Mechanosensing in cell-matrix adhesions – converting tension into chemical signals

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Dedicated to Ruth Chiquet-Ehrismann

Abstract

Cell-matrix adhesions have since long been recognized to be critical for the survival and proliferation of cells. In fact, these adhesive structures do not only physically anchor cells, but they also induce vital intracellular signaling at cell-matrix adhesion sites. Recent progress in the cell adhesion field is now starting to provide data and ideas how this so far enigmatic signaling process is induced and regulated by intracellular acto-myosin tension, or stiffness of the extracellular matrix. Understanding how cells are using this mechanosignaling system will be key to control biological processes such as development, cancer growth, metastasis formation and tissue regeneration. In this review, we illustrate and discuss the mechanosignaling mechanisms important in the regulation of cell-matrix adhesions at the molecular level.

Introduction

Chemical reactions are essential for biological functions, and molecular interactions are needed for the chemical reactions to take place. Compartmentalization or restriction to membrane surfaces increases the local concentration and thus facilitates protein-protein or protein-peptide interactions, creating protein complexes with specific cellular functions. For adherent cells, fluid shear forces and mechanical cues generated by motor proteins and tissue deformation are important elements of the signaling machinery, constantly modifying the structure and function of the proteins. In turn, mechanical strain on a protein network requires proteins to act as cross-linkers, or softeners to either reinforce or destabilize the mechanical scaffold. Cells have mechanisms to sense the strain or compliance of the extracellular scaffold, as well as to detect shear forces acting on its force-bearing structures such as cytoskeleton, cell-cell and cell-matrix adhesions. A critical aspect of such sensing mechanisms is the conversion of a mechanical force into local (chemical) signaling, that will induce cell behavior to counteract the mechanical challenge or to modulate cell-anchorage, creating survival signals important for the healthy state of cells.

Integrin-receptor containing cell-matrix adhesion sites are among the most complex but also highly versatile mechanosensing systems. Cell-matrix adhesions enable cells to monitor their adhesive state, as well as rapidly respond to changes in mechanical tension or shear forces by modulation of cellular adhesion and migration. All multicellular organisms require this sensing system to maintain the architecture and homeostasis of tissues and organs. Thus we may predict that any relevant signaling system involved in the regulation of cell growth, shape and migration is interfacing with the integrin-dependent mechanosignaling found in cell-matrix adhesion sites. So far, only a small fraction of its regulatory potential has been identified and for only a few of its components, the details at the molecular level are known.

Here we would like to describe emerging concepts, explaining the cellular mechanosensing in cell-matrix adhesions (aka focal adhesions) at the molecular level. Our aim is not only to describe the well-proven functions, but also to speculate about possible poorly characterized mechanisms involved.

Biological phenomena in mechanosensing and how mechanosignaling controls the cell behavior

Mechanosensing at cell-matrix adhesion sites is at the origin of durotaxis, a mechanism describing the ability of cells to migrate towards a stiffer surface [1]. A potentially similar effect is seen when cells are plated on a nanostructured gradient of integrin peptide ligands [2], where a cellular response is directed uphill. Cells also respond to externally applied stress by changing their adhesion sites [3] and stress fiber orientation [4]. For example, endothelial cells exposed to a fluid shear stress reorient their cytoskeleton, which is associated with Rho-family GTPase-mediated intracellular signaling [5]. Cyclic stretching of fibroblasts plated on an elastic membrane induces

remodeling of the actin cytoskeleton, but also stress induced expression of the non-adhesive extracellular matrix protein tenascin-C [6]. Interestingly, this stress-mediated response requires mechanical coupling of cells to fibronectin, but leads to the expression of tenascin-C that in turn reduces cell-adhesion and enhances motility of cells [7]. This demonstrates that the ECM and its specific composition is an integral part of the integrin-dependent mechanosensing of cells. The role of the ECM in the mechanosensing also includes growth factors, such as TGF- β that can be released from their latent and matrix-bound forms in response to integrin-mediated tension [8]. This indirect mechanosignaling pathway provides further insights into signaling mechanisms that require integrin-mediated stretching and remodeling of ECM proteins. Alternatively, matrix-bound but not soluble growth factors induce robust wound healing [9], or synergies with integrin-dependent adhesion [10], suggesting that mechanosignaling is also very important for the regulation of the integrin/receptor tyrosine kinase interface.

During cancer initiation, growth and migration, extracellular matrix deposition and stiffness modifies cell fate and behavior. While a stiffer ECM induces dedifferentiation of mammary gland epithelial cells [11], cancer cells can use amoeboid like movements, potentially induced by paxillin stimulated, RhoA-mediated contractility [12], making them less dependent on the physical state of the extracellular scaffold. On the other hand, melanoma cells are also able to escape inhibitor signaling by initiating a rigidity sensing-response involving focal adhesion kinase (FAK) [13]. Recently, another mechanism of tension-mediated signaling has been proposed to be implemented by the increased expression of cell surface mucins. A highly hydrated mucin layer on the cell surface is acting like a hydrodynamic spring to push cells away from the ECM scaffold, therefore reinforcing the mechanical link and signaling output of integrin-mediated cell-matrix adhesions [14].

