Matrix mechanics and receptor–ligand interactions in cell adhesion

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Cell adhesions to both soluble and insoluble extracellular matrix ligands are critical in inter and intra-cellular signaling that mediates numerous physiological processes. These adhesions are complex structures composed of many scaffolding and signaling proteins. There are four distinct types of cell–matrix adhesions: focal complexes, focal adhesions, fibrillar adhesions, and 3D cell–matrix adhesions, which vary in composition, organization and function. The primary mediators of cell–matrix adhesions are integrins, which are mechanosensitive transmembrane receptor proteins that directly bind to matrix ligands to initiate adhesion formation. The development of cell–matrix adhesions is affected by a number of factors including matrix properties such as dimensionality and rigidity, and forces, both internally and externally generated, exerted on the adhesion sites. In this article, we discuss how matrix mechanics and forces affect the assembly and maturation of cell–matrix adhesions.

Introduction: matrix mechanics regulate cellular processes

Cell–cell adhesions and cell–matrix adhesions are involved in a myriad of signaling pathways and are crucial to maintaining the structural integrity of tissues. The mechanical properties of the cell's environment, including the surrounding extracellular matrix (ECM) and neighboring cells, play a major role in modulating adhesion assembly and consequently cell function, since they affect how forces are detected and transmitted. Cells are subject to both internally generated forces as well as external forces, such as fluid shear stress in the vasculature and tensile pulling by a rigid microenvironment. The effect of substrate rigidity on cell behavior in particular has perhaps been most thoroughly investigated. Matrix stiffness has been demonstrated to affect cell morphology,[1] migration,[2,3] differentiation,[4] and proliferation and apoptosis.[5] Different responses in cell motility to stiffness have been observed for cells in two-dimensional (2D) and three-dimensional (3D) matrices,[6] indicating that dimensionality is also a major physical factor. However, the mechanisms of mechanotransduction, or how the cell transduces a mechanical signal into a biochemical response, are not yet fully understood. In this Emerging Area, we focus on the effects of force and matrix mechanics on receptor–ligand interactions and the formation of cell–matrix adhesions.

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Integrins are the primary mediators of cell–matrix adhesions

Cell–matrix adhesions are primarily mediated by integrins, which are transmembrane receptors that link the cytoskeleton to the ECM.[7] The ECM *in vivo* is a complex, varied network structure,

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composed of collagen and other structural fibrous proteins in addition to non-fibrous elements including adhesive molecules such as fibronectin, and proteoglycans which maintain network hydration to resist compressive forces.[8,9] ECM architecture and composition varies from tissue to tissue, and consequently the matrix exhibits a range of mechanical properties depending on the tissue of interest. For example, brain tissue is more compliant than muscle, which in turn is more compliant than skin.[10]

Matrix stiffness is a significant factor in the detection and transmission of forces and subsequent cellular responses, as briefly discussed earlier. The elastic modulus E is a measure of a material's resistance to stress, given by the force applied divided by the observed displacement (or the observed force exerted in response to a given displacement), and is a parameter frequently varied by modifying the cross-linking and density of a substrate to evaluate the effect of matrix mechanics on cell behavior. Cells exert a contractile force against the matrix, known as a traction force, in response to tensile loading. The force is transmitted through the cytoskeleton by way of Rho-activated actomyosin machinery. Cells on more rigid substrates are more contractile and produce traction forces of greater magnitude than cells on flexible matrices,[11] perhaps due to the greater resistance stiffer matrices pose to deformation. Consequently, a greater contractile force may then be required to maintain matrix strain.[10]

Integrins are in large part responsible for sensing the mechanical properties of the cell's environment. Integrins are heterodimers, composed of non-covalently bound α and β subunits. There have been 18 α and 8 β subunits discovered in mammalian cells, comprising 24 different integrin structures.[12,13] The extracellular domain binds to ECM ligands, including fibronectin, vitronectin, laminin, and collagen,[12,13] resulting in integrin clustering due to cytoskeletal reorganization. The cytoplasmic domains of the integrins then bind to proteins at the site of clustering to assemble complexes involved in cytoskeletal linkage and signaling.[14] Proteins that directly bind to the cytoplasmic domain of integrins include talin, which in turn binds adaptor scaffolding proteins, such as vinculin, paxillin, and α-actinin, that link to the cytoskeleton; enzymes are also recruited to adhesion sites, specifically kinases and phosphatases such as focal adhesion kinase (FAK) and receptor protein tyrosine phosphatase-α (RPTP-α), which are crucial in signal transduction.[14]

