Cell Biology International ISSN 1065-6995
doi: 10.1002/cbin.10563

REVIEW

Role of vinculin in cellular mechanotransduction

Wolfgang H. Goldmann*

Department of Biophysics, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany

Abstract

Cell–matrix adhesion and cell–cell contacts are essential for the metabolism, protein synthesis, survival, and cancer metastasis of cells. Major transmembrane receptors are the integrins, which are responsible for cell–matrix adhesions, and the cadherins, which are important for cell–cell adhesions. Adherent cells anchor via focal adhesion proteins to the extracellular matrix, whereas cell–cell contacts connect via focal adherens junction proteins. The temporal formation of these connections is greatly strengthened either through externally applied stresses on the cell or by myosin-driven cell contractility. The mechanism by which protein(s) within these connections sense, transmit, and respond to mechanochemical signaling is currently strongly debated and various candidates have been named. Vinculin has been described as one of the key players in cell–matrix and cell–cell adhesions that build a strong physical connection for transmitting forces between the cytoskeleton, the extracellular matrix, and cell–cell connections.

Keywords: alpha-actinin; cell–matrix adhesions and cell–cell contacts; mechanosensing; mechanotransduction; p130Cas; vinculin

History of vinculin

Vinculin was first identified and isolated by Geiger et al. (1980) and soon after, it was discovered in a variety of cells and tissues (Otto, 1986). The primary sequence of this 116kDa protein was published for chicken, nematode, and human species (Barstead and Waterston, 1989). The organization of the entire human vinculin gene, including its promoter sequence, was finally reported by Moiseyeva et al. (1993). For many years, vinculin was assumed to be an actin-binding protein and, indeed, the vinculin sequence contains actin-binding sites that can be blocked by specific antibodies (Isenberg et al., 1982; Ruhnau and Wegner, 1988; Ruddies et al., 1993). The molecular shape of vinculin was at that time described by the "balloon-on-a-string" model (Eimer et al., 1993).

Vinculin is a typical amphitropic protein that is present in the cell as a soluble cytoplasmic and a membrane-bound protein. Its association with negatively charged phospholipids (PA, PI, PG) has been reported; however, neutral lipids (PC, PE) do not promote vinculin binding (Ito et al., 1983; Niggli et al., 1986, 1994). Vinculin has also been reconstituted into lipid monolayers (Goldmann et al., 1992). This study reported a moderate dissociation constant for vinculin–phospholipid interaction depending on temperature, surface pressure, different lipid compositions, and ratios. At that time, it was not clear whether posttranslational modification of vinculin was important for lipid bilayer interactions. The degree of posttranslational lipid modification was believed to be due to the phosphorylation state. A significant increase in vinculin phosphorylation induced by the purified *src* gene product (Ito et al., 1982, 1983) showed a markedly lower level of palmitylated vinculin in Rous sarcoma virus-infected fibroblasts. Vinculin was reported to be one of the rare examples for which hydrophobic labeling by a lipid analog could be applied (Götter et al., 1995; Isenberg and Goldmann, 1995; Isenberg et al., 1996).

*Corresponding author: e-mail: wgoldmann@biomed.uni-erlangen.de

This review is dedicated to Andrea Feuerstein who died in a tragic accident in 2015.

Abbreviations: Ajuba, LIM domain-containing protein; ECM, extracellular matrix; Eplin, epithelial protein lost in neoplasm; ERK, extracellular signal-regulated kinase; FAs, focal adhesions; FAJs, focal adherens junctions; FAK, focal adhesion kinase; IpaA, invasin from shigella flexneri; MAP, mitogen-activated protein; MEK, mitogen extracellular signal-regulated kinase; MLC, myosin light chain; PA, phosphatidylacid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-biphosphate; Rap1, Ras-proximate-1; ROCK, Rho kinase; SH3, SRC homology 3 domain; VBS3, vinculin binding site 3; Vh, vinculin head; Vt, vinculin tail

The vinculin molecule

The following binding partners of vinculin have been reported: talin (Burridge and Mangeat, 1984), α-actinin (Small, 1985), catenin α/β (Watabe-Uchida et al., 1998), vinexin α/β, c-Cbl-associated protein (CAP), nArgBP2 (Kawabe et al., 1999), vasodilator-stimulated phosphoprotein (VASP) (Huttelmaier et al., 1998), actin-related protein complex (Arp2/3) (Higgs and Pollard, 2001), paxillin (Wood et al., 1994), Hic-5 (Thomas et al., 1999), F-actin (Ruddies et al., 1993), PKC-α (Ziegler et al., 2002), synemin, calpain (Liu and Schnellmann, 2003), polycystin-1 (Wilson et al., 1999), raver1 (Lee et al., 2008), phosphatidylinositol (4,5)-biphosphate (PIP_2) (Johnson et al., 1998), and lipid membrane (Goldmann et al., 1995b, 1996; Tempel et al., 1995; Diez et al., 2008, 2009). All of these binding partners can also interact with other proteins that link vinculin to the cellular signaling network (Zaidel-Bar et al., 2007). The ligand-binding sites of these proteins on vinculin are hidden by an intramolecular interaction between the vinculin head and tail domains (Ziegler et al., 2006). The vinculin molecule is believed to be in equilibrium between active and inactivate states (Goldmann, 2010; Diez et al., 2011). In the activated state, α-catenin, α-actinin, and talin can bind to the head domain of vinculin (Vh), whereas actin, paxillin, and PIP_2 associate with the tail domain of vinculin (Vt), and VASP, Arp2/3, vinexin, as well as ponsin (Zamir and Geiger, 2001; DeMali et al., 2002) bind to the flexible neck region.

Protein interactions with vinculin

Vinculin binding to F-actin

Previously, it was reported that vinculin associates with F-actin on two regions in the tail (Goldmann and Guttenberg, 1998; Huttelmaier et al., 1998; Cohen et al., 2005, 2006). Therefore, researchers suggested a model whereby actin binding of vinculin may weaken the interaction between the head and tail domain, allowing talin (in cell–matrix interactions) or α-catenin (in cell–cell adhesions) to fully bind to active vinculin (Janssen et al., 2006). It was proposed from molecular dynamics studies that the D1 region of the Vh stericly clashes with F-actin, preventing the linkage between the Vt and F-actin (Golji and Mofrad, 2013). Results showed that F-actin does not form a complex with vinculin in the closed conformation, only in the open conformation of vinculin. In the closed conformation, the charged residues of the head are linked to the Vt. The binding interface of F-actin was predicted near R1008 on the Vt. According to their computer simulation work (Golji and Mofrad, 2013), the open conformation of vinculin facilitates the binding of vinculin with the actin filament by removing the steric hindrance of the Vt–F-actin interaction. They could show that the Vt interacts favorably with F-actin when isolated from the head region (D1). Conformational changes subsequently lead to the opening of the vinculin molecule. They hypothesized that mechanical stresses transduced through vinculin's linkage with talin could enhance the movement of the head from the tail (Vt).

Full-length vinculin can bind, nucleate, cap, and crosslink F-actin (Jockusch and Isenberg, 1981; Goldmann and Guttenberg, 1998; Le Clainche et al., 2010). Vinculin's tail residues, R1049 and T1050, are proposed to be involved in dimer formation, suggesting that the D4 contacts in the head domain are released from the Vt upon actin binding (Golji and Mofrad, 2013). If, for instance, F-actin and the α-catenin fragment (cell–cell contact) are simultaneously mixed with full-length vinculin, the affinity of F-actin as well as α-catenin for vinculin increases significantly. This finding clearly indicates that vinculin's actin binding activity is enhanced by α-catenin, supporting the combinatorial input hypothesis (Goldmann et al., 2013).

Detailed understanding of the association of vinculin with F-actin is important for cellular mechanical stability. At focal adhesions (FAs), the actin cytoskeletal network is connected via integrins to the extracellular matrix (ECM). Forces originating from within or from outside the cell can only be mechanically re-enforced if the actin network is properly coupled to FAs. Golji et al. (2011) predicted from their computer simulation work that vinculin in maturing nascent adhesions could facilitate the F-actin linkage when mechanical stresses are present. During this process, the stress around vinculin would increase, as well as activate the molecule and by the same token intensify the linkage with actin filament and strengthen FAs. Using state-of-the-art techniques, Claire Waterman's group recently showed that FA maturation promotes vinculin activation and physical reinforcement of FAs (Case et al., 2015).

Supporting evidence for this notion comes also from studies elucidating the formation of an actin arc structure (Möhl et al., 2009; Küpper et al., 2010; Thievessen et al., 2013; Thompson et al., 2014). Actin polymerization leads to cell membrane protrusion, and rearward movement correlates with the retraction of the cell membrane (Plotnikov et al., 2012). During retrograde flow of the actin filaments, actin filament slippage occurs (Kanchanawong et al., 2010). These researchers interpret this event as integrin-FA disconnection, whereby vinculin stays connected which would allow vinculin to bind to another FA structure, stopping the actin flow. The slogan of vinculin acting as a molecular clutch was termed (Thievessen et al., 2013; Case et al., 2015).

Vinculin reinforces FAs by crosslinking actin filaments to the talin molecule. This is a critical step in cellular mechanics linking the cell to its substrate. Vinculin binds actin filaments to growing FAs and by capping them, regulates actin dynamics (Le Clainche et al., 2010).

Computer simulations of vinculin showed that it could cap actin filaments by interacting with them in the open conformation. Three binding sites on F-actin with vinculin were proposed, and their interaction is dependent on conformation and mechanical load according to Golji and Mofrad (2010). A function for vinculin in regulating actin dynamics has been proposed (Huveneers et al., 2012). Golji and Mofrad (2013) tested this in molecular dynamics simulations. They could show that cell migration, as well as FA formation, is controlled by vinculin (Goldmann et al., 1995a; Thievessen et al., 2015) and that vinculin–talin interaction plays a key role in regulating FA formation (Humphries et al., 2007).

Vinculin–actin interaction is important for the mechanics of FAs. Janssen et al. (2006) investigated this and found that basic residues on the surface of the vinculin tail match with acidic residues of F-actin. It remained, however, unclear whether the conformation of activated vinculin would be capable of that. Recent studies indicate that the Vt can inhibit actin polymerization when using a *Shigella flexneri* effector, IpaA (Ramarao et al., 2007). It is assumed, when using IpaA, actin polymerization is stopped by simultaneously activating vinculin tail's capping activity of F-actin. More evidence of Vt-capping activity comes from an in vitro study by Le Clainche et al. (2010). They showed that residues 1044–1066 of the Vt are responsible for the capping of F-actin by vinculin.

