Mechanosensitive systems at the cadherin–F-actin interface

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Summary

Cells integrate biochemical and mechanical information to function within multicellular tissue. Within developing and remodeling tissues, mechanical forces contain instructive information that governs important cellular processes that include stem cell maintenance, differentiation and growth. Although the principles of signal transduction (protein phosphorylation, allosteric regulation of enzymatic activity and binding sites) are the same for biochemical and mechanical-induced signaling, the first step of mechanosensing, in which protein complexes under tension transduce changes in physical force into cellular signaling, is very different, and the molecular mechanisms are only beginning to be elucidated. In this Commentary, we focus on mechanotransduction at cell–cell junctions, aiming to comprehend the molecular mechanisms involved. We describe how different junction structures are associated with the actomyosin cytoskeleton and how this relates to the magnitude and direction of forces at cell–cell junctions. We discuss which cell–cell adhesion receptors have been shown to take part in mechanotransduction. Then we outline the force-induced molecular events that might occur within a key mechanosensitive system at cell–cell junctions; the cadherin–F-actin interface, at which α-catenin and vinculin form a central module. Mechanotransduction at cell–cell junctions emerges as an important signaling mechanism, and we present examples of its potential relevance for tissue development and disease.

This article is part of a Minifocus on Adhesion. For further reading, please see related articles: 'Cadherin adhesome at a glance' by Ronen Zaidel-Bar (*J. Cell Sci.* **126**, 373-378). 'Cycling around cell–cell adhesion with Rho GTPase regulators' by Jessica McCormack et al. (*J. Cell Sci.* **126**, 379-391). 'E-cadherin–integrin crosstalk in cancer invasion and metastasis' by Marta Canel et al. (*J. Cell Sci.* **126**, 393-401).

Key words: Focal adherens junction, Cadherin, Actin, Myosin, Mechanotransduction, Catenin, Vinculin

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Introduction

Physical forces can influence tissues not only by directing changes in cell shape, but also by affecting a variety of cellular processes, such as polarity, division and differentiation (Ingber, 2003; Jaalouk and Lammerding, 2009; Schwartz and DeSimone, 2008; Vogel and Sheetz, 2006). These cellular processes entail complex biochemical signaling pathways and much of the current research aims at understanding how physical forces control cellular signaling. Cells experience changes in force that are either derived externally by deformations of the extracellular matrix (ECM) or by neighboring cells, or changes in internal force, for instance induced by actomyosin contractility (Maruthamuthu et al., 2010). Many of the generated forces concentrate at adhesions between cells and the extracellular matrix, or at homotypic cell–cell adhesions. Consequently, the transformation of physical stimuli into intracellular biochemical signaling (mechanotransduction) occurs within the multi-protein complexes at these adhesion sites (Gomez et al., 2011; Leckband et al., 2011; Lecuit et al., 2011; Moore et al., 2010).

To understand the molecular mechanisms that underlie mechanotransduction, it is important to distinguish between force-transmission and force-sensing. A protein that takes part in force-transmission is part of a chain of proteins that connects one physically restricted entity, e.g. a focal adhesion (FA), in which integrins bind to rigid ECM, to another physically restricted entity, e.g. a cadherin-based cell–cell junction complex. Changes in force that occur anywhere in the force chain will be transmitted through these proteins and result in an altered force distribution across cells and tissue. As proteins are not completely rigid structures, changes in force cause conformational deformations across the force chain. In the past decades, it became clear that several specialized protein machineries utilize such deformations to sense changes in the magnitude of force and induce proportional biochemical responses. These protein machineries are called force-sensors or mechanosensors (Bershadsky et al., 2006; Vogel and Sheetz, 2006).

In this Commentary we will discuss the force-sensing systems that might function in mechanotransduction at cell–cell junctions. Classical cadherins and the cytoplasmic protein complex that connects them to the actomyosin cytoskeleton are central elements in this response. We describe the organization of the actomyosin cytoskeleton at different junction structures and how it is integrated with local physical forces. We then outline which molecular events might occur at the cadherin–F-actin interface in response to force, and discuss their possible function in mechanotransduction at cell–cell junctions.

Different conformations of cell–cell junctions during formation, maturation and remodeling

Classical cadherins, and their connection to the actin cytoskeleton are crucial for all stable cell–cell adhesions to be formed (Gumbiner et al., 1988; Nagafuchi and Takeichi, 1988). The core

protein complex needed for cadherin stability and for linking cadherins to actin, consists of p120-catenin, β-catenin and α-catenin. These proteins are always present at cadherin-dependent cell–cell junctions at a level comparable to that of the cadherin itself (Gumbiner, 2005). In addition to these proteins, other F-actin-associated proteins localize to the cadherin complex, but their levels vary and appear to depend on the conformation of the cell–cell junctions and that of the associated actomyosin cytoskeleton (see below). There is a strong relationship between the organization and contractility of the actomyosin cytoskeleton and the formation, maturation and remodeling of cell–cell junctions (Cavey and Lecuit, 2009; Gomez et al., 2011; Yonemura, 2011). In this section, we will discuss the different conformations of cadherin-containing cell–cell junctions observed by microscopic analyses, and we will indicate what is known about the interaction with the actomyosin cytoskeleton at these different stages of adhesion.

