Mechanosensitivity and compositional dynamics of cell–matrix adhesions

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Cells perceive information about the biochemical and biophysical properties of their tissue microenvironment through integrin-mediated cell–matrix adhesions, which connect the cytoskeleton with the extracellular matrix and thereby allow cohesion and long-range mechanical connections within tissues. The formation of cell–matrix adhesions and integrin signalling involves the dynamic recruitment and assembly of an inventory of proteins, collectively termed the ‘adhesome’, at the adhesive site. The recruitment of some adhesome proteins, most notably the Lin11-, Isl1- and Mec3-domain-containing proteins, depends on mechanical tension generated by myosin II-mediated contractile forces exerted on cell–matrix adhesions. When exposed to force, mechanosensitive adhesome proteins can change their conformation or expose cryptic-binding sites leading to the recruitment of proteins, rearrangement of the cytoskeleton, reinforcement of the adhesive site and signal transduction. Biophysical methods and proteomics revealed force ranges within the adhesome and cytoskeleton, and also force-dependent changes in adhesome composition. In this review, we provide an overview of the compositional dynamics of cell–matrix adhesions, discuss the most prevalent functional domains in adhesome proteins and review literature and concepts about mechanosensing mechanisms that operate at the adhesion site.

Keywords: adhesome; focal adhesion; quantitative proteomics; integrins; extracellular matrix

EMBO reports (2013) 14, 509–519; published online 17 May 2013;
doi:10.1038/embor.2013.49

See the Glossary for abbreviations used in this article.

Introduction

Mechanobiology at cell adhesions is crucial for development, tissue homeostasis and the outcome of many diseases [1]. Cell adhesions connect to the cytoskeleton through the cytoplasmic domains of adhesion receptors, which allow cells to pull on other cells or their extracellular matrix (ECM) substrate by using myosin II-mediated contractile force. This force allows cells to probe their environment

constantly and to translate mechanical tension at cell adhesion sites into biochemical signals in a process called mechanosensing. Mechanosensing enables cells to respond rapidly to whole tissue parameters such as ECM stiffness, which influences decisions regarding the form, function and fate of cells [2]. Cell–matrix adhesions are mainly mediated by the 24 members of the integrin family, whereas cell–cell adhesions are mainly executed with the approximately 80 members of the cadherin superfamily [3,4]. Both integrin- and cadherin-mediated adhesions connect to the filamentous (F-) actin cytoskeleton by using a variety of adaptor and signalling proteins. These proteins assemble into a dense and highly dynamic network visible as a protein plaque at the plasma membrane, which we refer to as the adhesome [5,6]. The recruitment of several plaque proteins to the adhesome requires myosin II-mediated mechanical tension [7,8]. Force on cell–matrix adhesions can change the conformation of proteins and expose cryptic-binding sites within mechanosensitive adhesome proteins leading to the recruitment of further proteins, rearrangement of the cytoskeleton, reinforcement of the adhesive site and finally the induction of signalling [1,9–15]. Several important adaptor molecules and kinases of the adhesome such as talin, vinculin, Src and p130Cas are stretched by mechanical forces on stiff substrates [16–19], which in the case of Src and Fak can directly regulate their catalytic activity through force-induced structural rearrangements of the protein backbone [16].

Progress in the field of mechanobiology has revealed that mechanical cues perceived at cell adhesions have an important influence on cell and tissue architecture and long-term fate decisions, which are comparable to biochemical cues induced by growth factors. In addition, there is evidence emerging that these two types of signalling mechanism regulate each other—for instance, the inability of cells to respond to growth factor treatment in suspension or in soft compliant matrices shows that mechanosensing at cell–matrix adhesions and growth factor receptor signalling act synergistically [20]. Also, cell survival is regulated by adhesion signalling and therefore cells often undergo cell detachment-induced apoptosis, a process termed ‘anoikis’ [21]. To overcome anoikis and anchorage-dependent growth, tumour cells must uncouple cell–matrix adhesion signalling intermediates from adhesive events and mechanical tension, which might be achieved by mutations or hyperactivity of other cell surface receptor-mediated signalling pathways. Indeed, many kinases and adaptor proteins recruited to cell–matrix adhesions such as Src [22], Fak [23,24], p130Cas [25], c-Cbl [26], caveolin 1 and