Tension-induced signaling within focal adhesions is best seen when staining cells with phosphotyrosine antibodies [15]. Subsequent signaling-mediated maturation of cell-matrix adhesions, has long been observed in adherent cells in culture. When plated on rigid surfaces, the stronger the intracellular tension, the bigger the cell-matrix adhesions become [16]. Alternatively, maturation of focal or nascent adhesions occurs in response to extracellular as well as intracellular acto-myosin tension [3, 17]. An increase in mechanical tension on cell-matrix adhesions induces the recruitment of paxillin and FAK, a process dependent on the focal adhesion scaffold, occurring even after extraction of membranes [18]. More recent proteomics studies [19] confirm this tension-mediated recruitment of LIM-domain containing proteins such as paxillin and zyxin, suggesting that LIM-domains form a molecular structure sensitive to tension induced revelation of binding sites in the actin cytoskeleton [20] or within cell-matrix adhesion sites [21]. Although paxillin and Hic-5 are differentially orchestrating intracellular signaling and cytoskeletal remodeling [12], a mechanism to their tension-mediated recruitment to cell-matrix adhesions has only recently been proposed [21] (see below).

The combination of cell surface patterning and stem cell research has opened a new dimension of mechanosensing, where an apparent match

between substrate stiffness and physiological contractility of cells is searched [22]. The amount of available surface for cellular attachment has also an impact on differentiation towards osteoblasts or adipocytes [23], which is apparently linked to the activation of the YAP/TAZ transcription factors [24].

A key role in mechanosignaling is seen during directed cell migration. Cell detachment and steering at the cell rear requires myosin contraction [25]. Apparently, induction of paxillin-mediated recruitment of FAK/src kinases at cell-matrix adhesions regulates adhesion turnover and cell detachment, which allows cell migration [26, 27].

In order to illustrate the molecular mechanisms involved in cellular mechanosensing, we focus on a few proteins important for this process, namely fibronectin, integrin, paxillin/FAK and talin. With these examples it is possible to get an insight about the repertoire of functions employed in the mechanoregulation.

Talin – primary mechanosensor or just a structural scaffold?

Talin is a cytoplasmic adapter protein capable of directly connecting integrins to the actin cytoskeleton. Talin consists of N-terminal head domain (~residues 1-430) responsible for integrin binding, which has homology to FERM proteins. Talin rod domain (~residues 430-2500) consists solely of α -helices, which are packed into bundles of 4-5 helices [28]. The rod domain contains actin binding site as well as dimerization domain at the C-terminal end of the protein. The rod has also up to 13 binding sites for vinculin, up to 3 binding sites for RIAM and also putative binding sites for integrin, paxillin and possibly also for several other proteins [29]. One could thus consider talin a large scaffolding protein.

Talin has however unique mechanosensory characteristics: the activity and functions of talin are regulated at several levels and mechanical cues appear to play significant role in talin interactions and function in cell. First of all, talin autoinhibition is holding the protein in a resting, inactive state until released by synergistic actions of RIAM binding [30] and lipid interactions [31, 32] (Figure 1). Subsequent talin-integrin interaction is tightly linked to integrin activation, a process essential for the formation of early adhesive structures called focal complexes. At this stage, talin forms a relatively weak bond to integrin [33, 34], which, however is essential for the mechanosignaling and cellular functions, including migration.

The second level of regulation involves mechano-controlled opening of the binding sites within the talin rod domain. This process has been studied by using steered molecular dynamics, applying mechanical stress to talin rod fragments [35, 36] by magnetic tweezers studying the vinculin binding to talin rod under applied force [37] by AFM exploring the unfolding pathways [37] and by high-resolution fluorescence microscopy determining the length of talin molecule in living cells [38]. These studies have revealed that the length of the talin rod increases due to mechanical stress and that extension of talin

rod exposes binding sites for other proteins, including vinculin, which is the best characterized talin partner so far. Therefore, talin appears to be a key molecule in focal adhesion maturation, capable of turning mechanical cues to biochemical signals via exposure of binding sites (Figure 1).

Finally, talin is also involved in the dissociation of cell-matrix adhesions. This may involve unbinding of interaction partners and release of integrin and actin interactions, post-translational modifications, but also proteolytic relaxation of the system. Cleavage of talin in between head and rod domains by calpain appears to have relevance at least in cell culture [39].