Forming cell–cell junctions adopt a punctate morphology and are connected to radial actin bundles that extend from a network of circumferential actin bundles (Adams et al., 1998; Yonemura et al., 1995). Many actin regulatory proteins are recruited to these punctate adhesions, including vinculin, zyxin, ena/VASP proteins, formins and members of the ARP2/3 complex (Kobielak et al., 2004; Kovacs et al., 2002; Vasioukhin et al., 2000). These proteins are likely to control the extent and direction of F-actin polymerization at these sites (Fig. 1A). Activity of Rho-associated protein kinase (ROCK) signaling, which promotes actomyosin contraction, is required to form punctate adhesions (Vaezi et al., 2002), and α-catenin is a key linker between F-actin and these adhesion structures (Huveneers et al., 2012; Twiss et al., 2012). Besides cadherins, forming junctions contain nectins (Meng and Takeichi, 2009; Ogita et al., 2010), which can also bind to α-catenin and can thus form a physical link to F-actin. Both cadherins and nectins are crucial for junction formation (Mizoguchi et al., 2002; Tachibana et al., 2000; Takai and Nakanishi, 2003) and it has, therefore, remained unclear whether cadherins or nectins form the mechanical connection to actomyosin that organizes junction formation. Recently, we found that, in the absence of endogenous α-catenin, expression of an E-cadherin-α-catenin fusion protein completely restored cell–cell junction formation (Twiss et al., 2012). This observation argues that a physical connection between cadherin and F-actin is sufficient for junction formation and suggests that nectins perform a different function at this stage.

After initial junction formation, cell–cell contact expansion occurs through membrane protrusions and lateral distribution of cadherins to form linear adherens junctions (Adams et al., 1998; Ehrlich et al., 2002) (Fig. 1B). At these stages of junction maturation, the activity of myosin II is particularly high at the contact edges near the so-called actin arches and is thought to drive the expansion of cell–cell junctions (Krendel et al., 1999; Yamada and Nelson, 2007). Underscoring the close relationship between cell–cell junctions and actomyosin, E-cadherin engagement induces myosin II activity, which in turn promotes the concentration of cadherins at sites of cell–cell adhesion (Shewan et al., 2005). Once linear junctions have fully expanded, the actin cytoskeleton in their vicinity has also remodeled to form peri-junctional parallel actin bundles (Zhang et al., 2005). The α-catenin- and F-actin-binding protein EPLIN (also known as LIMA1) is needed to maintain linear adherens junctions, as in its absence, junctions show a punctate morphology (Abe and Takeichi, 2008). In addition, other actin regulatory proteins, including cortactin, ARP2/3 (Helwani et al., 2004) and neural Wiskott-Aldrich syndrome protein (N-WASP) (Kovacs et al., 2011), are present. Cadherins, nectins and tight junction proteins are all localized in close vicinity to linear cell–cell junctions. Whether and how parallel peri-junctional actin bundles connect to cell–cell junction complexes in this junction structure is not clear. Relatively faint F-actin signals have been shown to colocalize with linear cell–cell junctions in immunofluorescent images, whereas thicker F-actin bundles run in close vicinity, but do not seem to contact junctions directly (Fig. 1B), or move coordinately with the junctions in time-lapse experiments [see movie 2 (Huveneers et al., 2012) for a clear example].

In many epithelial cell lines, upon cell–cell junction formation, cells polarize apico-basally, grow strongly in height and form an apical zonula adherens (ZA) (Fig. 1C). In this conformation, most cell–cell adhesion complexes are localized near the apical surface. Cadherins and nectins concentrate just below the tight junctions to form strong actin contacted adhesions, although cadherin complexes lower down the lateral border are also described (Smutny et al., 2010). Thick, myosin-II-dependent actin bundles align tightly with ZA junctions, and this adhesion structure is dependent on the actin linkers α-catenin, EPLIN and vinculin (Abe and Takeichi, 2008; Miyake et al., 2006; Miyoshi and Takai, 2008; Watabe-Uchida et al., 1998; Yamazaki et al., 2008). In 2D culture, flat cells, such as endothelial cells, do not form a ZA and maintain linear adherens junctions. Also, several epithelial cell lines, including Madin Darby canine kidney (MDCK)-II cells do not form a clear apical ZA under normal 2D culture conditions. The fact that not all E-cadherin-positive epithelial cell lines form the same type of E-cadherin-containing cell–cell junctions might explain some of the current discrepancies in the field, including whether or not vinculin is present at cell–cell junctions and whether or not its presence is regulated by tension (see below).

Interestingly, during hepatocyte growth factor (HGF)-induced disruption of epithelial cell sheets, and during the collective cell movements induced by scratch wounding of epithelial monolayers, the steps of cell–cell adhesion maturation appear to occur in reversed order: cells flatten, there is a loss of apical junctional F-actin, the contractility of cytoplasmic actomyosin increases and the actomyosin cytoskeleton reorganizes to form radial bundles that are connected to remaining punctate cell–cell junctions, which then eventually are broken (de Rooij et al., 2005; le Duc et al., 2010; Mangold et al., 2011; Taguchi et al., 2011). A clear analogy exists between HGF-induced junction disruption and vascular hormone-driven junction remodeling in endothelial cells, which also encompasses a force-dependent formation of punctate cell–cell junctions (Huveneers et al., 2012). We have recently proposed to collectively name the punctate cell–cell junctions that are observed during the formation, remodeling and disruption of cell–cell adhesion, focal adherens junctions (FAJ) (Fig. 1A), to underscore the fact that they depend on classical cadherins and to highlight their analogy to integrin-based FAs, which also connect to radial F-actin bundles and depend on tension (Huveneers et al., 2012). In summary, different conformations of cadherin-containing cell–cell junctions exist that are interchangeable, and each subtype is characterized by a specific local organization of the actomyosin cytoskeleton and associates with a different set of intracellular proteins. What this means for the organization of and response to forces will be discussed below.