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Glossary

Arp2/3	actin-related protein 2/3 (-complex)
β -pix	PAK-interacting exchange factor beta
c-Cbl	casitas B-lineage lymphoma proto-oncogene
cDNA	complementary DNA
CH	calponin homology
Crp	cysteine-rich protein
Ena	protein-enabled
Erk	extracellular signal-regulated kinase
F-actin	filamentous actin
Fak	focal adhesion kinase
FERM	4.1 protein, ezrin, radixin and moesin
FHL	four and a half LIM
Fyn	proto-oncogene c-Fyn
Gef	guanine nucleotide exchange factor
GFP	green fluorescent protein
Ilk	integrin-linked kinase
Larg	leukaemia-associated RhoGEF
LD-motif	leucine-aspartate repeat motif
LIM	Lin11, Isl1, Mec3
Lpp	lipoma-preferred partner
mDia	diaphanous-related formin 1
MLP/CSRP3	cysteine and glycine-rich protein 3
Mypt1	myosin phosphatase-targeting subunit 1
p130Cas	CRK-associated substrate
PAK1	p21-activated kinase 1
PDLIM	PDZ and LIM domain protein
PKA	protein kinase A
SH2/3	src homology 2/3
Src	proto-oncogene c-Src (SaRComa)
Vasp	vasodilator-stimulated phosphoprotein

Fyn [27], Ilk [28], PKA and Pak1 [29] were shown to mediate anchorage-dependent proliferation and survival. As soon as they become constitutively active by activating mutations or by an elevated activity of alternative signalling pathways, they exert oncogenic activities and allow growth and survival without anchorage to the ECM, which is an important step during tumour progression to metastasis. Alternatively, tumour progression can be driven by increased matrix stiffness at tumour sites, which was shown to promote malignancy by inducing focal adhesion growth and growth factor receptor signalling [30]. Another intriguing example for the importance of mechanosensing was shown in two landmark studies, which have demonstrated that the differentiation of mesenchymal stem cells into different lineages is controlled by ECM substrate stiffness [9] and involves the mechanosensing-dependent activation of the transcription factor Yap1 [14].

Although the molecular compositions of adhesomes at cell–cell and cell–matrix adhesion sites differ [31], they are both complex, and common principles of structural and functional organization certainly exist between the two adhesive structures. We focus this review on the assembly of proteins that are recruited to integrin-mediated cell–matrix adhesions, which we refer to as the ‘integrin adhesome’. The molecular composition of the integrin adhesome was extensively studied over the past 20 years by generating specific antibodies, expressing GFP-fusion proteins and by performing yeast two-hybrid screens and protein pull-downs. More than 150 proteins have been reported to be stably or transiently associated with focal adhesions. They were summarized in a literature meta-study that also coined the

term ‘adhesome’ for the entire subproteome associated with integrin adhesions [32,33]. However, this number was derived from hundreds of studies that used different cell lines and experimental conditions. Proteomic analysis of the integrin adhesome shed light on the molecular composition of defined classes of cell–matrix adhesion structures in defined cell types [7,8,34]. We discuss the functional architecture and mechanisms of signal transduction at cell–matrix adhesions with a particular emphasis on force-induced integrin signalling. We review the proteomic studies that addressed the compositional dynamics of integrin adhesomes when mechanical forces are applied, putative mechanisms underlying force-mediated recruitment of proteins to cell–matrix adhesions and the most prevalent functional domains present in proteins of the integrin adhesome.

Integrin-mediated mechanosensing and myosin II

The concept of adhesion maturation entails that newly formed cell–matrix adhesions change their size and composition over time, which is crucially influenced by chemical as well as physical properties of the ECM-bound substrate. Cells use their actin–myosin II network to generate force and to pull on the ECM, which alters the supramolecular structure and physical properties of the ECM through the formation of fibrils and the stretching of pre-existing fibrils and networks [35,36]. The physical properties of the ECM in turn feed back on the cell through a process called adhesion-mediated ‘rigidity sensing’, which involves the reorganization of cell–matrix adhesions, induction of new signalling activities and finally a readjustment of the myosin II-mediated cell contractility (Fig 1). Integrin-mediated cell–matrix adhesions are usually categorized into different classes on the basis of their size, morphology and lifetime. The progression from newly formed nascent adhesions to mature focal adhesions (Fig 2) and fibronectin–matrix-remodelled fibrillar adhesions can be conceptualized as a myosin II-dependent maturation process from small cell–matrix adhesions with high turnover rate (nascent adhesion) to progressively larger and enduring adhesion sites (focal adhesion and fibrillar adhesion) stabilized by the connection to the cytoskeleton. The formation of nascent adhesions requires Arp2/Arp3-mediated actin polymerization, whilst their maturation to large focal adhesions depends on myosin II-mediated cell contractility, the activity of the formin mDia and ECM substrate stiffness [37]. The protrusive as well as the contractile activity of a cell is regulated precisely by cell–matrix adhesion signalling events. The balance of these two signalling outputs is crucially affected by ECM substrate stiffness and myosin II activity, which further accelerates adhesion maturation in a positive feedback system. Consequently, adhesion maturation decreases on soft ECM substrates and is blocked when cells grown on stiff substrates are treated with the specific myosin II inhibitor blebbistatin. Blebbistatin treatment leads to a burst of cell protrusions and newly formed nascent adhesions, which in a self-amplifying process promote Arp2/Arp3-driven actin polymerization in the cell periphery and further membrane protrusions and cell movement [38]. Mature adhesions stabilize the cell–ECM contacts and slow down cell movement.

