell–cell adhesion function.

Next, we sought to assess whether EcadTSMod was under tension in MDCK cells. The intensities of the signals emitted from the TSMod fluorophores monomeric teal fluorescent protein (mTFP) and Venus were used to compute the FRET index, a measure of TSMod stretching and tension transmitted through the cytoplasmic domain of E-cadherin: the lower the FRET index, the higher the tension (Fig. 1 *B* and *C*; and *SI Materials and Methods*). We calibrated the FRET index to FRET efficiency by measuring the FRET index of the 229aa tumor necrosis factor receptor associated factor flanked with mTFP and Venus (mTFP-TRAF-Venus) and mTFP-5aa-Venus expressed in MDCK cells (Fig. S2 and *SI Materials and Methods*), two constructs with known FRET efficiencies (39). We used a previously published FRET efficiency–force calibration to infer molecular tension (36).

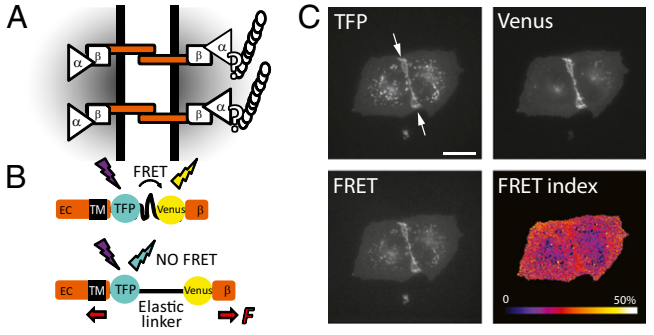

Fig. 1. (*A*) Working model for mechanotransduction through the E-cadherin/catenin complex. E-cadherin transmits mechanical tension between cells via transinteracting extracellular (EC) domains and to the actin cytoskeleton through β-catenin, αE-catenin, and possibly other proteins. (*B*) The tension sensitive module (TSMod) consists of the mTFP/Venus FRET pair separated by an elastic linker $(GPGGA)_8$ derived from spider silk. TSMod was inserted into the cytoplasmic domain of E-cadherin, where it can sense forces transmitted between the transmembrane domain (TM) and the β-catenin-binding domain (β). High and low FRET indices correspond to low and high tension, respectively. (*C*) Fluorescence imaging of two adherent MDCK cells expressing the EcadTSMod construct in the mTFP, Venus, and FRET (mTFP excitation; Venus emission) channels, and the corresponding map of FRET index = $I_{FRET}/(I_{FRET} + I_{mTFP})$, where I is the fluorescence intensity of the subscript channel corrected for background and spectral bleed-through. (Scale bar: 20 μm.)

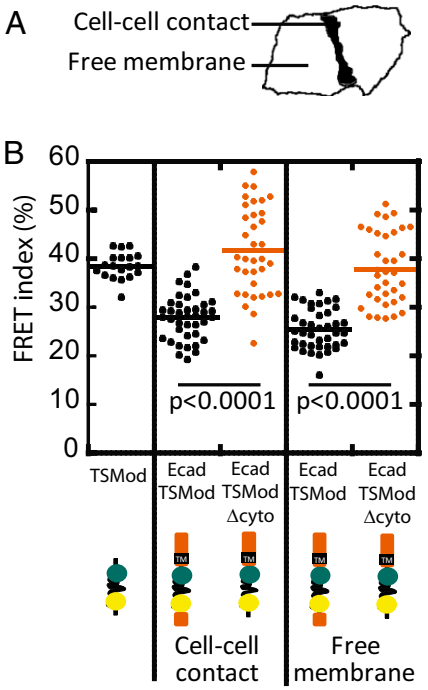

Fig. 2. (*A*) FRET index measured at cell–cell contacts and at the free membrane of expressing cells revealed that the EcadTSMod is under constitutive tension. (*B*) FRET index for TSMod in the cytoplasm, EcadTSMod and EcadTSModΔcyto at cell–cell contacts, and EcadTSMod and EcadTSModΔcyto at free membrane in MDCK cells. EcadTSMod had a lower FRET index than either cytoplasmic TSMod or EcadTSModΔcyto regardless of their subcellular localization, indicating that it was under tension. *P* values are calculated using a two-tailed Mann–Whitney test.