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Forces at cell–cell junctions

Transitions between the different junctional conformations, and the concomitant changes in cytoskeletal organization must lead to alterations in the magnitude and directions of forces exterted on cell–cell junctions. The magnitude of forces at cadherin-based junctions has been measured at different scales. Analysis of force-induced dissociation of single cadherin–cadherin interactions in atomic force microscopy shows that they can resist forces ranging from ~10 to 157 pN for E-cadherin, 35 to 55 pN for VE-cadherin and 17 to 40 pN for N-cadherin bonds (Baumgartner et al., 2000; Panorchan et al., 2006; Perret et al., 2004; Shi et al., 2008). The amount of tension that E-cadherin- or N-cadherin based cell–cell junctions between cell doublets in suspension can resist before breakage ranges between ~1 and 200 nN (Chu et al., 2004; Stockinger et al., 2011). Intriguingly, force-induced disruption of the cadherin complex between cell doublets appears to occur at the interaction between β-catenin and α-catenin, leaving cadherin and β-catenin at the plasma

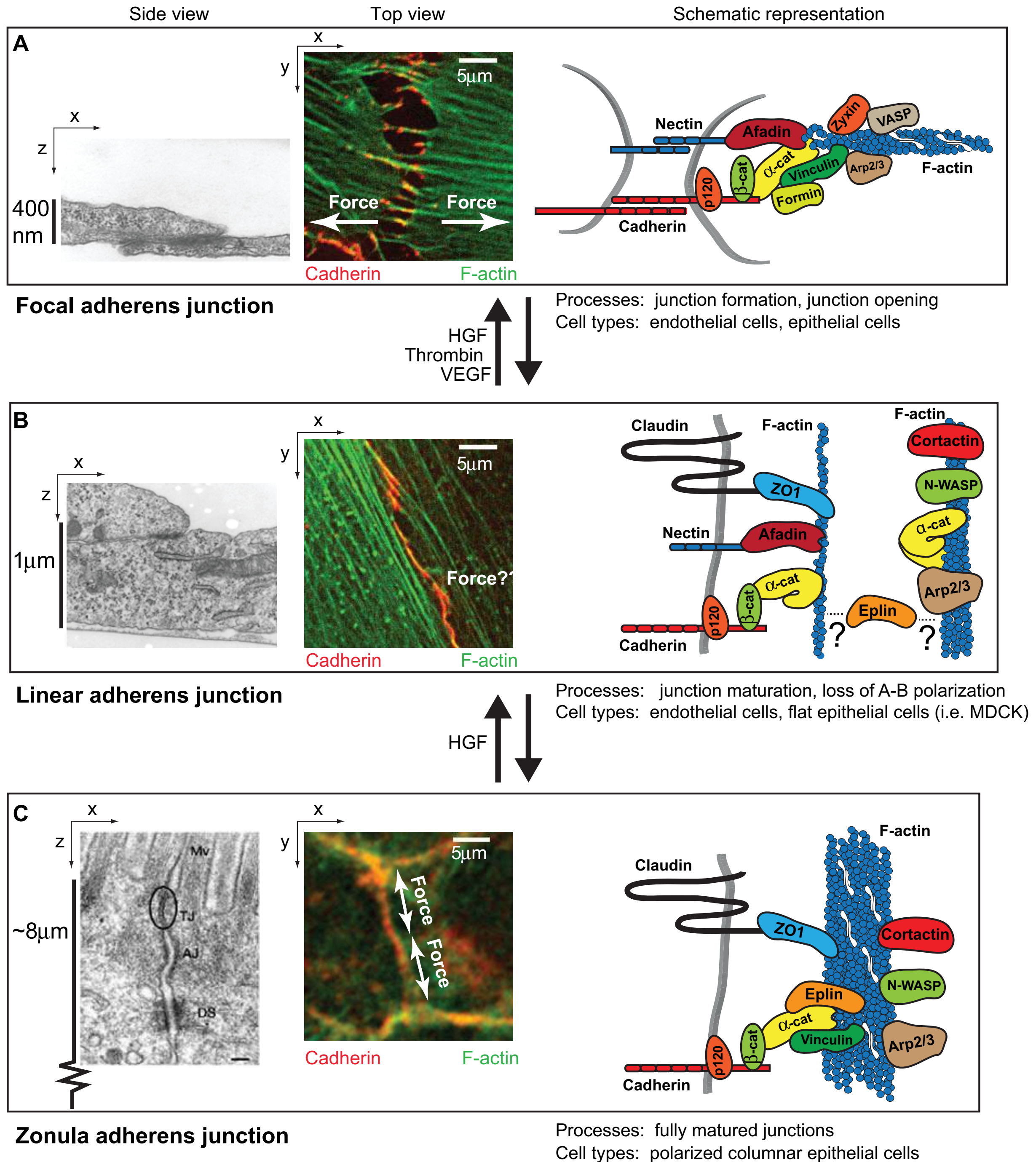


Fig. 1. See next page for legend.

membrane, whereas α-catenin and actin are disassembled in the cytoplasm (Maître et al., 2012). The pulling forces that are exerted on cell–cell adhesions in small groups of cells have recently been calculated from traction force measurements to range between ~40 nN to ~150 nN (Ganz et al., 2006; Liu et al., 2010b; Maruthamuthu et al., 2011). Experiments using the fluorescence resonance energy transfer (FRET)-based 'TSMod' tension sensors indicate that tension on single cadherins at cell–cell junctions are in the low pN range, similar to forces across vinculin in FAs [(Borghi et al., 2012; Grashoff et al., 2010) and M. A. Schwartz, personal communication], and the fluctuations of single filament forces in the cytoskeleton appear to be in the same range (5–7 pN) as indicated by the use of the spectrin-based sstFRET probe in cytoskeletal proteins (Meng and Sachs, 2011). It should be noted that the FRET-based tension measurements in adhesion complexes are on the basis of the average of FRET values in a large amount of clustered proteins. It is possible that only a fraction of the cadherin molecules in such clusters is in fact exposed to cytoskeletal pulling forces. This would mean that the actual range of forces that cadherins that are connected to the cytoskleleton experience, has been highly underestimated in these studies. As the average tension on single proteins in cell–cell junctions appears to be about four orders of magnitude lower than the tension on the entire cell–cell junctions, it would be interesting to test whether these values can indeed be related. This could be achieved, for instance, by measuring force on single molecules, total junction forces and the number of cadherin proteins within one single unit of cell–cell adhesion (e.g. a FAJ).