Integrins can respond to intracellular stimuli (inside-out signaling, resulting in changes to integrin conformation and subsequently ligand binding affinity) or extracellular stimuli (outside-in signaling, resulting in signal transduction through the cytoplasm).[13,14] $\alpha_v\beta_3$ integrins, for instance, can bind either vitronectin or fibronectin with different conformations of their extracellular domain.[15] $\alpha_v\beta_3$ integrin more strongly binds fibronectin, with vitronectin binding induced only once the β_3 subunit has been phosphorylated on the cytoplasmic side, which occurs upon cytoskeletal binding to β_3.[15] Therefore, vitronectin–$\alpha_v\beta_3$ integrin binding is reinforced upon cytoskeletal protein recruitment to β_3 domains in an example of inside-out signaling.[15] Similarly, it has been experimentally demonstrated that the conformation of $\alpha_5\beta_1$ integrin changes upon ligand binding,[16] initiating an internal cellular response to extracellular stimuli. In another example of outside-in signaling, application of a twisting force through magnetized ligand-coated beads to β_1 integrins results in focal adhesion formation, and the subsequent stiffening of the cytoskeleton is directly proportional to the force exerted, clearly illustrating the mechanosensory function of integrins.[17] Integrins are therefore bidirectional conduits for biochemical and mechanical information.

Force and matrix mechanics affect the assembly and maturation of cell–matrix adhesions

The process of cell–matrix adhesion formation and maturation has been discussed extensively.[7,14,18–24] Integrins bind to their respective ECM ligands, which leads to integrin clustering and the activation of enzymes including tyrosine and serine/threonine kinases and phosphatases that propagate the signal. A variety of molecules are then recruited to sites of integrin clustering to form large multimolecular adhesion complexes. In 2D systems, adhesions develop from early focal complexes into focal adhesions, which then may mature into fibrillar adhesions. Cells in 3D matrices develop 3D cell–matrix adhesions, which are distinct from those formed on 2D substrates.

Focal complexes

There are a number of different definitions of focal complexes as discussed by Geiger *et al.*: spatial (small adhesions ~1 μm^2 in area composed of integrins and other molecules localized to the lamellipodium edge), temporal (earliest focal adhesions), and signaling (Rac-inducible adhesions).[25] We assume here that focal complexes encompass all three definitions. Early matrix adhesions known as focal complexes are assembled in the ruffling lamellipodium at the leading edge in a process regulated by the small G protein Rac, a mediator of actin polymerization.[26] Focal complexes, also referred to as nascent adhesions,[18] are small, ephemeral, dot-like structures characterized by the colocalization of $\alpha_v\beta_3$ integrin, paxillin, talin, vinculin, FAK, and phosphotyrosine.[7,19,27,28]

Focal complex formation can be induced by applying forces to integrins,[17,26,29,30] either externally or internally.[23] Matrix stiffness promotes integrin clustering[11] and early integrin–cytoskeletal linkages are reinforced proportionally on rigid substrates, as more resistance and correspondingly increasing force is applied.[31] Talin1,[29] RPTP-α,[30] and vinculin[26,32] are important in the force-dependent strengthening of integrin–cytoskeletal linkages and the stabilization of initial cell–matrix contacts into focal complexes. Talin1, which can directly bind to integrins and actin, also contributes to the recruitment of paxillin and vinculin at initial adhesion sites.[29] Vinculin adhesion site localization increases with force,[26,33] which is not surprising given that cryptic vinculin binding sites on talin are exposed upon stretching of the talin rod.[34] Inhibiting myosin kinase inhibits vinculin recruitment,[26] suggesting that cell contractility is involved in generating the forces involved in exposing vinculin binding sites. Cells must consequently be seeded on stiff substrates to induce vinculin binding to integrin adhesion sites.[11] Once bound and activated, vinculin then may induce actin cytoskeletal reorganization to upregulate traction force generation by the cell at adhesion sites.[34]