Vinculin binding to talin

Talin is believed to be important for vinculin's localization to FAs (Zhang et al., 2008). Its spherical head with an elongated rod domain (Goldmann et al., 1994) is suitable for linking integrin and the actin cytoskeleton. The head domain binds β-integrin and F-actin and the rod domain have interaction sites for F-actin and vinculin. Previously, PtdIns(4,5)P_2 and actin were considered the preferred partners for talin as both binding sites overlap (Gilmore and Burridge, 1996; Weekes et al., 1996; Huttelmaier et al., 1998). Simultaneous binding of both partners, however, is unlikely (Steimle et al., 1999). Talin–vinculin association is of low affinity (Goldmann et al., 1992) and only the presence of PtdIns(4,5)P_2 increases its affinity significantly. Therefore, a two-step model of vinculin activation was proposed: (i) binding of PtdIns(4,5)P_2 weakens the interaction between the Vh and Vt and (ii) locking vinculin in the open conformation allows the binding of additional proteins such as F-actin.

Izard et al. (2004) demonstrated that talin–vinculin interaction leads to conformational changes in the Vt and that the Vh and a short talin peptide (e.g., VBS3) encompass a single vinculin-binding site in the head region (D1). Further binding sites for vinculin on talin are located in the rod domain, which are believed to be activated under tension (Coll et al., 1995; Volberg et al., 1995; Galbraith et al., 2002; Zaidel-Bar et al., 2003; Hytonen and Vogel, 2008). Indeed, Galbraith et al. (2002) could show in traction force experiments that vinculin is recruited in integrin-mediated adhesions, and Del Rio et al. (2009) reported that stretching of single talin rods exposed binding sites for vinculin. More recently, Ciobanasu et al. (2014) confirmed that activation of vinculin by stretched talin reinforces actin anchoring. Therefore, force-dependent binding of vinculin to talin is believed to facilitate F-actin binding to FAs in cells (Hirata et al., 2014b). Moreover, Austen et al. (2015) addressed ECM rigidity sensing of talin in the context of integrin, vinculin, and F-actin. The association of talin with vinculin could also influence α5β1-integrin clustering (Humphries et al., 2007; Alonso and Goldmann, 2012). Together, all these observations allowed for the view that FA dynamics is probably regulated by integrin–ECM linkages when cross-linked by talin and vinculin (Humphries et al., 2007). Further, talin was reported to be the first protein that is recruited to integrin clusters, that is, it provides a binding site for vinculin, which subsequently triggers adhesion maturation during cell spreading and assembly of FAs (Sen et al., 2012).

Vinculin binding to α-actinin

A number of other binding partners for vinculin, including α-actinin, have been suggested. Bois et al. (2006) showed that talin and α-actinin bind the Vh (D1) of autoinhibited vinculin and this binding can then lead directly to vinculin activation. Simulations showed that vinculin binding sites (VBS) from α-actinin and talin all bind vinculin (the Vh) by the same mechanism. Simulation of VBS from α-actinin by Lee et al. (2008), however, suggested that α-VBS binds with an inverted orientation. Their results demonstrate a significant difference between binding to lower-VBS and upper-VBS. Therefore, the nature of α-actinin binding to full-length vinculin might be a topic for future investigations (Shams et al., 2012).

Previously, Izard et al. (2004) claimed that the binding of α-actinin peptide can by itself displace the Vt from the vinculin head effectively. Further, vinculin might also be activated by a combinatorial mechanism (Ziegler et al., 2006; Goldmann et al., 2013). This requires at least two binding partners, the high-affinity VBS in talin and α-actinin. Analysis of intensity profiles of a vinculin construct (aa 1-880, head) cotransfected with FAK showed differences between vinculin and α-actinin. It is, therefore, assumed that α-actinin does not have an important role in vinculin-induced FA growth. More likely candidates are talin and paxillin, but not FAK, α-actinin, or phosphotyrosine. Meanwhile, several other research groups have also investigated whether α-actinin directly links integrins to

F-actin. It is believed that α-actinin competes with talin to bind integrins, and thus triggering adhesion maturation (Zaidel-Bar et al. 2003; Roca-Cusachs et al. 2013). More recently, Case et al. (2015) described in their super-resolution study that FAs are stratified vertically into three layers and that vinculin is distributed between all of them.

Vinculin binding to paxillin

In the recruitment of paxillin to vinculin, tyrosine phosphorylation does not play a role in hypertrophic FAs. However, when expressing Y31/118E phosphomimetic paxillin, it rescued vinculin recruitment into focal complexes and reduced FAK activity (Zaidel-Bar et al. 2003). In vinculin knockout cells and mutants expressing the Vt region (Y822F), the phosphorylation and FAK–paxillin interaction were increased (Subauste et al., 2004). This correlated with an upregulation of ERK activity, which suppressed apoptosis. It was, therefore, assumed that the Vt competes with FAK for paxillin binding regulating paxillin phosphorylation (Subauste et al., 2004; Klemm et al., 2009, 2010). This was not mediated by enhanced FAK phosphorylation but rather by p21-activated kinase (PAK) and MAP/ERK kinase (MEK) upstream of ERK (Subauste et al., 2004). Inhibition of ERK resensitized the vinculin-deficient cells to apoptosis. On the other hand, phosphorylation of FAK on Tyr397 and paxillin on Tyr118 was increased in cells lacking vinculin, and a Vt polypeptide (residues 811–1066) suppressed both phosphorylation of FAK and paxillin and resensitized the vinculin-null cells to apoptotic stimuli (Subauste et al., 2004). Recent observations showed that both cell stiffness and FA-associated paxillin and vinculin markedly and quickly change (Gomez et al., 2011).

Vinculin binding to p130Cas

Several recent studies have shown that stretching of proteins in vitro can produce a biochemical change either by uncovering tyrosine phosphorylation sites in p130Cas (Janostiak et al., 2011, 2014) or VBS in talin. Vinculin's recruitment is assumed to be mediated by paxillin phosphorylation (Saez et al., 2004). Specifically, the phosphorylation of the p130Cas substrate domain is important in stretch-dependent activation of GTPase Rap1. Local tension in focal adhesions leads to an extension of p130Cas substrate domain, making phosphorylation sites easily accessible for kinases, which subsequently increases p130Cas substrate domain phosphorylation. The anchorage of p130Cas in focal adhesions is mediated by its C-terminal and SH3 domain, predominantly through the interactions with vinculin as well as FAK (Goldmann, 2014b). It is likely that each event enhances the probability of activation and together lead to vinculin activation (Sawada et al., 2006).

Vinculin binding to phospholipids

In vinculin, the C-terminal arm binds to acidic phospholipids (Scott et al., 2006; Diez et al., 2008, 2009; Wirth et al., 2010) causing a conformational change in the tail that potentially relieves inhibition by the head domain, which may activate proteins such as talin. Biochemical evidence from in vitro studies describe that talin or α-actinin, either as a single binding component (Izard et al., 2004; Bois et al., 2006) or together with PIP_2 or actin, are able to disrupt the head–tail interaction and activate vinculin, which is regarded as a "combinatorial model" (Gilmore and Burridge, 1996; Izard et al., 2004; Bois et al., 2006; Janssen et al., 2006). Whether PIP_2 (Weekes et al., 1996; Huttelmaier et al., 1998) or actin (Chen et al., 2006) is the preferred partner for talin is still unknown. Steimle et al. (1999) believed that PIP_2 and F-actin binding are mutually exclusive, thus Brown and Izard (2015) recently reported that simultaneous binding of PIP_2 and F-actin is structurally possible.

Vinculin activation

Phosphorylation of vinculin head/tail

Intramolecular interaction of vinculin's head and tail domains mask phosphorylation sites and their ability to bind to target proteins. It is believed that phosphorylation of vinculin is important for the mechanocoupling function of vinculin (Subauste et al., 2004; Golji et al., 2012). Removing the phosphorylation site at position Y822 resulted in upregulation of p-ERK and in the reduction of cell migration (Subauste et al., 2004; Peng et al., 2011). More recently, Bays et al. (2014) reported that phosphorylation at Y822 is only increased when forces are applied to cell–cell junctions (E-cadherin), but not at cell–matrix adhesions, which points to a regulatory function of Y822. Furthermore, c-*src*-dependent vinculin phosphorylation at positions Y100 and Y1065 has been reported to affect cell spreading and migration, indicating that the phosphorylation of vinculin might stabilize the active/open conformation (Zhang et al., 2004; Moese et al., 2007). The hypothesis of vinculin activation by phosphorylation is not necessarily a competing hypothesis to vinculin activation by a stretching force. It is, therefore, conceivable that phosphorylation enhances the ability of vinculin to be activated by a stretching force, or perhaps a stretching force enhances the ability of vinculin to be activated by phosphorylation.

The phosphorylation of vinculin contributes to vinculin activation by enhancing the cooperative binding of actin and talin to vinculin. Although mechanical stretch does not affect integrin expression levels, it alters the intracellular distribution and induces the phosphorylation of several FA proteins, including paxillin, p130Cas, and FAK. Recently,

Golji et al. (2012) proposed in a simulation study that pS1033 and pS1045 of vinculin at the interface between the tail and head (D1) domains could impact its activation and that phosphorylated vinculin requires less activating force on D1. Auernheimer and Goldmann (2014a) confirmed that force transmission is dependent on the phosphorylation of vinculin at position S1033, and that vinculin must be in an activated conformation incorporated into the FA complex to transmit forces via the actin network.

Combinatorial activation of vinculin

In the autoinhibited full-length vinculin structure, F-actin might act as part of a combinatorial input framework together with other binding partners, such as α-catenin (cell–cell contacts) or talin (FAs), to induce Vh–Vt dissociation promoting vinculin activation (Goldmann et al., 2013). The interaction between the head region (D4) the Vt of vinculin plays an important role in regulating binding between the Vh and talin (Cohen et al., 2005). Interactions of full-length vinculin with α-catenin (Bakolitsa et al., 1999), talin (Cohen et al., 2005), and IpaA, a protein that binds to vinculin and competes with talin, are all weaker than the respective interactions with the Vh. This implicates that full head–tail dissociation does not generally occur due to the binding of these ligands alone.

Because tryptophan fluorescence and increase of protease sensitivity support a conformational change in the Vt upon actin binding, an unfurling of the bundle was proposed as part of the actin binding mechanism as well (Bakolitsa et al., 1999). However, data are incompatible with unfurling of the five-helix bundle upon actin binding and clearly show that there are no large-scale changes upon binding. The nature of the conformational change triggered by actin binding is probably subtler, involving local rearrangements in the strap and C-terminal loop regions.

One of the actin binding sites identified by Golji et al. (2011) is partially occluded by Vt–Vh interactions in the autoinhibited form, and steric hindrance between the Vh domain D1 and F-actin prevent the second binding site to make full contact with F-actin. The recombinant fragment VD153, where the 153 residues of D1 are removed, does not contain the residues that occlude one of the Vt's actin binding sites or the residues that would clash with F-actin (Janssen et al., 2006; Golji et al., 2012; Auernheimer and Goldmann, 2014b). Each of the two actin-binding surfaces of the Vt contacts a different actin monomer along the filament. The binding surface at the bottom of the helix bundle, which is exposed in the autoinhibited form, contacts a highly charged area on top of subdomain 1 of actin. The combined body of data suggest that binding of vinculin to F-actin potentiates dimerization not by triggering a large-scale conformational change in the Vt, such as bundle unfurling, but by presenting two monomers on opposing filaments in the correct position and orientation. The low-binding affinity of actin to full-length vinculin is insufficient to trigger head–tail dissociation to fully expose the occluded actin-binding site and to eliminate the steric clashes of the actin filament and the N-terminal Vh residues (Janssen et al., 2006). According to these authors, the steric occlusion prevents the formation of a stable vinculin–F-actin complex.

