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REVIEW

TITLE

Mechanosensitivity of integrin adhesion complexes: role of the consensus adhesome

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ABBREVIATIONS

ECM, extracellular matrix; FAK, focal adhesion kinase; FN, fibronectin; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GTPase, guanosine triphosphatase; IAC, integrin-associated adhesion complex; ILK, integrin-linked kinase; IPP, ILK-PINCH-Parvin complex; IQGAP1, IQ motif-containing GTPase-activating protein 1; LIM, Lin-11, Isl1, and Mec-3; MS, mass spectrometry; PINCH, particularly interesting new cys-his protein 1; SYNCRIP, synaptotagmin binding, cytoplasmic RNA interacting protein; VASP; vasodilator-stimulated phosphoprotein; VCAM-1, vascular cell adhesion molecule-1.

KEYWORDS

Cell adhesion; Consensus adhesome; Extracellular matrix; Focal adhesions; Integrins; Mass spectrometry-based proteomics; Mechanosensing

ABSTRACT

Cell and tissue stiffness have been known to contribute to both developmental and pathological signalling for some time, but the underlying mechanisms remain elusive. Integrins and their associated adhesion signalling complexes (IACs), which form a nexus between the cell cytoskeleton and the extracellular matrix, act as a key force sensing and transducing unit in cells. Accordingly, there has been much interest in obtaining a systems-level understanding of IAC composition. Proteomic approaches have revealed the complexity of IACs and identified a large number of components that are regulated by cytoskeletal force. Here we review the function of the consensus adhesome, an assembly of core IAC proteins that emerged from a meta-analysis of multiple proteomic datasets, in the context of mechanosensing. As IAC components have been linked to a variety of diseases involved with rigidity sensing, the field is now in a position to define the mechanosensing function of individual IAC proteins and elucidate their mechanisms of action.

Cell adhesion, integrin adhesion complexes and mechanotransduction

A requirement for a multicellular existence is the ability of cells to form higher order structures, tissues and organs [1]. To do this, cells must organise and integrate themselves with regard to each other and their external microenvironment, a feat that is achieved in part via cell surface adhesion receptors. Whilst cell-cell adhesion is mediated mainly by cadherins, cell adhesion to the extracellular matrix (ECM) is predominantly orchestrated by integrins. A multitude of integrin-ECM associations have been described [2], which provide direct physical connections between the ECM and the intracellular actomyosin cytoskeleton [3]. Integrins rely on the recruitment of multi-protein integrin adhesion complexes (IACs) to their cytoplasmic domains to mediate their functions, and transduce bidirectional signals with wide-ranging effects on development and disease [4]–[7].

Mechanotransduction, the ability of cells to sense force, whether generated externally via the extracellular matrix or internally by actomyosin-based contractility, is firmly established to play a key role in differentiation and proliferation [8]–[11]. Variation of cell and tissue stiffness results in altered transcriptional programming, thereby affecting stem cell lineage decision-making, and is also associated with diseases such as cancer and fibrosis [10], [12]. Cells sense force via a variety of plasma membrane receptors including integrins [13], [14]. As integrins and IACs form linkages between cells and their microenvironment, they can act as mechanochemical signalling centres [14], [15]. Indeed many IAC components are linked to a variety of diseases involved with rigidity sensing [16] and proteins such as vinculin, talin and p130Cas transmit forces to actin via conformational changes [17]–[20]. Moreover IACs relay force to the ECM to regulate durotaxis [21], and integrins themselves act as mechanosensory components of IACs. Force affects the strength of the interaction between $\alpha 5 \beta 1$ and its ECM ligand fibronectin (FN) [22], and $\alpha 5 \beta 1$ forms force-stabilised catch bonds that undergo cyclic mechanical reinforcement [23], [24]. Together, these studies demonstrate that the ECM-integrin-IAC axis is a key regulatory component of the mechanosignalling pathways that determine cell and tissue fate.