We measured FRET indices for TSMod (expressed in the cytoplasm), EcadTSMod, and EcadTSModΔcyto at cell–cell contacts and EcadTSMod and EcadTSModΔcyto at the contact-free plasma membrane (Fig. 2*A*). Within cell–cell contacts, the EcadTSMod FRET index was ∼30%, compared with ∼40% for either EcadTSModΔcyto or cytoplasmic TSMod (Fig. 2*B*). Therefore, EcadTSMod appeared to be under tension, and this tension required the catenin-binding domain of E-cadherin. Surprisingly, the EcadTSMod FRET index was lower than that of EcadTSModΔcyto at plasma membrane not in contact with other cells (Fig. 2*B*). Thus, E-cadherin appears to be under constitutive tension at the plasma membrane whether or not it is localized to cell–cell contacts.

We sought to test whether the tension exerted on E-cadherin was generated by filamentous actin and myosin II activity. To test whether filamentous actin was required, cells were incubated in cytochalasin B, an inhibitor of actin polymerization (40). This treatment resulted in an increase in the EcadTSMod FRET index at both cell–cell contacts and the contact-free plasma membrane (Fig. 3*A*). We tested whether myosin II activity was required by

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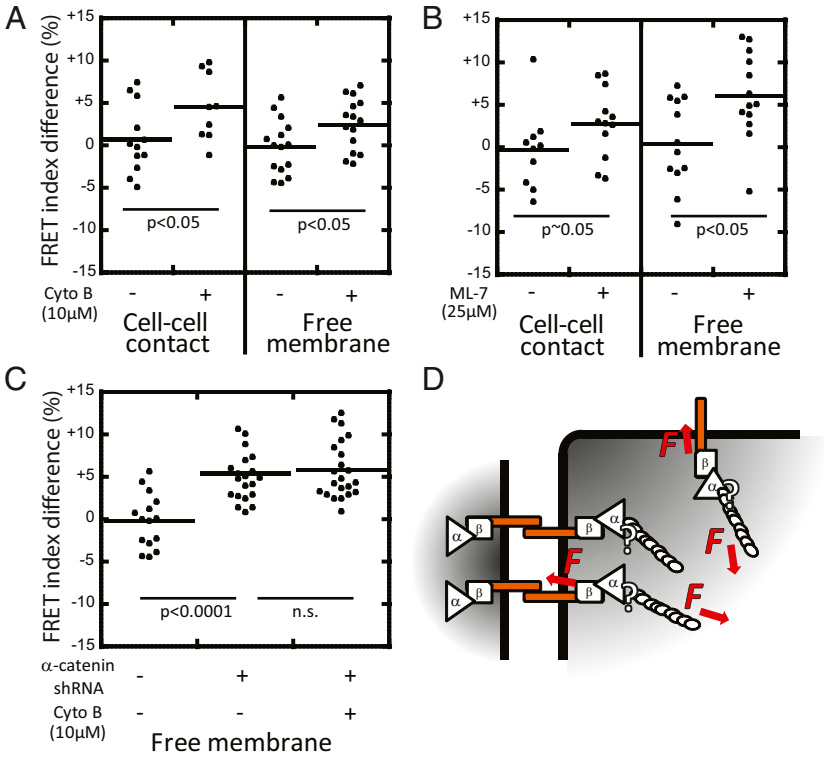

Fig. 3. Tension on EcadTSMod requires actomyosin activity and αE-catenin. FRET index difference before and after perturbation with: actin polymerization inhibitor cytochalasin B (*A*); myosin II activity inhibitor ML-7 (*B*); and αE-catenin depletion (*C*). Measurements were performed at cell–cell contacts (*A* and *B*) and at contact-free plasma membrane (*A*–*C*). Lack of E-cadherin recruitment to cell–cell contacts upon αE-catenin depletion prevented the measurement of a cell–cell contact FRET index in *C*. All three disruptions resulted in increased FRET index. Combined αE-catenin depletion and cytochalasin B addition did not increase the FRET index further, suggesting that either disruption interrupts force transmission through E-cadherin. (*D*) Proposed model: the cytoplasmic domain of E-cadherin is under constitutive tension generated by the actomyosin cytoskeleton and mediated by αE-catenin at cell–cell contacts and at contact-free membranes. *P* values are calculated using a two-tailed Mann–Whitney test.

incubating cells with ML-7, an inhibitor of myosin light chain kinase (41), which also resulted in an increase in the EcadTSMod FRET index at both cell–cell contacts and the contact-free plasma membrane (Fig. 3*B*).