Focal adhesions

Focal complex maturation into focal adhesions requires the GTPase Rho, and like focal complexes can also be induced by the application of force.[17,35] Focal adhesions, also known as

focal contacts, are marked by paxillin, vinculin, phosphotyrosine, FAK, phosphorylated FAK, $\alpha_v\beta_3$ integrin, tensin, and zyxin.[19,27] Applying force induces RhoA/Rho kinase (ROCK) signaling pathway-dependent α-actinin expression[36] and phosphorylation of FAK and extracellular signal-regulated kinase (ERK) 1/2.[37] Additionally, force application triggers the local assembly of actin and myosin into stress fibers that exert contractile force and cytoskeletal tension,[35,36] and focal adhesion formation and elongation centripetally in response to the force.[11,35] Myosin IIa is required for focal adhesion stabilization but not for nascent adhesion formation.[18] Adhesion size corresponds to force, either cell-generated[33] or external[11] when above 1 μm^2; below the 1 μm^2 area threshold, there was no correlation in force and adhesion size.[38] The area of focal adhesions in turn corresponds proportionally to adhesion strength, integrin–ligand binding, and vinculin and talin recruitment up to a point before plateauing.[39] Perturbing actomyosin results in the shrinkage of adhesion sites, indicating the importance of contractile force in focal adhesion formation.[33] Vinculin has been shown to be crucial to the formation of contractile force-bearing stress fibers, and a factor in the recruitment of α-actinin and paxillin.[32] Zyxin also plays a major role in facilitating actin polymerization in response to force at adhesion sites.[40] In migrating cells, focal adhesions exert weaker forces than focal complexes, suggesting that maturing adhesions serve more as anchorage sites rather than propulsive sources of force, a function that focal complexes, localized to the leading edge, apparently serve.[41,42] Recently, "supermature" focal adhesions that are larger (8–30 μm long in contrast to classical focal adhesions which are 2–6 μm long) and are capable of transmitting four times greater stress (~12 nN μm^{-2}) were found to form on deformable substrates and to recruit α-smooth muscle actin (α-SMA) to stress fibers in response to high tension.[43] Supermature focal adhesion formation and α-SMA recruitment is constrained by seeding the cells on soft substrates and on non-deformable 6 μm micropatterned islands.[43]

On stiffer substrates, cells form stable focal adhesions,[10,11,26,44] have upregulated expression of integrins[1] and stress fibers,[1,11] phosphorylate FAK,[11] and exert greater traction forces.[11] In contrast, cells on compliant substrates instead form dynamic adhesions[2,10,41] with downregulated phosphotyrosine levels,[2] resulting in cells that migrate faster[2] and are not as spread.[1,2] Inhibiting tyrosine phosphatase stabilizes focal adhesions on flexible matrices while inhibiting myosin and cell contractility results in reduced recruitment of vinculin and decreased phosphotyrosine.[2] A recently proposed model suggests that the size of focal adhesions and the timescale of focal adhesion formation scales with matrix compliance, with small focal adhesions forming quickly on flexible substrates.[45] It should be noted, however, that cellular response to matrix mechanics is also dependent on cell phenotype. For example, neutrophils, which normally are flowing in blood rather than adhering to stiff substrates, can spread as well on stiff as on very soft (~2 Pa) surfaces.[1] Most of the work on cell adhesion though has been performed using fibroblasts, which have been shown to be highly sensitive to matrix mechanics.

