

Mechanosensing at integrin-mediated cell-matrix adhesions: from molecular to integrated mechanisms.

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Abstract

Integrin-mediated adhesions between cells and the extracellular matrix are fundamental for cell function, and one of their main roles is to sense and respond to mechanical force. Here we discuss the different mechanisms that can confer mechanosensitivity to adhesions. We first address molecular mechanisms mediated by force-induced changes in molecular properties, such as binding dynamics or protein conformation. Then, we discuss recent evidence on how these mechanisms are integrated with cellular and extracellular parameters such as myosin and actin activity, membrane tension, and ECM properties, endowing cells with an exquisite ability to both detect and respond to physical and mechanical cues from their environment.

Introduction

The molecular nature of the structures conferring adhesion between cells and their surrounding extracellular matrix (ECM) began to be elucidated over 40 years ago, when Abercrombie et al. [1] identified dense “plaques” at the cell-substrate interface of migrating cells. Since then, the study of cell-matrix adhesions has flourished, leading to the identification of multiple types of adhesive structures, and of their roles in cell migration [2], signaling [3], and disease progression [4]. Cell-matrix adhesions can be classified according to their shape and molecular complexity, and for instance focal complexes, focal adhesions, and fibrillar adhesions progressively increase in size and complexity. Despite this diversity, most types of cell-matrix adhesions share a common basic architecture, characterized on one end by the transmembrane proteins, integrins, that bind the ECM, and on the other end by actin fibers that indirectly bind to integrins via a set of adaptor proteins (Fig. 1A). These adaptor proteins can include over a hundred different types of molecules, which function as a major hub for biochemical signaling events [5]. However, beyond mediating biochemical signaling, one of the main functions of cell-matrix adhesions is to detect, transmit, and respond to mechanical signals. Such signals include the rigidity of the ECM or forces transmitted from it, as well as mechanical cues originating within the cell, like cytoskeletal contraction or changes in membrane tension.

Cell-matrix adhesions are described as mechanosensitive because they grow and mature in response to applied forces or increased substrate rigidity, and shrink or disassemble in the absence of such stimuli [6,7]. Once formed, mature focal adhesions connect to the cytoskeleton via the formation of stress fibers. Focal adhesions and stress fibers in turn affect nuclear shuttling and the activity of different transcriptional regulators, for instance by changing their binding affinity to either focal adhesions [8] or actin [9], or by transmitting force to the nucleus, opening nuclear pores, and promoting their nuclear entry [10]. Thus, mechanosensitive adhesion growth and maturation can directly drive gene transcription. Due to the complexity of cell-matrix adhesions, the mechanosensing process is mediated by a rich network of molecular interactions and biochemical pathways, which have been the subject of recent extensive reviews [11,12]. Given the concise nature of this review, we will not cover those interactions in detail. Instead, we will focus on a more fundamental question: what are conceptually the different types of mechanisms that can confer mechanosensitivity to cell-matrix adhesions, and what is the experimental evidence for them?

Mechanosensitivity through regulation of binding dynamics

Force applied to cell-matrix adhesions is transmitted through their molecular elements, from ECM-integrin bonds, to adaptor proteins, and finally to the actin cytoskeleton (Fig. 1A). For this force to be detected, it needs to trigger some sort of event in the affected molecules. The most common type of molecular event effected by force is bond dissociation. In the most intuitive scenario, known as “slip bond”, force applied to a bond weakens it, promoting dissociation. However, some bonds behave as “catch bonds”, which strengthen under force up to a given threshold, and only weaken if this threshold is surpassed. Catch bonds have been mostly described for integrin-ECM interactions such as the bond between fibronectin and integrins, $\alpha 5\beta 1$ and $\alpha v\beta 3$ [13,14], but they have recently been found also in adaptor protein interactions, such as the bond between vinculin and actin [15]. Regardless of whether they operate as catch or slip bonds, in both cases force is a key regulator of bond lifetime. In the case of integrin-ECM adhesion, this means that the number of ligand-bound

integrins, and the stability of the binding, will depend on force. In turn, this will determine subsequent integrin clustering (Fig. 1B), and the likelihood of triggering downstream integrin-mediated events which depend on integrin ligation and clustering, such as activation of FAK and Src [16], or recruitment of adaptor proteins like vinculin or zyxin [17].

Force-dependent bond lifetimes provide sensitivity not only to externally applied forces, but also to passive ECM mechanical properties such as its rigidity. Indeed and as predicted by the molecular clutch model [18], as cells pull on the ECM through contraction of the actomyosin cytoskeleton, different matrix rigidities lead to different regimes of force transmission. This then affects the dynamics of cell-matrix bond formation and rupture, regulating force transmission itself and subsequently, cell migration, actin dynamics, and adhesion formation [7,18-20]. Adhesion-dependent mechanosensitivity, particularly for integrins, can be further tuned by cells through several strategies, conferring the ability to respond to specific mechanical conditions. These strategies include binding to the matrix through different integrin types with different mechanosensitivities [21,22], the action of different integrin binding partners such as talin [23], sharpin [24], shank [25], kank [26], kindlin [27], ICAP-1 [28], or ZO-1 [29], which regulate integrin activation and thereby force-dependent ligand binding [13], or the regulation of the steric hindrance to ligand binding provided by the glycocalyx [30] (discussed below).