Although talin is studied extensively, the mechanosignaling of talin is still poorly understood. The presence of multiple binding sites for several different proteins within talin rod makes it a fascinating example of a force-modulated protein: talin appears to be capable of adopting several different functions and thus acting as a primary mechanosensor in living cells.

Figure 1 Regulation of protein conformation and interactions in focal adhesions by mechanical tension.

The figure illustrates how three proteins central to the assembly of the cell-matrix contact are structurally and functionally modified by mechanical tension. (1) In the extracellular space, fibronectin adopts an autoinhibited globular shape until it gets in contact with other ECM proteins, or is bound by integrins. Applied mechanical stress leads to fibronectin extension and lateral polymerization into FN-fibers [40] and also enables binding of growth factors to fibronectin fibers. (2) Integrin exists in a bent,

inactive state until it becomes activated by intracellular (talin binding) and extracellular ligands [41-43]. Application of lateral forces appears to open the integrin legs, inducing the high-affinity conformation, thereby reinforcing the connection to the ECM [44, 45]. (3) Talin is released from the autoinhibited state by a combination of lipid interactions and RIAM binding, which drives talin-integrin complex formation [30]. Force applied on talin, while bridging the ECM and actin-myosin cytoskeleton, opens binding sites for vinculin [37] simultaneously weakening the interaction with RIAM [28].

Integrins are mechanosensory receptors on cell surfaces

As discussed above, talin takes a key position within cell-matrix adhesions, providing a dynamic link to the actin cytoskeleton, while connecting to cell surface receptors of the integrin family, thereby enabling mechanical linkage to the ECM scaffold. Once the ECM/integrin/talin/F-actin link is formed, signaling competent adapter proteins, such as paxillin are recruited in a tension-dependent manner. To understand how this relay is working we need to discuss both the ECM-integrin, as well as integrin-talin linkage in detail.

Integrins are heterodimeric, allosterically controlled cell surface receptors that exist in different conformational states (Figure 1). These conformational states are influenced by ECM-ligand binding to the ectodomain, as well as talin and kindlin binding to the cytoplasmic tail of the β -integrin tail. Additional regulatory mechanisms have evolved to control the ECM and talin linkage that are described elsewhere. In the case of the β 3-integrin receptors α v β 3 and α IIb β 3, three conformational states have been identified. The bent, or inactive conformation exists for example in resting platelets. Integrin extension can be induced by binding of the talin-head domain, suggested to act on a juxtamembrane clasp formed between the α - and β -integrin tails [43] (Figure 1). Alternatively, replacing Ca^{2+} with Mn^{2+} also induces this extended, flexible, but still low-affinity conformation [41]. ECM-ligand binding to the extended, low-affinity conformation, will lead to the conversion into the high-affinity state by an allosteric movement involving the ligand-binding β -I-like domain and the connected hybrid domain, in a process termed hybrid-domain swing-out [45]. The hybrid-domain swing-out is allosterically linked to enhanced ligand-binding affinity and straitening of the tensional vector along the ECM/integrin/talin axis (Figure 1). It is therefore likely that the observed catch-bond behavior of integrins [46] is linked to the tension-mediated hybrid-domain swing-out. This elegant system would suggest that tensile forces exert allosteric effects on integrin receptors.

During platelet activation, α IIb β 3-integrin receptors switch into their fibrinogen-binding mode, during a process termed inside-out activation of integrins. Talin and kindlin recruitment to the cytoplasmic tail of integrins are crucial for this step [42, 47]. When reconstituted in nanodiscs, the head-domain of talin induces a 2-fold increase in the extension of integrins [43], but fails to induce the open, high-affinity conformation in the absence of ECM-ligands [48]. This in fact suggests that stable ECM/integrin/talin linkages can

only be obtained if the system is put under tensile stress. Indeed, FRAP experiments have demonstrated a rapid integrin-exchange dynamics when integrins are activated and clustered by talins in the absence of a link to the actin cytoskeleton. In contrast integrin dynamics is strongly reduced when the integrin is blocked in the high-affinity hybrid-domain swing-out conformation [49]. Although some information may still be missing to understand the formation and clustering of the integrin/talin/kindlin-module, it is accepted that the mechanical coupling between the ECM and the actin cytoskeleton allows the recruitment of signaling adapters to induce intracellular signaling. This step will be discussed in the next chapter.

Tension-dependent recruitment of paxillin and the link to tyrosine kinases

Paxillin, discovered over 20 years ago, and its family members have been identified as signaling hubs, regulating processes such as actin-polymerization, cell proliferation, spreading and cell migration [50]. Paxillin is a member of the superfamily of LIM-domain containing proteins, which includes pinch (from the ILK/pinch/parvin complex), zyxin and many other proteins. In paxillin, the C-terminal LIM-domains are responsible for recruitment to cell-matrix adhesion sites [51] (Figure 2). Interestingly, LIM-domain containing proteins are enriched in cell-matrix adhesions in response to augmented mechanical tension [19]. Although some members of the LIM-domain family can bind Tyr-containing peptide motifs such as NPXY, found in the cytoplasmic tail of integrins [52], paxillin interaction was only seen with membrane proximal integrin tail residues, but not NPXY-motifs of integrins [51, 53]. Therefore, it is intriguing that paxillin is recruited to membrane extracted focal adhesions when put under mechanical tension [18], proposing that LIM-domains can target tyrosine-containing peptides only, when presented in a tensioned configuration (Figure 2).