Qualitative studies using laser ablations to disrupt junction-connected actin networks revealed that, at the ZA, actomyosin-based tension is oriented laterally along the junction (Fig. 1C) (Cavey et al., 2008; Farhadifar et al., 2007), whereas tensile radial actin bundles that are connected to FAJs create tension that is oriented perpendicular to the junctions towards the cytoplasm (Fig. 1A) (Huveneers et al., 2012; Liu et al., 2010a; Liu et al., 2010b). The mechanosensing protein vinculin is localized to FAJs as well as at ZA junctions, but it is mostly absent from the linear junctions that are found in flat cells, such as Human umbilical vein endothelial cells (HUVECs) and MDCKs (Huveneers et al., 2012; le Duc et al., 2010; Yamada et al., 2005). This could mean that there is less tension on the cadherin complex at linear junctions than at other junction stages; a notion that is corroborated by the recent study that used E-cadherin–TSMod in MDCK cells to show that tension on E-cadherin in linear AJs is comparable with tension experienced by E-cadherin molecules that are in the plasma membrane outside of cell–cell junctions (Borghi et al., 2012). The tension on E-cadherin increases about threefold when external stretch is applied to cells, and junctions remodel from linear into FAJ-like structures (Borghi et al., 2012). Generally speaking, force measurement experiments at cell–cell junctions have been limited thus far. It is, therefore, unclear how the magnitude and directions of forces fluctuate during cell–cell adhesion and tissue remodeling. It will be interesting to use these newly developed FRET-based tension sensors to systematically measure forces, or at least their fluctuations, that associate with the different stages and conformations of cell–cell junctions.

Fig. 1. Adherens junction structures associated with the actomyosin cytoskeleton. Cell–cell junctions exist in different conformations depending on the maturity of the junction, the cell polarization state or junction remodeling, which is regulated by hormones. Depicted here are three different junction types typically observed in cell culture. FAJs, linear adherens junctions and ZA junctions, which are distinct with regard to the organization of the associated actomyosin network, the amount and direction of tension applied to them, and their molecular composition. (**A**) FAJs: during the formation of cell–cell junctions or the remodeling of existing cell–cell junctions, junctions adopt a punctate morphology, and contain cadherin and nectin adhesion complexes (Huveneers et al., 2012; Taguchi et al., 2011; Takai et al., 2008; Twiss et al., 2012). Cadherin adhesions are physically pulled by perpendicular actomyosin bundles (shown in green in the immunofluorescence image). This probably brings α-catenin under tension, and alters its conformation (as indicated by the elongated yellow shape) to allow recruitment of vinculin to reinforce cell–cell adhesions (Huveneers et al., 2012; Yonemura et al., 2010). In addition, several other actin regulatory proteins are recruited to FAJs, such as zyxin, VASP, formin and ARP2/3, which might be linked to cadherin or nectin complexes directly, or are part of the actin cytoskeleton that is associated with FAJs. (**B**) After expansion of FAJs to linear junctions, thick actomyosin bundles run in parallel with the cell–cell contacts, whereas only thin F-actin structures exactly colocalize with junction markers. Linear junctions contain only very little vinculin, suggesting that they are not under tension (Huveneers et al., 2012; le Duc et al., 2010) and depend on actomyosin activity to recruit EPLIN (Taguchi et al., 2011). It is currently unclear whether EPLIN is interacting with the cell–cell adhesion complex, or the peri-junctional F-actin, or both (see question marks). EM and IF images from HUVECs in A and B were kindly provided by Adam Grieve and Joppe Oldenburg, Hubrecht Institute, Utrecht, The Netherlands. (**C**) In fully polarized cells, such as in epithelial monolayers, linear junctions further mature into ZA junctions (Watabe-Uchida et al., 1998). In this conformation, tight junction and cadherin–adhesion complexes are closely connected to thick actomyosin bundles, which constrict the apical region of the cell by applying forces that run in parallel to cell–cell junctions (Miyoshi and Takai, 2008). The formation of a ZA depends on the α-catenin interacting proteins vinculin and EPLIN (Abe and Takeichi, 2008; Watabe-Uchida et al., 1998). Actin regulatory proteins, such as cortactin, N-WASP and Arp2/3, are now in close proximity to the cell–cell adhesions complexes. The IF image of DLD1 colon carcinoma cells was kindly provided by Floor Twiss, Hubrecht Institute, Utrecht, NL. The EM image has previously been published by Tsukita and colleagues (Tsukita et al., 2001) and was used with permission. Mv, microvillus; DS, desmosome; AJ, adherens junction; TJ, tight junction.

Mechanosensing at cell–cell junctions

Adhesion receptors currently implicated in mechanosensing

In recent years, classical cadherin-based cell–cell adhesion complexes have clearly emerged as mechanosensors. N-cadherin has been implicated to act as a mechanosensor in experiments that use micropillars to show a co-dependence of pillar stiffness and N-cadherin-based traction forces (Ladoux et al., 2010). Direct evidence for mechanosensing by E-cadherin has been obtained from magnetic twisting cytometry of E-cadherin-coated magnetic beads, which demonstrates that E-cadherin-based cell–bead adhesions are stiffened in response to prolonged shear forces, in a manner that is proportional to their magnitude (le Duc et al., 2010). In endothelial cells, exertion of increased force on cell–cell junctions by direct mechanical tugging or myosin contraction increases junction size without a loss of tension on the junction structure itself, suggesting the activation of a mechanoresponse at the VE-cadherin complex that enhances cell–cell adhesion (Liu et al., 2010b). The most recent example of mechanosensing by a classical cadherin is that of the pulling of beads coated with C-cadherin adhering to *Xenopus* cells; this results in a polarized cell protrusion activity in the direction opposite to the pulling force (Weber et al., 2012). This

mechanoresponse was shown to be dependent on intermediate filaments that connect to cadherin-based cell–cell junctions through plakoglobin, whereas, in the other cases described above, the measured cadherin mechanoresponses depend on the actomyosin cytoskeleton. It should be noted that in most of these studies, no direct biochemical readout of cadherin mechanotransduction was used. Hence, the involvement of biochemical signals (which is part of the definition of mechanotransduction) in these studies was only inferred from the apparent downstream cellular responses, and the challenge now is to delineate the biochemical pathways involved. Although recent work clearly implicates classical cadherins in cell–cell mechanosensing, several other cell–cell junction receptors that physically connect to cytoskeletons exist, and it is highly possible that additional mechanosensitive adhesion receptor complexes are present at cell–cell interfaces. In this context, interestingly, platelet endothelial cell adhesion molecule 1 (PECAM1) has been identified to be a mechanosensor in shear-flow alignment of endothelial cells, whereas VE-cadherin merely exerts an adaptor role in these cells (Tzima et al., 2005). It is likely that the current models of mechanosensing at cell–cell junctions will need to be adapted in order to accommodate the contribution of multiple adhesion complexes in their appropriate cellular context.