Fibrillar adhesions

Focal adhesions can further develop into fibrillar adhesions, which mediate fibronectin assembly into a fibrillar matrix. Fibrillar adhesions are characterized by $\alpha_5\beta_1$ integrins and tensin.[19,46] Since β_1 integrin binding to fibronectin is not as stable as α_v integrin binding to vitronectin, which is a comparatively more rigid, fixed ligand, actomyosin contractile activity pulls on fibronectin fibers and β_1 integrins move from focal adhesions to the ECM to promote fibrillogenesis.[20,46] When force is applied to fibronectin, the protein unfolds partially to expose self-recognition sites,[47] facilitating fibronectin fibril assembly in a Rho-requiring contractility-dependent process.[48]

3D cell–matrix adhesions

3D matrix adhesions are distinct from the previously discussed adhesions, which have primarily been observed in 2D studies. 3D adhesions are marked by paxillin, vinculin, phosphotyrosine, FAK, $\alpha_5\beta_1$ integrin, and tensin,[19,49] and are formed at ECM sites containing fibronectin.[50] In 3D adhesions, only paxillin is phosphorylated while in 2D systems, as mentioned earlier, FAK is strongly phosphorylated too.[50] Vinculin is downregulated in 3D adhesions as well.[51] Blocking α_5 and β_1 integrins results in 2D-like focal adhesions.[50]

On stiffened 3D substrates, 2D-like adhesions are formed,[50] showing FAK phosphorylation.[11] Furthermore, inducing cells in 3D matrices to constitutively express activated RhoA to increase contractility (which has previously been linked to matrix stiffness,[11] though in this study, the correlation between contractility and matrix rigidity was only found at high RhoA levels) results in the formation of 2D-like focal adhesions.[51] Inhibiting Rho kinase prevents the formation of adhesions altogether.[52] In 2D systems, the stiffness of the substrate is significantly greater than the compliance of the cell while in contrast, in 3D matrices, the ECM is comparatively much less rigid, and so it is likely that the difference in relative stiffness is a factor in the differences observed in 2D and 3D adhesions.

A summary and comparison of protein components (a by no means exhaustive list given that more than 50 adhesion-associated molecules have been identified[20]) for the different adhesion types is provided in Table 1 and illustrated in Fig. 1.

Table 1 Markers for different types of adhesions

Marker	Focal complex[27]	Focal adhesion[27,53]	Fibrillar adhesion[53]	3D cell–matrix adhesion[50,51]
α_v integrin	+++	+++		
α_5 integrin		+/−	+++	+++
β_1 integrin		+/−	+++	+++
β_3 integrin	+++	+++		
α-actinin	+	+++	+/−	+[a]
FAK	+	+++		+[a]
Paxillin	++	+++	+/−	+++
Phosphotyrosine	+++	+++	+/−	+[a]
Talin	++	+++	+/−	+[a]
Tensin	+	++	+++	+[a]
Vinculin	+	+++	+/−	+
Zyxin	+/−	+++		+[a]

Degree of site localization: blank: 0%; +/−: <20%; +: 20–50%; ++: 50–80%; +++: >80%; +[a] present but limited data on degree of localization.