So far, there is no formal proof for this hypothesis, but recent data show that tyrosine residues of the integrin tail are crucial for the (direct or indirect) recruitment of paxillin to cell-matrix adhesions [21]. Interestingly, the tyrosine residue in the membrane-proximal NPXY-motif has a double function, as it is required for talin-head domain binding in the first place and then also assuring the subsequent recruitment of paxillin [21]. Because paxillin-mediated signaling requires talin-mediated integrin activation, it is possible that the two proteins bind to the integrins in a sequential manner. However, we think that this is not likely, since an increase in the affinity (50-fold) of the integrin-peptide to the talin-head domain, does not affect the recruitment of paxillin and subsequent cell spreading. This suggests that paxillin is recruited to the NPLY-motif in a talin-bound, and mechanically tensioned state (Figure 2). Interestingly, when the talin-head/integrin association is tested in the context of ligand-bound Mn^{2+} activated integrin, the Tyr-residue in the NPLY-motif is no longer required for association and integrin clustering [32]. This suggests that the tyrosine side-chain can be released from the talin surface, without affecting the mechanical stability of the ECM/integrin/F-actin linkage,

making it available for alternative binding partners, such as paxillin. In order to expose the tyrosine residue, binding of kindlin to the proximal NPXY and inter-NPXY region might be essential. Such a scenario would explain the critical signaling role of kindlin, for example seen during cell and platelet spreading [54, 55].

As indicated above, paxillin enables the recruitment and establishes of multiple links to other focal adhesion adapter proteins, among them a link to vinculin, the PIK/GIT/PAK complex and to parvin [50]. The N-terminal LD-motifs are also responsible for pTyr-dependent binding to the FAT-domain of focal adhesion kinase [26].

mechanical stability of the ECM/integrin/F-actin linkage. This talin/kindlin/integrin assembly offers a suitable landing pad for paxillin (lower panel), which is a key scaffolding protein, recruiting FAK, c-Src, ILK/pinch/parvin complex as well as the GIT/PIX/PAK complex, involved in regulating Rho-family and ARF-GTPases [50]. Paxillin phosphorylation regulates the association with FAK, which kinase activity is regulated by acidic lipid-binding to its N-terminal FERM domain and pTyr-dependent association with c-Src. The localized activation of the FAK-kinase is critical for the turnover of cell-matrix adhesions during the retraction of the cell rear, proposing in turn, tyrosine-motifs in the integrin-tail as targets for kinase-mediated feedback control (see text for references).

Tensional sensing by tyrosine kinases

FAK consists of an N-terminal FERM-domain that can interact with acidic lipids of the plasma membrane. The linker to the kinase domain contains the autophosphorylated Tyr-motif responsible for the recruitment of c-Src. Interestingly, the C-terminal focal adhesion targeting-domain of FAK, binds the N-terminal LD-motifs in paxillin. Thus the kinase domain of FAK is mechanically coupled to the membrane and the N-terminal domain of paxillin, which in turn can associate with F-actin associated proteins such as vinculin, or the ILK/pinch/parvin complex [50]. Because of this mechanical linkage, it is likely that FAK-kinase activity is influenced by the mechanical tension between the N- and C-terminal domain of FAK. Interestingly, FAK has been crystallized in an autoinhibited conformation, in which the c-Src-binding Tyr-motif is sequestered on the surface of the FERM domain [56]. In order to autophosphorylate this Tyr-motif, considerable domain-movements in FAK are required, which can in part be induced by the PIP2-mediated binding of the N-terminal FERM domain to membranes [57]. In addition, a mechanical link to the N-terminal LD-motifs in paxillin could further accelerate the full activation of the FAK-kinase. The critical role of the FAK-kinase activity is shown in knock-in mice expressing kinase-dead FAK. FAK kinase activity is required for recruitment of c-Src via pTyr397, as well as blood vessel formation and the turn-over of cell-matrix adhesions [58]. This demonstrates that FAK activation is an integral part of integrin-dependent mechanosignaling, locally affecting cell-matrix adhesions and cell motility. Whether FAK is a bona fide mechanoregulated kinase needs to be shown in the future.