Proteomic analysis of adhesion complexes – definition of a consensus integrin adhesome

To understand how mechanical links are formed between the ECM and the intracellular environment, the molecular composition of IACs has been studied extensively using candidate-based microscopy and biochemical approaches. The wealth of information gained from these studies, which includes work on different cell types and under different experimental conditions, has been curated into a hypothetical integrin adhesome [16], [25], [26]. To provide an unbiased view of IAC composition and function [27], the global composition of IACs has recently been characterised using protocols to isolate the adhesion nexus biochemically coupled with mass spectrometry (MS)-based proteomic analysis [28], [29]. These methods have been used to analyse IAC composition from a variety of cell types and conditions, including mesenchymal stem cells [30], [31], and the effects of different ECM ligands or integrin heterodimers [32], [33], [34], [35], microtubule polymerisation inhibition by nocodazole [36], [37], and myosin-II inhibition by blebbistatin [35], [38], [39] on IAC composition. In addition, the phosphorylation profile of isolated IACs has been reported [40], which identified novel

phosphorylation sites on IAC components and regulators of adhesion signalling. These datasets provide context-dependent compositional snapshots of IACs at particular time points and have suggested an underestimation of the scale and complexity of IAC organisation at the molecular level.

To create a resource for further analysis of IACs, we have computationally integrated several IAC proteomes. These datasets were generated from multiple cell types, using diverse methods from different laboratories [41]. The resulting experimentally defined 'meta-adhesome' database contains 2,412 proteins that are enriched to IACs recruited to FN in at least one of the seven MS datasets [41]. Along with functions classically associated with cell adhesion, the meta-adhesome database contains proteins linked to a wide variety of cellular functions that have not currently been firmly connected to adhesion. Furthermore, an emergent property of the meta-adhesome is the definition of an IAC core of 60 commonly identified proteins, termed the consensus adhesome. Analysis of the protein-protein interaction network of the consensus adhesome identifies four interconnected axes that form the integrin-actin structural connection. These axes centre on the established IAC components of kindlin-ILK (integrin-linked kinase)-PINCH (particularly interesting new cys-his protein 1), FAK (focal adhesion kinase)-paxillin, talin-vinculin and α -actinin-zyxin-VASP (vasodilator-stimulated phosphoprotein) (Fig. 1). Proteins that directly linked integrins with actin are α -actinin, filamin, talin, tensin and, via a low-evidence $\alpha 5\beta 1$ integrin interaction, FHL3. Other associated molecules may function to stabilise the connection of these integrin-actin linkers, such as PDLIM1 and PDLIM5, in facilitating the integrin- α -actinin-actin connection, or migfilin in bridging the connection between kindlin and actin via filamin.

The consensus adhesome represents commonly identified IAC proteins in the context of cellular attachment to FN via the $\alpha 5\beta 1$ and $\alpha V\beta 3$ FN-binding integrins (Fig. 1). One outstanding question is whether the composition of the consensus IAC changes in an ECM ligand- or integrin subunit-dependent manner? Since only 10 out of 60 consensus adhesome proteins were identified in two vascular cell adhesion molecule-1 (VCAM-1)-induced IAC datasets [32], [34], it appears likely that the consensus composition is determined by the specific integrin-ligand combination (proteins common to both FN and VCAM-1 datasets were α -actinin-4, annexin A1, filamin C, IQGAP1 (IQ motif-containing guanosine triphosphatase (GTPase)-activating protein 1), $\beta 1$ integrin, SYNCRIP (synaptotagmin binding, cytoplasmic RNA interacting protein), talin, VASP, vinculin and zyxin). In addition, ADP-ribosylation factor 1 (ARF1) was not detected in any FN-induced IAC datasets used to construct the meta-adhesome, but was identified in VCAM-1-induced IACs [32], [34]. Additional studies that analyse IAC composition from cells exposed to different ECM microenvironments will help to determine ECM-dependent changes in IAC composition. Another unanswered question is what is the composition of IACs from cells in longer-term cell culture or *in vivo*? IAC structures derived from cells in longer-term culture may be more similar to IACs from cells *in vivo* as cells are exposed to a complex cell-derived ECM and not one ECM substrate. However, an alternative view could be that cells in long-term culture are still cultured on rigid substrates and the composition of IACs is likely to be different to their counterparts *in vivo*, where rigidities are several orders of magnitude below plastic [42], [43]. Future studies that investigate the composition of IACs *in vivo* and from cells in 3D

environments [44] will be useful in determining the functional contribution of IACs in their native environments.