As vinculin lacks enzymatic activity and functions by interacting with other proteins, it is important to understand how the autoinhibited conformation is disrupted. From the crystal structures and biochemical analyses, we know that vinculin is held in the autoinhibited conformation by at least two head–tail interfaces (Bakolitsa et al., 2004; Cohen et al., 2005). Based on the fact that no vinculin ligand binds to full-length vinculin with the same affinity as that of the head–tail interaction (<1 nM), it was proposed that two ligands are required to disrupt both interfaces leading to vinculin activation (Bakolitsa et al., 2004). To directly test this hypothesis, Susan Craig's laboratory applied the Foerster resonance energy transfer (FRET) system that reports vinculin conformational changes (Chen et al., 2005, 2006). This system was extensively used to study conformational changes in vinculin as well as to test the ability of talin to activate vinculin. Neither the talin rod, which contains at least three VBS, nor a short talin peptide containing a single VBS induced a change in FRET signal (Chen et al., 2006). These findings are consistent with the notion that two or more ligands are required for vinculin activation. Indeed, when F-actin was applied alongside either the talin rod or the peptide, vinculin was activated in a dose-dependent manner (Chen et al., 2006). These data support a mechanism whereby at least two proteins, talin and F-actin, are required to activate vinculin.

Vinculin activation by binding to talin

The interaction between talin and vinculin is a critical cellular process that involves mechanotransduction and mechanical interaction with the cellular environment via FAs. First mechanistic evidence came from a biochemical study that the bipartite autoinhibitory state of vinculin (comprising of D1-Vt and D4-Vt contacts) masks the binding site for talin (Cohen et al., 2005). Crystal structures of vinculin in its inactive and talin-activated state proved that talin induces a conformational change in the Vh allowing for direct cytoskeletal assembly of vinculin in FAs (Izard et al., 2004). More recently, Golji et al. (2011) simulated the association by using talin's VBS and vinculin's D1 before and after vinculin activation. They found that the recruitment of talin and vinculin to FAs is directly correlated with a mechanical stimulus applied to the site of FA

formation. These results suggested a mechanical sensation by talin and vinculin (Auernheimer et al., 2015).

These observations led to two specific assertions concerning vinculin recruitment to FAs: (i) It is possible that talin is not the only mechanosensor at FAs, but vinculin might also be dependent on a mechanical environment for its activation. Considering that the suggested mechanism for vinculin activation is that actin and talin cooperatively interact with vinculin to cause its activation, this proximity of talin to vinculin by the weaker hydrophobic interaction at the lower VBS could allow for vinculin activation. (ii) Furthermore, phosphorylation of vinculin could also contribute to vinculin activation by enhancing the cooperative binding of actin and talin to vinculin. With D1 weakly interacting with the lower VBS, an electrostatic interaction between the Vt and actin could then stretch vinculin and cause its activation. The stretching of vinculin could lead to a conformational change in which D1 moves away from the Vt and vinculin becomes activated. Next, talin's VBS would be able to fully insert into D1 to solidify and strengthen the talin–vinculin link. The strengthening of the interaction between vinculin and talin after vinculin activation allows vinculin to take a larger mechanical load at FAs, reflecting its role as a reinforcing agent. With vinculin activation by this mechanism, it is possible that the number of actin filaments linked to each talin rod can be multiplied via vinculin (Golji and Mofrad, 2010; Golji et al., 2011).

Vinculin in FA formation

Vinculin interaction with talin clusters integrins in an active form to induce FA enlargement, and its interaction with talin and actin leads to FA growth (Humphries et al., 2007). This interaction also drives the recruitment and release of core FA components and regulates the transmission of mechanical signals from the ECM (Carisey et al., 2013; Hirata et al., 2014a). Talin is a key molecule regulating integrin activation (Gingras et al., 2006, 2009). However, Peng et al. (2011) proposed that vinculin activity may be the important driving force for FA growth. It might, therefore, be that active vinculin locks talin in FAs in an active conformation, resulting in the growth of the adhesion site, thus providing an ideal platform for the recruitment of other FA components and a link to the actin cytoskeleton. Other proteins, such as talin and paxillin, but not FAK, α-actinin, or phosphotyrosine, correlated highly with localization of vinculin in FAs, and their formation and enlargement.

Vinculin in diseased and cancer cells

Vinculin expression is commonly lost in cancer cells and mutations in vinculin are linked to a variety of diseased states such as cardiomyopathies. Efforts to better understand the role of vinculin have included the development of animal models. This has confirmed and expanded our knowledge of vinculin's role in cell–cell and cell–matrix adhesions and has underscored the importance of vinculin in vivo (Lifschitz-Mercer et al., 1997; Somiari et al., 2003; Goldmann et al., 2013).

The loss of vinculin leads to the disruption of cell adhesion and cell migration, both of which are processes crucial for embryonic development. Disrupting vinculin expression causes cellular abnormalities. Xu et al. (1998) tested this directly by deleting the vinculin gene in mice, which resulted in lethality at embryonic day E10. Most prominent were defects of the neural folds and head structures in the ventral cranial midline as well as malformation of the heart. In the vinculin null embryo, the heart is only about half the size of the thin walls (Rodriguez Fernandez et al., 1992, 1993). All these defects could potentially arise from improper cell–cell and cell–matrix adhesion and actin remodeling.

In cardiac myocytes, vinculin is detected at the intercalated disks and costameres (Volk and Geiger, 1984). These heart-specific structures are similar to cell–matrix adhesions (Samarel, 2005). Costameres, like FAs, organize myofilaments into a three-dimensional structure and link them to the ECM. These structures can transduce mechanical forces across the cell membrane. Intercalated disks are similar to adherens junctions, desmosomes, and gap junctions (Noorman et al., 2009). These structures are important for the mechanical coupling of cardiac myocytes. Besides cadherin and catenin in adherens junctions, vinculin is a major component of the mechanical transduction system (Chen et al., 2004). These findings demonstrate that vinculin is essential for proper heart function, owing to its effects on cell–cell adhesion in intercalated disks (Zemljic-Harpf et al., 2004). More recently, an interesting study has been performed to elucidate vinculin's function on cytoskeletal remodeling in an aging heart (Kaushik et al., 2015).

FAs in cellular mechanotransduction

Mechanotransduction in cells starts locally, but its effects are transmitted globally in the cell via phosphorylation cascades or diffusion of second messengers such as calcium ions that activate complex signaling pathways (Goldmann, 2014a). At FAs, mechanotransduction not only involves talin and vinculin but also membrane-bound integrins (Goldmann et al., 1996; Ezzell et al., 1997). The process of integrin clustering at FAs can be force-induced, and the binding of integrin to the ECM can be mechanosensitive. Results so far come from simulations (Golji and Mofrad, 2010). Other studies have assumed a talin conformation in which VBS is rotated out of its hydrophobic groove in the rod domain during VBS activation (Ziegler et al., 2008; Auernheimer et al., 2015). It might also be possible that talin

mechanosensation is the result of external stress (Golji et al., 2011).

Vinculin recruitment to FAs

Talin has been proposed to be the protein that recruits vinculin to FAs due to its early engagement with integrin (Horwitz et al., 1986). However, recent studies have shown that the vinculin mutant, A50I, which blocks talin binding, is also able to localize to FAs, leaving the question of how vinculin is recruited unanswered (Bakolitsa et al., 2004; Cohen et al., 2006; Humphries et al., 2007; Diez et al., 2011; Peng et al., 2011). Some evidence suggests that paxillin may be responsible for this recruitment via its ability to bind both integrins and vinculin directly. According to Pasapera et al. (2010), nascent adhesions are bound to talin and paxillin and an increase in myosin-mediated FAK phosphorylation results in elevated paxillin phosphorylation, and phosphorylated paxillin then promotes vinculin recruitment to the adhesion site.

Vinculin's involvement in force transmission pathways

Integrin, talin, and vinculin are directly involved in mechanotransduction (Goldmann, 2002, 2010, 2012). Talin and vinculin are among the numerous FA proteins that link the cytoplasmic domains of integrin subunits to F-actin filaments (Goldmann et al., 1995, Goldmann and Guttenberg, 1998). Vinculin binding to talin stabilizes the talin–integrin complex, locking integrins in an active conformation, and increased stability of talin in active vinculin-containing FAs has been observed (Dumbauld et al., 2013). A model in which tension-activated vinculin stabilizes the talin–integrin complex leading to enforcement of integrin adhesion and stabilization of integrin-proximal proteins of FAs has been proposed by (Puklin-Faucher and Sheetz, 2009). Using a FRET probe to measure mechanical tension across single molecules it was reported that vinculin is under ~2.5 pN of tension in vivo when reinforcing FAs. It is likely that this tension stretches vinculin to a conformation similar to the one tested by (Grashoff et al., 2010). Further, vinculin has been reported to control the intra- and extracellular transmission of mechanical cues that are important for the reorganization of FAs (Carisey et al., 2013), and Chang and Kumar (2013) reported on vinculin tension distribution of individual stress within cell–matrix adhesions. More recently, Hernandez-Varas et al. (2015) investigated vinculin-mediated tension and adhesion complex area demonstrating a plastic, context-dependent relationship.

Vinculin, ECM, integrin, and forces

The fibronectin receptors and integrins establish initial adhesion between the cell and the ECM. Talin and vinculin are transiently recruited to the adhesion to reinforce integrin binding to form a structural link between the ECM and the actin cytoskeleton. This results in cellular stiffening and requires both actomyosin integrity and cellular contractility (Faull and Ginsberg, 1995).

In vitro, cellular level forces can stretch adhesion proteins that link the ECM to the actin cytoskeleton, exposing hidden binding sites. There is, however, little direct evidence that in vivo forces produce significant in vivo stretching to cause domain unfolding (Sawada et al., 2006; Janostiak et al., 2014). It has been reported that talin is repeatedly stretched by 100–350 nm in vivo by actomyosin contraction (talin's in vitro length is only between 50 nm and 60 nm) (Goldmann et al., 1994).

FA formation and mechanotransduction

Phosphorylation of vinculin also contributes to vinculin activation by enhancing the cooperative binding of actin and talin to vinculin (Golji et al., 2012; Auernheimer and Goldmann, 2014a; Auernheimer et al., 2015). With vinculin activation in this way, it might be possible that the number of actin filaments linked to each talin rod can be crosslinked via vinculin. In force-induced FA formation, as the load on the developing focal complex increases, one would expect more vinculin activation and recruitment of activated vinculin to crosslink talin and actin filaments. Golji et al. (2011) investigated the interaction of activated vinculin with talin. It was suggested that electrostatic forces might be important for driving the binding of vinculin to actin.