Mechanosensitivity through activation of mechano-chemical switches

Beyond affecting bond lifetime, another major molecular effect of force is to regulate protein conformation (Fig. 1C). The best known example of this effect is with talin, an adaptor molecule linking actin to integrins that is submitted to cell-ECM forces [31,32]. Upon force application, several domains of talin unfold, exposing previously hidden binding sites to vinculin [33,34]. Subsequent vinculin binding then reinforces the adhesion site, possibly through interactions with both integrins and actin [15,35], and leads to adhesion maturation. Whereas the detailed mechanism by which vinculin binding leads to adhesion maturation is unclear, this constitutes a prime example of a mechano-chemical switch, in which a mechanical signal (force) is converted into a biochemical one (a protein-protein interaction). Several other examples of mechano-chemical switches in adaptor proteins have been described. For instance, force-induced events include changes in filamin crosslinking angles affecting integrin binding [36], stretching of p130Cas leading to its phosphorylation [37], and activation of focal adhesion kinase by dissociating auto-inhibitory interactions [38], although this latter effect remains to date a computational prediction. Interestingly, talin has recently been reported to respond to force not only by unfolding but also by cleaving, producing a rod domain needed for cell growth [39]. Outside of adaptor proteins, mechanical stretching can also induce chemical events in ECM proteins such as fibronectin (by promoting the binding of interleukin 7 [40]), or in integrins, by inducing conformational changes that in turn regulate ECM binding [14].

Mechanosensitivity integration through contractility and ECM properties.

In cells, the different types of molecular mechanosensing mechanisms described above are coupled to each other, to biochemical signaling pathways, and to higher order force regulation, giving rise to complex feedback mechanisms (Fig. 2A). Some fundamental design rules, however, are beginning to emerge. For instance, cytoskeletal structure, dynamics, and integrity will affect how force is transmitted to adhesions, in turn regulating mechanosensing [41]. Some recent examples of this

principle include the regulation of actin stress fiber mechanics by zyxin, which tunes force transmission [42], the alignment and re-orientation of integrins by actin flows, which affects their clustering and thereby their function [43], and the re-ordering of the actin cytoskeleton induced by stiffness [44]. The collective behavior of ensembles of several ECM-integrin-adaptor protein-actin links (often referred to as “clutches”) is also affected by force, as observed and predicted theoretically by the molecular clutch theory [18,45]. This framework could explain the mechanosensitive oscillations observed in force transmission in focal adhesions [46,47], and potentially also the nano-scale force contraction cycles observed in maturing adhesions [48]. Such nano-scale contractions depend on substrate rigidity [49] and are associated with altered activity of receptor tyrosine kinases [50]. Further, they may sense rigidity through the duration of the force cycles applied, although the underlying mechanisms are unclear.

Additionally, a fundamental aspect that determines adhesion mechanosensing is the coupling between binding dynamics and mechano-chemical switches. In our recent work, we have determined that the differential response to force of talin unfolding and integrin-fibronectin unbinding enables cell sensing of the rigidity [7], ligand density, and ligand distribution [51] of the ECM. If we consider a given actin-talin-integrin-fibronectin clutch, this differential response entails that a low applied force will tend to trigger integrin-fibronectin unbinding first, disengaging the clutch and preventing talin unfolding (Fig. 2B). In contrast, applying force above a given threshold (of about 5 pN) will first lead to talin unfolding, enabling vinculin binding and subsequent mechanosensing. Thus, this coupled system allows talin unfolding only above a threshold force, conferring sensitivity to force, but also to substrate rigidity and ligand distribution. This is because clutch forces are increased both by high substrate rigidity and low ligand density, since total cell applied force is distributed among fewer ligands (Fig. 2C). Interestingly, this framework confirms that cell spatial sensing of ligands is also mediated by mechanosensing, and not by a direct length measurement as previously hypothesized [51]. This concept of differential mechanosensing has so far been explored by using the talin-integrin system, but it could apply in several other cases. A particularly interesting example is the complex between glycoprotein Ib, and the ECM protein Willebrand factor, which contains three mechanosensitive elements in series: a catch bond between both proteins, and two unfoldable domains within glycoprotein Ib [52].