Biochemical and biophysical mechanism of rigidity sensing

Although the molecular mechanisms of feedback between integrin signalling, myosin II motor activity and ECM rigidity are incompletely resolved, there is convincing evidence for the

Fig 1 | Reciprocal feedback connections at cell-matrix adhesions involve mechanosensitive modules. Both the chemistry and the mechanical properties of the ECM are controlled by pericellular mechanisms such as matrix crosslinking, fibrillogenesis and proteolysis, affecting integrin use and activation. These properties, and the integrins that become engaged, influence the composition of connectors and signal transducers that are recruited to cell-matrix adhesion sites leading to specific signalling and regulation of cytoskeletal components. Myosin II-mediated pulling on stiff ECM generates tension that is distributed along ECM fibrils, the cell-matrix adhesome and the cytoskeletal filaments, which promotes the partial unfolding or conformational rearrangement of mechanosensitive adhesome proteins and ECM ligands. Such allosteric mechanisms lead to the recruitment of new proteins into tensioned cell-matrix adhesions resulting in the activation of signalling pathways as well as structural reinforcement and stabilization of adhesion sites, which in turn feed back on various signalling events. Furthermore, biophysical mechanisms such as catch-bonding of, for example, integrin-ligand and F-actin-myosin II interactions are involved in cellular mechanosensing. ECM, extracellular matrix; Erk, extracellular signal-regulated kinase; F-actin, filamentous actin; GAP, GTPase-Activating Protein; GEF, guanine nucleotide exchange factor; GPV, platelet glycoprotein V; Larg, leukaemia-associated RhoGEF; RHAMM, receptor for hyaluronan-mediated motility; Src, proto-oncogene c-Src.

involvement of biochemical as well as biophysical signalling events. Rigidity sensing of ECM microenvironments requires a dynamic quantitative, as well as qualitative, adjustment of the composition of the integrin adhesome to induce the accurate strength of feedback signalling to myosin II and to efficiently couple the newly produced forces to the ECM substrate. Integrin signalling can control myosin II activity through various pathways that all impinge on the phosphorylation of the regulatory myosin light chain (MLC) at Thr18/Ser19 (reviewed in [39]). To mediate feedback between ECM stiffness and myosin II activity, specific mediators of integrin-signalling pathways should be activated by high tension at the cell-matrix adhesion site. This was indeed the case when force was applied to cell-matrix adhesions—the recruitment and activities of the RhoA activators Gef-H1 and Larg increased [40]. Whilst force activates Larg through the Src family kinase Fyn, the activity of Gef-H1 is triggered by force-induced Erk phosphorylation [40]. Interestingly, the recruitment of Erk to cell-matrix adhesions is Src-dependent [41], which suggests that force-mediated activation of Src and Erk represents a crucial step

in activating myosin II through Larg-Gef-H1-RhoA. When the RhoA pathway is successfully coupled to its downstream effector kinase Rock, myosin II activity is increased through direct phosphorylation and subsequent dissociation of the MLC phosphatase targeting subunit Mypt1 from actomyosin fibres [42,43]. However, phosphorylation of MLC can also be mediated directly by the MLC kinase (Mlck), which is regulated by Ca^{2+} [44] and acts preferentially on peripheral cell-matrix adhesions when high tension is applied [45].

In addition to these signalling pathways that lead to myosin II activation, there is also evidence for the existence of direct biophysical mechanisms by which myosin II-produced forces couple to the actomyosin-focal adhesion structure. The phosphorylation of MLC does not change the affinity of myosin II for actin filaments [39], and therefore other mechanisms must operate that change myosin II function. Interestingly, it has been shown that mechanical stretching of actin filaments enhances myosin II motor domain binding to F-actin [46]. In agreement with this observation, reducing mechanical tension of actomyosin cables by using laser-mediated actin

A Adhesion maturation from nascent adhesions to focal adhesions **B** Recruitment of LIM domain proteins goes along with adhesion maturation