Fibronectin – contribution to mechanosignaling from extracellular space

As discussed above, adhesion structures are “hot spots” for cellular mechanosignaling. But how are cellular adhesions connected to the extracellular environment? Fibronectin is an extensively studied protein of the ECM, and demonstrates how ECM proteins can contribute to converting mechanical signals into chemical cues.

Fibronectin consists of several globular domains and may be described as a pearl chain of FnI, FnII and FnIII repeats [59]. Extracellular matrix is under mechanical tension and it has been shown that fibronectin molecules vary significantly in their conformation when cells apply force to their environment [60]. Comparison of the mechanical stability of fibronectin domains shows that they differ significantly in the force required for unfolding [61]. Therefore, it is possible that the number of (partially) unfolded domains vary dependent on the mechanical load of the cellular environment, and this causes changes in chemical signaling due to variation in the availability of the binding sites for other binding partners.

A recent study by Kubow et al. [62] demonstrated that collagen I is preferentially co-localized with relaxed fibronectin fibers in cell culture (Figure 1). In addition, the collagen peptide corresponding to the fibronectin-binding domain in collagen I was demonstrated to bind more efficiently to relaxed in vitro manufactured fibronectin fibers as compared to extended fibers. Similarly, stretching of fibronectin has been found to control the binding of bacterial adhesins [63], demonstrating that fibronectin-interaction mediated over several consecutive domains is very sensitive to the tensile forces modifying the spacing of these fibronectin domains.

We have recently expressed GFP-labeled VEGF, which was found to incorporate into ECM at sites of integrin-containing fibrillar adhesions [15]. Thus, it appears that a certain degree of FN stretching is required to expose binding sites for growth factors (Figure 1). Interestingly, ECM fibers are maintained under a certain viscoelastic stress, which can be sensed by cells and which is critical for maintaining a pool of ECM-bound growth factors. While enhanced forces are required to release TGF- β from ECM fibers, matrix-bound VEGF is released upon dissection of ECM-fibers [64]. This would consist of an elegant mechanism to release large amount of VEGF-upon injury induced released of ECM tension.

Summary and outlook

Mechanosensing plays a key function during the adhesive response of cells towards the extracellular matrix. In turn the extracellular matrix provides an elastic scaffold to which integrin receptors are anchored. Cytoplasmic integrin adapter proteins assure that the ECM/integrin/F-actin connection is maintained and tuned to the specific contractile capacity of each cell type. Tensile forces along this mechanical link are activating a mechanosensitive switch that can be recognized by signaling adapter proteins, such as paxillin. This creates a signaling network, controlling the actin cytoskeleton, as well as adhesive and motile functions of the cell.

A better understanding of the mechanosensory machinery might help to develop novel molecular tools to treat and prevent diseases associated with altered mechanosignaling at cell-matrix adhesions, such as tumor growth, metastasis formation or fibrosis. Tension-induced exposure of cryptic binding sites within these proteins may open targetable binding sites for peptides and small molecules to block mechanically activated signaling pathways at the level of cell-matrix adhesions.

Acknowledgements

This work has been supported by the Academy of Finland (projects 290506 and 136288) (VPH) the Swiss Foundation for Research on Myopathies (BWH), the Swiss National Science foundation (31003A-130742) (BWH) and the Ligue Genevoise contre le Cancer (BWH).

References

- [1] C.M. Lo, H.B. Wang, M. Dembo, Y.L. Wang, Cell movement is guided by the rigidity of the substrate, *Biophys J* 79 (2000) 144-152.
- [2] M. Arnold, V.C. Hirschfeld-Warneken, T. Lohmuller, P. Heil, J. Blummel, E.A. Cavalcanti-Adam, M. Lopez-Garcia, P. Walther, H. Kessler, B. Geiger, J.P. Spatz, Induction of cell polarization and migration by a gradient of nanoscale variations in adhesive ligand spacing, *Nano Lett* 8 (2008) 2063-2069.
- [3] D. Riveline, E. Zamir, N.Q. Balaban, U.S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, A.D. Bershadsky, Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK- independent mechanism, *J Cell Biol* 153 (2001) 1175-1186.
- [4] C. Tamiello, C.V. Bouten, F.P. Baaijens, Competition between cap and basal actin fiber orientation in cells subjected to contact guidance and cyclic strain, *Sci Rep* 5 (2015) 8752.
- [5] E. Tzima, M.A. del Pozo, S.J. Shattil, S. Chien, M.A. Schwartz, Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment, *EMBO J* 20 (2001) 4639-4647.
- [6] R. Lutz, T. Sakai, M. Chiquet, Pericellular fibronectin is required for RhoA-dependent responses to cyclic strain in fibroblasts, *J Cell Sci* 123 (2010) 1511-1521.
- [7] R. Chiquet-Ehrismann, R.P. Tucker, Tenascins and the importance of adhesion modulation, *Cold Spring Harb Perspect Biol* 3 (2011).
- [8] F. Klingberg, M.L. Chow, A. Koehler, S. Boo, L. Buscemi, T.M. Quinn, M. Costell, B.A. Alman, E. Genot, B. Hinz, Prestress in the extracellular matrix sensitizes latent TGF-beta1 for activation, *J Cell Biol* 207 (2014) 283-297.

