Cadherin Adhesion and Mechanotransduction

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Annu. Rev. Cell Dev. Biol. 2014. 30:291–315

First published online as a Review in Advance on July 9, 2014

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi: 10.1146/annurev-cellbio-100913-013212

Keywords

α-catenin, actin, cytoskeleton, morphogenesis, allostery

Abstract

Cadherins are the principal adhesion proteins at intercellular junctions and function as the biochemical Velcro that binds cells together. Besides this mechanical function, cadherin complexes are also mechanotransducers that sense changes in tension and trigger adaptive reinforcement of intercellular junctions. The assembly and regulation of cadherin adhesions are central to their mechanical functions, and new evidence is presented for a comprehensive model of cadherin adhesion, which is surprisingly more complex than previously appreciated. Recent findings also shed new light on mechanisms that regulate cadherin junction assembly, adhesion, and mechanotransduction. We further describe recent evidence for cadherin-based mechanotransduction, and the rudiments of the molecular mechanism, which involves α-catenin and vinculin as key elements. Potential roles of a broader cast of possible force-sensitive partners are considered, as well as known and speculative biological consequences of adhesion and force transduction at cadherin-mediated junctions.

Contents

INTRODUCTION

Cadherins are a large superfamily of calcium-dependent adhesion proteins that constitute the principal adhesion proteins mediating cell-cell cohesion in all soft tissues (Niessen et al. 2011). The classical cadherins, which are the most extensively studied, typically form homophilic, adhesive bonds with cadherins on adjacent cells. The adhesive function is crucial for maintaining the integrity of many multicellular tissues. The mechanical linkages are also dynamically regulated to enable rapid disruption of intercellular contacts, as in leukocyte extravasation, or programmed changes in cell positioning during tissue morphogenesis (Gumbiner 2005, Niessen et al. 2011). Both the mechanism of cadherin adhesion and its regulation are key determinants of tissue homeostasis.

Adhesion: the force required to separate a bond or disrupt junctions between two surfaces, such as between two cells

In addition to this mechanical function, cadherin ligation activates signaling cascades that regulate cytoskeletal organization and broader functions, such as cell cycle progression and differentiation (Niessen et al. 2011). Recent findings (Ladoux et al. 2010, Leckband et al. 2011, le Duc et al. 2010, Liu et al. 2010, Yonemura et al. 2010) revealed that cadherin complexes are also force sensors that transduce fluctuations in intercellular tension into intracellular signals that regulate such tissue functions as barrier permeability and tissue remodeling.

This review provides a comprehensive examination of cadherin mechanics with particular emphasis on recent findings pertaining to the mechanical functions of cadherin complexes. Because

adhesion and its regulation are central to the mechanobiology of cadherin adhesions, we review new evidence for a comprehensive model of cadherin adhesion, which is surprisingly more complex than the classical description of simply zipping cells together. Recent evidence is presented for additional mechanisms that regulate cadherin adhesion and junction assembly. We also emphasize cadherin-based mechanotransduction and the rudiments of the underlying molecular mechanism, by which cadherin complexes interpret tensional changes to alter both local and global mechanical properties of cells and tissues. We consider potential roles of a cast of possible mechanotransduction partners that are directly or indirectly associated with cadherin complexes, as well as known and speculative biological consequences of adhesion and force transduction at cadherin-mediated junctions.

Mechanotransduction: the conversion of changes in tension into biochemical perturbations (typically conformational changes) that alter molecular interactions or reactions in the cell

CLASSICAL CADHERIN STRUCTURE AND ITS RELATIONSHIP TO ADHESION AND SIGNALING FUNCTIONS

Classical Cadherin Structures

Classical cadherins are the most extensively studied of the cadherin superfamily. Their overall structure is composed of an extracellular domain, which embeds the adhesive function, and folds into five beta-barrel extracellular domains, with three calcium-binding sites at each interdomain junction. The extracellular domains are numbered one through five, beginning with the N-terminal domain, which embeds the primary adhesion site. In addition, there are a single-pass transmembrane domain and a cytoplasmic domain, which binds different cytoplasmic signaling and cytoskeletal proteins (**Figure 1**). The structural details are reviewed elsewhere (Shapiro & Weis 2009).

Cytoplasmic Domain and Its Interactions

Catenins are principal cytoplasmic binding partners and consist of α-, β-, γ-, and p120-catenin (p120ctn). There are other cadherin-associated proteins, but these are the principal (known) components in the mechanical chain between cadherin bonds and actomyosin. Of these, both β- and γ-catenin, or plakoglobin, bind to cadherin cytodomains and couple the complex to the actin cytoskeleton and possibly to intermediate filaments, respectively, through linker proteins (Shapiro & Weis 2009). This review focuses on the cadherin/β-catenin/α-catenin complex and actin. The actin-binding protein α-catenin binds β-catenin and mechanically links type-I classical cadherins to actin. This mechanical chain can directly influence cadherin adhesion strength by maintaining robust connections to the cytoskeleton, but the catenins can also regulate junction mechanics indirectly. First, p120ctn binding to cadherin cytodomains masks a ubiquitination sequence, which signals cadherin endocytosis. p120ctn thus modulates cell cohesion by regulating cadherin abundance at the membrane (Niessen et al. 2011). Second, p120ctn is a postulated master regulator of cadherin-dependent Rho GTPase signals, which in turn regulate cytoskeletal organization (Niessen et al. 2011). As discussed in this review, a third function of p120ctn is the inside-out regulation of cadherin adhesion (Petrova et al. 2012).

MECHANISM AND REGULATION OF CADHERIN-MEDIATED INTERCELLULAR ADHESION

The transduction of mechanical signals between cells and through the cytoskeletal network of tissues necessarily requires intercellular adhesion. Despite this seemingly simple mechanical

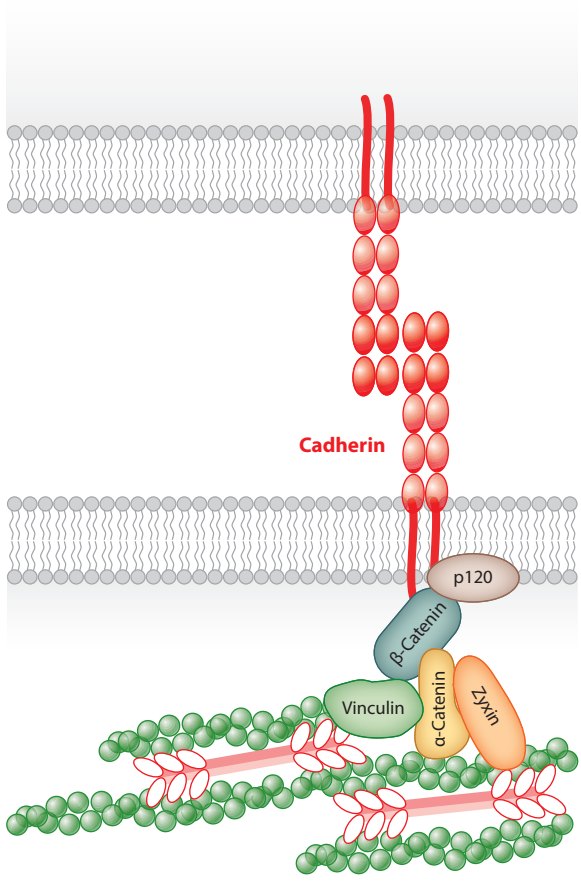


Figure 1

Architecture of classical cadherin junctions. Type-I classical cadherins form adhesive contacts through the N-terminal domains of the extracellular region. The main interactors with the cytodomain include p120ctn, β-catenin, and α-catenin, which can bind directly to F-actin or indirectly through other actin-binding proteins, such as vinculin.

function, the mechanism of cadherin-mediated cell adhesion is not simple. The following sections discuss current evidence for the mechanism of cadherin binding, factors that regulate this function, and their biological consequences.

Cadherins Form Multiple Bonds with Different Adhesive and Kinetic Properties

A widespread view for many years was that cadherin-based adhesion involved a single binding interface between the N-terminal domains. However, several studies with different experimental approaches more recently have demonstrated that cadherin adhesion involves the formation of multiple, different cadherin-cadherin bonds, which involve different structural regions and exhibit different kinetic and mechanical properties (Ciatto et al. 2010, Harrison et al. 2010, Leckband & Prakasam 2006, Leckband & Sivasankar 2012b, Rakshit et al. 2012).