The consensus adhesome is enriched in actin-binding proteins, and the force-sensitive LIM (Lin-11, Isl1, and Mec-3) domain [45] was the most significantly enriched protein module [41], emphasising a potential role for the consensus adhesome in linking integrins to actin (Fig. 1). This structural connection is required for dynamic exchange of both chemical and mechanical signals across the plasma membrane [5], [46]. In contrast, the consensus adhesome contains a low proportion of signalling molecules (kinases, phosphatases, guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and GTPases) from the literature-curated integrin adhesome [16], [25], [26]. These differences may be methodological, as structural components are likely to be more stable in IACs and are therefore isolated more easily, while signalling components interact transiently and may be lost upon IAC isolation. This raises an important issue regarding the nature of the consensus adhesome and, ultimately, how IACs are defined. Many studies use canonical IAC components such as vinculin or paxillin to define IAC areas, and proteins were characterised as literature-curated adhesome components if they colocalised with integrins and/or were functionally involved in adhesion [26], [47]. However, IACs do not have a clear boundary, are highly dynamic and should not be considered a classical organelle, so it is difficult to define exactly where an IAC begins and ends [27], [47]. Our analyses suggest that the IAC isolation methods isolate the cellular environment proximal to IACs, which may include short struts of actomyosin that are linked to IACs. Therefore, it is the IAC together with proteins in the vicinity of the IAC that is isolated and analysed by MS. We conclude that the consensus adhesome represents the intrinsic, core components of IACs that regulate the integrin-actin structural connection, while the non-consensus meta-adhesome proteins likely represent additional associated proteins located in the vicinity of this core connection.

Effects of myosin-II-generated force on the consensus adhesome

IACs are highly dynamic structures whose maturation and turnover is dependent on force generated by the actomyosin machinery [48]. This dynamism is manifested in different types of IAC structures such as nascent adhesions, focal complexes, focal adhesions, fibrillar adhesions, invadopodia and podosomes [49]–[52]. Cells generally contain a varied array of these IAC structures at any one time and therefore analysis of isolated IACs by MS results in the combined analysis of a heterogeneous population of different types of IAC depending on their size, lifetime and maturation state.

Several studies have sought to investigate the global effects of IAC maturation state and tension on IAC composition by treating cells with the myosin II inhibitor blebbistatin, which causes IACs to disassemble [35], [38], [39,], [41]. IACs isolated from blebbistatin-treated cells represent a more homogeneous population of smaller nascent adhesions and focal complexes. These studies revealed that the number and abundance of proteins recruited to IACs was reduced in myosin II-inhibited cells. Examination of the functional roles and structural domains of the myosin II-sensitive proteins revealed significant enrichment for proteins containing the LIM domain [45], which was in agreement with other studies that implicated the LIM domain-containing IAC proteins zyxin and paxillin in force sensing [53],

[54]. Conversely, a small proportion of proteins identified by MS are enriched in IACs after myosin II-inhibition, and one such protein, the Rac GEF β -PIX, negatively regulates IAC maturation and cell migration [38]. To examine further the effects of force on IACs and the roles of different integrins during IAC maturation, IACs have been isolated from cells expressing α V-, β 1-, or α V and β 1- class integrins that were treated with blebbistatin [35]. Expression of α 5 β 1 integrin resulted in the recruitment of the canonical IAC proteins talin, kindlin and ILK to IACs, but not the LIM domain-containing proteins upon blebbistatin treatment, which is consistent with studies that isolated IACs from blebbistatin-treated cells [38], [39]. However, in cells that did not express α 5 β 1 integrin but uniquely expressed α V integrins, both canonical and LIM domain-containing IAC components are reduced upon blebbistatin treatment, which indicated that α 5 β 1 integrins, but not α V integrins, are able to recruit IAC molecules in the absence of myosin II-mediated tension [35]. These data suggested that integrins have distinct functional roles during IAC maturation and in force sensing [45]. This view is supported by a recent study that demonstrated that cells mainly using α V β 3 integrins are more able to respond to ECM-generated force modulation and stiffness changes than cells mainly using α 5 β 1 integrins, which therefore suggests that regulation of the relative amounts of these integrin heterodimers can affect force transmission and mechanosensing at sites of cell-ECM adhesion [55].