Many other actin regulatory proteins are also recruited to punctate adhesions, which include vinculin, zyxin, ena/VASP, formins, and Arp2/3 proteins (Kovacs et al., 2002). They are likely to control the extent of F-actin polymerization at these sites. In addition, Rho-asociated protein kinase (ROCK) signaling is required to form punctate adhesions. A study by Hu et al. (2007) showed that integrins and proteins, such as paxillin, zyxin, and FAK, without direct interaction sites for actin have a low correlation with actin flow. Other FA proteins, such as α-actinin, also influence the flow of actin. This points to different molecular hierarchies of the actomyosin force machinery (Kanchanawong et al., 2010; Thievessen et al., 2013; Giannone, 2015). According to Hemmings et al. (1996) and Lee et al. (2004), talin colocalizes precisely with vinculin in FAs, but this is not an effective link to F-actin. Therefore, vinculin might act as a principle connector of the FA core to actin filaments and as a major transmitter of forces (Goldmann and Ingber, 2002). A vinculin mutation (A50I) inhibited talin binding and the FA growth-promoting activity as well as force generating activity were lost (Cohen et al., 2006; Diez et al., 2011). Cohen et al. (2006) examined the dynamic effect of active vinculin on other proteins localized in FAs. Reduced

mobility of vinculin affected the turnover rate of talin and integrins in FAs.

It has been proposed that the Vh and Vt regions of vinculin have distinct functions, that is, the Vh is involved in talin binding and subsequent cellular actin binding, whereas the Vt influences the coupling with the mechanotransduction force machinery. According to Möhl et al. (2009), nascent adhesions form first and as the mechanical stress intensifies, the talin molecules become activated for interaction with vinculin. This results in vinculin changing from its closed to its open conformation with complete binding of F-actin and talin as well as dramatic FA growth (Hu et al., 2007). The complete activation of vinculin gives direct access of talin and α-actinin to the head; ponsin, vinexin, VASP, and Arp2/3 to the neck; and actin, PIP_2, and paxillin to the tail (Zamir and Geiger, 2001; Ziegler et al., 2006; Goldmann et al., 2013). More recently, Auernheimer et al. (2015) showed that the phosphorylation of vinculin is an essential step for the binding to talin and FA formation.

Various research groups proposed several steps of vinculin activation in cellular mechanotransduction: (i) low-affinity binding to talin or neck-binding proteins to focal complexes (Chen et al., 2005), (ii) possible association with PIP_2 or actin (Huttelmaier et al., 1998; Bakolitsa et al., 1999, 2004; Chen and Dokholyan, 2006; Janssen et al., 2006), (iii) when activated, rapid adhesion complex turnover, and (iv) conformational change of vinculin leads to stabilization of vinculin-talin-integrin binding in FAs. A recent report revealed that PIP2 binding to vinculin is necessary for maintaining FAs, actin organization, cell migration, and spreading (Chinthalapudi et al., 2014).

Focal adherens junctions (FAJs) in cellular mechanotransduction

Role of vinculin in FAJs

Vinculin is an integral part of adherens junctions in various tissues and has been the focus of various researchers (Bloch and Hall, 1983; Koteliansky and Gneushev, 1983; Pardo et al., 1983; Drenckhahn and Franz, 1986). Their efforts have uncovered three roles for vinculin: (i) vinculin acts downstream of myosin VI, a minus end-directed motor necessary for the E-cadherin-dependent process of border-cell migration (Maddugoda et al., 2007), (ii) vinculin regulates E-cadherin surface expression in cadherin-based adhesion (Peng et al., 2009), and (iii) vinculin plays a role in the mechanosensory response of E-cadherin (Le Duc et al., 2010). The responses require the contractile actin cytoskeleton. Cells lacking vinculin showed a significant reduction in stiffness and exhibited a dramatic decrease in the recruitment of phosphorylated myosin light chains (MLCs) to cell–cell junctions when stimulated with a cell growth factor. Further, α-catenin binding to vinculin was reported to be dependent upon myosin activity by Yonemura et al. (2010).

Vinculin is not required for the assembly of cadherin-mediated cell–cell junctions; however, it is believed to strengthen the mechanical links between adhesion complexes (containing E-cadherin, β-catenin, and α-catenin) and the actin cytoskeleton. 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. It remains to be elucidated which of these possible events is indeed elicited by an increase in tension at cell–cell junctions and thus contributes to cadherin mechanotransduction.

Vinculin recruitment to adherens junctions

Vinculin recruitment to adherens junctions is still controversial. α-Catenin is required for vinculin recruitment and localization to intercellular junctions according to Watabe-Uchida et al. (1998) and Sheikh et al. (2006). Myosin VI has also been implicated in vinculin recruitment to cell–cell junctions. Specifically, myosin VI is necessary for the incorporation of vinculin into stable cadherin-containing adhesions (Sheikh et al., 2006).

FAJ formation and mechanotransduction

Cell–cell adhesions are sites where cells experience and resist tugging forces that may serve in mechanotransduction. Le Duc et al. (2010) provided direct evidence that E-cadherin participates in a mechanosensing pathway that regulates the actomyosin cytoskeleton to modulate cell stiffness in response to pulling forces. Further, these authors demonstrated that cellular stiffening in response to twisting force is reduced in vinculin-deficient cells. As α-catenin/Eplin (epithelial protein lost in neoplasm) bind vinculin directly (Le Duc et al., 2010; Yonemura et al., 2010; Peng et al., 2012), it was proposed that force transmission to α-catenin/Eplin might alter its conformation. Thus, vinculin might not be the sole mediator of the cytoskeletal response in controlling actin filament dynamics and organization.

FAs and FAJs exhibit many striking similarities (Figures 1a and 1b). Both (i) consist of a cluster of transmembrane receptors, (ii) provide a highly dynamic mechanical link to the actin cytoskeleton, and (iii) consists of a large number of signaling and structural molecules that cluster at the junction through multiple, redundant protein–protein interactions (Nagafuchi et al., 1991). We and others have pointed out that there must be a link between FAs and FAJs controlling cellular behavior (Lauffenburger and Wells, 2001; Lange et al., 2013). For instance, increasing cell–cell adhesion mechanically competes with and decreases cell–substrate adhesion (Lauffenburger and Wells, 2001) and leads to selective

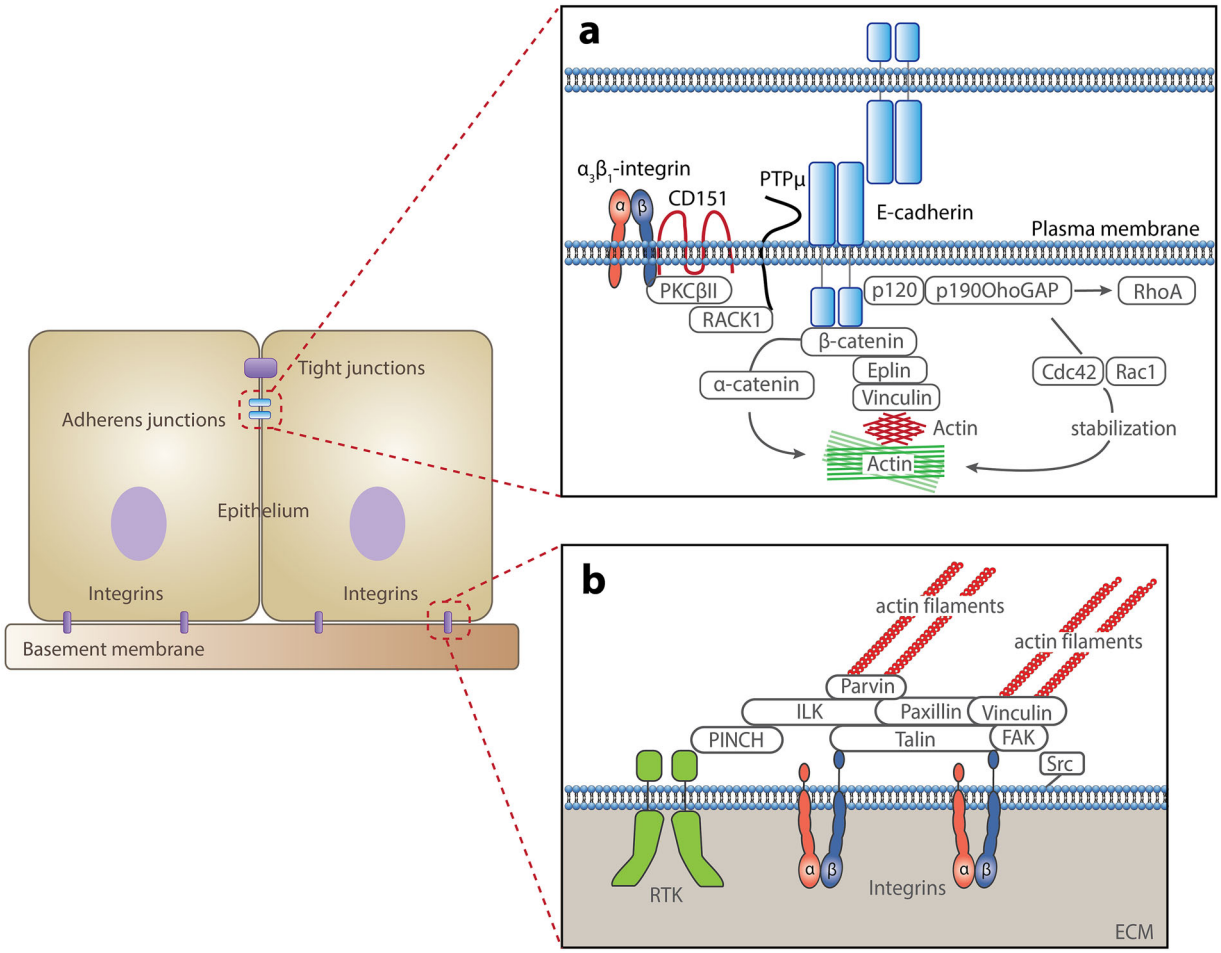

Figure 1 A schematic diagram indicating the cell–cell junction (a) and cell–matrix junction (b). Adapted from Yilmaz M. and Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev. 2009;28:15–33.

reinforcement of FAs and FAJs (Levenberg et al., 1998). Thus, the extent to which this interplay results from the redistribution of cytoskeletal tension is unknown.

It has also been reported that cadherin-mediated cell–cell contacts decrease cell-ECM adhesion. Chen and his group could show that cadherin-mediated cell–cell contact affects cell-ECM adhesion (Legant et al., 2012), using a lithographic method. They could show by preventing contact inhibition of cell spreading, cadherin-mediated cell–cell contact no longer inhibited proliferation, but acted as a stimulatory signal for cell proliferation in vascular cells. These results imply that FAJs can generate two opposing signals that affect cell proliferation (McCain et al., 2012; Mertz et al., 2012). Recently, Huveneers et al. (2012) reported that vinculin protects the force-dependent remodeling of FAJs. However, the open question of how FAs, FAJs, ECM, integrins, and cell–cell adhesion molecules interact still remains.