Generally, experimental investigations of cadherin binding can be classified into three categories: structure determinations, solution-binding measurements, and adhesion- or force-based

measurements. Importantly, these approaches probe different properties of protein bonds, in part because of differences between protein interactions in crystals, association between freely diffusing proteins in solution, or protein binding within narrow intermembrane gaps (Wu et al. 2011, Zhu et al. 2000). Additionally, parameters determining the complex stability in solution may not determine the strength of bonds under tension, as at adhesive junctions (Marshall et al. 2003, Rakshit et al. 2012). Consequently, different approaches generated results that in some cases appeared to be contradictory, and the mechanism of cadherin binding remained controversial for nearly two decades. However, recent theoretical and biophysical studies appear to largely reconcile these differences and shed new light on the mechanism of cadherin adhesion (summarized in **Figure 2**).

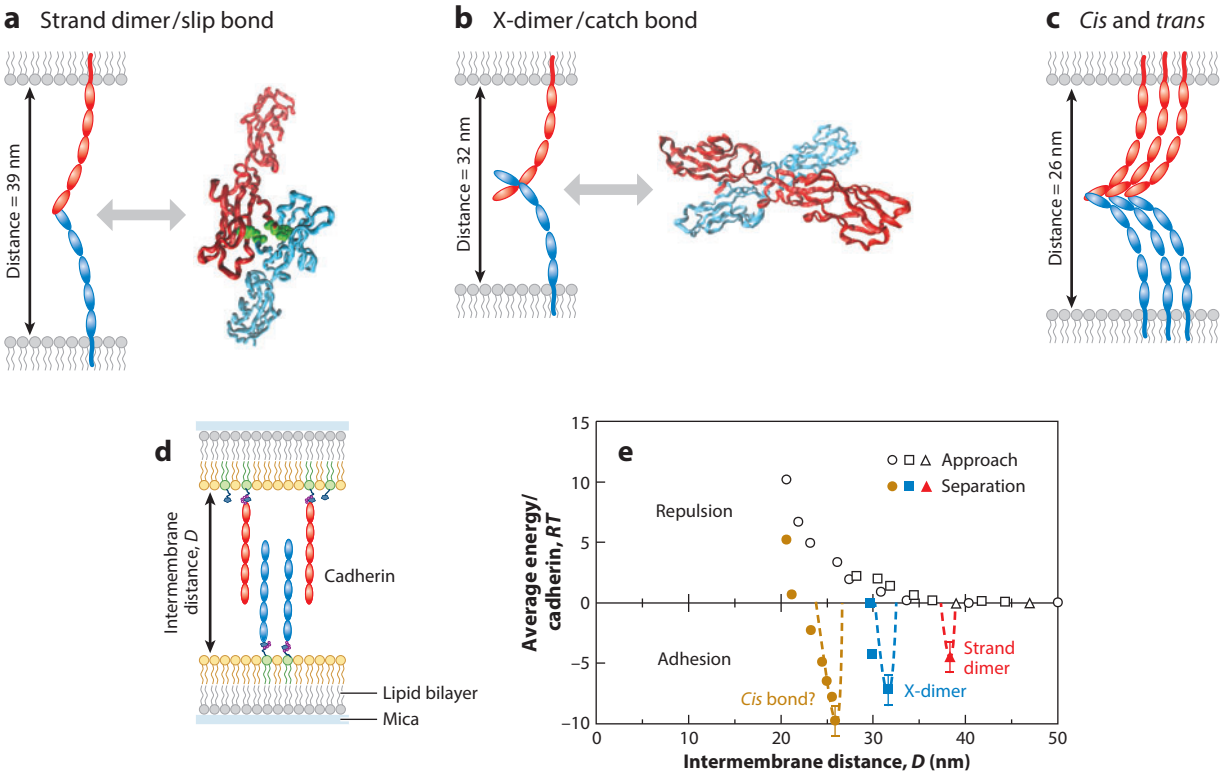
EC: extracellular domain in the cadherin extracellular region

Strand dimerization and *trans* adhesion. The primary adhesive interface of type-I classical cadherins initially identified in crystal structures of binding-competent extracellular domain fragments involves the mutual exchange of tryptophans at position 2 (Trp2) and their insertion into hydrophobic pockets on EC1 of the apposing protein (**Figure 2*a***) (Shapiro & Weis 2009). This is referred to as a strand dimer, and all biophysical studies, including crystallographic and NMR structures, solution-binding data, and adhesion-based measurements, confirmed that this strand dimer is crucial for cadherin adhesion (Leckband et al. 2011). The most compelling evidence is that mutating the conserved Trp2 residue to alanine (W2A) nearly abolishes cadherin-mediated adhesion, although W2A mutants localize to cell-cell junctions (Kitagawa et al. 2000, Tamura et al. 1998), and mutant ectodomains weakly aggregate beads (Prakasam et al. 2006).

Despite the critical role of the strand dimer interface in cadherin adhesion, experimental evidence has argued for a more complex binding mechanism that involves additional interactions. Despite the critical role of the strand dimer interface, the EC12 fragment was shown to be the minimal functional unit required for cell adhesion (Shan et al. 2004). However, E-cadherin mutations in the strand-dimer (EC1) interface ablate cell adhesion (Berx et al. 1998).

From a biophysical perspective, force versus distance measurements, which quantify repulsive and attractive forces between membrane-bound cadherin ectodomains as a function of the intermembrane distance (**Figure 2*d***), demonstrated that the cadherin ectodomains adhere at three different membrane separations (**Figure 2*e***) (Leckband & Prakasam 2006, Leckband & Sivasankar 2012a). Extracellular domain–deletion mutants mapped these spatially distinct bonds to different regions of the extracellular domain (Leckband & Prakasam 2006).

Subsequent determinations of the force needed to rupture single cadherin-cadherin bonds similarly identified multiple, distinct types of cadherin bonds (**Figure 2*f–h***) (Leckband & Sivasankar 2012a). The single bond studies determine the force to rupture individual molecular bonds. These are not equilibrium measurements, and the rupture force is related to the dissociation rate (Evans & Calderwood 2007, Evans & Ritchie 1997). In comparison, force-distance measurements (**Figure 2*d,e***) reflect many protein interactions and quantify both intersurface separation distances and the population-averaged adhesion energies of the bonds (Leckband & Israelachvili 2001). Conventional single bond rupture measurements steadily increase the force on a bond until it fails, and analyses of histograms of the rupture forces (see **Figure 2*g,h***) identify different bonds and quantify their strengths and dissociation rates (reviewed in Evans & Calderwood 2007, Leckband & Israelachvili 2001, Leckband & Prakasam 2006). Analyses of histograms of single cadherin bond rupture forces revealed that the ectodomains formed three distinct bonds with different kinetic and mechanical properties (**Figure 2*g***) (Leckband & Prakasam 2006). As in force versus distance measurements, each of these bonds required different regions of the cadherin extracellular domain (Shi et al. 2010). Recently reported structures of different adhesive complexes, in addition to the strand dimer, as well as new biophysical data now appear to reconcile these different experimental findings. These recent findings are described in detail below.


a Strand dimer/slip bond
Distance = 39 nm
b X-dimer/catch bond
Distance = 32 nm
c *Cis* and *trans*
Distance = 26 nm
d
Intermembrane distance, *D*
Cadherin
Lipid bilayer
Mica
e
Approach
Separation
Repulsion
Adhesion
Cis bond?
Strand dimer
X-dimer
Average energy/ cadherin, *RT*
Intermembrane distance, *D* (nm)
f
AFM tip
Cadherin
Glass surface
g
EC12 versus EC12
X-dimer
Trans dimer
Probability (10^{-2})
Rupture force (pN)
h
EC1–4 versus EC1–4
Strand dimer
X-dimer
Cis bond?
Rupture force (pN)
i
Test cell
Red blood cell
j
Test cell membrane
Cadherin
IgG Fc
Anti-Fc antibody
Red blood cell membrane
k
Cis bond?
P2
P1
Strand dimer
Cell binding probability
Cell-cell contact time (s)

X-dimer interface and its importance in force transmission. A second cadherin-binding interface was revealed in structures of the EC12 fragment of the nonclassical, truncated T-cadherin and of binding-incompetent EC12 fragments of E- and N-cadherins (Ciatto et al. 2010, Nagar et al. 1996). T-cadherin lacks the Trp2 essential for the strand-swapped dimer between type-I classical cadherins (Dames et al. 2008, Shapiro & Weis 2009). Nevertheless, it is adhesion competent, as demonstrated by the fact that it facilitates neurite outgrowth (Ranscht & Dours-Zimmermann 1991). In crystals, T-cadherin EC12 fragments associate in an antiparallel, crossed configuration (**Figure 2*b***), referred to as the X-dimer, which involves extensive interprotein contacts at the EC1–EC2 junction (Ciatto et al. 2010). Nagar et al. (1996), who crystallized an inactive form of EC12 of N-cadherin with an unprocessed N-terminal methionine, which inhibits strand dimerization, first reported an X-dimer structure, but its significance was not realized initially because the crystallized fragment contained the unprocessed N-terminal Met and was presumed inactive. The W2A mutant of E-cadherin similarly forms X-dimers in crystals (Ciatto et al. 2010).