Fig 2 | Myosin II-mediated adhesion maturation involves the zinc-finger type LIM domain. **(A)** A mouse fibroblast plated on fibronectin immunostained for the focal adhesion protein paxillin, the lamellipodium protein cortactin and F-actin. Nascent adhesions (arrows) form in the protruding, cortactin-positive lamellipodium and are not linked to F-actin bundles. By contrast, focal adhesions (arrowheads) are stabilized by their linkage to contractile F-actin bundles. **(B)** In the course of adhesion maturation, myosin II-mediated pulling through F-actin bundles induces the recruitment of specific proteins such as LIM-domain-containing proteins to cell–matrix adhesions. The box in the panel on the right shows proteins that are enriched in nascent adhesions (gene names in blue) or focal adhesions (gene names in red; LIM-domain proteins are marked with asterisks). Data selected from references [8,52]. FA, focal adhesion; F-actin, filamentous actin; LIM, Lin11, Isl1, Mec3; NA, nascent adhesion.

filament cutting led to fast dissociation of myosin II, indicating that tension is both necessary and sufficient to localize myosin II to actin fibres [47]. Thus, high tension along actomyosin increases the bond lifetime between the myosin motor domain and the actin filament resulting in increased processivity at higher force loads [48]. These bonds are called ‘catch bonds’, and are defined as chemical bonds with an increased lifetime on force application—a property that is also inherent to $\alpha 5 \beta 1$ integrin–fibronectin bonds [49,50]. This implies that the processivity of actomyosin cables and the lifetime of the integrin–ECM adhesion can be modulated by the mechanical

load. One study used atomic force microscopy (AFM) to uncover a new mechanism of adhesion reinforcement; it was shown that application of cyclic forces to the $\alpha 5 \beta 1$ -integrin–fibronectin bonds produces a 100-fold increase in bond lifetime (1–100 s; [51]). Surprisingly, this increased bond lifetime did not require sustained forces, as is the case for ‘classic’ catch bonds. This so-called ‘cyclic mechanical reinforcement’ of integrin–ECM–ligand bonds, together with biophysical and biochemical-signalling mechanisms discussed above, allows cells to memorize their past force experience and helps to optimally mature focal adhesions.

Integrin-specific adhesomes in rigidity sensing

As focal adhesions always contain combinations of several integrins, it is unknown whether and to what extent individual integrin classes bound to the same ECM ligand use special pathways to sense force. This important question has been addressed by reconstituting integrin-null fibroblasts with αv - and/or $\beta 1$ -family integrin cDNAs; [52]). Combining quantitative proteomics with traction force microscopy and phenotypic characterization of $\alpha 5\beta 1$ -, $\alpha v\beta 3$ - and $\alpha 5\beta 1/\alpha v\beta 3$ -expressing cells seeded on fibronectin micropatterns allowed correlating cell shape, F-actin stress fibre formation and rigidity sensing of their microenvironment with integrin subtype-specific focal adhesion composition and signalling [52]. The results have demonstrated that fibroblasts can only sense the rigidity of fibronectin-based microenvironments when they express both the αv -class and $\alpha 5\beta 1$ -integrins. Furthermore, several of the above mentioned biochemical and biophysical-signalling mechanisms were coordinated in a specific and differential manner by $\alpha 5\beta 1$ - and $\alpha v\beta 3/5$ -integrins. In particular, the coupling of RhoA to downstream effectors was differentially affected by the two integrin classes. Whilst αv -class-mediated RhoA activation is coupled with mDia-mediated stress fibre formation and therefore structural adhesion reinforcement, $\alpha 5\beta 1$ -integrin couples RhoA to Rock and subsequent myosin II-mediated force generation [52]. Therefore, it requires the cooperation of both integrin classes to induce an efficient feedback amplification of myosin II on stiff substrates (Fig 3).

The study of the single integrin class-expressing cells also uncovered striking differences in the ability of $\alpha 5\beta 1$ - and αv -class integrins to adjust adhesion lifetime and strength to mechanical forces. Whereas $\alpha 5\beta 1$ adhered efficiently to fibronectin after blebbistatin-induced myosin II inhibition, αv -class integrin-mediated adhesion is strongly reinforced by myosin II, and with time the αv -class integrins accumulate in adhesion areas exposed to the highest traction forces [52]. These findings are reminiscent of single protein tracking experiments of $\beta 1$ - and $\beta 3$ -integrins, which showed that $\beta 3$ -integrins are immobilized in large focal adhesions, whilst $\beta 1$ -integrins are more mobile [53]. Interestingly, $\alpha v\beta 3$ -integrin has been shown to be needed for cell stiffening on force application, which might be consistent with a role in structural reinforcement of the adhesion [54]. Together, these observations predict that the extracellular domains of $\alpha 5\beta 1$ -integrin and $\alpha v\beta 3/5$ -integrin evolved functional specifications for adjusting adhesion lifetime and strength to mechanical tension. This hypothesis needs to be tested in future experiments by systematically studying the influence of force on the on- and off-rates of $\alpha 5\beta 1$ - and αv -class integrins. The fact that $\alpha 5\beta 1$ - and αv -class integrins require cooperation to achieve rigidity sensing of fibronectin-based microenvironments [52] suggests that the cooperation is equally necessary for cell migration towards a rigidity gradient—a process called 'durotaxis'. Durotaxis contributes to several developmental and pathophysiological processes and seems to depend on a specific type of focal adhesion able to exert dynamically fluctuating ('tugging') tractions in a Fak-phosphopaxillin-vinculin-signalling-dependent manner [55].