Actomyosin perturbation induced a smaller increase of the FRET index than deletion of the cytoplasmic domain (Fig. 2*B*). We tested whether this could result from increased intermolecular FRET rather than tension relaxation. Intermolecular FRET may arise between proximal fluorescent E-cadherins, especially if they cluster at the plasma membrane. To directly quantify the contribution of intermolecular FRET, we measured the FRET index of EcadTSModΔcyto, which is not under tension, as a function of its expression level. We found a weak dependence of the FRET index on fluorescent protein expression level, consistent with small differences in the chance of encounters between fluorescent proteins over large variations in surface density (Fig. S3*A*). Perturbation of cells with ML-7 or cytochalasin B, however, did not induce significant or quantitative changes in EcadTSMod surface density (Fig. S3*B*) despite significant increases in EcadTSMod FRET index (Fig. 3 *A* and *B*). Thus, intermolecular FRET is unlikely to account for the increase in the FRET index observed upon actomyosin perturbation.

We next tested whether αE-catenin was required to transmit actomyosin-generated tension to E-cadherin. EcadTSMod was cotransfected with a shRNA against αE-catenin (42–44). Cells expressing EcadTSMod and depleted of αE-catenin did not recruit EcadTSMod or β-catenin between closely apposed neighboring cells (Fig. S4). Nevertheless, depletion of αE-catenin caused an increase in the EcadTSMod FRET index at the contact-free membrane (Fig. 3*C*). Significantly, addition of cytochalasin B to αE-catenin–depleted cells did not induce a further increase in the EcadTSMod FRET index (Fig. 3*C*). Together, these results show that actin filaments and myosin II activity require αE-catenin to exert constitutive tension on the cytoplasmic domain of E-cadherin (Fig. 3*D*).

Finally, we sought to assess whether stretching pairs of cells increased tension in the cytoplasmic domain of E-cadherin. We used micromanipulation to apply increasing steps of uniaxial stretch across cell–cell contacts between adhering cells (Fig. 4*A* and *SI Materials and Methods*) and measured the EcadTSMod FRET index over time (Fig. 4*B*). We found that the FRET index at the cell–cell contact decreased as a function of cell extension (Fig. 4*C*). In contrast, the FRET index at contact-free regions of the plasma membrane exhibited no correlation with cell extension (Fig. 4*C*). On average, the change in the FRET index per unit change in extension ratio at cell–cell contacts was significantly different from that at contact-free plasma membrane (Fig. 4*D*). Thus, the cytoplasmic domain of E-cadherin in stretched cells transmits increased tension within cell–cell contacts, but not at contact-free regions of the plasma membrane.

Discussion

Mechanotransduction is ubiquitous during embryonic development, tissue regeneration, and disease (1, 4). Cell adhesion proteins are structural and functional hubs between the cytoskeleton and the extracellular environment and hence are strategically positioned to transduce mechanical signals (45). Mechanotransduction through integrin adhesion proteins to the extracellular matrix is well studied (46). Protein components in these adhesion complexes respond directly to mechanical forces by changing conformation, which