SPR: surface plasmon resonance

AUC: analytical ultracentrifugation

Solution-binding studies of the X-dimer and its mutants identified one possible function of X-dimers. Surface plasmon resonance (SPR) measurements quantified the impact of X-dimer mutations on association and dissociation rates between soluble and immobilized EC12 fragments, and analytical ultracentrifugation (AUC) measurements quantified their impact on the equilibrium dissociation constants (K_d) (Leckband & Sivasankar 2012b). The K14E mutation at this interface disrupted homotypic interactions by both EC12 fragments of T-cadherin and E-cadherin W2A mutants, which cannot strand dimerize. However, the K14E mutant of EC12 increases the activation barrier in the dimerization reaction pathway, and thus affects the rates of strand-dimer formation and dissociation but not K_d (Leckband & Sivasankar 2012b). This mechanism also appears to operate at cell junctions. Live-cell fluorescence recovery after photobleaching studies showed that the K14E mutant retards cadherin exit from intercellular junctions, consistent with slowed strand-dimer dissociation (Hong et al. 2011). The X-dimer thus appears to be an important intermediate in the strand-dimerization reaction pathway that lowers the activation energy and accelerates the formation of more stable (lower-K_d) strand dimers. Which complex predominates at cell-cell junctions is unknown, but the X-dimer K_d (0.92 mM) relative to the strand-dimer K_d (0.099 mM) of E-cadherin EC12 fragments suggests that some X-dimer would be present.

Despite its higher K_d, the X-dimer adhesion was stronger than the strand dimer in biophysical measurements. In force-distance measurements (**Figure 2*e***), adhesion at the intermembrane distance of 32 nm is stronger than the strand-dimer adhesion at 39 nm. The intermediate bond is due to the X-dimer, because (*a*) 32 nm compares with the X-dimer length; (*b*) W2A mutants

Figure 2

Comprehensive biophysical and structural model of cadherin adhesion. (*a*) Cartoon and crystal structure of the *trans* dimer, which forms a slip bond. (*b*) Crystal structure of the X-dimer, which forms a catch bond, and cartoon depicting the intermembrane configuration of the complex. (*c*) Proposed formation of *cis* and *trans* dimers. (*d*) Cadherin configurations in (*e*) force-distance measurements between oriented ectodomain monolayers. Cadherin ectodomains adhere at three membrane distances indicated by the three force minima ($F < 0$). (*f*) Schematic of a single bond rupture atomic force microscopy (AFM) experiment. (*g*) Histogram of single-bond rupture forces (pN) between EC12 fragments measured at a pulling rate $R = 800$ pN/s. The larger peak at ~50 pN is due to X-dimers, and the small peak at ~30 pN corresponds to the rupture of the *trans* dimer. (*h*) Histogram of single-bond rupture forces between EC1–4 fragments showing three distinct peaks (bonds), due to rupture of the *trans* dimer, X-dimer, and possibly *cis* bonds. (*i*) Configuration of test cell expressing cadherin and a red blood cell modified with oriented cadherin extracellular domains, as shown in *j*. (*k*) Cell-binding probability versus cell-cell contact time between a test cell and modified red blood cell, as shown in *i*. A rapid initial increase in binding probability to $P1 = 0.45$ is followed by a delay and then a second, slower increase to a higher binding probability at $P2 = 0.68$ (*dashed line*). The solid line is a fit of the initial binding data to a kinetic model for strand dimerization.

SLIP BONDS AND CATCH BONDS

The mechanical strength of a noncovalent bond is determined by steadily increasing the force on a bond until it fails at the rupture force, F_r, which determines the bond strength. The increase in force is rapid enough to rupture the bond quickly—typically within 1 ms. Because bonds dissociate more slowly in the absence of force, force acts to increase the rate of receptor-ligand dissociation. This is described mathematically by $k_{off} = k_0 \exp[-(E_{act} - F \cdot \gamma)/kT]$. Here, F is the force, k_0 is the dissociation rate in the absence of force, E_{act} is the activation energy, and γ is an empirical parameter that describes how the bond responds to force. Values of γ can be positive, negative, or zero (Dembo et al. 1988). For typical bonds, such as antibody-antigen bonds, the dissociation rate increases with the magnitude of the applied force. In such cases, $\gamma > 0$, and these bonds are referred to as slip bonds. However, if $\gamma < 0$, then the dissociation rate decreases with increasing force, and the bonds appear to be more stable at higher force. Such bonds are catch bonds. For example, selectin catch bonds slow leukocyte rolling under high shear forces in the bloodstream but are weaker at low shear, to enable cells to roll along the endothelium at lower fluid shear (Marshall et al. 2003, Takeichi 1990, Thomas 2008). Ideal bonds are force independent. Experimentally, these bond types are distinguished by measuring their lifetimes $\tau = 1/k_{off}$ when subjected to a constant applied force (Marshall et al. 2003, Niessen et al. 2011).

How are bond lifetimes related to bond strengths? The rupture forces are measured at different rates of increasing force, R. The relationship between the rupture force F_r, the pulling rate R, and the intrinsic bond lifetime τ_0 is given by $F_r = \beta \cdot \mathrm{Ln}((R \cdot \tau_0)/\beta)$, where β depends on the length of the bond and on temperature (Evans & Ritchie 1997).

formed the intermediate bond but not the strand dimer at 39 nm; and (*c*) Asp mutations near the X-dimer interface ablate the intermediate bond at 32 nm (Prakasam et al. 2006). In single-bond rupture measurements, the W2A mutant of E-cadherin EC12 eliminated the weak bond (peak at ~20 pN) but retained the stronger bond (peak at ~60 pN) (**Figure 2*g***) (Rakshit et al. 2012, Shi et al. 2010). Conversely, the X-dimer mutant K14E eliminated the stronger EC12 bond but retained the weaker bond (Rakshit et al. 2012). The resulting conundrum was that, although the X-dimer was mechanically stronger than the strand dimer, the strand dimer K_d was lower. Because K_d generally determines adhesion strength (Li & Leckband 2006), results from solution-binding (SPR and AUC) and adhesion [atomic force microscopy (AFM) and force-distance] measurements appeared to be inconsistent with each other.

Recent single-bond rupture measurements (AFM; **Figure 2*f–h***) now appear to both reconcile the seeming discrepancies between solution binding and adhesion measurements and identify a distinct mechanical function for the X-dimer. Findings based on measured lifetimes of single cadherin bonds between EC12 fragments subject to constant applied force demonstrated that the X-dimer forms a catch bond, but that the strand dimer is a slip bond (see sidebar, Slip Bonds and Catch Bonds) (Rakshit et al. 2012). In a classical signature of catch bonds, the X-dimer lifetime increased with increasing applied force up to ~29 pN and then decreased as the bond converted to slip-bond behavior at higher force. Thus, the relative apparent strengths of the X-dimer and strand dimer could depend on intercellular tension.

This catch-bond behavior explains the greater mechanical strength of the X-dimer relative to the strand dimer in adhesion measurements (**Figure 2*e,g,h***). Because this behavior is a consequence of how the bonds respond to force, solution affinities would not predict their relative mechanical strengths at cell-cell junctions. These differences could influence intercellular cohesion, such that the strand dimer and X-dimer might play different roles in contractile versus static tissues, for example.

AFM: atomic force microscopy

Catch bond: catch bonds rupture more slowly (lifetime increases) when under tension and therefore resist force more effectively (become stronger) than when they experience smaller or zero forces

Slip bond: slip bonds rupture more quickly (lifetime decreases) when a force is applied; they resist tension less effectively (become weaker) at high forces relative to low forces

FRET: fluorescent resonance energy transfer

Cis* dimers and cadherin clustering.** The X- and strand dimers facilitate *trans* adhesion, but lateral (*cis*) cadherin interactions (**Figure 2*c) may also contribute to the assembly and stability of intercellular junctions. Monoclonal antibodies against EC3 and EC4 disrupt VE-cadherin junctions (Lampugnani et al. 1992), and function-disrupting mutations linked to diffuse gastric cancer are clustered near the EC2–EC3 domains of E-cadherin (Berx et al. 1998, Prakasam et al. 2006). Moreover, biophysical measurements of EC1–5 fragments detected three distinct bonds (**Figure 2*e,b***), compared with the two bonds identified with EC12 fragments (**Figure 2*e,g***) (Leckband & Sivasankar 2012b). The kinetics of cadherin-mediated cell-cell binding (**Figure 2*i–k***) provided functional evidence that the full ectodomain contributes to intercellular adhesion (Chien et al. 2008).