Myosin II-driven maturation of the integrin adhesome

Reinforcement of cell-matrix adhesions by mechanical tension, as described above, might also require qualitative or quantitative changes of adaptor proteins in the integrin adhesome. Such changes could occur on the level either of protein recruitment or the post-translational modification of proteins. Whilst the effect

Fig 3 | Integrin-specific adhesomes cooperate for rigidity sensing. $\alpha 5\beta 1$ -integrins adhere to fibronectin, assemble kindlin 2- and Ilk-rich small peripheral adhesions in a myosin II-independent manner. The protein assembly in $\alpha 5\beta 1$ -containing adhesions activates Rac1, Wave and Arp2/Arp3-driven actin polymerization to induce membrane protrusions, and RhoA/Rock-mediated myosin II activation to induce tension. This tension increases the adhesion lifetime of αv -class integrins bound to ligand on stiff substrates, which reinforces and stabilizes focal adhesions. GEF-H1 is recruited by αv -class integrins to focal adhesions, which reinforces RhoA/myosin II in an $\alpha 5\beta 1$ -dependent manner, and increase RhoA activity to promote mDia-mediated stress fibre formation. The combination of αv -class integrin-mediated structure (focal adhesion anchoring and stress fibre formation) with the $\alpha 5\beta 1$ -mediated force generation (myosin II activity) constitutes a synergistic system, which is important for adapting cellular contractility and architecture to the rigidity of fibronectin-based microenvironments. Scheme adapted from reference [52]. Arp2/3, actin-related protein 2/3; Erk, extracellular signal-regulated kinase; GEF-H1, guanine nucleotide exchange factor H1; IPP, Ilk, Pinch, Parvin.

of mechanical tension on the phosphorylation landscape of the integrin adhesome and cytoskeleton is still elusive, we and others have analysed the changes in protein recruitment as a function of myosin II activity by using high-resolution quantitative mass spectrometry [7,8]. Although previous studies have shown that the transition from nascent adhesion to focal adhesion involves a differential abundance of certain integrin-adhesome proteins [56–58], they were limited to only a few proteins. The unbiased mass spectrometry approach used by two laboratories allowed, for the

first time to our knowledge, a comprehensive bird's eye view of the adhesion maturation process of fibroblasts seeded on fibronectin [7,8, 52]. Relative quantification of adhesome components before and after myosin II inhibition with blebbistatin revealed that many 'core adhesome' components did not change their stoichiometry relative to $\beta 1$ -integrin on adhesion maturation [8]. Interestingly, these proteins were mainly integrin proximal factors such as the kindlins and talins, which are known to bind directly to integrin cytoplasmic tails [59]. By contrast, other adhesome proteins were either reduced or increased relative to $\beta 1$ -integrin in the course of myosin II-driven adhesion maturation (Fig 2). Intuitively, the factors that were reduced could be considered either as initial phase components involved in forming nascent adhesions and promoting actin polymerization and membrane protrusions, or as early negative regulators of nascent adhesion turnover that stabilize the structure. Indeed, the Rac-specific Gef β -pix at nascent adhesions was identified as an important mediator of lamellipodial protrusions [7]. Proteins, the relative amounts of which increased on adhesion maturation, could potentially be involved in feedback loops controlling adhesion maturation and turnover, myosin II activity and reinforcement of the cell–matrix interactions. Interestingly, this group of proteins contained several plausible candidates that could be involved in such a task [7,8]. Vinculin and filamin, for instance, can stabilize the link between integrins and F-actin [60,61] and thereby increase tension at the adhesion site. Gef-H1 and testin were shown to activate RhoA, leading to increased myosin II activation [40,62]. Cytoskeletal adaptors and cross-linkers such as the α -actinins, the PDLIMs, the FHLs and the zyxin family proteins were shown to stabilize the actomyosin system and the Ena/Vasp proteins are believed to promote actin polymerization at adhesion sites [63].