Taken together, these studies have generated valuable insights into the identity of mechanosensitive proteins in IACs, such as LIM domain-containing proteins [45]. We analysed the effects of blebbistatin treatment that were reported in three datasets generated in different laboratories [38], [39], [41] on the full complement of consensus adhesome components (Fig. 2). Unsurprisingly, these analyses support previous observations regarding the force-sensitive nature of LIM domain-containing proteins in IAC proteomes (bold text, Fig. 2). However, these analyses also reveal that the majority of the consensus adhesome is at least partially reduced upon inhibition of actomyosin-generated contractility (red nodes, Fig. 2). In contrast, the larger cohort of meta-adhesome proteins was less affected by blebbistatin treatment than the consensus adhesome (average Ctrl/Blebb fold change, 1.2 vs. 2.1, respectively). In particular, proteins centred on the α -actinin-zyxin-VASP axis are most sensitive to blebbistatin treatment, and α -actinin and VASP are two of the most blebbistatin-sensitive proteins that do not contain a LIM domain (Fig. 2). Proteins in the kindlin-ILK-PINCH and talin-vinculin axes are also reduced, but to a much lesser extent (Fig. 2). We conclude that the network view of commonly identified proteins in the consensus adhesome represents a structural core IAC composition in the context of FN-mediated cell adhesion that is affected by force modulation. However, it is not known how much of the true mechanosensitive IAC proteome is captured by using blebbistatin as a readout of IAC mechanosensing and it will be interesting to assess the role of ECM stiffness and other mechanical cues on IAC composition in future studies. In the remaining part of this review we will address the evidence for the roles of some of the commonly identified consensus adhesome components in mechanosensing and force transmission at adhesive contacts and how they regulate cell function. Vinculin and talin will be covered elsewhere in this special issue; therefore, they are not discussed further here.

Examples of consensus adhesome links to mechanosensing and force transduction

The α -actinin, zyxin and VASP axis

Zyxin relocates to actin stress fibres (SFs) in response to cyclic stretch in fibroblasts, resulting in thickening of the SFs, an effect that is lost in zyxin-null cells [56]. VASP also relocates to SFs after stretching, in a zyxin-dependent manner. Estimation of the unbinding rate for zyxin at IACs after pharmacological inhibition of cytoskeletal tension, changes in matrix compliance, and laser ablation of individual SFs in endothelial cells, confirm its mechanosensitive behaviour [57]. Based on correlated changes in forces within laser ablated SFs, mathematical modelling and zyxin localisation, zyxin appears to localise at sites of force application *in vivo* in *Drosophila* [58]. Myosin II activation is required for localisation of zyxin at force-bearing sites [54], [57], whereas the three LIM domains of zyxin are necessary for its mechanosensitive function [54]. In addition, zyxin has been shown to allow actin polymerisation at adhesion sites [59] and to be required for SF maintenance and repair [60]. Zyxin also promotes VASP localisation at strain sites along the SFs, while interaction with VASP is required for zyxin to rescue SF repair defects in zyxin-null cells [60] and to induce SF thickening after cyclic stretch [61]. These observations highlight the relevance of the zyxin-VASP interaction for cell mechanosensing. In addition, zyxin has been shown to translocate to the nucleus and mediate mechanosensitive gene expression after cyclic stretch in vascular smooth muscle cells [62] and endothelial cells [63], and to mediate the contractile response after force application by atomic force microscopy using FN-coated beads [64].

Mechanotransduction roles for α -actinin have also been proposed, acting as a scaffold for tension-sensitive proteins [65]. A recent study using magnetic tweezers and FN-coated beads showed that α -actinin is required for reinforcement of adhesion sites in fibroblasts, and its depletion led to increased applied force on FN-coated polydimethylsiloxane pillars [66].

The FAK and paxillin axis

Although paxillin localisation is insensitive to myosin II inhibition or matrix compliance [67], phosphorylation at Y31/Y118 is reduced after blebbistatin treatment, and a phosphomimetic version of paxillin rescues vinculin localisation to small adhesions after myosin II inhibition, indicating that paxillin phosphorylation is required for vinculin recruitment at immature adhesions [67]. These data suggest a role for paxillin phosphorylation in mechanosensing, recruiting adhesome components and possibly mediating intracellular signalling in response to mechanical cues. In agreement with this, cyclic stretch induced phosphorylation of paxillin and paxillin-dependent endothelial cell permeability [68]. However, shear forces have been shown to reduce paxillin phosphorylation and turnover [69]. Therefore, different types of force may differentially regulate paxillin phosphorylation and its mechanosensing activity.