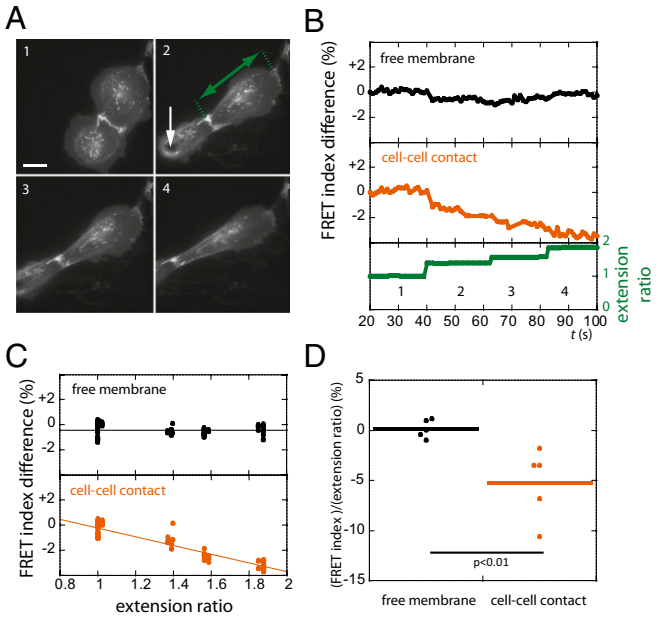

Fig. 4. Externally applied uniaxial stretch between pairs of cells decreased the FRET index of EcadTSMod at cell–cell junctions but not at the contact-free plasma membrane. (*A*) Fluorescence images from a sequence of increasing steps (1, 2, 3, 4; ~20-s intervals) of stretch applied to a cell doublet using a microneedle. (In image 2, the white arrow shows the point of contact between the needle and the cell, and the green arrow shows the cell extension.) (*B*) Extension ratio (cell length in the direction of the stretch normalized by cell length at rest) and EcadTSMod FRET index at the cell–cell contact and at the free membrane for the cell that is not directly in contact with the needle in *A*. FRET decreased at the junction but not at the free membrane. (*C*) EcadTSMod FRET index vs. extension ratio from cell in *A*. Solid lines show linear fits. At the cell–cell contact: $R^2 = 0.88$ and $\Delta y/\Delta x = -3.5\% \pm 0.1\%$ (SEM); at contact-free membrane: $R^2 = 8.10^{-5}$ and $\Delta y/\Delta x = 0\% \pm 0.1\%$ (SEM). Slopes are significantly different with $P < 0.001$, *t* test. (*D*) Change in FRET index per unit change in extension ratio ($n = 6$ cells). FRET index decreases with increasing extension ratio at the cell–cell contact but not at the free membrane. *P* value is calculated using two-tailed Mann–Whitney test. (Scale bar: 20 μm.)

exposes cryptic binding sites to putative binding partners and phosphorylation (47, 48). Recently, a FRET-based tension sensor, which we used here, revealed that vinculin experiences pN forces at integrin-based focal adhesions in cultured cells (36).

In contrast to studies of mechanotransduction at integrin-based adhesions, relatively little is known about the mechanical features of the cadherin/catenin adhesion complex at the molecular level. Recent studies have reported that cadherins transmit actomyosin-generated forces to the extracellular environment (32, 33). External forces applied to cells through cadherins reveal a mechanical coupling between the membrane and the cytoskeleton that requires the cadherin cytoplasmic domain (24) and may cause changes in cellular mechanical properties or protrusive activity (25, 26). Conformational changes in α-catenin and/or the recruitment of cytoskeleton-binding proteins to sites of cadherin-mediated adhesion may be involved (17, 18, 27–29). However, there is no direct evidence at the molecular level that cadherins are under actomyosin-dependent tension or that cadherins transmit extracellular forces to the cytoskeleton through the catenin-binding cytoplasmic domain. Here, we addressed this problem directly by using variants of E-cadherin (Fig. S5) that contain a tension sensor module (TSMod), and showed that: (*i*) the E-cadherin cytoplasmic domain is under tension that is dependent on an intact actin cytoskeleton, αE-catenin and myosin II activity; (*ii*) tension generated by the actomyosin cytoskeleton on E-cadherin is constitutive, regardless of whether E-cadherin is localized within or outside of cell–cell contacts at the plasma membrane; and (*iii*) tension in the cytoplasmic domain of E-cadherin is increased specifically at cell–cell contacts upon externally applied uniaxial stretch.

The force sensitivity of the flagelliform linker in the TSMod construct was determined previously by single-molecule stretching using optical tweezers (36). Based on those measurements and FRET index calibration (Fig. S2), we estimate that a 1-pN increase in force corresponds to a ~6% decrease in FRET index on our experimental setup (*SI Materials and Methods*). The force sensor TSMod was inserted into a region of the E-cadherin juxtamembrane domain that is unstructured even when E-cadherin binds to β-catenin (49, 50). Previous results indicate that E-cadherin at the plasma membrane is constitutively bound to β-catenin (51, 52), whether or not the E-cadherin/β-catenin complex is associated with αE-catenin or actin (15). Thus, the observed changes in FRET index are unlikely to reflect conformational changes in the E-cadherin juxtamembrane region. Moreover, we showed directly that changes in intermolecular FRET between fluorescent E-cadherins are unlikely to account for the FRET index differences observed upon actomyosin perturbation (Fig. S3). Taken together, these data indicate that the E-cadherin cytoplasmic domain is under 1- to 2-pN constitutive load from the cytoskeleton (Figs. 2 and 3). This value is similar to that observed for vinculin in focal adhesions between integrins and the extracellular matrix (36), indicating that the forces exerted across proteins of both adhesion complexes are of the same order of magnitude. It is noteworthy, however, that this value likely masks a range of forces that are averaged out over many molecules and over time and space. This may explain why tension on E-cadherin was only partially reduced upon pharmacological perturbation of actin polymerization, myosin II activity, and shRNA-mediated depletion of αE-catenin (Fig. 3). It is possible that low levels of αE-catenin and actomyosin activity suffice to exert some tension on E-cadherin after drug treatment and/or protein depletion. In addition, E-cadherin may interact with intermediate filaments through desmosomal linker proteins (22, 23, 26, 53). Intermediate filaments might constitutively exert tension on E-cadherin or compensate for reduced αE-catenin–mediated actomyosin tension following drug treatment or αE-catenin depletion.