- [9] M.M. Martino, P.S. Briquez, E. Guc, F. Tortelli, W.W. Kilarski, S. Metzger, J.J. Rice, G.A. Kuhn, R. Muller, M.A. Swartz, J.A. Hubbell, Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing, *Science* 343 (2014) 885-888.
- [10] S. Tabone-Eglinger, Z. Calderin-Sollet, P. Pinon, N. Aebischer, M. Wehrle-Haller, M.C. Jacquier, D. Boettiger, B. Wehrle-Haller, Niche anchorage and signaling through membrane-bound Kit-ligand/c-kit receptor are kinase independent and imatinib insensitive, *FASEB J* 28 (2014) 4441-4456.
- [11] M.J. Paszek, N. Zahir, K.R. Johnson, J.N. Lakins, G.I. Rozenberg, A. Gefen, C.A. Reinhart-King, S.S. Margulies, M. Dembo, D. Boettiger, D.A. Hammer, V.M. Weaver, Tensional homeostasis and the malignant phenotype, *Cancer Cell* 8 (2005) 241-254.
- [12] N.O. Deakin, C.E. Turner, Distinct roles for paxillin and Hic-5 in regulating breast cancer cell morphology, invasion, and metastasis, *Mol Biol Cell* 22 (2011) 327-341.
- [13] E. Hirata, M.R. Girotti, A. Viros, S. Hooper, B. Spencer-Dene, M. Matsuda, J. Larkin, R. Marais, E. Sahai, Intravital imaging reveals how BRAF inhibition generates drug-tolerant microenvironments with high integrin beta1/FAK signaling, *Cancer Cell* 27 (2015) 574-588.
- [14] M.J. Paszek, C.C. DuFort, O. Rossier, R. Bainer, J.K. Mouw, K. Godula, J.E. Hudak, J.N. Lakins, A.C. Wijekoon, L. Cassereau, M.G. Rubashkin, M.J. Magbanua, K.S. Thorn, M.W. Davidson, H.S. Rugo, J.W. Park, D.A. Hammer, G. Giannone, C.R. Bertozzi, V.M. Weaver, The cancer glycocalyx mechanically primes integrin-mediated growth and survival, *Nature* 511 (2014) 319-325.
- [15] B. Geiger, A. Bershadsky, R. Pankov, K.M. Yamada, Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk, *Nat Rev Mol Cell Biol* 2 (2001) 793-805.
- [16] N.Q. Balaban, U.S. Schwarz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, B. Geiger, Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates, *Nat Cell Biol* 3 (2001) 466-472.
- [17] M. Vicente-Manzanares, J. Zareno, L. Whitmore, C.K. Choi, A.F. Horwitz, Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells, *J Cell Biol* 176 (2007) 573-580.
- [18] Y. Sawada, M.P. Sheetz, Force transduction by Triton cytoskeletons, *J Cell Biol* 156 (2002) 609-615.
- [19] H.B. Schiller, C.C. Friedel, C. Boulegue, R. Fassler, Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins, *EMBO Rep* 12 (2011) 259-266.
- [20] M.A. Smith, E. Blankman, N.O. Deakin, L.M. Hoffman, C.C. Jensen, C.E. Turner, M.C. Beckerle, LIM domains target actin regulators paxillin and zyxin to sites of stress fiber strain, *PLoS One* 8 (2013) e69378.
- [21] P. Pinon, J. Parssinen, P. Vazquez, M. Bachmann, R. Rahikainen, M.C. Jacquier, L. Azizi, J.A. Maatta, M. Bastmeyer, V.P. Hytonen, B. Wehrle-Haller, Talin-bound NPLY motif recruits integrin-signaling adapters to regulate cell spreading and mechanosensing, *J Cell Biol* 205 (2014) 265-281.
- [22] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, *Cell* 126 (2006) 677-689.