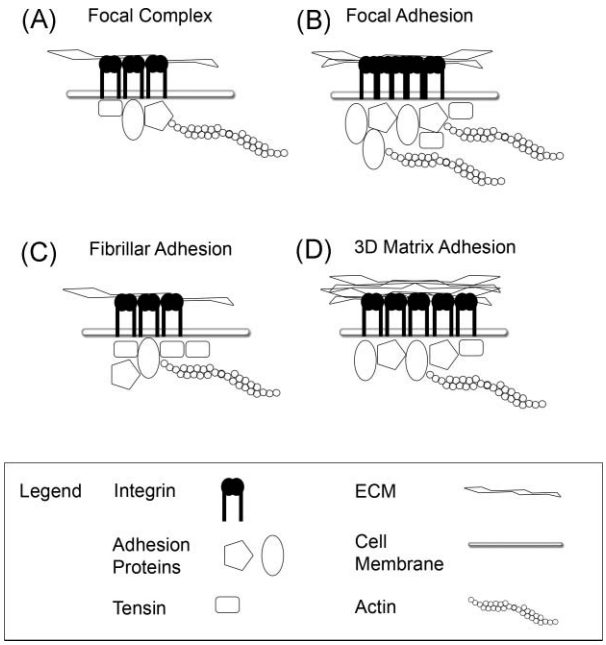

Fig. 1 (A) Focal complexes are formed initially as integrin clusters and adaptor scaffolding proteins are recruited to clustering sites. (B) Focal adhesions mature from focal complexes when force is applied. Focal adhesions are characterized by increased levels of many adhesion proteins. (C) Fibrillar adhesions involve the translocation of β_1 integrins and the pulling of fibronectin fibers to induce fibrillogenesis. (D) 3D matrix adhesions develop when cells are seeded in 3D matrices and are distinct in composition and structure from the adhesions observed in 2D.

Force and matrix mechanics have implications for receptor–ligand interactions

As we have already seen, cell–matrix adhesions are driven in large part by integrins reversibly binding to their respective ECM ligands. Bond formation, strength, and lifetime depend on receptor–ligand reaction rates and binding afffinities.[54] Bell proposed a model of receptor-mediated cell adhesion over 30 years ago, suggesting that integrin–ligand binding is a balance of attractions due to specific receptor–ligand binding affinities and repulsions due to electrostatic, osmotic, and other non-specific interactions, and that applying force will affect the rate of bond dissociation.[55] It has been experimentally found, for instance, that the dissociation rate constant k_{OFF} for zyxin in focal adhesions increases in response to reduced traction forces, achieved by decreasing matrix rigidity, inhibiting the contractile machinery, or cutting stress fibers with a laser.[56] In a thermodynamics-based model developed to predict the effect of force on bonds by simulating receptors and ligands with elastic springs, Dembo predicted the existence of catch bonds, which have extended bond lifetimes upon force application, in contrast to more intuitive slip bonds, which have reduced bond lifetimes in response to force.[57] Catch bonds have recently been demonstrated experimentally using atomic force microscopy, showing that bonds formed between $\alpha_5\beta_1$ integrin and fibronectin have longer lifetimes when 10–30 pN forces are applied due to force-induced alterations in the integrin headpiece.[58]

The magnitude of the forces applied is not the only important factor in bond dissociation. Bond strength, or the force at which bonds repeatedly fail, is dependent on loading rate and the length of time of the loading, suggesting that the timescale of force application is significant.[59] Under slow loading, bond strength increases slowly with loading rate; at intermediate loading rates, bond strength grows logarithmically with loading rate; and at ultrafast loading rates, only frictional drag holds the bond together as forces escalate very quickly.[59] Consequently, bond strengths should be reported with loading rates. For example, the bond between fibronectin and the cytoskeleton, which requires talin, ruptures at 2 pN when loaded at 60 nm/s.[60] A consideration that should be made when examining biomechanical data is that consecutive measurements on bond formation and breakage may not have independent identically distributed distributions.[61] It has been shown, for instance, that C-cadherin homotypic binding and T cell receptor binding to antigens bound to major histocompatibility complexes demonstrate memory, with negative feedback and positive feedback with repeated binding events over time, respectively.[61]

Bell's early model of cell adhesion also suggested the importance of the spatial distribution of bonds on a ligated substrate.[55] The critical bond spacing for integrin–ligand bonds needed for focal adhesion formation has been experimentally determined (maximum bond spacing: 58–73 nm),[62] and then calculated theoretically (maximum bond spacing: 39–89 nm).[63] The quantitative analysis of the critical bond-spacing applies the supposition that excessive ligand spacing rather than integrin spacing is constraining since membrane receptor density is very fluid.[63] The critical bond spacing was derived from the assumption that focal adhesion development depends on membrane deformation and thermal undulations to bring ligands and their respective receptors in contact for binding, and that sustaining a focal adhesion requires a balance of energies due to attractions with receptor–ligand binding and thermal undulations, and repulsions due to bulge pressure and membrane deformation.[63] The distribution and density of integrins and ligands therefore has a major effect on adhesion formation and strength. The spacing between arginine–glycine–aspartic acid ligands (RGD ligands, being the sequence integrins recognize on several ECM proteins) was found to regulate integrin clustering and the formation of stable focal adhesions, with cells failing to achieve persistent migration on substrates with ligands distributed 108 nm apart in contrast to cells on substrates with ligands 58 nm apart.[64] In a study using synthetic polymer substrates, RGD ligands that are highly clustered and densely packed induced adhesion that is strengthened when applied forces increased within a certain range (between 70 to 150 pN/cell) before weakening as forces continued to escalate.[65] When both receptor and ligand densities increase, the strength of a given adhesion site increases proportionally, with adhesion strength shown to be directly related to the number of receptor–ligand bonds formed.[66]