Key members of the cadherin–adhesion complex

In adherens junctions, the major cell–surface adhesion receptors are the cadherins (Niessen and Gottardi, 2008; Ogita et al., 2008). The extracellular domains of cadherins mediate strong cell–cell adhesion by binding to cadherins on adjacent cells. The cytoplasmic tail of cadherin interacts with several proteins, including β-catenin (via the distal portion of the cadherin cytoplasmic tail) and p120-catenin (via a more proximal region of the cadherin cytoplasmic tail (Perez and Sanderson, 2005). β-catenin in turn binds α-catenin, and both β-catenin/Eplin bind to vinculin (Figure 1).

All evidence to date suggests that vinculin is required to maintain the integrity of adherens junctions. The first hint of vinculin's importance came from the observation that cell–cell adhesion is lost in numerous cancer cells during the initial stages of tumor formation (Lifschitz-Mercer et al., 1997; Somiari et al., 2003). Later it was found that defects in

mice lacking vinculin were consistent with a role for vinculin in regulating adherens junction function. Vinculin's affinity for cell–matrix adhesions, where only small amounts of vinculin are needed to maintain adhesion, is higher than its affinity for cell–cell adhesions (Xu et al., 1998; Bakolitsa et al., 2004; Bays et al., 2014).

Vinculin is a coactivator of αE-catenin and its binding to αE-catenin has been described to be force-dependent at cell–cell contacts. Under low-stress conditions, an inhibitory region of αE-catenin blocks the VBS. Upon F-actin binding to the C-terminus of αE-catenin and subsequent actomyosin contraction, this inhibitory region is displaced and vinculin is able to bind (Maiden et al., 2013). The C-terminus of α-catenin modulates the F-actin binding. Interactions between α-catenin and the actin cytoskeleton occur through both direct and indirect mechanisms, depending on the cellular context. Modeling studies demonstrated that conformational changes in the whole protein are not sufficient to affect actin binding.

The N-terminal domain of α-catenin is accessible and its central domain recruits vinculin and probably α-actinin through force-dependent changes of its conformation (Maiden et al., 2013). This correlates with the discovery that an increasing number of actin-associated proteins, such as α-actinin, formin, and Ajuba, are able to associate with α-catenin. α-Catenin alters its conformation under tension to allow recruitment of vinculin to reinforce cell–cell adhesions (Yonemura et al., 2010; Huveneers et al., 2012). The recruitment of vinculin upon stretching of α-catenin could strengthen cell–cell adhesion by providing additional bonds between the cadherin complex and F-actin. The binding of α-catenin to the Vh enhances the binding of the Vt to F-actin (Choi et al., 2012; Peng et al., 2012; Bays et al., 2014). This has also been shown for many factors that bind to the Vh (Ziegler et al., 2006). By placing vinculin into the force chain between F-actin and cadherin, any tension across the cadherin-F-actin linkage could affect its conformation. Further, bundling of F-actin to the cadherin complex could reinforce cell–cell adhesion. Vinculin might also bring other factors to cadherin adhesions, such as the VASP (Brindle et al., 1996) and ponsin (Kioka et al., 1999; Mandai et al., 1999), as well as the Arp2/3 complex, (DeMali et al., 2002; Tang et al., 2012) to regulate F-actin remodeling. Moreover, there is evidence that vinculin itself is an actin nucleator (Ruddies et al., 1992; Le Clainche et al., 2010; Wen and Janmey, 2011). The force-regulated domain of α-catenin can also bind to α-actinin and its tail domain to Eplin, which is somehow regulated by myosin activity (Taguchi et al., 2011). Finally, the actin nucleator formin-1 also binds to the D3 domain of α-catenin (Vasioukhin and Fuchs, 2001) and might contribute to force-induced actin-polymerization at cell–cell junctions. For α-catenin, there is also evidence for an allosteric regulation of its actin-binding activity (Drees et al., 2005), as well as for its vinculin-binding activity (Yonemura et al., 2010; Choi et al., 2012).

Forces at the zonula adherens (ZA) and FAJ are different

Adhesive structures form different connections to F-actin (Yonemura et al., 2010). 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. The exact mechanisms that recruit vinculin and Eplin to the distinct junction structures are still unclear, and might involve both recruitment by force-induced changes in α-catenin and changes in the F-actin cytoskeleton. The question of the links between the cadherin complex and actin cytoskeleton is more complex than previously envisioned because α-catenin is a multimodular molecule that undergoes, under the action of mechanical strengths, conformational changes unmasking cryptic ligand binding sites. A model exists in which Eplin primarily connects the vascular–endothelial (VE)–cadherin–catenin complex to F-actin by interacting with the closed conformation of α-catenin. When forces are applied by the actin filament-Eplin network, α-catenin adopts a stretched conformation, unmasking a cryptic binding domain for vinculin. Depending on the tension amplitude exerted by the cytoskeleton, variable amounts of vinculin are recruited to cell–cell junctions.

Interplay between cell–cell and cell–matrix interaction

Cell adhesions are important for many morphogenic processes, including cell sorting, cell rearrangement, and cell movement (Gumbiner, 2005). It is, therefore, not surprising that the disruption of cell adhesion components occurs in numerous diseased states. As described above, the loss of vinculin leads to the disruption of cell adhesion and cell migration, both of which are processes crucial to embryonic development.

The global vinculin knockout provides key insights into vinculin function and suggests that vinculin plays a crucial role in heart function. The heart-specific structures are similar to cell–matrix adhesions and circumferentially align with the Z disk of the myofibrils (Samarel, 2005). They share many components of FAs, which include integrins, talin, vinculin, α-actinin, and FAK. These structures connect neighboring myocytes in a staggered fashion and are important for the mechanical and electrical coupling of cardiac myocytes. Vinculin's expression is upregulated in response to loading, and its localization in costameres is disrupted upon the

unloading of mechanic force (Sharp et al., 1997). Although vinculin knockout mice appear healthy initially, 50% died suddenly before reaching 14 weeks of age due to ventricular tachycardia and disruptions in electrical conductance (Zemljic-Harpf et al., 2007). An analysis of tissues harvested prior to the loss of ventricular function showed highly serrated intercalated disks that were detached from the myofibrils (Zemljic-Harpf et al., 2007). The disruptions in intercalated disks arose from a loss of cadherin at this site, a phenomenon that is recapitulated in epithelial cells in which vinculin expression is silenced (Peng et al., 2009).

Conclusions

Mechanical forces are essential for the organization, growth, maturation, and function of living tissues. At the cellular level, many of the biological responses to external forces originate from specialized microscale structures. Force transduction through cell–matrix (FAs) and cell–cell contacts (FAJs) controls the maturation or assembly/disassembly of these adhesions and triggers intracellular signaling cascades that influence cellular behaviors. Upon force application, many cell types actively rearrange the organization of the contractile apparatus.

What the integrin receptors accomplish as mechanosensors/transducers between the ECM and the actomyosin cytoskeleton, cadherin complexes may do at cell–cell junctions. To date, the exact molecular composition of the link between cadherin and actin is not completely resolved. However, in recent years, researchers have addressed the question of how α-catenin bridges E-cadherin–catenin complexes to the actin cytoskeleton. It is now believed that the protein Eplin establishes a link between the cadherin–β-catenin complex and actin, which acts as a mechanotransmitter (Peng et al., 2012). Thus, colocalization of Eplin and vinculin appears restricted to areas where cell–cell junctions are mature. The mechanism of mechanosensing/transduction at E-cadherin junctions may differ from integrin-dependent force sensing/transduction. More recent findings broadly implicate E-cadherin as playing a central role in the development and maintenance of epithelial tissues.

Acknowledgments and funding

This work was supported by grants from the Staedtler Stiftung, L'Agence nationale de la recherché (ANR), and Deutsche Forschungsgemeinschaft (FOR1228-Z2). I thank Dr. Victoria Jackiw for proofreading and copyediting the manuscript and Dr. Navid Bonakdar for redrawing Figure 1.

References

Alonso JL, Goldmann WH (2012) Influence of divalent cations on the cytoskeletal dynamics of K562 cells determined by nanoscale bead tracking. Biochem Biophys Res Commun 421: 245–8.

Auernheimer V, Goldmann WH (2014a) Serine phosphorylation on position 1033 of vinculin impacts cellular mechanics. Biochem Biophys Res Commun 450: 1095–8.

Auernheimer V, Goldmann WH (2014b) Vinculin E29R mutation changes cellular mechanics. Biochem Biophys Res Commun 452: 661–4.

Auernheimer V, Lautscham LA, Leidenberger M, Friedrich O, Kappes B, Fabry B, Goldmann WH (2015) Vinculin phosphorylation at residues Y100 and Y1065 is required for cellular force transmission. J Cell Sci 128:3435–43.

Austen K, Ringer P, Mehlich A, Chrostek-Grashoff A, Kluger C, Klinger C, Sabass B, Zent R, Rief M, Grashoff C (2015) Extracellular rigidity sensing by talin isoform-sensitive mechanical linkages. Nat Cell Biol 17:1597–606.

Bakolitsa C, Cohen DM, Bankston LA, Bobkov AA, Cadwell GW, Jennings L, Critchley DR, Craig SW, Liddington RC. (2004) Structural basis for vinculin activation at sites of cell adhesion. Nature 430: 583–6.

Bakolitsa C, de Pereda JM, Bagshaw CR, Critchley DR, Liddington RC (1999) Crystal structure of the vinculin tail suggests a pathway for activation. Cell 99: 603–13.

Barstead RJ, Waterston RH (1989) The basal component of the nematode dense-body is vinculin. J Biol Chem 264: 10177–85.

Bays JL, Peng X, Tolbert CE, Guilluy C, Angell AE, Pan Y, Superfine R, Burridge K, DeMali KA. (2014) Vinculin phosphorylation differentially regulates mechanotransduction at cell-cell and cell-matrix adhesions. J Cell Biol 205: 251–63.

Bloch RJ, Hall ZW (1983) Cytoskeletal components of the vertebrate neuromuscular junction: vinculin, alpha-actinin, and filamin. J Cell Biol 97: 217–23.

Bois PR, O'Hara BP, Nietlispach D, Kirkpatrick J, Izard T (2006) The vinculin binding sites of talin and alpha-actinin are sufficient to activate vinculin. J Biol Chem 281: 7228–36.

Brindle NP, Holt MR, Davies JE, Price CJ, Critchley DR (1996) The focal-adhesion vasodilator-stimulated phosphoprotein (VASP) binds to the proline-rich domain in vinculin. Biochem J 318: 753–7.

Brown DT, Izard T (2015) Vinculin-cell membrane interactions. Oncotarget 6: 34043–4.

Burridge K, Mangeat P (1984) An interaction between vinculin and talin. Nature 308: 744–6.

Carisey A, Tsang R, Greiner AM, Nijenhuis N, Heath N, Nazgiewicz A, Kemkemer R, Derby B, Spatz J, Ballestrem C (2013) Vinculin regulates the recruitment and release of core focal adhesion proteins in a force-dependent manner. Curr Biol 23: 271–81.