In dual-micropipette measurements (**Figure 2*i***), which were also used to determine molecular mechanisms of receptor-mediated cell binding in immune recognition and integrin-based adhesion (Chesla et al. 1998, Huang et al. 2010, Jiang et al. 2011), two cells are repetitively brought into contact for defined intervals. The measured binding probability—the number of detected binding events divided by the total number of cell-cell contacts (see **Figure 2*k***)—reflects the number of bonds formed in the time interval (Chesla et al. 1998). The kinetics measured with full-length cadherins differed from the expected kinetic time course for strand dimerization, which predicts that the binding probability will increase with cell-cell contact time to a limiting value P_1 (see **Figure 2*k***). Cell-binding kinetics measured with EC12 fragments, which form X- and strand dimers, exhibit the predicted kinetic profile (as in the solid line in **Figure 2*k***) (Chien et al. 2008). However, with full-length extracellular domains, the initial increase in binding probability to P_1 is followed by a 2–5-s delay and then a further increase to a higher binding probability, P2 (**Figure 2*k***). The initial increase is due to *trans* dimerization and is described by a kinetic model for *trans* dimerization (solid line, **Figure 2*k***), but the more complex kinetic behavior requires the full ectodomain for all classical cadherins thus studied (Barry et al. 2014, Chien et al. 2008, Tabdili et al. 2012).

Structural studies of full-length extracellular domains identified a third potential binding interface that may account for the more complex binding behavior of full extracellular domains, compared with EC12 fragments. In densely packed structures, extracellular domains form antiparallel strand dimers, which are bent sufficiently far to contact adjacent cadherins through a postulated *cis* interface between EC1 and a region spanning the EC2–3 junction on the adjacent protein (**Figure 2*c***) (Shapiro & Weis 2009). Mutations in this region disrupt cadherin ordering at vesicle-vesicle contacts in vitro without altering the strand-dimerization K_d. However, attempts to characterize this *cis* interaction through solution-binding measurements, including NMR, AUC, single-molecule FRET, and SPR, were unsuccessful (Harrison et al. 2011, Haussinger et al. 2002, Zhang et al. 2009). The absence of detectable *cis* complexes, even in concentrated solutions, suggests that the K_d of this putative *cis* interface is below the ambient thermal energy (background). It remains to be determined whether this putative *cis* interface accounts for biophysical data and whether it contributes to intercellular junction integrity.

Confinement may reveal protein interactions that are not detected in solution. To investigate why the *cis* interface seen in structures might not occur in solution, Wu et al. (2011) carried out simulations of cadherin binding within narrow intermembrane gaps (Wu et al. 2010, 2011). These and subsequent studies suggested that restricting cadherin (and membrane) fluctuations within narrow gaps could reduce the entropy barrier for forming weak protein interactions, and that this effect could facilitate lateral cadherin interactions and ordering at junctions between rigid or flexible membranes (Hu et al. 2013, Wu et al. 2011). Confinement could also induce cooperativity between *cis* and *trans* bonds, because proteins bound to their

neighbors are less likely to diffuse out of the contact region and could more readily reform *trans* bonds (Wu et al. 2010). The simulations might thus reconcile differences between structures, solution-binding data, and adhesion measurements where cadherins are confined to narrow gaps, as in the simulations (Leckband & Prakasam 2006). Further study is needed to test this.

VASP: vasodilator stimulated phosphoprotein

N-Glycosylation Impedes Putative *Cis* Interactions and Junction Organization

Posttranslational modifications of cadherin extracellular domains also impact junction architecture, possibly by perturbing lateral cadherin interactions. *N*-glycosylation sites on cadherin ectodomains are distributed along the backbone at sites away from the strand-dimer interface. Kinetics studies of cadherin-mediated cell binding (**Figure 2*i***) demonstrated that *N*-glycans affect the cell-binding kinetics but not the strand dimer affinity (Langer et al. 2012). The removal of *N*-glycans on N-cadherin did not affect the strand dimer affinity but accelerated the rise to the higher-probability binding state (P2 in **Figure 2*k***). One interpretation is that the second step (P2 in **Figure 2*k***) reflects lateral cadherin association, which is sterically impeded by the bulky carbohydrates. Glycan removal would thus accelerate junction assembly as observed.

Aberrant ectodomain *N*-glycosylation is associated with oral cancers, abnormal junction organization, loose epithelial tissue structure in *Drosophila*, and increased epithelial barrier permeability, possibly owing to disrupted lateral packing (Jamal et al. 2009, Liwosz et al. 2006, Nita-Lazar et al. 2009). Conversely, hypoglycosylated E-cadherin forms tighter junctions (Nita-Lazar et al. 2010). How then could cadherin glycoproteins cluster in vivo? The extent of *N*-glycosylation decreases with increasing cell density (Nita-Lazar et al. 2010), so that reduced *N*-glycosylation in dense tissues might enable clustering at cell junctions.

Cadherin Clusters In Vivo Are Regulated by Actin and Endocytosis

In vivo, the role of *cis* interactions and cadherin clustering also remains to be established. Cadherin clusters at intercellular junctions in tissues are well documented, but the requirement for actin coupling (Cavey et al. 2008, Hong et al. 2013, Rauzi et al. 2010), myosin II (Shewan et al. 2005), and Ena/VASP (Scott et al. 2006) suggests that the organizing machinery is more complex than the passive self-assembly modeled in simulations (Wu et al. 2011) and in cell-free systems (Harrison et al. 2011). Cluster size distributions in *Drosophila* epithelia appear to be regulated by dynamic competition between cluster fusion, breakup, and endocytosis. Superresolution imaging and mathematical modeling of the kinetics of cluster formation revealed that cadherin association with actin stabilizes clusters against breakup, whereas endocytosis removes clusters above a critical size (Truong Quang et al. 2013). The study did not address how clusters form initially, and small clusters were observed on free (nonadhering) cell membranes. Although ectodomain interactions might facilitate initial cadherin oligomerization, evidence for *cis* dimers on cells is mixed (Chtcheglova et al. 2007, Troyanovsky et al. 2003, Tsuiji et al. 2007, Zhang et al. 2009).

Whether cadherin ectodomains form *cis* bonds that contribute to junction assembly in vivo remains to be established. The ubiquity of larger cadherin clusters at junctions may be important for controlling cell shape and tissue organization (Lecuit et al. 2011), but the in vivo role of *cis* bonds as observed (see **Figure 2*c***) remains to be determined.

Allosteric Regulation of Cadherin Adhesion and Signaling Functions

Inside-out signaling and the allosteric regulation of cadherin-mediated adhesion. Several processes regulate cadherin-mediated adhesion, including proteolytic shedding, endocytosis, downregulation of cadherin expression, or disruption of cytoskeletal coupling (reviewed in

Niessen et al. 2011). That soluble factors such as TNFβ or activin alter cell adhesion without changing cadherin surface expression suggests possible inside-out regulation (Gumbiner 2005). Such changes could result either from altered interactions with the cytoskeleton or other membrane proteins or from allosteric inside-out signaling, similar to integrins (Hynes 2002). Although biophysical studies suggested allosteric coupling between extracellular domains (Shi et al. 2010), they did not demonstrate such allostery in membrane-bound cadherin.

With antibodies that activate E-cadherin adhesion, Petrova et al. (2012) demonstrated the allosteric regulation of cadherin adhesion and intracellular signaling. Colo205 cells express a full complement of E-cadherin but do not aggregate in solution unless treated with protease or the kinase inhibitor staurosporine (Aono et al. 1999). A screen identified anti-E-cadherin antibodies that activated Colo205 adhesion (Petrova et al. 2012). The antibody epitopes mapped to junctions between EC domains away from the strand dimer interface. Importantly, antibody binding activated Colo205 cell adhesion and altered both the exposure of a cytoplasmic domain epitope and the phosphorylation state of p120 catenin. This effect of antibody binding demonstrated allosteric coupling between the cytodomain conformation, interactions with a cytosolic protein, and cadherin-mediated cell adhesion.