FAK and paxillin act together to mediate the migration of cells across a gradient of ECM rigidity, a mechanosensitive process known as 'durotaxis' [21]. When traction forces exerted by mature focal adhesions (FA $\geq 1.5 \mu\text{m}$) are measured using traction force microscopy in mouse fibroblasts cultured on FN-coated polyacrylamide (PA) gels, two populations of adhesions are found, and 'tugging' FAs

show an increase in traction forces. Soft substrates (8.6 kPa PA gels) are associated with increased tugging FAs, whereas more stable FAs are seen on rigid substrates (32 and 55 kPa gels). This observation is interesting, since rigidities of some tissues *in vivo* are below 30 kPa [70]. This study also showed that the FAK-paxillin axis is required for FA traction at higher rigidities (8.6 kPa), but reduction of extracellular stiffness is sufficient to trigger FA dynamics. Significantly, perturbation of paxillin phosphorylation results in reduction of the range of ECM rigidities where durotaxis occurred. Therefore, FAK and paxillin are required for cells to sense and respond to the rigidity of the ECM, at least within a specific range.

Paxillin is also recruited earlier than zyxin to strain sites in SFs [71], [72]. Interestingly, paxillin is recruited to, and stabilises, sites of SF strain independently from zyxin [71]. The role of SFs in mechanosensing has been reviewed recently [73]; however, the relevance of stress fibre repair and the role of zyxin and paxillin in the context of mechanosensing *in vivo* remain to be addressed.

The role of FAK in mechanosensing has been well characterised, responding to cyclic stretch, shear stress and substrate rigidity, among other cues [74]. Recent evidence has revealed a role for FAK in mechanosensitive cell cycle progression [11]. Substrate stiffness regulates cell proliferation and cyclin D1 expression and FAK is activated on stiff substrates. However, active FAK is unable to rescue cyclin D1 expression in soft substrates, while inhibiting FAK abrogates cyclin D1 induction in stiff substrates [11]. Inhibition of Rac also reduces cyclin D1 levels downstream of FAK. However, activation of Rac was again unable to rescue cyclin D1 induction in soft substrates, suggesting additional effects of ECM stiffness on the cell cycle [11]. A second study from the same group further defined this mechanosensitive pathway, involving FAK, p130Cas, Rac and the protein lamellipodin, regulating S phase entry and cyclin D1 expression [75]. Arterial stiffening was observed after injury [11]. FAK is phosphorylated in smooth muscle cells after arterial injury, whereas deletion of FAK decreases the number of cells in S phase, highlighting the relevance of the FAK/Rac pathway *in vivo* [76]. FAK is also required for mitotic spindle orientation by a mechanism depending on FAK-paxillin interaction, and to reorientate the mitotic spindle in response to mechanical compression in *Xenopus* embryos [77].

FAK has also been implicated in mechanosensing during fibrosis, where forces are exerted by the hypertrophic response accompanying cutaneous injury. FAK transduces mechanical forces in this context, activating ERK and inducing the secretion of the chemokine MCP-1 [78]. FAK is phosphorylated at Y397 after cutaneous injury, and is further activated on mechanical loading [78]. In a model of injury repair in the presence of mechanical loading, surface scarring is reduced in mice with FAK-null dermal fibroblasts; interestingly, this correlates with decreased proliferation [78]. Static strain applied to wild type and FAK null fibroblasts corroborates the mechanosensitive activation of the FAK/ERK axis, whereas pharmacological inhibition of FAK reduces scar formation [78]. Subsequent experiments from the same group confirmed the relevance of FAK for mechanosensing in keratinocytes and in wound healing *in vivo* [79]. In summary, these studies highlight the relevance of FAK mechanosensing in cell and tissue homeostasis and repair.

The ILK, PINCH and parvin (IPP) axis

The IPP complex has not been as well characterised in the context of mechanosensing. However, evidence from *in vivo* and clinically relevant *in vitro* models suggests a mechanosensing role for these proteins. A mutation in zebrafish ILK that abrogates its association with β -parvin impairs cardiac function without affecting cardiac morphology or ILK localisation at the sarcolemma, placing ILK as a putative component of the cardiac stretch sensor [80]. Loss of ILK also affected cardiac structure and function in mice [81] and, more recently, ILK has been implicated as a mechanosensitive force transduction in cardiomyocytes, by regulating the Ca^{2+} ATPase SERCA-2a [82]. Furthermore, when high pressure is applied to carotid arteries, ILK expression is increased and the ILK-paxillin interaction is induced [83]. ILK is also required for cell migration in Caco-2 cells subjected to cyclic mechanical deformation in scratch assays [84], and its deficiency leads to decreased traction forces generated by VSMCs in response to stiffness on PDMS micropillars [85]. PINCH is stabilised in VSMCs subjected to stretch [86], whereas loss of function of PINCH in zebrafish led to ILK instability, cardiac failure and decreased expression of stretch-responsive genes [87]. Despite the growing evidence from cardiac cells, evidence from other systems, as well as studies assessing the response of these proteins to different mechanical cues, are needed to support a role of the IPP complex in mechanotransduction.