- [23] R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, *Dev Cell* 6 (2004) 483-495.
- [24] S. Dupont, L. Morsut, M. Aragona, E. Enzo, S. Giulitti, M. Cordenonsi, F. Zanconato, J. Le Digabel, M. Forcato, S. Bicciato, N. Elvassore, S. Piccolo, Role of YAP/TAZ in mechanotransduction, *Nature* 474 (2011) 179-183.
- [25] M. Vicente-Manzanares, K. Newell-Litwa, A.I. Bachir, L.A. Whitmore, A.R. Horwitz, Myosin IIA/IIB restrict adhesive and protrusive signaling to generate front-back polarity in migrating cells, *J Cell Biol* 193 (2011) 381-396.
- [26] C.K. Choi, J. Zareno, M.A. Digman, E. Gratton, A.R. Horwitz, Cross-correlated fluctuation analysis reveals phosphorylation-regulated paxillin-FAK complexes in nascent adhesions, *Biophys J* 100 (2011) 583-592.
- [27] C. Lawson, S.T. Lim, S. Uryu, X.L. Chen, D.A. Calderwood, D.D. Schlaepfer, FAK promotes recruitment of talin to nascent adhesions to control cell motility, *J Cell Biol* 196 (2012) 223-232.
- [28] B.T. Goult, T. Zacharchenko, N. Bate, R. Tsang, F. Hey, A.R. Gingras, P.R. Elliott, G.C. Roberts, C. Ballestrem, D.R. Critchley, I.L. Barsukov, RIAM and vinculin binding to talin are mutually exclusive and regulate adhesion assembly and turnover, *J Biol Chem* 288 (2013) 8238-8249.
- [29] D.A. Calderwood, I.D. Campbell, D.R. Critchley, Talins and kindlins: partners in integrin-mediated adhesion, *Nat Rev Mol Cell Biol* 14 (2013) 503-517.
- [30] H.S. Lee, C.J. Lim, W. Puzon-McLaughlin, S.J. Shattil, M.H. Ginsberg, RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences, *J Biol Chem* 284 (2009) 5119-5127.
- [31] E. Goksoy, Y.Q. Ma, X. Wang, X. Kong, D. Perera, E.F. Plow, J. Qin, Structural basis for the autoinhibition of talin in regulating integrin activation, *Mol Cell* 31 (2008) 124-133.
- [32] F. Saltel, E. Mortier, V.P. Hytonen, M.C. Jacquier, P. Zimmermann, V. Vogel, W. Liu, B. Wehrle-Haller, New PI(4,5)P₂- and membrane proximal integrin-binding motifs in the talin head control beta3-integrin clustering, *J Cell Biol* 187 (2009) 715-731.
- [33] G. Jiang, G. Giannone, D.R. Critchley, E. Fukumoto, M.P. Sheetz, Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin, *Nature* 424 (2003) 334-337.
- [34] S. Kukkurainen, J.A. Maatta, J. Saeger, J. Valjakka, V. Vogel, V.P. Hytonen, The talin-integrin interface under mechanical stress, *Mol Biosyst* 10 (2014) 3217-3228.
- [35] V.P. Hytonen, V. Vogel, How force might activate talin's vinculin binding sites: SMD reveals a structural mechanism, *PLoS Comput Biol* 4 (2008) e24.
- [36] S.E. Lee, R.D. Kamm, M.R. Mofrad, Force-induced activation of talin and its possible role in focal adhesion mechanotransduction, *J Biomech* 40 (2007) 2096-2106.
- [37] A. del Rio, R. Perez-Jimenez, R. Liu, P. Roca-Cusachs, J.M. Fernandez, M.P. Sheetz, Stretching single talin rod molecules activates vinculin binding, *Science* 323 (2009) 638-641.
- [38] F. Margadant, L.L. Chew, X. Hu, H. Yu, N. Bate, X. Zhang, M. Sheetz, Mechanotransduction in vivo by repeated talin stretch-relaxation events depends upon vinculin, *PLoS Biol* 9 (2011) e1001223.