Studies with p120ctn confirmed the bidirectionality of this allosteric regulation. Aggregation and adhesion tests with Colo205 cells transfected with p120ctn mutants in which the six phosphorylation sites were mutated either to alanine (6S,T > A) or to the phosphomimetic glutamate (6S,T > E) confirmed that p120ctn phosphorylation regulates cadherin-dependent cell aggregation. The 6S,T > A mutant phenocopies staurosporine treatment, but cells with the 6S,T > E mutant do not aggregate (Petrova et al. 2012). A current question is whether activating antibodies and p120ctn phosphorylation alter the strand dimer affinity or activate adhesion through other mechanisms.

Allostery and outside-in signaling. Cadherin ligation activates RhoGTPase and Src signaling (Braga 2002, McLachlan & Yap 2007), but this is only circumstantial evidence for allosteric outside-in signaling. However, the ligand dependence of cadherin-based mechanotransduction may provide evidence for outside-in signaling. Cadherin-based mechanotransduction requires homophilic cadherin ligation such that cells responded to force on cadherin bonds only when probed with the homophilic ligand. The inability to elicit a mechanoresponse by tugging on cadherins with anti-cadherin antibodies or heterophilic cadherin ligands suggests that mechanotransduction (discussed in the following section) requires a ligand-specific conformational change to engage the cytosolic mechanotransduction machinery (Tabdili et al. 2012).

Inside-out signaling: cytoplasmic domain interactions with cytosolic proteins or posttranslational modifications allosterically induce a conformational change in the extracellular region that alters the intrinsic binding or enzymatic activity of the protein

Allostery: conformational changes induced in a protein structure that are triggered by ligand binding away from the site of the induced change

Outside-in signaling: the ligation of a protein or its association with other proteins induces a conformational change in the cytoplasmic domain that alters its biochemical reactivity or interactions with cytosolic binding partners; this direct allosteric regulation is distinct from perturbing other membrane proteins that could themselves signal, such as growth factor receptors or tension-sensitive ion channels

CADHERINS IN MECHANOTRANSDUCTION

The adhesive connections formed by cadherin bonds (see previous sections) intrinsically convey mechanical information to cells by resisting force generated by endogenous contractile forces or by exogenous forces from, for example, fluid shear stress, tissue rigidity, or compressive and extensive forces. Cadherin ligation alone is sufficient to trigger biochemical signaling (reviewed in Wheelock & Johnson 2003 and Yap & Kovacs 2003), but cadherin complexes also connect the cytoskeletons of adjacent cells and are thus part of a linear mechanical chain that experiences fluctuations in tensile forces owing to dynamic cytoskeletal deformations. Deformations in cytoskeletal elements are crucial in the embryonic development of tissues (morphogenesis), in tissue repair, and in pathologies such as cancer. Whereas cadherins were previously regarded as passive structural elements in force transmission between adjacent cells, recent findings demonstrated that cadherin complexes actively sense fluctuations in tension and elicit proportional biochemical responses that direct cell

behavior. This specific function of cadherins is called mechanotransduction, and its mechanisms and emerging relevance in tissue development and disease are reviewed in the next sections.

Adhesion strengthening: the increase in adhesion with time; in cells, this could result from several possible mechanisms, such as, but not restricted to, changes in intrinsic receptor binding properties

Classical Cadherin Complexes Are Mechanosensors

Direct evidence for mechanosensing by F-actin-associated classical cadherin complexes was obtained for E-cadherin, N-cadherin, and VE-cadherin in diverse experimental settings. Broadly, evidence for cadherin-based mechanotransduction includes force-dependent remodeling of intercellular junctions (Liu et al. 2010); altered mechanical properties, such as adhesion strength and cell traction (Ladoux et al. 2010, Tabdili et al. 2012, Thomas et al. 2013); and junction stiffness (le Duc et al. 2010).

Cadherin junctions remodel in response to both endogenous and exogenous forces. In endothelial cells, endogenous tugging forces on junctions between cell doublets on patterned micropillar arrays triggered an increase in tension across the junction and a consequent increase in the size (width) of the VE-cadherin-containing cell-cell contact. This tension was produced by actomyosin contractility in the two cells and was controlled by diverse chemical and genetic manipulations (Liu et al. 2010). In epithelial cells, the stiffness of E-cadherin-based junctions between cells and Fc-E-cadherin-coated magnetic beads proportionally increased in response to exogenous twisting torque exerted on the magnetic beads (le Duc et al. 2010). The latter stiffening response correlated with actin accumulation at the beads (Barry et al. 2014). External mechanical stimuli also trigger changes in cadherin-mediated adhesion. In dual micropipette measurements, the adhesion between cell doublets increased after pulling on cell pairs, and this correlated with actomyosin remodeling at the junctions (Thomas et al. 2013). Adhesion strengthening could result from inside-out signaling, cadherin recruitment, or reinforced cytoskeletal linkages, but the force-triggered, cadherin-mediated adhesion strengthening appears to be due to reinforced actin anchoring (Barry et al. 2014).

Substrate rigidity sensing is another manifestation of mechanotransduction, and evidence for N-cadherin rigidity sensing was obtained by studying cellular responses to differences in the stiffness of N-cadherin-coated micropillars. Traction forces exerted by muscle cells on N-cadherin-based adhesions (Ladoux et al. 2010), as well as by epithelial cells on E-cadherin coated substrates, increased with the substrate stiffness (Barry et al. 2014, Tabdili et al. 2012). In rigidity sensing, the endogenous actomyosin contractility generates force against deformable substrata via adhesive bonds. The fact that, through an undefined sensory and positive feedback loop, endogenous contractile forces increase in proportion to extracellular matrix rigidity demonstrates that the cadherin complex is an active mechanosensor.

The above findings dealt with actomyosin-dependent junction proximal processes, but a unique case of mechanotransduction was reported for the classical compaction C-cadherin in *Xenopus* (Weber et al. 2012). In this study, pulling on C-cadherin-coated beads adhered to C-cadherin on the surface of *Xenopus* embryo mesendoderm cells resulted in a concentration of keratin filaments near the pulled contact and the consequent induction of cell polarity and migration away from the applied force (Weber et al. 2012). In this case, plakoglobin was needed as a physical linker between C-cadherin and intermediate filaments (Weber et al. 2012). However, plakoglobin itself was recruited to C-cadherin junctions only after pulling. Exactly how this increase in mechanical forces was initially sensed remains unclear. The role of actomyosin was not investigated in this system. Nevertheless, the findings of Weber et al. (2012) reveal that classical cadherin mechanotransduction occurs not only at adherens junctions but also in other types of cadherin adhesions. This hints at possible mechanotransduction in other cadherin-based structures, such as hemidesmosomes or desmosomes, although there has been little mechanobiological research on the latter systems.