Serial LIM domains mediate adhesome maturation

One particular protein domain, namely the zinc-finger type LIM domain, was found to correlate with force-dependent recruitment of proteins to cell–matrix adhesions [8], which suggests that the LIM domain might be involved in the tension-dependent localization of proteins to cell–matrix adhesions. LIM-domain proteins of the adhesome such as the zyxin, Pdlim, Fhl, paxillin and Crp families have diverse molecular functions such as the regulation of actin and focal adhesion organization and signalling (reviewed in [64]). The LIM domain is a tandem zinc-finger structure that mediates protein–protein interactions in the cytoplasm and nucleus. Several protein–protein interactions in focal adhesions have already been previously assigned to this domain [64–66], and the recruitment of several LIM-domain adhesome proteins to focal adhesions formed on stiff substrates has been mapped to their carboxy-terminal LIM domains [67–68]. However, the search for a consensus protein-binding interface for LIM domains on the interacting proteins remains unsuccessful [64]. One example for a LIM-domain-mediated protein–protein interaction at cell–matrix adhesions is the interaction of migfilin with kindlin 2 [70]. Migfilin is believed to act as a molecular switch by disconnecting filamin from integrin tails, thereby regulating integrin activation and the dynamics of the ECM–actin link [71]. Mice lacking the Migfilin gene, however, developed normally [72] apart from experiencing mild effects on bone remodelling by osteoblasts [73]. Migfilin belongs to the zyxin-family of focal adhesion proteins, which could compensate for migfilin loss. Furthermore, stress situations

might be required to unravel the specialized functions of migfilin, as exemplified by the tumour suppressor function of the zyxin-family member Limd1 in a lung cancer model [74]. In contrast with its binding partner kindlin 2, the localization of migfilin to cell–matrix adhesions was highly sensitive to myosin II inhibition. In total, 22 out of 24 identified LIM-domain proteins found in the integrin adhesome were significantly reduced on myosin II inhibition, which led to the proposal that LIM domains might represent a biosensor for mechanical tension at cell–matrix adhesion sites [8]. Some previous observations support this hypothesis. For instance, it was shown that in stretched cells the LIM protein zyxin is predominantly found at focal adhesion or stress fibres bearing the highest force loads [75–77] and MLP (CSRP3) was shown to function as a stretch sensor at muscle Z-discs [78]. Zyxin family proteins, as well as most other LIM proteins in cell–matrix adhesions, have serial LIM domains present in a modular arrangement with defined linker distances between the LIM domains. Interestingly, single LIM-domain constructs of zyxin-family members never localized to cell–matrix adhesions, instead a cooperation of two or three domains is needed for targeting zyxin, Lpp and migfilin to focal adhesions [67–69]. It was suggested that the different LIM domains in those proteins cooperate in focal adhesion targeting by separately mediating interactions with different proteins within focal adhesions [64]. On the basis of the particularly interesting observation that changing the length of the linker sequence between LIM1 and LIM2 in Lpp was sufficient to destroy its focal adhesion localization [68], we speculate that alternative mechanisms might anchor serial LIM modules in proteins to putative structures that become apparent at adhesion sites only when they are under mechanical tension. Possible modes for the generation of a LIM-docking structure are exemplified by the stretch-induced binding of vinculin to talin or the stretch-induced phosphorylation of p130Cas [17,18], in which force-mediated exposure of cryptic motifs in proteins generate new high-affinity binding sites. Alternatively, polymeric structures, such as cross-linked F-actin filaments comprising regularly spaced binding sites for proteins, could induce an allosteric change of its structure under mechanical tension. As several LIM proteins bind to actin or actin-binding proteins [64], it is also conceivable that the disappearance of LIM proteins on blebbistatin treatment reflects an altered actin filament organization. Therefore, it might also be possible that LIM proteins are not directly recruited to integrin adhesion complexes, but rather to a focal adhesion-specific combination of F-actin and actin cross-linkers such as α -actinin, and that serial LIM domains act as a 'molecular ruler' for the distance of serial LIM-domain-binding sites. Future studies are needed to judge whether this putative strain-dependent docking structure exists and to characterize the precise molecular mechanism of force-induced recruitment of LIM-domain proteins to focal adhesion sites.

Prevalent functional domains in the integrin adhesome

Apart from the LIM domain the integrin adhesome is enriched for several other protein domains that are involved in protein–protein interactions at cell–matrix adhesions. Here, we briefly present the most prominent domains identified by proteomic analysis of the integrin adhesome. By using the proteomic workflow outlined in Fig 4A we analysed the differential adhesome composition of wild-type and integrin-deficient fibroblasts plated on different

Fig 4 | Proteomic analysis of cell-matrix adhesomes. (A) Workflow for quantitative mass spectrometry and bioinformatic analysis of purified cell-matrix adhesions, as described in [8]. (B) The adhesome components determined by proteomics were assessed for the abundance of distinct domains (PFAM, PROSITE). The pie chart depicts the quantitative distribution of protein domains. CAMSAP, calmodulin-regulated spectrin-associated protein; EGF, epidermal growth factor; FA, focal adhesion; FERM, 4.1 protein, ezrin, radixin, moesin; GAP, GTPase-activating protein; IRS-1, insulin receptor substrate 1; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC, liquid chromatography; LIM, Lin11-, Isl1- and Mec3; MS, mass spectrometry; PAGE, polyacrylamide gel electrophoresis; PDZ, PSD95, Dlg1, zo-1; PFAM, protein family; PH, Pleckstrin homology; PTB, phosphotyrosine binding; SDS, sodium dodecyl sulfate; SH2/3, src homology 2/3; SILAC, stable isotope labeling by/with amino acids in cell culture; WD, tryptophan-aspartic acid 40 repeat; WH1, WASP homology 1.