α-catenin and vinculin – a central protein pair in cadherin mechanotransduction

Clearly, to function in mechanosensing, cell–cell adhesion receptors must be present in a force chain that connects the cytoskeletal networks of two neighboring cells. The existence of a linear physical connection between cadherins and F-actin has been debated in recent years, because α-catenin that has been purified from cell lysates was found to be unable to bind to F-actin and β-catenin simultaneously (Drees et al., 2005; Yamada et al., 2005). The studies described above – which identified cadherins as mechanosensors – nevertheless argued that such a physical connection does exist in intact cells. Furthermore, recent studies of α-catenin indicate that, in intact cells, it exists in a conformation that allows it to directly bridge β-catenin and F-actin (Kwiatkowski et al., 2010; Twiss et al., 2012; Yonemura et al., 2010). Thus, the current models still favour a minimal core structure consisting of cadherin, β-catenin and α-catenin that is sufficient to form cadherin-based junctions that are connected by actin. These three proteins would be the core force-bearing unit at cadherin-based junctions (Fig. 2). The key mechanosensing event in this unit then is the force-induced stretching of α-catenin, which opens up a binding site for vinculin (Yonemura et al., 2010). The resulting recruitment of vinculin is needed for the mechanotransduction response (cell-stiffening), which has been observed in magnetic twisting cytometry experiments with cadherin-coated beads (le Duc et al., 2010; Twiss et al., 2012). Moreover, by preventing vinculin recruitment (by replacing the vinculin binding site in α-catenin), we showed that force-dependent reinforcement of cell–cell adhesion is a function of junctional vinculin. Vinculin that is recruited to FAJs during thrombin-induced endothelial junction remodeling protects junctions from breaking, and when it is recruited to FAJs during *de novo* formation of epithelial cell–cell junctions, it enhances sealing of the epithelial sheet (Huveneers et al., 2012; Twiss et al., 2012). A role for vinculin in the tightening of epithelial cell–cell junctions and the formation of the ZA has been shown previously (Maddugoda et al., 2007; Watabe-Uchida et al., 1998), but it remains unknown how this role relates to its force-dependent recruitment to α-catenin and its localization to FAJs. Thus, α-catenin and vinculin form a central functional module in cadherin mechanosensing, and investigating the role and regulation of their diverse interactions with other proteins will be important to delineate the details of this process. We have summarized the current knowledge about the structure, conformational regulation and interactors of α-catenin and vinculin in Box 1.

Exactly how vinculin propagates the mechanoresponse to bring about the reinforcement of cell–cell junctions is not known. On the basis of biochemical studies, the recruitment of vinculin upon

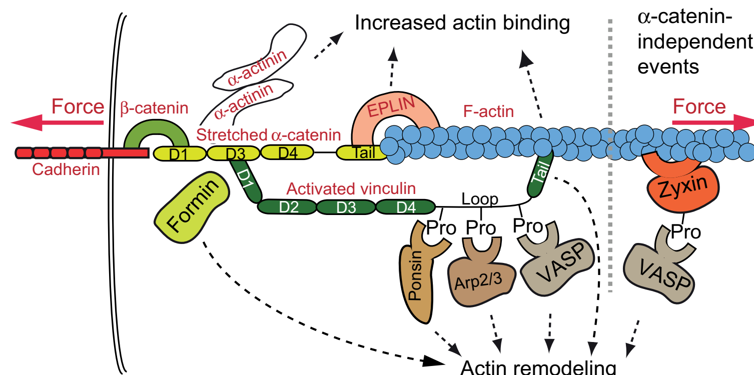
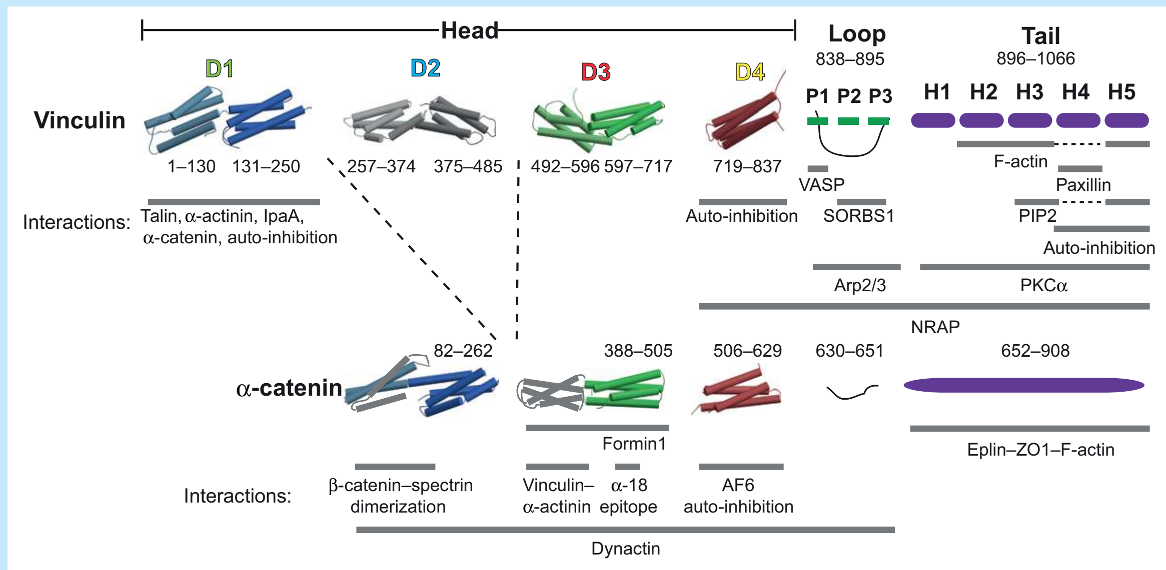