CONCLUSIONS AND FUTURE PERSPECTIVES

Here we review the role of consensus adhesome components in mechanotransduction. It should be noted that the consensus adhesome represents commonly identified components from the MS analysis of IACs isolated on FN [41]. As a consequence it is enriched for adaptor and actin-binding proteins of the literature curated adhesome and likely represents a stable core integrin-actin linkage isolated from the plasma membrane and cytoplasmic environment of integrins, and captures only a smaller complement of the transiently-associated signalling adhesion components. In this regard, it will be interesting to integrate consensus proteins with the contractome [88], a recently described systems view of actomyosin contractility in non-muscle cells, to assess how adhesion and contraction regulation are integrated. Several outstanding questions remain: Does the consensus composition reflect the heterogeneity of adhesion structures (fibrillar adhesions, focal adhesions, focal complexes and nascent adhesions) observed in cells? Is the same complement of proteins recruited to other ECM ligands or via alternative integrin heterodimers? How does the consensus differ in 3D environments or *in vivo*? Does matrix elasticity regulate consensus composition in a different way to cytoskeletal tension? These issues, along with the roles that the force-sensing consensus components play in disease, will no doubt be addressed by future experiments. In addition, as cells propagated in culture acquire a memory of their past mechanical environment [89] a detailed understanding of the mechanisms of force sensing may find applications for mesenchymal stem cells used in cell therapies or tissue engineering as these approaches require an understanding of how to maintain and differentiate MSCs both *in vitro* and *in vivo* [90], [91].

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FIGURE LEGENDS

Figure 1. The consensus integrin adhesome. A schematic representation of the consensus adhesome: proteins that were at least two-fold enriched to FN-induced IACs compared with complexes isolated from negative control ligand conditions in at least five IAC datasets (excluding ECM components). The resulting 60 proteins identified represent the consensus adhesome [41]. The schematic shows the main interactions between 42 proteins that interact with each other, or actin, based upon interaction evidence reported in the literature [41]. Thick black node border indicates literature-curated adhesome protein [16]. Interactions were manually validated and scored (high, medium, low) according to the level of experimental evidence for that interaction shown by the thickness of the grey edges [41]. Bold text indicates LIM domain-containing protein and yellow node indicates actin-binding protein, as reported in InterPro [92]. Actin was not present in the consensus adhesome but is depicted for illustrative purposes. While two α -actinin isoforms (α -actinin-1 and -4) were incorporated in the consensus adhesome, α -actinin is depicted as one node. Unconnected consensus adhesome components not shown are: ALYREF, BRIX1, DDX18, DDX27, DMT1, DNAJB1, FAU, FEN1, H1FX, HP1BP3, LIMD1, MRTO4, P4HB, POLDIP3, PPIB, RPL23A, SIPA1 and SYNCRIP.

Figure 2. The force-sensitive consensus adhesome. Proteins identified in the consensus adhesome were analysed in the context of three datasets that reported changes in IAC composition upon inhibition of actomyosin contractility by blebbistatin treatment before mass spectrometry. **(A)** The $\log_2$ fold change in the abundances of consensus adhesome components identified by mass spectrometry in IACs isolated from untreated cells (Ctrl) and blebbistatin-treated cells (Blebb) in each study is plotted as coloured circles. The median $\log_2$ ratio is also displayed (black circle). Proteins are indicated by protein name and gene name. LIM domain-containing proteins are indicated by bold text. MEF, mouse embryonic fibroblast cells (red; [41]); MKF, mouse kidney fibroblast cells (blue; [39]); HFF, human foreskin fibroblast cells (green; [38]). **(B)** The median $\log_2$ (Ctrl/Blebb) ratio was mapped onto the consensus adhesome interaction network (as depicted in Fig. 1) to reveal the force-sensitive nature of the consensus adhesome. Node colour is proportional to the median $\log_2$ (Ctrl/Blebb) ratio.

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