Sidebar A | In need of answers

- (i) Adhesome interactions—how is the formation of a cell–matrix adhesion initiated? What are the dynamics of protein–protein interactions at cell–matrix adhesion sites and what is the structure of these supra-molecular assemblies?
- (ii) Mechanotransduction—how is mechanical force translated into biochemical signals at cell–matrix adhesions? How are signals from cell–matrix adhesions spatially propagated to the actomyosin cytoskeleton? How does mechanical tension trigger the recruitment of proteins to cell–matrix adhesion sites? Is there a common force-dependent docking site recognized by serial LIM domains within cell–adhesome proteins?
- (iii) Signalling, specificity and diversity—how diverse is cell–matrix adhesion signalling in different cell types and tissues? Which proteins of the adhesome mediate integrin-subtype-specific functions? What are the molecular mechanisms of integrin crosstalk? Where and how are integrin and growth factor receptor signals combined? How and why do distinct cell–matrix signals sometimes promote and inhibit tumour progression?

substrates or treated with inhibitors. We used unsupervised learning methods, such as hierarchical cluster analysis [8], to correlate mass spectrometry-intensity profiles of the isolated proteins with the profiles of previously known focal adhesion proteins. By using this approach, we generated a list of candidate integrin-adhesome components [52]. In Fig 4B, we analysed this list for the abundance of protein domains and found that the most abundant protein domains in integrin-adhesome proteins were SH3, LIM, CH, SH2, protein kinase, PH and FERM domains. With the exception of the protein kinase domain, all of these domains have functions in protein–protein interactions and recruitment to cell–matrix adhesions. Interestingly, only the presence of a LIM domain gives proteins a statistically significant bias towards force-dependent recruitment to focal adhesions. Although this does not exclude the involvement of other domains in mechanotransduction processes, it indicates that LIM domains are unique in their function to determine force-dependent subcellular localization of proteins. Here, we describe the other prevalent protein family domains in the integrin adhesome and discuss possible roles in mechanosensing.

The SH3 domain is a small β -barrel fold of approximately 60 amino acids, which mediates protein–protein interactions. Typically, the SH3 domain binds to a polyproline type II helix within proline-rich motifs or to hydrophobic motifs in other proteins [79]. The affinity of SH3-domain-mediated interactions is rather weak—in the micromolar range—and therefore these protein–protein interactions are transient and flexible in nature, with the SH3 domain scanning multiple binding interfaces on the target protein [80]. Consequently, SH3 domains allow proteins to recognize multiple ligands. However, SH3 domain interactions are flexible and can act as a switchboard within which selective interactions are determined by either structural rearrangements of the whole protein or by post-translational modifications within the domain [32]. One example for such a selectivity is the observation that tyrosine phosphorylation within the SH3 domain of p130Cas inhibits its interaction with Fak and recruitment of Fak to focal adhesions [81]. As the SH3-fold is also regulated by allosteric mechanisms, it would be of particular interest to analyse the influence of force-mediated structural rearrangements on SH3-mediated protein–protein interactions.

The CH domain is an approximately 100-amino-acid long F-actin-binding domain. CH domains in adhesome proteins either come alone (for example, calponin and IQGAP), in tandem (for example, spectrins, filamins, α -actinins, plectins and parvins) or in two tandems with a total of four CH domains (for example, fimbrin and plastins; [82]). Apart from mediating actin binding, the CH domain also facilitates several actin-independent protein–protein interactions. For instance, Erk was shown to bind to the CH domain of several actin-binding proteins [83]. It remains to be seen whether these interactions are required for focal adhesion targeting of Erk. Another example is the CH2 domain of α -parvin, which binds to the LD1 motif of paxillin [84]. The tandem CH-domain proteins α -actinin and plectin were significantly enriched in focal adhesions relative to nascent adhesions [8], indicating that both are involved in adhesion maturation. Indeed, the focal adhesion resident plectin 1f isoform was shown to stabilize cell–matrix adhesion by connecting to the intermediate filament vimentin network [85].