Fig. 2. Possible force-induced events at the cadherin–F-actin interface. This schematic shows the events that might be induced by an increase in tension on cadherin-based cell–cell junctions. Stretching of α-catenin results in the release of intramolecular interactions and an increase in the availability of protein-binding sites. The resultant binding of vinculin results in its allosteric activation (see Box 1) releasing the F-actin binding site. Tension across vinculin, which is now in the cadherin–F-actin force-chain, might further affect its conformation. Recruitment of α-actinin and EPLIN by α-catenin might further reinforce the cadherin–F-actin linkage, and recruitment of formin-1 induces actin remodeling. Vinculin might also bring actin remodelers, including VASP, ponsin and the Arp2/3 complex, to the cadherin adhesion. Force-induced deformation of F-actin itself, which is independent of α-catenin or vinculin regulation, can result in the recruitment of zyxin and VASP to further regulate F-actin remodeling. It remains to be elucidated which of these possible events elicited by an increase in tension on cell–cell junctions is really involved in cadherin mechanotransduction. Pro, proline residue.

Box 1. α-catenin, vinculin and their interaction partners – a central hub in cadherin mechanotransduction

α-catenin and vinculin are structurally homologous proteins, with a relatively low sequence homology. Overall structural information comes from free full-length vinculin, which is comprised of multiple helical-bundle modules (Bakolitsa et al., 2004). The smallest modules are four-helix bundles, which form the characteristic domains D1–4 that build the vinculin head domain (see Figure). Structural information of free α-catenin has been limited to the fragments shown in full color (amino acid residues 82–262 and 388–629) (Pokutta et al., 2002; Pokutta and Weis, 2000), but indicates that its overall organization is similar to that of vinculin (although the D2 domain is not present in α-catenin). Gray rods (in α-catenin) represent domains for which no structural information is available. The tail domain of vinculin consists of five α-helices (schematically drawn for a clear display of interactions) and is separated from the head domain by a flexible loop that contains several proline-rich elements. In free, full-length vinculin, the D1 domain is interacting with the tail domain, which shields it from its binding partners (Johnson and Craig, 1995). The D4 domain also impairs D1 interactions (Cohen et al., 2005). Interactions with the D1 or tail domain of vinculin influence the interactions of its other domains, and this allosteric regulation is referred to as 'activation' of vinculin. For α-catenin, there is also evidence for an allosteric regulation of its actin-binding activity (Drees et al., 2005), as well as its vinculin-binding activity (Choi et al., 2012; Yonemura et al., 2010). Intermolecular shielding of the vinculin-binding domain is taking place within the head domain through an interaction between the α-catenin D3 and D4 domains. This interaction is relieved in a myosin-dependent manner, probably by stretching of the protein (Yonemura et al., 2010). Moreover, the vinculin-binding portion of α-catenin is linearized when it is co-crystallized with vinculin, showing directly that extensive unfolding events occur (Choi et al., 2012; Rangarajan and Izard, 2012). Additional interactions besides those with vinculin are likely to be influenced by force-dependent unfolding of α-catenin, and protein interactions with vinculin itself might be affected as well if tension across vinculin increases. Taken together, α-catenin and vinculin are proteins that consist of helical modules. Intermolecular autoinhibitory interactions occur between these modules, and their force-dependent disruption appears to be central to mechanotransduction at cell–cell junctions. PIP2, phosphatidylinositol 4,5-bisphosphate.



stretching of α-catenin could simply strengthen cell–cell adhesion by providing an additional bond between the cadherin complex and F-actin (Fig. 2). Indeed, binding of α-catenin to the head domain of vinculin enhances the binding of the vinculin tail to F-actin (Choi et al., 2012; Peng et al., 2012), as has been shown for many of previously identified factors that bind to the head domain of vinculin (Ziegler et al., 2006). This scenario would place vinculin into the force chain, between F-actin and cadherin, and any tension across the cadherin-F-actin linkage might therefore affect its conformation. Because vinculin contains two actin-binding sites at its C-terminal tail (Hüttelmaier et al., 1997), it might further reinforce cell–cell adhesion by bundling of F-actin that is attached to the cadherin complex. In addition to increasing the binding of F-actin, vinculin might bring other factors to cadherin adhesions, such as members of the vasodilator-stimulated phosphoprotein (VASP) (Brindle et al., 1996) and ponsin families (Kioka et al., 1999; Mandai et al., 1999), as well as the ARP2/3 complex (DeMali et al., 2002; Tang and Brieher, 2012), to regulate F-actin remodeling. Moreover, there is evidence that vinculin itself has an actin-polymerization activity in its tail domain (Le Clainche et al., 2010; Wen et al., 2009). Therefore, a force-dependent increase in actin polymerization at cell–cell junctions might serve to counteract the pulling force that is exerted by contractile F-actin. In addition to binding vinculin, the force-regulated D3 domain of α-catenin can bind to α-actinin (Nieset et al., 1997) and its tail domain to EPLIN, which is somehow regulated by myosin activity (Taguchi et al., 2011). These interactions might further reinforce the linkage between cadherin and F-actin (Fig. 2). Finally, the actin nucleator formin-1 also binds to the D3 domain of α-catenin (Vasioukhin et al., 2000) and might, thus, contribute to force-induced actin-polymerization at cell–cell junctions. However, it remains to be elucidated which of these possible events is indeed elicited by an increase in tension on cell–cell junctions and thus contributes to cadherin mechanotransduction.