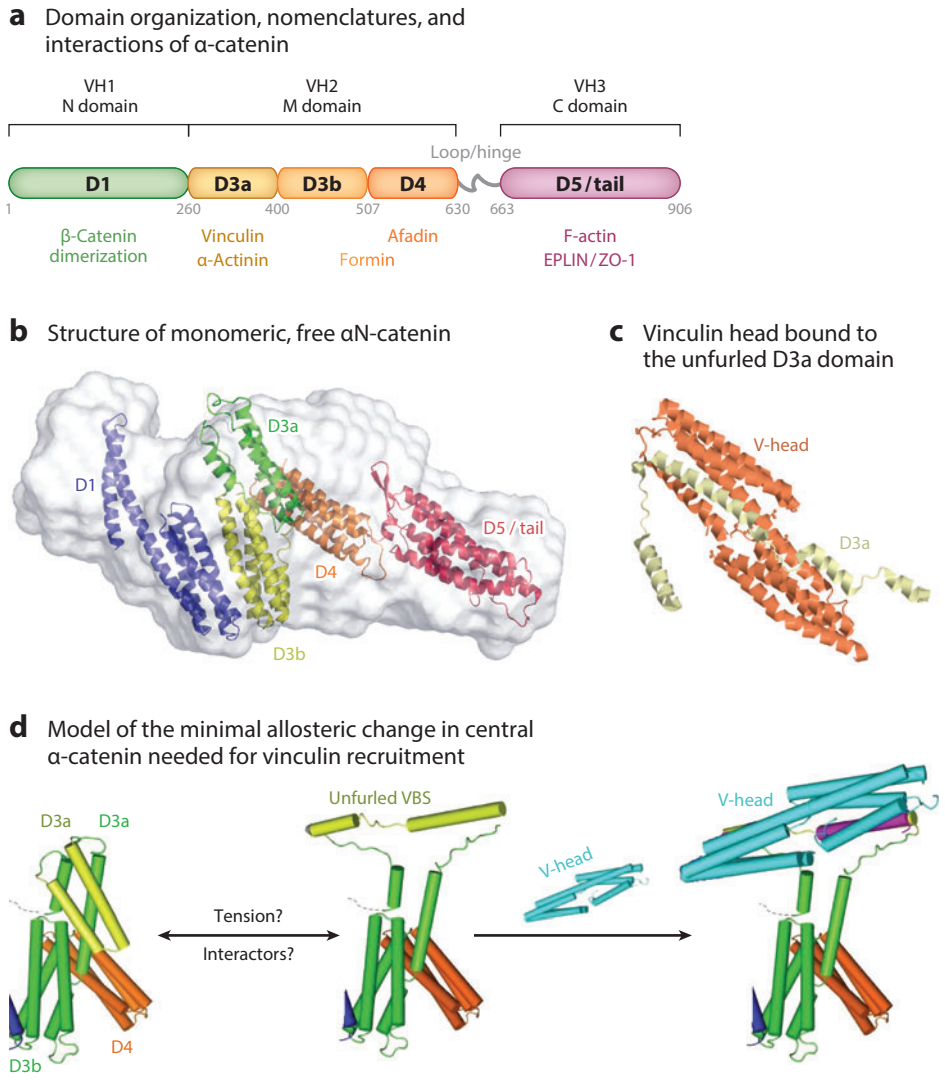


Figure 3

α-Catenin structure and interactions possibly implicated in mechanotransduction. (*a*) Domain organization and nomenclature and selected binding partners that could be involved in cadherin mechanotransduction. Illustrations show (*b*) how monomeric α-catenin adopts a conformation that can bridge β-catenin and F-actin, (*c*) how α-catenins' D3a domain unfurls to interact with the head domain of vinculin (V-head), and (*d*) how this could minimally affect the structure of the central effector domains (D3a/b and D4). *b* adapted from Ishiyama et al. 2013, *c* adapted from Rangarajan & Izard 2012, *d* adapted from Choi et al. 2012. Abbreviation: VBS, vinculin binding site.

α-Catenin Is a Central Molecule in Cadherin Mechanotransduction

Several studies identified early molecular events involved in cadherin mechanosensing, and a rudimentary model for the initial steps is emerging (**Figure 3**). The central molecule in this system is α-catenin, which links E-cadherin-associated β-catenin to F-actin (Desai et al. 2013) (**Figure 1**). Based on a seminal study that used an epitope-specific, anti-α-catenin antibody to

Focal adherens junctions (FAJs): distinct cadherin-mediated intercellular junctions that are characterized by punctate vinculin staining and radial actin fibers

detect conformational changes following myosin II activation, the initial postulate was that the central effector domains (see **Figure 3*a***) of α-catenin are masked by an intramolecular interaction, which is released when α-catenin undergoes a conformational change under tension (Yonemura et al. 2010). Releasing the autoinhibition of the D3a domain (**Figure 3*a,c,d***) results in vinculin recruitment (see below) to stressed cell-cell junctions as the immediate downstream effector of α-catenin mechanosensing (Yonemura 2011, Yonemura et al. 2010).

Crystal structures of (parts of) α-catenin obtained under different conditions, and in combination with other proteins, then revealed that the central domains of α-catenin (**Figure 3*b***) associate through multiple electrostatic interactions (Ishiyama & Ikura 2012, Ishiyama et al. 2013, Rangarajan & Izard 2013), which likely affect the unfurling of the D3a domain that exposes the vinculin-binding site (Choi et al. 2012, Rangarajan & Izard 2012) (**Figure 3*c,d***). Tension, in cooperation with additional proteins that bind the D3b and D4 domains, could thus influence the dynamics of α-catenin opening and vinculin binding. **Figure 3** summarizes current models of α-catenin interactions and their conformational control.

Vinculin Is a Key Effector of α-Catenin Mechanosensing

As indicated above, the best-characterized effector of the tension-dependent conformational regulation of α-catenin is its closest homolog, vinculin (**Figure 4**). Several studies demonstrated that vinculin is recruited to cadherin-based cell-cell junctions in response to both endogenous myosin II–dependent contractility (Huveneers et al. 2012, Yonemura 2011) and externally applied tension (Barry et al. 2014, Thomas et al. 2013). The loss of vinculin or the mutation of the D3a domain of α-catenin reduces cadherin mechanotransduction, as measured in bead-twisting and cell-tugging studies (Barry et al. 2014, le Duc et al. 2010, Thomas et al. 2013, Twiss et al. 2012).

In 2D cultures, the formation and remodeling of cadherin-based cell-cell adhesion involve a population of punctate cadherin junctions that we named focal adherens junctions

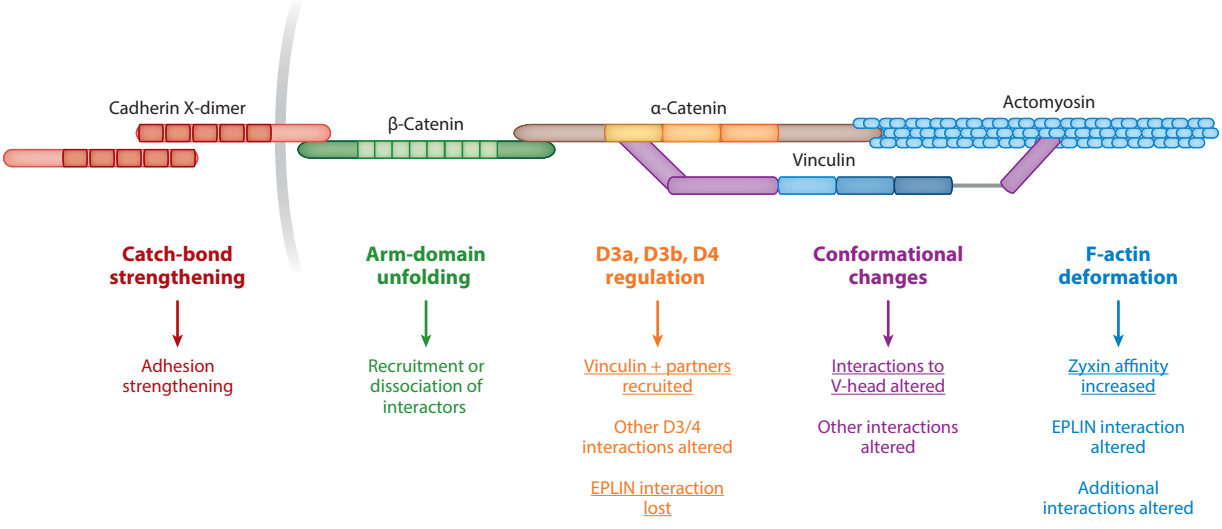


Figure 4

Force-induced alterations in and consequences of the cadherin-F-actin chain. Schematic overview of the components at the cadherin-F-actin chain that may undergo conformational or physical changes in response to increased intercellular tension. Putative and proven (*underlined*) immediate molecular consequences are indicated per component in comparable color.

(FAJs) (reviewed in Huveneers & de Rooij 2013). The formation, stability, and dynamics of these FAJs are governed by actomyosin-dependent mechanical forces and are impaired in vinculin-depleted cells or when the D3a domain of α-catenin is perturbed (Barry et al. 2014, Huveneers et al. 2012, Taguchi et al. 2011). Vinculin mediated the compaction of cell-cell junctions in breast cancer cells (Maddugoda et al. 2007), and the D3a domain of α-catenin was required for the formation of the apical zonula adherens in colon cancer cells (Huveneers & de Rooij 2013, Imamura et al. 1999, Watabe-Uchida et al. 1998), both of which are actomyosin-dependent processes. Notably, zonula adherens junctions and FAJs are distinctly different structures, and it is unclear whether vinculin regulation and function are comparable between them. How vinculin brings about its junction-stabilizing effects is unclear, but it could depend on its F-actin binding capacity (Ziegler et al. 2006), its actin remodeling activity (Le Clainche et al. 2010, Wen et al. 2009), or its ability to bind and recruit actin regulators (Carisey et al. 2013).