The SH2 domain is approximately 100-amino-acids long and specifically recognizes peptide sequences with a phosphorylated tyrosine. As the cell–matrix adhesome is a major hotspot of phosphotyrosine signalling and SH2–phosphotyrosine interactions are crucial switches to assemble multiprotein complexes reversibly, it is important to analyse which SH2 switches are a direct result of force on cell–matrix adhesions. In contrast to serine/threonine and lipid kinases that existed before integrins evolved, tyrosine phosphorylation by tyrosine kinases, most notably Fak [86], and recognition of phosphotyrosine by SH2 domains evolved concomitantly with integrin-mediated adhesion signalling [87]. The protein tyrosine kinase domain is, similarly to all other protein kinase domains, composed of two lobes—an amino-terminal ATP-binding domain and a C-terminal substrate-binding groove and catalytic pocket, which are connected by a coiled peptide linker [88]. Kinase domain activity is controlled by allosteric mechanisms, as exemplified by the inactivation of Src kinase by the auto-inhibited globular protein conformation, which results in the misalignment of crucial residues in the kinase domain. The stability of the auto-inhibition of Src requires the cooperation of the SH2 and SH3 domains of the kinase. It was shown to be reduced by both mechanical tension at cell–matrix adhesions and integrin cytoplasmic tail binding [16,89], indicating that force-induced allosteric changes might directly activate kinases and thereby activate and contribute to integrin signalling.

The PH and FERM domains are implicated in locating adhesome proteins to the plasma membrane and to integrins. The PH domains are 100–200-amino-acids long and mediate binding to negatively charged phosphoinositides of the plasma membrane through their positively charged β -sandwich fold [90]. The FERM domain connects adhesome proteins with plasma membranes by binding lipids as well as β -integrin cytoplasmic tails [91]. FERM domains consist of three lobes with the phosphotyrosine binding fold in the F3 lobe mediating the interaction of the FERM-domain proteins talin, kindlin and Fak with integrins. FERM domains also mediate other protein–protein interactions at the adhesome as exemplified by the FERM-dependent binding of Fak to the Arp2/Arp3 complex [92]. Interestingly, the FERM domain of Fak can also fold back onto its kinase domain thereby inhibiting Fak activity [93], which might also be regulated by force-induced allosteric changes [94].

Concluding remarks

Despite the identification of such a plethora of focal adhesion proteins, many questions concerning their hierarchical organization, specificity, dynamics and mechanisms of molecular interactions, as well as their abilities to sense force and transduce biochemical signals remain unresolved (Sidebar A). To address these issues, we require a detailed knowledge of the overall architecture of the various types of adhesive structure, the identity of the molecular components, their precise multidimensional localization, the force ranges applied to adhesome proteins, as well as the dynamics, stoichiometry and hierarchy of protein–protein interactions. Ultra-resolution microscopy and cryo-electron tomography studies have begun to address the nanoscale architecture and spatial distribution of single proteins within cell–matrix adhesions [5,6,95,96]. The force ranges on some focal adhesion proteins have been explored by using biophysical methods [19,97] and, finally, the identity and regulation of the integrin adhesome has been analysed and refined by means of quantitative mass spectrometry [7,8,34, 52]. It is clear that additional biophysical and structural analysis of integrins and adhesome protein complexes is necessary to capture and understand the dynamics and details of molecular events that result in force-induced activation of downstream signalling pathways. Future research into that field could shed light on local force-dependent post-translational modifications in the adhesome and cytoskeleton, and their correlation with the magnitude and spatio-temporal distribution of applied forces. Furthermore, the dynamics of protein–protein interactions as a function of force and the identification of the elusive LIM-domain-binding motif at cell–matrix adhesions are important research directions. As cells co-express many integrins, the identity of cell–matrix adhesions and associated integrin signalling is highly diverse and potentially subjected to complex cross-regulation as shown for fibronectin-binding integrins [52]. However, the idea that this type of functional specification and differential nature of signals derived from single integrin subtypes and the mechanisms of receptor crosstalk might also operate for non-fibronectin-binding integrins awaits future investigation.

ACKNOWLEDGEMENTS

We thank Caroline C. Friedel (Ludwig-Maximilians-Universität, Munich, Germany) for support with the bioinformatic analysis of protein–protein interaction network data, Kay Hofmann (Milteny Biotech, Germany) for discussions and Carsten Grashoff, Richard Scheltema (Max Planck Institute of Biochemistry, Martinsried, Germany) and Roy Zent (Vanderbilt Medical Centre, Nashville, Tennessee, USA) for critical reading of the manuscript. Herbert B. Schiller was supported by a long-term fellowship of the European Molecular Biology Organization and Reinhard Fässler by the European Research Council, the German Funding Agency and the Max Planck Society.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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