- [39] S.J. Franco, M.A. Rodgers, B.J. Perrin, J. Han, D.A. Bennin, D.R. Critchley, A. Huttenlocher, Calpain-mediated proteolysis of talin regulates adhesion dynamics, *Nat Cell Biol* 6 (2004) 977-983.
- [40] G. Baneyx, L. Baugh, V. Vogel, Fibronectin extension and unfolding within cell matrix fibrils controlled by cytoskeletal tension, *Proc Natl Acad Sci U S A* 99 (2002) 5139-5143.
- [41] E.T. Eng, B.J. Smagghe, T. Walz, T.A. Springer, Intact α IIb β 3 integrin is extended after activation as measured by solution X-ray scattering and electron microscopy, *J Biol Chem* 286 (2011) 35218-35226.
- [42] S. Tadokoro, S.J. Shattil, K. Eto, V. Tai, R.C. Liddington, J.M. de Pereda, M.H. Ginsberg, D.A. Calderwood, Talin binding to integrin beta tails: a final common step in integrin activation, *Science* 302 (2003) 103-106.
- [43] F. Ye, G. Hu, D. Taylor, B. Ratnikov, A.A. Bobkov, M.A. McLean, S.G. Sligar, K.A. Taylor, M.H. Ginsberg, Recreation of the terminal events in physiological integrin activation, *J Cell Biol* 188 (2010) 157-173.
- [44] J. Zhu, B.H. Luo, T. Xiao, C. Zhang, N. Nishida, T.A. Springer, Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces, *Mol Cell* 32 (2008) 849-861.
- [45] J. Zhu, J. Zhu, T.A. Springer, Complete integrin headpiece opening in eight steps, *J Cell Biol* 201 (2013) 1053-1068.
- [46] F. Kong, A.J. Garcia, A.P. Mould, M.J. Humphries, C. Zhu, Demonstration of catch bonds between an integrin and its ligand, *J Cell Biol* 185 (2009) 1275-1284.
- [47] Y.Q. Ma, J. Qin, C. Wu, E.F. Plow, Kindlin-2 (Mig-2): a co-activator of β 3 integrins, *J Cell Biol* 181 (2008) 439-446.
- [48] A. Dai, F. Ye, D.W. Taylor, G. Hu, M.H. Ginsberg, K.A. Taylor, The structure of a full-length membrane-embedded integrin bound to a physiological ligand, *J Biol Chem* (2015).
- [49] C. Cluzel, F. Saltel, F. Paulhe, J. Lussi, B.A. Imhof, B. Wehrle-Haller, The mechanisms and dynamics of α v β 3 integrin clustering in living cells, *J. Cell Biol.* 171 (2005) 383-392.
- [50] N.O. Deakin, C.E. Turner, Paxillin comes of age, *J Cell Sci* 121 (2008) 2435-2444.
- [51] M.C. Brown, J.A. Perrotta, C.E. Turner, Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding, *J Cell Biol* 135 (1996) 1109-1123.
- [52] R.Y. Wu, G.N. Gill, LIM domain recognition of a tyrosine-containing tight turn, *J Biol Chem* 269 (1994) 25085-25090.
- [53] M.D. Schaller, C.A. Otey, J.D. Hildebrand, J.T. Parsons, Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains, *J Cell Biol* 130 (1995) 1181-1187.
- [54] E. Montanez, S. Ussar, M. Schifferer, M. Bosl, R. Zent, M. Moser, R. Fassler, Kindlin-2 controls bidirectional signaling of integrins, *Genes Dev* 22 (2008) 1325-1330.
- [55] M. Moser, B. Nieswandt, S. Ussar, M. Pozgajova, R. Fassler, Kindlin-3 is essential for integrin activation and platelet aggregation, *Nat Med* 14 (2008) 325-330.

- [56] D. Lietha, X. Cai, D.F. Ceccarelli, Y. Li, M.D. Schaller, M.J. Eck, Structural basis for the autoinhibition of focal adhesion kinase, *Cell* 129 (2007) 1177-1187.
- [57] G.M. Goni, C. Epifano, J. Boskovic, M. Camacho-Artacho, J. Zhou, A. Bronowska, M.T. Martin, M.J. Eck, L. Kremer, F. Grater, F.L. Gervasio, M. Perez-Moreno, D. Lietha, Phosphatidylinositol 4,5-bisphosphate triggers activation of focal adhesion kinase by inducing clustering and conformational changes, *Proc Natl Acad Sci U S A* 111 (2014) E3177-3186.
- [58] S.T. Lim, X.L. Chen, A. Tomar, N.L. Miller, J. Yoo, D.D. Schlaepfer, Knock-in mutation reveals an essential role for focal adhesion kinase activity in blood vessel morphogenesis and cell motility-polarity but not cell proliferation, *J Biol Chem* 285 (2010) 21526-21536.
- [59] R. Pankov, K.M. Yamada, Fibronectin at a glance, *J Cell Sci* 115 (2002) 3861-3863.
- [60] M.L. Smith, D. Gourdon, W.C. Little, K.E. Kubow, R.A. Eguiluz, S. Luna-Morris, V. Vogel, Force-induced unfolding of fibronectin in the extracellular matrix of living cells, *PLoS Biol* 5 (2007) e268.
- [61] D. Craig, M. Gao, K. Schulten, V. Vogel, Tuning the mechanical stability of fibronectin type III modules through sequence variations, *Structure* 12 (2004) 21-30.
- [62] K.E. Kubow, R. Vukmirovic, L. Zhe, E. Klotzsch, M.L. Smith, D. Gourdon, S. Luna, V. Vogel, Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix, *Nat Commun* 6 (2015) 8026.
- [63] M. Chabria, S. Hertig, M.L. Smith, V. Vogel, Stretching fibronectin fibres disrupts binding of bacterial adhesins by physically destroying an epitope, *Nat Commun* 1 (2010) 135.
- [64] K. Egervari, G. Potter, M.L. Guzman-Hernandez, P. Salmon, M. Soto-Ribeiro, K. B. B. T., B. Wehrle-Haller, J.Z. Kiss, Astrocytes spatially restrict VEGF signaling by polarized secretion and incorporation of VEGF into the actively assembling pericellular matrix, *Glia* (2015) in press.