Additional α-Catenin Binders that May Be Involved in Cadherin Mechanotransduction

As argued above, additional protein binding to the central domains of α-catenin (**Figure 3*a***) is likely to attenuate the vinculin-dependent mechanoresponse by affecting the unfurling kinetics of the D3a domain (Choi et al. 2012, Ishiyama et al. 2013, Rangarajan & Izard 2012). Moreover, it could be involved in additional mechanoresponses invoked by tension across α-catenin. Besides vinculin, α-actinin also binds to the D3a domain (**Figure 3*a***) (Nieset et al. 1997). α-Actinin-4 regulates ARP2/3 activity to assemble and maintain F-actin at E-cadherin junctions (Tang & Brieher 2012), and it could thus link cadherin mechanotransduction to F-actin polymerization. A similar function in F-actin nucleation and polymerization could be envisioned for formin1, which interacts with the D3b and D4 domains (**Figure 3*a***), localizes to FAJs, and is implicated in junction formation and actin polymerization (Higashida et al. 2013, Kobielak & Fuchs 2004). The most interesting α-catenin binder in this respect may be AF-6 (afadin in *Drosophila*), which binds to the D4 domain (**Figure 3*a***) and was required during apical constriction and germ-band extension in *Drosophila* embryos (Sawyer et al. 2010, 2011). Both processes are characterized by large increases in actomyosin contraction, and the absence of afadin caused the actomyosin network to detach from cell-cell junctions during these processes (Sawyer et al. 2009, 2011). Thus, multiple protein-binding events at the central domains of α-catenin could be involved in cadherin mechanotransduction.

Besides interactions with the central domains of α-catenin, the localization of EPLIN, which binds to the tail domain of α-catenin (**Figure 3*a***), at cell-cell junctions is also mechanosensitive (Taguchi et al. 2011). Because EPLIN localizes to F-actin structures outside of junctions and is absent from vinculin-containing FAJs (Taguchi et al. 2011), it is currently unclear whether tension in actin structures or tension in the cadherin complex determines EPLIN localization. Whether EPLIN plays a functional role in cadherin mechanotransduction is also unclear.

Possible Mechanosensitive Events Beyond α-Catenin

Importantly, once recruited to α-catenin, vinculin and other F-actin binders, such as EPLIN and ZO-1, also become part of the force chain between actomyosin and cadherin junctions. Their conformational regulation may impart additional functionality or additional effector recruitment and could thus constitute secondary, tension-sensitive events at the cadherin-actomyosin interface. As an example of such a secondary event, at integrin adhesions, the coupling of tension-recruited vinculin to F-actin enhanced its affinity for talin, resulting in increased integrin affinity for external ligands (Carisey et al. 2013).

Focal adhesion: specialized adhesive junctions at sites of integrin attachment between the cell surface and extracellular matrix ligands

Besides α-catenin and associated proteins, E-cadherin, β-catenin, and F-actin also experience increased tension, which could deform or otherwise alter their biochemical properties. The strength of E-cadherin catch bonds increases with force from ~10–30 pN (Rakshit et al. 2012). Under low-pN forces (~20–40), isolated β-catenin also stretches and refolds in discrete steps that represent the unfolding of individual armadillo repeats (Valbuena et al. 2012). These unfolding events could mechanically regulate interactions of β-catenin domains with other cytosolic proteins to contribute to cadherin-based mechanotransduction. Interestingly, the recent tension-sensing FRET probes report junctional forces approximately tenfold lower than the catch-bond force regime of X-dimers or the unfolding forces of β-catenin (Borghi et al. 2012, Conway et al. 2013). This is not unexpected because FRET sensors report the ensemble-averaged tension on sensors in a junction, as opposed to single proteins, and both the force and tension sensors are likely heterogeneously distributed in junctions. In α-catenin- or cadherin-depleted systems, E-cadherin-α-catenin fusion proteins, which bypass β-catenin in the force-chain, can rescue junction regulation (Watabe-Uchida et al. 1998) and tissue development (Pacquelet & Rorth 2005, Sarpal et al. 2012)—at least in the tested contexts. Thus, the actual importance of β-catenin in mechanotransduction remains to be proven.

Specific proteins may also sense tension-dependent conformational changes in junction-proximal F-actin. An example of force-dependent protein recruitment to F-actin is the zyxin localization to stressed F-actin, either at focal adhesions or within F-actin fibers (Colombelli et al. 2009, Hirata et al. 2008, Smith et al. 2010). Zyxin in turn recruits VASP, as well as possible additional binders, to regulate actin polymerization (Smith et al. 2010). Both zyxin and VASP were implicated in the regulation of F-actin dynamics and organization at cell-cell junctions (Nguyen et al. 2010, Scott et al. 2006, Sperry et al. 2010), and VASP affects force-dependent reinforcement of adhesion between VE-cadherin-coated beads and endothelial cells (Kris et al. 2008). Deformations of the cortical actin network, rather than individual fibers, might also trigger junction-proximal biochemical reactions, such as conformational changes in filamin A or actin remodeling (Ehrlicher et al. 2011, Higashida et al. 2013, Shemesh et al. 2005). The force-dependent conformational regulation of F-actin may also contribute to mechanotransduction at cadherin adhesions (Higashida et al. 2013). **Figure 5** summarizes the putative main biophysical events induced by increased tension across the cadherin-actomyosin connections and their immediate downstream effects.

POSSIBLE CELLULAR CONSEQUENCES OF CADHERIN MECHANOTRANSDUCTION

Local Effects: Adhesion Strength and Junction Morphology

The common theme among the effects of mechanically stimulating cadherin complexes is a positive feedback that remodels cell-cell junctions. As summarized in **Figure 5*a***, changes observed in a diversity of assays include adhesion strengthening (Thomas et al. 2013), junction growth (Liu et al. 2010), altered junction stiffness (le Duc et al. 2010), and the dependence of cadherin-based traction forces on the rigidity of cadherin substrata (Ladoux et al. 2010, le Duc et al. 2010) (see section on cadherins as mechanosensors, above). Actomyosin inhibitors interfere with cadherin-based mechanotransduction in all of these assays, as does elimination of essential adhesion components, such as α-catenin. **Figure 5*b*** summarizes events that could occur sequentially or in parallel with increased tension and accommodates different observations from these complementary assays of junctions.

Correlations between force fluctuations and intercellular junction remodeling are also observed in vivo. In *Drosophila* and *Caenorhabditis elegans*, actomyosin-generated tension at cadherin

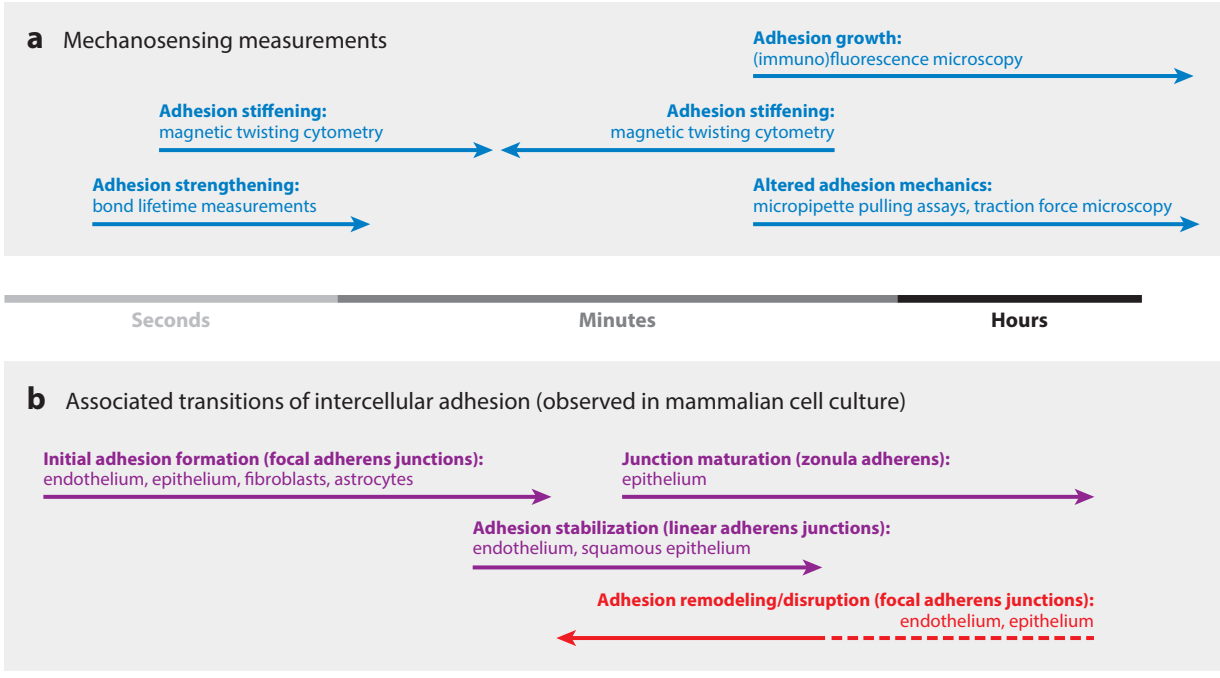


Figure 5

Experimentally observed evidence of cadherin mechanotransduction and corresponding transitions at intercellular junctions in mammalian cell culture. (*a*) Different measurements that suggest cadherin-based mechanotransduction. (*b*) Changes in junctional composition associated with different dynamic transitions at cell-cell adhesions, the type (phenotype) of adhesion observed during those transitions, and the cell types in which they occur.