Case LB, Baird MA, Shtengel G, Campbell SL, Hess HF, Davidson MW, Waterman CM (2015) Molecular mechanism of vinculin activation and nanoscale spatial organization in focal adhesions. Nat Cell Biol 17: 880–92.

Chang CW, Kumar S (2013) Vinculin tension distributions of individual stress fibers within cell-matrix adhesions. J Cell Sci 126: 3021–30.

Chen CS, Tan J, Tien J (2004) Mechanotransduction at cell-matrix and cell-cell contacts. Annu Rev Biomed Eng 6: 275–302.

Chen H, Choudhury DM, Craig SW (2006) Coincidence of actin filaments and talin is required to activate vinculin. J Biol Chem 281: 40389–98.

Chen H, Cohen DM, Choudhury DM, Kioka N, Craig SW (2005) Spatial distribution and functional significance of activated vinculin in living cells. J Cell Biol 169: 459–70.

Chen Y, Dokholyan NV (2006) Insights into allosteric control of vinculin function from its large scale conformational dynamics. J Biol Chem 281: 29148–54.

Chinthalapudi K, Rangarajan ES, Patil DN, George EM, Brown DT, Izard T (2014) Lipid binding promotes oligomerization and focal adhesion activity of vinculin. J Cell Biol 207: 643–56.

Choi HJ, Pokutta S, Cadwell GW, Bobkov AA, Bankston LA, Liddington RC, Weis WI (2012) AlphaE-catenin is an autoinhibited molecule that coactivates vinculin. Proc Natl Acad Sci USA 109: 8576–81.

Ciobanasu C, Faivre B, Le Clainche C (2014) Actomyosin-dependent formation of the mechansensitive talin-vinculin complex reinforces actin anchoring. Nat Commun 5: 3095.

Cohen DM, Chen H, Johnson RP, Choudhury B, Craig SW (2005) Two distinct head-tail interfaces cooperate to suppress activation of vinculin by talin. J Biol Chem 280: 17109–17.

Cohen DM, Kutscher B, Chen H, Murphy DB, Craig SW (2006) A conformational switch in vinculin drives formation and dynamics of a talin-vinculin complex at focal adhesions. J Biol Chem 281: 16006–15.

Coll JL, Ben-Ze'ev A, Ezzell RM, Rodriguez Fernandez JL, Baribault H, Oshima RG, Adamson ED (1995) Targeted disruption of vinculin genes in F9 and embryonic stem cells changes cell morphology, adhesion, and locomotion. Proc Natl Acad Sci USA 92: 9161–5.

Del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz MP (2009) Stretching single talin rod molecules activates vinculin binding. Science 323: 638–41.

DeMali KA, Barlow CA, Burridge K (2002) Recruitment of the Arp2/3 complex to vinculin: coupling membrane protrusion to matrix adhesion. J Cell Biol 159: 881–91.

Diez G, Auernheimer V, Fabry B, Goldmann WH (2011) Head/tail interaction of vinculin influences cell mechanical behavior. Biochem Biophys Res Commun 406: 85–8.

Diez G, Kollmannsberger P, Mierke CT, Koch TM, Vali H, Fabry B, Goldmann WH (2009) Anchorage of vinculin to lipid membranes influences cell mechanical properties. Biophys J 97: 3105–12.

Diez G, List F, Smith J, Ziegler WH, Goldmann WH (2008) Direct evidence of vinculin tail-lipid membrane interaction in beta-sheet conformation. Biochem Biophys Res Commun 373: 69–73.

Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI (2005) Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. Cell 123: 903–15.

Drenckhahn D, Franz H (1986) Identification of actin-, alpha-actinin-, and vinculin-containing plaques at the lateral membrane of epithelial cells. J Cell Biol 102: 1843–52.

Dumbauld DW, Lee TT, Singh A, Scrimgeour J, Gersbach CA, Zamir EA, Fu J, Chen CS, Curtis JE, Craig SW, Garcia AJ (2013) How vinculin regulates force transmission. Proc Natl Acad Sci USA 110: 9788–93.

Eimer W, Niermann M, Eppe MA, Jockusch BM (1993) Molecular shape of vinculin in aqueous solution. J Mol Biol 229: 146–52.

Ezzell RM, Goldmann WH, Wang N, Parasharama N, Ingber DE (1997) Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. Exp Cell Res 23: 14–26.

Faull RJ, Ginsberg MH (1995) Dynamic regulation of integrins. Stem Cells 13:38–46.

Galbraith CG, Yamada KM, Sheetz MP (2002) The relationship between force and focal complex development. J Cell Biol 159: 695–705.

Geiger B, Tokuyasu KT, Dutton AH, Singer SJ (1980) Vinculin, an intracellular protein localized at specialized sites where microfilament bundles terminate at cell membranes. Proc Natl Acad Sci USA 77: 4127–31.

Giannone G (2015) Super-resolution links vinculin localization to function in focal adhesions. Nat Cell Biol 17: 845–7.

Gilmore AP, Burridge K (1996) Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4-5-bisphosphate. Nature 381: 531–5.

Gingras AR, Vogel KP, Steinhoff HJ, Ziegler WH, Patel B, Emsley J, Critchley DR, Roberts GC, Barsukov IL (2006) Structural and dynamic characterization of a vinculin binding site in the talin rod. Biochemistry 45: 1805–17.

Gingras AR, Ziegler WH, Bobkov AA, Joyce MG, Fasci D, Himmel M, Rothemund S, Ritter A, Grossmann JG, Patel B, Bate N, Goult BT, Emsley J, Barsukov IL, Roberts GC, Liddington RC, Ginsberg MH, Critchley DR (2009) Protein structure and folding: structural determinants of integrin binding to the talin rod. J Biol Chem 284: 8866–76.

Goldmann WH (2002) Mechanical aspects of cell shape regulation and signaling. Cell Biol Int 26: 313–7.

Goldmann WH (2010) Correlation between the interaction of the vinculin tail domain with lipid membranes, its phosphorylation and cell mechanical behaviour. Cell Biol Int 34: 339–42.

Goldmann WH (2012) Mechanotransduction and focal adhesions. Cell Biol Int 36: 649–52.

Goldmann WH (2014a) Mechanosensation: a basis cellular process. Prog Mol Biol Transl Sci 126: 75–102.

Goldmann WH (2014b) Vinculin-p130Cas interaction is critical for focal adhesion dynamics and mechano-transduction. Cell Biol Int 38: 283–6.

Goldmann WH, Auernheimer V, Thievessen I, Fabry B, Vinculin (2013) Cell mechanics, and tumor cell invasion. Cell Biol Int 37: 397–405.

Goldmann WH, Bremer A, Haner M, Aebi U, Isenberg G (1994) Native talin is a dumbbell-shaped homodimer when it interacts with actin. J Struct Biol 112: 3–10.

Goldmann WH, Ezzell RM, Adamson ED, Niggli V, Isenberg G, Vinculin (1996) Talin and focal adhesions. J Muscle Res Cell Motil 17: 1–5.

Goldmann WH, Guttenberg Z (1998) Examination of temperature-induced 'gel-sol' transformation of alpha-actinin/cross-linked actin networks by static light scattering. FEBS Lett 426: 255–9.

Goldmann WH, Ingber DE (2002) Intact vinculin protein is required for control of cell shape, cell mechanics, and rac-dependent lamellipodia formation. Biochem Biophys Res Commun 290: 749–55.

Goldmann WH, Niggli V, Kaufmann S, Isenberg G (1992) Probing actin and liposome interaction of talin and talin-vinculin complexes: a kinetic, thermodynamic and lipid labeling study. Biochemistry 31: 7665–71.

Goldmann WH, Schindl M, Cardozo TJ, Ezzell RM (1995a) Motility of vinculin-deficient F9 embryonic carcinoma cells analyzed by video, laser confocal, and reflection interference contrast microscopy. Exp Cell Res 221: 311–9.

Goldmann WH, Senger R, Kaufmann S, Isenberg G (1995b) Determination of the affinity of talin and vinculin to charged lipid vesicles: a light scatter study. FEBS Lett 368: 516–8.

Golji J, Lam J, Mofrad MR (2011) Vinculin activation is necessary for complete talin binding. Biophys J 100: 332–40.

Golji J, Mofrad MR (2010) A molecular dynamics investigation of vinculin activation. Biophys J 99: 1073–81.

Golji J, Mofrad MR (2013) The interaction of vinculin with actin. PLoS Comput Biol 9: e1002995.

Golji J, Wendorff T, Mofrad MR (2012) Phosphorylation primes vinculin for activation. Biophys J 102: 2022–30.

Gomez GA, McLachlan RW, Yap AS (2011) Productive tension: force-sensing and homeostasis of cell-cell junctions. Trends Cell Biol 21: 499–505.

Götter R, Goldmann WH, Isenberg G (1995) Internal actin filament dynamics in the presence of vinculin: a dynamic light scattering study. FEBS Lett 359: 220–2.

Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, Schwartz MA (2010) Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nat Lett 466: 263–7.

Gumbiner BM (2005) Regulation of cadherin-mediated adhesion in morphogenesis. Nat Rev Mol Cell Biol 6: 622–34.

Hemmings L, Rees DJ, Ohanian V, Bolton SJ, Gilmore AP, Patel B, Priddle H, Trevithick JE, Hynes RO, Critchley DR (1996) Talin contains three actin-binding sites each of which is adjacent to a vinculin-binding site. J Cell Sci 109: 2715–26.

Hernandez-Varas P, Berge U, Stroemblad S (2015) A plastic relationship between vinculin-mediated tension and adhesion complex area defines adhesion size and lifetime. Nat Commun 6: 7524.

Hirata H, Chiam KH, Lim CT, Sokabe M (2014a) Actin flow and talin dynamics govern rigidity sensing in actin-integrin linkage through talin extension. J Royal Soc Interface 11: 20140734.

Hirata H, Tatsumi H, Lim CT, Sokabe M (2014b) Force-dependent vinculin binding to talin in live cells: a crucial step in anchoring the actin cytoskeleton to focal adhesions. Am Physiol Cell Physiol 306: C607–20.

Horwitz A, Duggan K, Buck C, Beckerle MC, Burridge K (1986) Interaction of plasma membrane fibronectin receptor with talin-a transmembrane linkage. Nature 320: 531–3.

Hu K, Ji L, Applegate KT, Danuser G, Waterman-Storer CM (2007) Differential transmission of actin motion within focal adhesions. Science 315: 111–5.

Humphries JD, Wang P, Streuli C, Geiger B, Humphries MJ, Ballestrem C (2007) Vinculin controls focal adhesion formation by direct interactions with talin and actin. J Cell Biol 179: 1043–57.

Huttelmaier S, Mayboroda O, Harbeck B, Jarchau T, Jockusch BM, Rudiger M (1998) The interaction of the cell-contact proteins VASP and vinculin is regulated by phosphatidylinositol-4,5-bisphosphate. Curr Biol 8: 479–88.

Huveneers S, Oldenburg J, Spanjaard E, van der Krogt G, Grigoriev I, Akhmanova A, Rehmann H, de Rooij J (2012) Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. J Cell Biol 196: 641–52.