Vinculin and EPLIN – sensors of cadherin–F-actin tension or sensors of F-actin organization?

The differences in the cadherin complex and the connected actomyosin cytoskeleton that have been observed for the various cell–cell junction conformations provoke the question whether the same mechanical mechanisms apply for all cell–cell junctions. The myosin-dependent recruitment of vinculin occurs both at the tensile FAJs and at the ZA, but EPLIN is only found at ZAs and is excluded from FAJs. Moreover, the release of tension on FAJs by ablating the connected F-actin bundles increases the amount of EPLIN at the resulting unstressed cell–cell contact sites (Taguchi et al., 2011). Thus, it appears that the presence of vinculin and EPLIN at FAJs is oppositely regulated by force, suggesting that they fulfill complementary functions. There are also differences in the organization of F-actin at FAJs and at the ZA that raise the question of whether they employ the same molecular mechanisms for vinculin recruitment. As indicated by laser ablation experiments at cell–cell junctions (Cavey et al., 2008; Farhadifar et al., 2007; Huveneers et al., 2012), the direction of force at ZA and FAJ is different, and ultrastructural analysis of the associated actomyosin cytoskeleton demonstrates that these adhesive structures form different connections to F-actin (Yonemura et al., 1995). The radial, contractile F-actin bundles that directly connect to FAJs can be easily envisioned to put tension on the cadherin complex (Fig. 1A). However, it is more difficult to imagine how the parallel actin bundles that align the ZA in monolayers and anchor at tri-cellular connections (Fig. 1C) exert tensile force on cadherin complexes that are located in the middle between two anchoring points. Interestingly, it has been shown that vinculin can localize to apical cell–cell junctions in a force-dependent and force-independent manner. The latter is mediated by an increased stabilization of F-actin, and could thus rely on the actin-binding activity of vinculin, rather than on its interaction with α-catenin (Sumida et al., 2011). EPLIN also localizes to the actin cytoskeleton outside of cell–cell junctions [(Abe and Takeichi, 2008; Song et al., 2002) and our unpublished observations]. On the basis of these findings, one could speculate that, at least in part, the localization of EPLIN and vinculin to the ZA is indirectly induced by myosin II activity through a stabilization of F-actin at cell–cell junctions (Shewan et al., 2005), whereas the localization of vinculin at, and the exclusion of EPLIN from FAJs, is directly regulated by force through its concomitant deformation of α-catenin. In the case of FAJs, we directly confirmed the importance of α-catenin for the recruitment of vinculin to junctions by replacing endogenous α-catenin with a mutant form (α-catenin–ΔVBS), which lacks the vinculin-binding sequence. In these cells, FAJs that are devoid of vinculin still form, indicating that vinculin is not required for junction formation per se (Huveneers et al., 2012). Experiments that address the recruitment of these proteins to the ZA will be more challenging, because a ZA is not formed at all in the absence of vinculin or EPLIN recruitment (Abe and Takeichi, 2008; Watabe-Uchida et al., 1998). In conclusion, the exact mechanisms that recruit vinculin and EPLIN to the distinct junctions structures are still unclear, and might involve both recruitment by force-induced changes in α-catenin and changes in the F-actin cytoskeleton.

Zyxin and VASP – potential cell–cell mechanosensing beyond vinculin

In addition to vinculin and EPLIN, other proteins have been found to accumulate at force-dependent cell–cell junctions, including Zyxin and VASP (Nguyen et al., 2010; Sperry et al., 2010). The molecular mechanisms underlying their recruitment to junctions have not been established and might be complex. Zyxin is recruited to tensile F-actin (Nguyen et al., 2010; Yoshigi et al., 2005), which is certainly present at cell–cell junctions, but the close Zyxin homologs Ajuba and lipoma preferred partner (LPP) are known to be recruited by α-catenin and α-actinin (Hansen and Beckerle, 2008; Marie et al., 2003), which are also present in mechanical force-chains. Moreover, Zyxin localization to cell–cell adhesions has recently been attributed to its interaction with nectin (Gregory Call et al., 2011). VASP is recruited to F-actin structures by Zyxin (Smith et al., 2010), and this may similarly mediate its recruitment to cell–cell junctions (Fig. 2). However, vinculin also contains a VASP-binding site (Brindle et al., 1996), providing another possible means of recruiting VASP to cell–cell junctions. Thus, potentially, the recruitment of Zyxin and VASP to tensile cell–cell junctions contributes to F-actin remodeling independent of α-catenin stretching, adding an additional layer to the mechanosensing machinery at cell–cell junctions.

Alternative mechanosensing mechanisms at cell–cell junctions

It is conceivable that alternative mechanosensing mechanisms are at play in cell–cell junctions besides the described force-induced stretching of α-catenin, although this has not been shown directly yet. In the case of integrin-mediated adhesion, both the stiffness of the ECM and actin flow rates determine the tension that proteins within this linkage experience, and a special subset of protein–protein bonds, the so-called 'catch bonds', directly respond to increases in tension by growing stronger (Moore et al., 2010; Vogel and Sheetz, 2006). Retrograde F-actin flow also occurs at the edge of cells when new junctions are formed or junctions are remodeling (Kametani and Takeichi, 2007). In these situations, it is likely that there is increased tension on proteins that connect cell–cell junction complexes and actin (Bard et al., 2008; Kametani and Takeichi, 2007), and perhaps tension-induced catch bonds exist within this linkage. Indeed, measurements of single cadherin molecules using atomic force microscopy indicate that cadherins themselves display catch bond properties in response to tension (Rakshit et al., 2012). Furthermore, mechanosensitive ion channels, such as the transient receptor potential cation channel subfamily V member 4 (TRPV4) that can interact with the cadherin complex through β-catenin, might localize at cell–cell contact sites (Janssen et al., 2011; Ko et al., 2001; Sokabe et al., 2010). This raises the possibility that force-dependent Ca^{2+} influxes are generated through classical cadherin complexes, similar to channel-dependent mechanosensing by the distantly related cadherin 23 in the inner ear (Müller, 2008). We expect that mechanosensing mechanisms at cell–cell junctions extend beyond α-catenin stretching, and a key challenge will be to delineate the hierarchy and interdependence of these multiple mechanosensitive systems.