We found that E-cadherin is not only under actomyosin-dependent tension within cell–cell contacts but also in regions of plasma membrane where there was no cell–cell adhesion. Thus, the cadherin/catenin complex may be a constitutive membrane anchor for the cortical actomyosin cytoskeleton. In this context, it is noteworthy that cadherin lateral mobility in the plasma membrane depends on the catenin-binding region of the cytoplasmic domain even outside of cell–cell contacts and is regulated by the underlying cortical actin cytoskeleton (54, 55). That the cadherin/catenin complex functions in membrane-to-cortex attachment may explain why this complex is important for regulating macropinocytosis in contact-free protruding membranes (56), single-cell wound closure in *Drosophila* (57), and plasma membrane blebbing during early embryogenesis in zebrafish (58). Plasma membrane blebbing involves functional crosstalk between the cadherin/catenin complex and ezrin–radixin–moesin (ERM) proteins (58), which also mediate membrane-to-cortex attachment (59). Although the molecular events directly downstream of E-cadherin tension remain unclear, E-cadherin, and likely αE-catenin, may be involved in a ubiquitous tension-sensing mechanism that regulates cortical cytoskeleton activity as a function of cell shape, size, or membrane activity.

Externally applied stretch on cell pairs increases tension in the cytoplasmic domain of E-cadherin at cell–cell contacts by ~1 pN (Fig. 4). Importantly, tension across the E-cadherin cytoplasmic domain appears to increase in proportion to the applied stretch, which may allow a graded signaling output from the adhesion complex. However, tension does not appear to be propagated to E-cadherin in the plasma membrane outside the cell–cell

contact, suggesting a lack of lateral mechanical coupling. Additionally, within the timescale of our experiments, the increased tension across E-cadherin induced by stretching cells did not relax to its original constitutive value, which may enable localized and sustained adhesion-specific signaling. Force-induced conformation changes within the cadherin/catenin complex could elicit downstream signaling, for example, by modulating binding affinity to direct or indirect binding partners such as EPLIN or vinculin (17–21, 25, 27–29). Protein accumulation at adhesion sites could thus promote changes in cell and tissue mechanical properties and cell adhesive and migratory behavior (18, 25, 26, 28, 29). Over longer timescales, this may also regulate gene expression and likewise have important roles during development and disease.

Conclusion

Recent studies suggested a role of the cadherin/catenin complex in mechanotransduction at cell–cell contacts. In this study, we provide direct evidence that mechanical tension is transmitted through the E-cadherin cytoplasmic domain as a consequence of actomyosin activity and involving αE-catenin and as a response to external mechanical stimulus through neighboring cells. These results therefore validate a critical condition for cadherins as bona fide mechanosensors at cell–cell contacts. Surprisingly, our results also show that the cytoplasmic domain of E-cadherin is subject to constitutive, actomyosin-generated pN-scale tension at the contact-free plasma membrane, revealing a constitutive function of E-cadherin as a link between the cell membrane and the actomyosin cytoskeleton. Although the signaling pathways downstream of mechanically stimulated cadherins remain to be characterized, our results suggest that cadherins function as adhesion-dependent mechanosensors during morphogenesis of multicellular assemblies and as adhesion-independent mechanosensors that adapt their signaling output in response to changes in cell size, shape, or membrane activity.

Materials and Methods

MDCK type II G cells stably or transiently expressing fluorescently tagged proteins were monitored on a wide-field epifluorescence inverted microscope 2–3 d after shRNA transfection or within an hour after drug treatment, depending on experiment. Image analysis was performed with Image J and publicly available plugins and macros.

Full materials and methods are available in *SI Materials and Methods*.

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