Hytonen VP, Vogel V (2008) How force might activate talin's vinculin binding sites: SMD reveals a structural mechanism. PLoS Comput Biol 4: e24.

Isenberg G, Goldmann WH (1995) Acting-binding proteins—lipid interactions. The cytoskeleton. In: Hesketh JE, Pryme IP, eds. Greenwich, Connecticut: JAI Press Inc, pp. 169–204.

Isenberg G, Leonard K, Jockusch BM (1982) Structural aspects of vinculin-actin interactions. J Mol Biol 158: 231–49.

Isenberg G, Niggli V, Pieper U, Kaufmann S, Goldmann WH (1996) Probing phosphatidylinositolphosphates and adenosinenucleotides on talin nucleated actin polymerization. FEBS Lett 397: 316–20.

Ito S, Richert N, Pastan I (1982) Phospholipids stimulate phosphorylation of vinculin by the tyrosine-specific protein kinase of Rous sarcoma virus. Proc Natl Acad Sci USA 79: 4628–31.

Ito S, Werth DK, Richert ND, Pastan I (1983) Vinculin phosphorylation by the src kinase. Interaction of vinculin with phospholipid vesicles. J Biol Chem 258: 14626–31.

Izard T, Evans G, Borgon RA, Rush CL, Bricogne G, Bois PR (2004) Vinculin activation by talin through helical bundle conversion. Nature 427: 171–5.

Janostiak R, Brabek J, Auernheimer V, Tatarova Z, Lautscham LA, Dey T, Gemperle J, Merkel R, Goldmann WH, Fabry B, Rösel D (2014) CAS directly interacts with vinculin to control mechanosensing and focal adhesion dynamics. Cell Mol Life Sci 71: 727–44.

Janostiak R, Tolde O, Bruhova Z, Novotny M, Hanks SK, Rösel D, Brabek J (2011) Tyrosine phosphorylation within the SH3 domain regulates CAS subcellular localization, cell migration, and invasiveness. Mol Biol Cell 22: 4256–67.

Janssen ME, Kim E, Liu H, Fujimoto LM, Bobkov A, Volkmann N, Hanein D (2006) Three-dimensional structure of vinculin bound to actin filaments. Mol Cell 21: 271–81.

Jockusch BM, Isenberg G (1981) Interaction of alpha-actinin and vinculin with actin: opposite effects on filament network formation. Proc Natl Acad Sci USA 78: 3005–9.

Johnson RP, Niggli V, Durrer P, Craig SW (1998) A conserved motif in the tail domain of vinculin mediates association with

and insertion into acidic phospholipid bilayers. Biochemistry 37: 10211–22.

Kanchanawong P, Shtengel G, Pasapera AM, Ramko EB, Davidson MW, Hess HF, Waterman CM (2010) Nanoscale architecture of integrin-based cell adhesions. Nature 468: 580–4.

Kaushik G, Spenlehauer A, Sessions AO, Trujillo AS, Fuhrmann A, Fu Z, Venkatraman V, Pohl D, Tuler J, Wang M, Lakatta EG, Ocorr K, Bodmer R, Bernstein SI, Van Eyk JE, Cammarato A, Engler AJ (2015) Vinculin network-mediated cytoskeletal remodeling regulates contractile function in the aging heart. Sci Transl Med 292: 292–99.

Kawabe H, Hata Y, Takeuchi M, Ide N, Mizoguchi A, Takai Y (1999) NArgBP2, a novel neural member of ponsin/ArgBP2/vinexin family that interacts with synapse-associated protein 90/postsynaptic density-95-associated protein (SAPAP). J Biol Chem 274: 30914–8.

Kioka N, Sakata S, Kawauchi T, Amachi T, Akiyama SK, Okazaki K, Yaen C, Yamada KM, Aota S (1999) Vinexin: a novel vinculin-binding protein with multiple SH3 domains enhances actin cytoskeletal organization. J Cell Biol 144: 59–69.

Klemm AH, Diez G, Alonso JL, Goldmann WH (2009) Comparing the mechanical influence of vinculin, focal adhesion kinase and p53 in mouse embryonic fibroblasts. Biochem Biophys Res Commun 379: 799–801.

Klemm AH, Kienle S, Rheinlaender J, Schaeffer TE, Goldmann WH (2010) The influence of Pyk2 on the mechanical properties in fibroblasts. Biochem Biophys Res Commun 393: 694–7.

Koteliansky VE, Gneushev GN (1983) Vinculin localization in cardiac muscle. FEBS Lett 159: 158–60.

Kovacs EM, Goodwin M, Ali RG, Paterson AD, Yap AS (2002) Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. Curr Biol 12: 379–82.

Küpper K, Lang N, Möhl C, Kirchgessner N, Born S, Goldmann WH, Merkel R, Hoffmann B (2010) Tyrosine phosphorylation of vinculin at position 1065 modifies focal adhesion dynamics and cell tractions. Biochem Biophys Res Commun 399: 560–4.

Lange J, Auernheimer V, Strissel PL, Goldmann WH (2013) Influence of focal adhesion kinase on the mechanical behavior of cell populations. Biochem Biophys Res Commun 436: 246–51.

Lauffenburger DA, Wells A (2001) Getting a grip: new insights for cell adhesion and traction. Nat Cell Biol 3: E110–2.

Le Clainche C, Dwivedi SP, Didry D, Carlie M-F (2010) Vinculin is a dually regulated actin filament barbed end-capping and side-binding protein. J Biol Chem 285: 23420–32.

Le Duc Q, Shi Q, Blonk I, Sonnenberg A, Wang N, Leckband D, de Rooij J (2010) Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. J Cell Biol 189: 1107–15.

Lee HS, Bellin RM, Walker DL, Patel B, Powers P, Liu H, Garcia-Alvarez B, de Pereda JM, Liddington RC, Volkmann N, Hanein D, Critchley DR, Robson RM (2004) Characterization of an actin-binding site within the talin FERM domain. J Mol Biol 343: 771–84.

Lee SE, Chunsrivirot S, Kamm RD, Mofrad MR (2008) Molecular dynamics study of talin-vinculin binding. Biophys J 95: 2027–36.

Legant WR, Choi CK, Miller JS, Shao L, Gao L, Betzig E, Chen CS (2012) Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proc Natl Acad Sci USA 110: 881–6.

Levenberg S, Katz BZ, Yamada KM, Geiger B (1998) Long-range and selective autoregulation of cell-cell or cell-matrix adhesions by cadherin or integrin ligands. J Cell Sci 111: 347–57.

Lifschitz-Mercer B, Czernobilsky B, Feldberg E, Geiger B (1997) Expression of the adherens junction protein vinculin in human basal and squamous cell tumors: relationship to invasiveness and metastatic potential. Hum Pathol 28: 1230–6.

Maddugoda MP, Crampton MS, Shewan AM, Yap AS (2007) Myosin VI and vinculin cooperate during the morphogenesis of cadherin cell-cell contacts in mammalian epithelial cells. J Cell Biol 178: 529–40.

Maiden SL, Harrison N, Keegan J, Cain B, Lynch AM, Pettitt J, Hardin J (2013) Specific conserved C-terminal amino acids of *Caenorhabditis elegans* HMP-1/alpha-catenin modulate F-actin binding independently of vinculin. J Biol Chem 288: 5694–706.

Mandai K, Nakanishi H, Satoh A, Takahashi K, Satoh K, Nishioka H, Mizoguchi A, Takai Y (1999) Ponsin/SH3 P12: an l-afadin- and vinculin-binding protein localized at cell-cell and cell-matrix adherens junctions. J Cell Biol 144: 1001–17.

McCain ML, Lee H, Aratyn-Schaus Y, Kleber AG, Parker KK (2012) Cooperative coupling of cell-matrix and cell-cell adhesions in cardiac muscle. Proc Natl Acad Sci USA 109: 9881–6.

Mertz AF, Che Y, Banerjee S, Goldstein JM, Rosowski KA, Revilla SF, Niessen CM, Marchetti MC, Dufresne ER, Horsley V (2012) Cadherin-based intercellular adhesions organize epithelial cell-matrix traction forces. Proc Natl Acad Sci USA 110: 842–7.

Moese S, Selbach M, Brinkmann V, Karlas A, Haimovich B, Backert S, Meyer TF (2007) The Helicobacter pylori CagA protein disrupts matrix adhesion of gastric epithelial cells by dephosphorylation of vinculin. Cell Microbiol 9: 1148–61.

Möhl C, Kirchgessner N, Schaefer C, Kuepper K, Born S, Diez G, Goldmann WH, Merkel R, Hoffmann B (2009) Becoming stable and strong: the interplay between vinculin exchange dynamics and adhesion strength during adhesion site maturation. Cell Motil Cytoskeleton 66: 350–64.

Moiseyeva EP, Weller PA, Zhidkova NI, Corben EB, Patel B, Jasinska I, Koteliansky VE, Critchley DR (1993) Organization of the human gene encoding the cytoskeletal protein vinculin and the sequence of the vinculin promoter. J Biol Chem 268: 4318–25.

Nagafuchi A, Takeichi M, Tsukita S (1991) The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. Cell 65: 849–57.

Niessen CM, Gottardi CJ (2008) Molecular components of the adherens junction. Biochim Biophys Acta 1778: 562–71.

Niggli V, Dimitrov DP, Brunner J, Burger MM (1986) Interaction of the cytoskeletal component vinculin with bilayer structures analyzed with a photoactivatable phospholipid. J Biol Chem 261: 6912–8.

Niggli V, Kaufmann S, Goldmann WH, Weber T, Isenberg G (1994) Identification of functional domains in the cytoskeletal protein talin. Eur J Biochem 224: 951–7.

Noorman M, van der Heyden MA, van Veen TA, Cox MG, Hauer RN, de Bakker JM, van Rijen HV (2009) Cardiac cell-cell junctions in health and disease: electrical versus mechanical coupling. J Mol Cell Cardiol 47: 23–31.

Ogita H, Ikeda W, Takai Y (2008) Roles of cell adhesion molecules nectin and nectin-like molecule-5 in the regulation of cell movement and proliferation. J Microsc 231: 455–65.

Otto JJ (1986) The lack of interaction between vinculin and actin. Cell Motil Cytoskeleton 6: 48–55.

Pardo JV, Siliciano JD, Craig SW (1983) Vinculin is a component of an extensive network of myofibril-sarcolemma attachment regions in cardiac muscle fibers. J Cell Biol 97: 1081–8.

Pasapera AM, Schneider IC, Rericha E, Schlaepfer DD, Waterman CM (2010) Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. J Cell Biol 188: 877–90.

Peng X, Cuff LE, Lawton CD, DeMali KA (2009) Vinculin regulates cell-surface E-cadherin expression by binding to beta-catenin. J Cell Sci 123: 567–77.

Peng X, Maiers JL, Choudhury D, Craig SW, DeMali KA (2012) Alpha catenin uses a novel mechanism to activate vinculin. J Biol Chem 287: 7728–37.