Adhesion strength in turn modulates cellular responses. Intermediate adhesivity, as quantified by ligand density and integrin density, facilitates motility the best, as a biphasic relation was found between cell motility, and adhesion strength and detachment force.[67] Similarly, matrices with intermediate collagen density yield the largest cell spreading areas, with cells seeded on rigid substrates spreading the most.[44] Cells seeded on the softest gels though are less sensitive to ligand density, suggesting that matrix stiffness and ligand density are strongly related variables.[44]

A recent discovery of a mechanism of how matrix mechanics and force can affect integrin–ligand interactions is the switching of $\alpha_5\beta_1$

integrin–fibronectin bonds between relaxed and tensioned states.[68] Contractile cytoskeletal force against a stiff matrix induces a tensioned state and more compliant substrates restrict the number of tensioned bonds formed. Both internal and external forces can trigger the switch from relaxed to tensioned. The $\alpha_5\beta_1$ integrin–fibronectin bond strength increases in the tensioned state as synergy sites on the ligand are engaged and stabilize the bond. The tensioned bond is important to phosphorylate FAK and so rigid matrices facilitate signaling. Perturbing contractile machinery disrupts the relaxed to tensioned bond switch and results in reduced FAK phosphorylation. This force-induced switch in bond states is an example of a catch bond in that applying tension has a strengthening effect on the adhesion, similar to what has been observed in the flow-enhanced adhesions formed between leukocyte selectins and the endothium, and the bacterial protein FimH and the intestinal epithelium.[69]

Conclusions

Cell–matrix adhesions are important in a variety of aspects of cell behavior. Since the development of adhesions and their subsequent biochemical and mechanical signaling activity is largely influenced by force, the physical properties of the cellular environment are significant factors in modulating cellular responses. However, there remains much to be learned about how cell–matrix adhesions function as mechanosensors and mechanotransducers. Much progress has been made as techniques have improved. Recently, spatio-temporal mapping of Src activation in cells in response to force application to ligand-coated beads bound to membrane integrins was accomplished using a reporter Src gene,[70] and traction force mapping with a resolution of ~1 μm was achieved,[71] suggesting that the field of mechanobiology is well on its way to developing an understanding of the dynamics of cell adhesion formation on a molecular scale.

However, there are still a number of questions left unanswered. Complete pathways of mechanotransduction, with the identification of all the necessary molecules and their respective functions, have yet to be determined. The distinctions between 2D and 3D adhesions—differences in how they are formed, their composition, and how they respond to matrix mechanics and forces—must be more fully investigated. It is also important to identify other mechanical parameters besides matrix stiffness that may be significant in adhesion formation and function in both 2D and 3D matrices. Another consideration is the influence of other cells on an individual cell's sensing of its mechanical environment. In one study, cell–cell contact eliminated dependence of cell spreading on substrate stiffness.[1] In another, it was found that cells can detect and respond to the traction forces generated by neighboring cells with the response depending on substrate rigidity.[72] On the most compliant substrates, cells that made contact remained touching after initially connecting; on intermediately rigid gels, cells in pairs touched and released, migrating close together; and on rigid substrates, cells migrated away from each other.[72]

With the increased use of synthetic micropatterned substrates with tunable mechanical properties, and high-resolution research tools such as optical tweezers, atomic force microscopy, fluorescence recovery after photobleaching (FRAP), and fluorescence resonance energy transfer (FRET) becoming more widely available, rapid progress is being made in unraveling the complexity of cell–matrix adhesions and their mediation of mechanotransduction.

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