Cadherin mechanosensing in development and disease

The cadherin mechanosensing systems discussed in this Commentary are likely to be involved in the biophysical control of tissue morphogenesis. As shown in *Drosophila melanogaster*, actomyosin-based forces applied at cadherin-based cell–cell adhesions further direct actomyosin contraction to control tissue elongation. These are clear examples of

developmental mechanosensing, but it is not known which proteins fulfill structural roles and which fulfill mechanosensing roles (He et al., 2010; Rauzi et al., 2010). In zebrafish cells, differential tension in the cortical cytoskeleton directs sorting of germline cells (Krieg et al., 2008). For this to occur, a mechanical coupling between E-cadherin-based cell–cell contacts and actin is required, and it was hypothesized that the strength of this coupling is set by the cortical actin tension through cadherin mechanosensing (Maître et al., 2012). In mammalian development, mechanical control mechanisms are likely to be similarly important (Papusheva and Heisenberg, 2010), and vinculin knockout mice indeed show severe defects in tissue morphogenesis, which are most notable in the development of the central nervous system and the heart (Xu et al., 1998). During tumor progression, physical parameters also control morphogenesis. It is well established that increased stiffness of the extracellular matrix, concomitant with intracellular actomyosin contractility, results in disruption of epithelial organization (DuFort et al., 2011; Paszek et al., 2005; Schedin and Keely, 2011). Large-scale gene transcription has been shown to be regulated by tumor tissue stiffness (DuFort et al., 2011; Paszek et al., 2005; Schedin and Keely, 2011), and the transcriptional regulators YAP and TAZ have been identified as key intermediates in mechanically-induced cell growth and differentiation processes (Dupont et al., 2011). Interestingly, YAP was found to be directly regulated by α-catenin in epidermal stem cells (Schlegelmilch et al., 2011; Silvis et al., 2011), and by the E-cadherin complex in epithelial cell lines (Kim et al., 2011), but it is unknown whether this relates to mechanical forces at cell–cell junctions. Thus, mechanical forces are driving embryonic and pathological tissue morphogenesis events, and a role for cadherin mechanosensing is anticipated, although any direct evidence has not yet been provided.

Many other examples that emphasize the physiological importance of sensing mechanical forces come from vascular studies (Hahn and Schwartz, 2009; Ingber, 2002), in which hemodynamic forces remodel vessels (Lucitti et al., 2007) or regulate growth factor-induced sprouting angiogenesis (Song and Munn, 2011). Here, mechanosensing mechanisms are clearly implicated in disease conditions. Endothelial responses to shear flow have atheroprotective functions, and a disturbance of blood flow, which leads to a drop in shear force and to different shear force patterns, is strongly associated with the development of atherosclerosis (Cunningham and Gotlieb, 2005; Hahn and Schwartz, 2009). Dynamic regulation of the cadherin–F-actin linkage is also important for transendothelial migration of leukocytes (Schulte et al., 2011). In this process, transient remodeling of cell–cell junctions is required (Vestweber, 2002) and thrombin is one of the hormones that regulates this. On the basis of our results that, during thrombin-induced junction remodeling, junctions are protected by force-induced recruitment of vinculin (Huveneers et al., 2012), we anticipate that cadherin mechanosensing is involved in this process *in vivo*. This would suggest that therapeutically stabilizing the interaction between α-catenin and vinculin might result in vascular stabilization, which will be useful to treat the many inflammatory-based diseases that show excessive leukocyte transmigration. Furthermore, excessive Rho-activity and prolonged actomyosin contraction, which leads to disruption of endothelial barrier function (Krishnan et al., 2011), is often observed in pulmonary endothelial dysfunction (Storck and Wojciak-Stothard, 2012). Here, a stabilization of the interaction between α-catenin and vinculin could be envisioned to provide protection against the loss of vascular barrier function, which causes a large part of the associated clinical conditions.

Conclusions and future perspectives

Mechanoresponsive adhesion systems emerge as signaling mechanisms in tissue development and disease, and increasing our understanding of the molecular machineries involved will enhance our knowledge of their relevance *in vivo*. Thus far, the best-documented downstream effect of cadherin mechanosensing is the protection of cell–cell junctions through recruitment of vinculin. Harnessing this effect through the stabilization of vinculin at junctions could be employed as a treatment for vascular diseases that entail junction instability. Further elucidation of mechanosensitive pathways and their underlying molecular mechanisms might thus lead to the discovery of therapeutic targets and intervention strategies.

It is likely that the full molecular details of the interaction between α-catenin and vinculin will be deciphered by structural biologists, and some structures of the interaction sites have already appeared in the literature (Choi et al., 2012; Rangarajan and Izard, 2012) (see Box 1). This will allow the development of specific mutants that abolish this particular interaction without perturbing any other crucial functions. The use of such α-catenin or vinculin mutants in animal models will allow to assess the importance of this interaction in tissue morphogenesis, inflammation and tumor progression. Apart from vinculin, several other interaction partners of α-catenin might be affected by its force-dependent conformational regulation. Further investigation of the composition of FAJs and the underlying regulatory mechanisms will increase our understanding of force-dependent junction remodeling, and our eventual ability to manipulate this process for therapeutic use or in tissue engineering approaches.

Finally, the recent development of FRET-based tension sensors, and the elucidation of force-dependent protein interactions and localizations allow to directly assess forces and their fluctuations by light microscopy. These tools will strongly benefit the investigation of the mechanical properties of cell–cell adhesions and their regulation in complex biological systems, such as developing tissues, embryos and tumors, which had previously been beyond our technical abilities. Therefore, new insights into the biophysical properties of tissues and tumors, and their relation to the state and progression of disease are expected to be available in the near future.

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