Peng X, Nelson ES, Maiers JL, DeMali KA (2011) New insights into vinculin function and regulation. Int Rev Cell Mol Biol 287: 191–231.

Perez JF, Sanderson MJ (2005) The contraction of smooth muscle cells of intrapulmonary arterioles is determined by the frequency of Ca2+ oscillations induced by 5-HT and KCl. J Gen Physiol 125: 555–67.

Plotnikov SV, Pasapera AM, Sabass B, Waterman CM (2012) Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. Cell 151: 1513–27.

Puklin-Faucher E, Sheetz MP (2009) The mechanical integrin cycle. J Cell Sci 122: 179–86.

Ramarao N, Le Clainche C, Izard T, Bourdet-Sicard R, Ageron E, Sansonetti PJ, Carlier MF, Tran Van Nhieu G (2007) Capping of actin filaments by vinculin activated by the Shigella IpaA carboxyl-terminal domain. FEBS Lett 581:853–57.

Roca-Cusachs P, del Rio A, Puklin-Faucher E, Gauthier NC, Biais N, Sheetz MP (2013) Integrin-dependent force transmission to the extracellular matrix by alpha-actinin triggers adhesion maturation. Proc Natl Acad Sci USA 110: E1361–70.

Rodriguez Fernandez JL, Geiger B, Salomon D, Ben-Ze'ev A (1992) Overexpression of vinculin suppresses cell motility in BALB/c 3T3 cells. Cell Motil Cytoskeleton 22: 127–34.

Rodriguez Fernandez JL, Geiger B, Salomon D, Ben-Ze'ev A (1993) Suppression of vinculin expression by antisense transfection confers changes in cell morphology, motility, and anchorage-dependent growth of 3T3 cells. J Cell Biol 122: 1285–94.

Ruddies R, Goldmann WH, Isenberg G, Sackmann E (1992) The viscoelastic moduli of actin/filamin solutions: a micro-rheologic study. Biochem Soc Trans 21: 37S.

Ruddies R, Goldmann WH, Isenberg G, Sackmann E (1993) The viscoelasticity of entangled actin networks: the influence of defects and modulation by talin and vinculin. Eur Biophys J 22: 309–21.

Ruhnau K, Wegner A (1988) Evidence for direct binding of vinculin to actin filaments. FEBS Lett 228: 105–8.

Saez AO, Zhang W, Wu Y, Turner CE, Tang DD, Gunst SJ (2004) Tension development during contractile stimulation of smooth muscle requires recruitment of paxillin and vinculin to the membrane. Am J Physiol Cell Physiol 286: C433–47.

Samarel AM (2005) Costameres, focal adhesions, and cardiomyocyte mechano-transduction. Am J Physiol Heart Circ Physiol 289: H2291–301.

Sawada Y, Tamada M, Dubin-Thaler BJ, Cherniavskaya O, Sakai R, Tanaka S, Sheetz MP (2006) Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 127: 1015–26.

Scott DL, Diez G, Goldmann WH (2006) Protein-lipid interactions: correlation of a predictive algorithm for lipid-binding sites with three-dimensional structural data. Theor Biol Med Model 3: 17.

Sen S, Ng WP, Kumar S (2012) Contributions of talin-1 to glioma cell-matrix tensional homeostasis. J Royal Soc Interface 9: 1311–7.

Shams H, Golji J, Mofrad MR (2012) A molecular trajectory of alpha-actinin activation. Biophys J 102:2050–9.

Sharp WW, Simpson DG, Borg TK, Samarel AM, Terracio L (1997) Mechanical forces regulate focal adhesion and costamere assembly in cardiac myocytes. Am J Physiol 273: H546–56.

Sheikh F, Chen Y, Liang X, Hirschy A, Stenbit AE, Gu Y, Dalton ND, Yajima T, Lu Y, Knowlton KU, Peterson KL, Perriard JC, Chen J (2006) Alpha-E-catenin inactivation disrupts the cardiomyocyte adherens junction, resulting in cardiomyopathy and susceptibility to wall rupture. Circulation 114: 1046–55.

Shewan AM, Maddugoda M, Kraemer A, Stehbens SJ, Verma S, Kovacs EM, Yap AS (2005) Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. Mol Biol Cell 16: 4531–42.

Small JV (1985) Geometry of actin-membrane attachments in the smooth muscle cell: the localisations of vinculin and alpha-actinin. EMBO J 4:45–9.

Somiari RI, Sullivan A, Russell S, Somiari S, Hu H, Jordan R, George A, Katenhusen R, Buchowiecka A, Arciero C, Brzeski H, Hooke J, Shriver C (2003) High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast. Proteomics 3: 1863–73.

Steimle PA, Hoffert JD, Adey NB, Craig SW (1999) Polyphosphoinositides inhibit the interaction of vinculin with actin filaments. J Biol Chem 274: 18414–20.

Subauste MC, Pertz O, Adamson ED, Turner CE, Junger S, Hahn KM (2004) Vinculin modulation of paxillin-FAK interactions regulates ERK to control survival and motility. J Cell Biol 165: 371–81.

Taguchi K, Ishiuchi T, Takeichi M (2011) Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. J Cell Biol 194: 643–56.

Tang X, Wen Q, Kuhlenschmidt TB, Kuhlenschmidt MS, Janmey PA, Saif TA (2012) Attenuation of cell mechanosensitivity in colon cancer cells during in vitro metastasis. PLoS ONE 7: e50443.

Tempel M, Goldmann WH, Isenberg G, Sackmann E (1995) Interaction of the 47-kDa talin fragment and the 32-kDa vinculin fragment with acidic phospholipids: a computer analysis. Biophys J 69: 228–41.

Thievessen I, Fakhri N, Steinwachs J, Kraus V, McIsaac RS, Gao L, Chen BC, Baird MA, Davidson MW, Betzig E, Oldenbourg R, Waterman CM, Fabry B (2015) Vinculin is required for cell polarization, migration and extracellular matrix remodeling in 3D collagen. FASEB J 29: 4555–67.

Thievessen I, Thompson PM, Berlemont S, Plevock KM, Plotnikov SV, Zemljic-Harpf A, Ross RS, Davidson MW, Danuser G, Campbell SL, Waterman CM (2013) Vinculin-actin interaction couples actin retrograde flow to focal adhesions, but is dispensable for focal adhesion growth. J Cell Biol 202: 163–77.

Thomas SM, Hagel M, Turner CE (1999) Characterization of a focal adhesion protein, Hic-5, that shares extensive homology with paxillin. J Cell Sci 112: 181–90.

Thompson PM, Tolbert CE, Shen K, Kota P, Palmer SM, Plevock KM, Orlova A, Galkin VE, Burridge K, Egelman EH, Dokholyan NV, Superfine R, Campbell SL (2014) Identification of an actin binding surface on vinculin that mediates mechanical cell and focal adhesion properties. Structure 22: 697–706.

Vasioukhin V, Fuchs E (2001) Actin dynamics and cell-cell adhesion in epithelia. Curr Opin Cell Biol 13: 76–84.

Volberg T, Geiger B, Kam Z, Pankov R, Simcha I, Sabanay H, Coll JL, Adamson E, Ben-Ze'ev A (1995) Focal adhesion formation by F9 embryonal carcinoma cells after vinculin gene disruption. J Cell Sci 108: 2253–60.

Volk T, Geiger B (1984) A 135-kd membrane protein of intercellular adherens junctions. EMBO J 3: 2249–60.

Watabe-Uchida M, Uchida N, Imamura Y, Nagafuchi A, Fujimoto K, Uemura T, Vermeulen S, van Roy F, Adamson ED, Takeichi M (1998) Alpha-catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. J Cell Biol 142: 847–57.

Weekes J, Barry ST, Critchley DR (1996) Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin, exposing actin-binding and protein kinase C phosphorylation sites. Biochem J 314: 827–32.

Wen Q, Janmey PA (2011) Polymer physics of the cytoskeleton. Curr Opin Solid State Mater Sci 15: 177–82.

Wilson PD, Geng L, Li X, Burrow CR (1999) The PKD1 gene product, polycystin-1 is a tyrosine-phosphorylated protein that colocalizes with α2β1-integrin in focal clusters in adherent renal epithelia. Lab Invest 79:1311–23.

Wirth VF, List F, Diez G, Goldmann WH (2010) Vinculin's C-terminal region facilitates phospholipid membrane insertion. Biochem Biophys Res Commun 398: 433–7.

Wood CK, Turner CE, Jackson P, Critchley DR (1994) Characterisation of the paxillin-binding site and the C-terminal focal adhesion targeting sequence in vinculin. J Cell Sci 107: 709–17.

Xu W, Baribault H, Adamson ED (1998) Vinculin knockout results in heart and brain defects during embryonic development. Development 125: 327–37.

Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M (2010) Alpha-catenin as a tension transducer that induces adherens junction development. Nat Cell Biol 12: 533–42.

Zaidel-Bar R, Ballestrem C, Kam Z, Geiger B (2003) Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. J Cell Sci 116: 4605–13.

Zaidel-Bar R, Itzkovitz S, Ma'ayan A, Iyengar R, Geiger B (2007) Functional atlas of the integrin adhesome. Nat Cell Biol 9: 858–67.

Zamir E, Geiger B (2001) Molecular complexity and dynamics of cell-matrix adhesions. J Cell Sci 114: 3583–90.

Zemljic-Harpf AE, Miller JC, Henderson SA, Wright AT, Manso AM, Elsherif L, Dalton ND, Thor AK, Perkins GA, McCulloch AD, Ross RS (2007) Cardiac-myocyte-specific excision of the vinculin gene disrupts cellular junctions, causing sudden death or dilated cardiomyopathy. Mol Cell Biol 27: 7522–37.

Zemljic-Harpf AE, Ponrartana S, Avalos RT, Jordan MC, Roos KP, Dalton ND, Phan VQ, Adamson ED, Ross RS (2004) Heterozygous inactivation of the vinculin gene predisposes to stress-induced cardiomyopathy. Am J Pathol 165: 1033–44.

Zhang X, Jiang G, Cai Y, Monkley SJ, Critchley DR, Sheetz MP (2008) Talin depletion reveals independence of initial cell spreading from integrin activation and traction. Nat Cell Biol 10: 1062–68.

Zhang Z, Izaguirre G, Lin SY, Lee HY, Schaefer E, Haimovich B (2004) The phosphorylation of vinculin on tyrosine residues 100 and 1065, mediated by SRC kinases, affects cell spreading. Mol Biol Cell 15: 4234–47.

Ziegler WH, Gingras AR, Critchley DR, Emsley J (2008) Integrin connections to the cytoskeleton through talin and vinculin. Biochem Soc Trans 36: 235–9.

Ziegler WH, Liddington RC, Critchley DR (2006) The structure and regulation of vinculin. Trends Cell Biol 16: 453–60.

Ziegler WH, Tigges U, Zieseniss A, Jockusch BM (2002) A lipid-regulated docking site on vinculin for protein kinase C. J Biol Chem 277: 7396–404.

Received 12 October 2015; accepted 14 November 2015.
Final version published online 3 January 2016.