junctions is associated with junction shrinkage in several developmental processes. In *Drosophila* ventral furrow formation and *C. elegans* gastrulation, preexisting actomyosin contractions drive apical constriction only when they are coupled to cadherin junctions and start to generate tension fluctuations (Roh-Johnson et al. 2012). In *Drosophila* germband extension, periodic actomyosin contractions coupled to dorsoventral-oriented cadherin junctions through α-catenin drive junction shrinkage to regulate polarized cell intercallations during tissue elongation (Lecuit et al. 2011). In the latter process, the junction shrinkage results from myosin-induced cadherin clustering, which in turn drives cadherin endocytosis (Levayer & Lecuit 2013). In zebrafish gastrulation, cell intercallation depends on α-catenin (Schepis et al. 2012). Thus, the coordination of force- and cadherin complex–dependent junction shrinkage is observed in vivo in lower organisms. How this relates to cadherin mechanotransduction events observed in vitro in higher organisms, and whether the same molecular mechanisms apply, remains to be established.

Distant Effects: Actomyosin Contractility and Organization

Besides actomyosin changes that are near the core-cadherin complex and that control local junction remodeling, tension-triggered events also occur at locations distant from the junctional complex. The directional cell migration and coincident front-rear asymmetry of keratin filaments induced by tension on the C-cadherin complex exemplify such a distant effect (Weber et al. 2012). Evidence suggesting that cadherin mechanotransduction alters global actomyosin organization includes differences in stress fiber morphology in cells on rigid versus soft cadherin-coated substrates (Ladoux

et al. 2010). Cardiomyocytes cultured on N-cadherin substrates of appropriate rigidity similarly induced stereotypical cytoskeletal organization (Chopra et al. 2011). Cell-cell junction formation also altered integrin-based tractions (Jasaitis et al. 2012). In *Drosophila* embryos, actomyosin coupling to the cadherin complex regulates the actin flows that determine the anisotropy of tension at cadherin junctions that in turn drive their shrinkage (Lecuit et al. 2011, Levayer & Lecuit 2013). Thus, actyomyosin coupling to cadherin complexes influences the organization and dynamics of different cytoskeletal systems, in order to control cellular responses to changes in tension across cell-cell junctions.

CADHERIN MECHANOTRANSDUCTION IN DEVELOPMENT AND DISEASE

The essential role of cadherin adhesion in morphogenesis and tissue homeostasis (Gumbiner 2005, Niessen et al. 2011, Tepass et al. 2000), as well as in diseases such as cancer (Berx & van Roy 2009, Jeanes et al. 2008) and cardiomyopathy (El-Amraoui & Petit 2013), is well established. Moreover, the regulation of cadherin adhesions is necessary for many biological processes, such as wound healing, endothelial junction disruption during leukocyte extravasation, and collective cell movements during morphogenesis (Gumbiner 2005). Cadherins are also involved in transcriptional regulation through the Wnt/β-catenin (Gumbiner 2005, Niessen et al. 2011, Tepass et al. 2000) and Hippo pathways (Kim et al. 2011). Cell-cell cohesion is obligatory in these contexts. Demonstrating that force fluctuations also modulate these cadherin-dependent processes is more challenging because force transduction requires intercellular cohesion, but the converse may not always hold. Despite the current scarcity of unambiguous demonstrations that mechanical force regulates cadherin-mediated functions, some evidence is emerging.

In *Drosophila* development, for example, where forces integrated at cadherin junctions feed back through cytoskeletal organization, there is evidence that force transduction orchestrates junction-remodeling processes involved in tissue morphogenesis (Lecuit et al. 2011, Levayer & Lecuit 2013). The role of cadherin-dependent force transduction may also be context dependent, as suggested by different effects of α-catenin mutations in different tissues and developmental processes in *Drosophila* embryos (Desai et al. 2013). In the cardiovascular system, the influence of fluid shear forces on changes in interendothelial tension and consequent shear alignment of endothelial cells is well documented (Hahn & Schwartz 2009, Tzima et al. 2005).

At the same time, examples abound that suggest forces on cadherin adhesions may alter biological processes, but in these cases, an essential role for cadherin-mediated force transduction has yet to be demonstrated. Forces generated by dynamic, contractile actin-myosin networks in *Drosophila* drive changes in tissue organization and signaling (Kasza & Zallen 2011). In the cardiovascular system in mammals, heart development requires oscillations in blood flow and pressure that result in fluctuating tension at cell-cell junctions (Granados-Riveron & Brook 2012), and cardiomyocyte-specific vinculin knockouts display developmental defects in the specialized cell-cell junctions of the intercalated disk (Zemljic-Harpf et al. 2014). Actomyosin contractility controls endothelial-barrier disruption during leukocyte transmigration. Thrombin-stimulated endothelial contractility correlates with increased paracellular permeability, and Rho-dependent arterial stiffening correlates with increased vascular leakage (Huynh et al. 2011). In many cases, the mechanics (stiffness) and topography of 2D- and 3D-tissue environments (Dupont et al. 2011, Engler et al. 2006, Gjorevski & Nelson 2010, Hsu et al. 2013, Lu et al. 2012, Ng et al. 2012, Serra-Picamal et al. 2012) regulate morphogenetic movements and tissue integrity, as well as transcriptional programs. Such processes undoubtedly result in altered intercellular tension (de

Rooij et al. 2005, Liu et al. 2010, Maruthamuthu et al. 2011). Unambiguously demonstrating that cadherin-based force transduction plays an essential role is the current challenge.

SUMMARY POINTS

1. Homophilic adhesion by type-I classical cadherins proceeds through a process that involves the formation of multiple, different cadherin-cadherin bonds, which involve different regions of the extracellular domain. Each of these bonds plays a different role in assembling cadherin adhesions and in resisting force.
2. Cadherins are allosterically regulated proteins. The phosphorylation state of p120 catenin, which binds to the cytoplasmic domain, regulates cadherin adhesion. Conversely, antibody binding to the ectodomain can alter p120 catenin phosphorylation.
3. Cadherin complexes are mechanosensors that sense fluctuations in cytoskeletal tension to trigger biochemical changes that alter the mechanical properties of cadherin junctions through a positive feedback loop.
4. α-Catenin and vinculin are the key elements in the mechanosensory chain linking cadherins and the actomyosin cytoskeleton. At cadherin adhesions, vinculin is the principal effector of force-induced changes in the conformation of α-catenin, which is the likely stretch-activated force sensor in the complex.
5. Besides force-induced changes in α-catenin, mechanical deformations of actin and other actin and/or cadherin-associated proteins near cadherin adhesions may contribute to cadherin-based force sensing.

FUTURE ISSUES

1. The role of cadherin catch bonds in intercellular junction formation and stabilization under tension remains to be established.
2. The involvement of specific lateral bonds between cadherin ectodomains in the assembly of junctions between cells, in vivo and in cell culture, remains an open question.
3. There are many examples in vivo and in vitro of biological processes that directly involve fluctuations in intercellular tension and cytoskeletal deformations, but a direct role for cadherin mechanotransduction in these processes has yet to be demonstrated.
4. Apart from the central elements of cadherin, α-catenin, and vinculin in mechanosensing, an unexplored question is whether other architectural proteins associated with cadherin adhesions contribute to cadherin-based force sensing.
5. Studies are needed to determine whether signaling proteins, such as Src kinases, RhoGTPases, or PI3kinase, are also activated by force at cadherin adhesions.
6. Investigations must establish how force-dependent changes in the sizes of cadherin adhesions in vivo relate to cadherin mechanotransduction events observed in vitro. Such studies would also determine whether the same molecular mechanisms apply in both contexts.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

D.E.L. was supported by the National Institutes of Health (NIH RO1 GM09743) and by the National Science Foundation (NSF CMMI 10-29871, NSF CBET 11-32116). J.dR. was supported by AICR Grant 13-0300. We acknowledge many contributions that were not included in this review owing to space restrictions.

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