Mechanosensitivity integration through actin dynamics and membrane tension

Any machinery adhering cells to the extracellular environment is intimately linked to the main guardian of cell integrity: the plasma membrane. The lipid bilayer supports all adhesive molecules and numerous studies show that membrane mechanics is also a key element in the mechanosensitivity of cell-substrate adhesions. Membrane tension, and particularly in our focus here, plasma membrane tension, is a mechanical property of the cell based on four driving sources: osmotic forces, cytoskeletal forces, membrane bending rigidity (stiffness, composition) and attachment to the underlying cytoskeleton through different lipids and proteins (see our recent review [53]). These four elements are linked to the physical properties of the lipid bilayer as a fluid, but barely stretchable material (about 4% before lysis). Recent work has shown that membrane tension should be seen as a master regulator of cell functions, as it can be rapidly transmitted across the cell [54-60]. As membrane tension is closely linked to actin cytoskeleton dynamics [53,61], it was postulated through mathematical modeling that adhesion dynamics could be influenced by fluctuations in membrane tension [62]. Indeed, older observations clearly linked membrane tension

to cell protrusions [63], a key feature of motile cells dependent on both cytoskeletal and adhesion dynamics. From several recent studies, it emerges that membrane mechanics can influence adhesion mechanosensing at three different levels. First, it can influence adhesion formations [60] by controlling protrusion rate and actin behavior [60,64]. Second, it can potentially influence adhesion clustering [30,65]. Finally, it can directly [30,66] and more surprisingly, indirectly [67,68] induce integrin activation, although its role here is less clear.

By combining evidence from membrane tension changes in spreading and migrating fibroblasts [60] and migrating keratocytes [64], a consensus model for the influence of membrane tension in actin dynamics and adhesion positioning can be built (Fig. 3A). At low membrane tension, a fast protruding lamellipodia is observed [60,64] with unbranched actin filaments perpendicular to the cell edge [64]. At this stage, adhesions are at the back of the lamellipodial actin, sustaining the leading edge structure by forming and disassembling in a staggered manner [60]. As the cell runs out of membrane area, rising membrane tension induces an increased load on actin, resulting in a slower lamellipodia with a more branched and less perpendicular actin network [64]. Adhesions at the back react to this increasing load by growing and progressively organizing as a single row [60]. At very high membrane tension, actin polymerization is potentially pushed upward due to buckling [60]. This leads to the force-induced maturation of a single adhesion row at the back of this buckled region, as well as the creation of a preferential nucleation site for new adhesions at the front region [60]. This explains how cells can form adhesions along the leading edge in a synchronous manner, in response to force generated by actin polymerization rather than myosin-based contractility [60]. While the buckling was not investigated in the keratocyte-based study [64], older reports showed potential evidence of it [69] pointing to a potentially universal mechanism.

As for adhesion positioning during motility, the influence of membrane mechanics for integrin clustering was first proposed through mathematical modeling [65], then supported experimentally [30]. The basic principle of this mechanism is a competition between relatively short molecules, integrins (about 25 nm in the non-extended configuration), and long and bulky glycoproteins in the glycocalyx (up to >100 nm). While not directly proven, the influence of membrane mechanics and potentially membrane tension is easy to explain. As both the glycocalyx and integrins are supported by the plasma membrane, the long glycocalyx hinders the accessibility of integrins to the matrix, strongly reducing binding rates (Fig. 3B). Once an integrin binds, however, it bends the membrane to overcome the glycocalyx barrier, generating a region where diffusing integrins and the ECM are in close proximity, forming a “kinetic trap” where binding rates are increased. This leads to larger and highly activated adhesions. Interestingly, a long glycocalyx is a hallmark of cancer cells, pointing to a central role of mechanical forces originated from the membrane in cancer progression [30].

The third mechanism where membrane mechanics is implicated is the potential activation of integrins by non-integrin ECM ligands, such as the urokinase-type plasminogen activator (uPAR), independently of integrin-ECM engagement [67,68]. The mechanism proposed is based on the fact that the binding between uPAR and the ECM protein vitronectin was sufficient to trigger integrin signaling. Fascinatingly, this effect seems to require membrane tension, but not integrin-ECM binding. The working model for this cross activation could be close to the glycocalyx “kinetic trap” [30] in the sense that uPAR binding to the substrate will induce an out of plane membrane deformation, dragging nearby integrins away from actin and adaptor proteins (Fig. 3C). If integrins are bound to adaptor proteins such as talin (even if not to the ECM), this would then generate a force that would increase with membrane tension, favoring the activation of mechano-chemical

switches. This mechanism, while still unclear, points towards a central role for membrane-mediated mechanotransduction in integrin activation, as previously suggested [66].

Conclusion

At the molecular level, cell-matrix adhesion mechanosensitivity is enabled by mechanisms which are reasonably clear, such as force regulation of binding dynamics and protein conformation. However, such mechanisms are coupled to each other, and to both cellular and extracellular parameters such as myosin contractility, ECM properties, and membrane tension. This leads to integrated mechanisms providing fine-tuned sensitivity to the cell physical environment. Even though some mechanisms are now understood, how mechanosensing is integrated across scales remains largely unexplored. Future work should resolve, for instance, how mechanosensing is regulated in complex, three-dimensional multi-cellular environments, or why adhesion mechanosensing fails to operate in certain conditions [70].

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Figure captions.

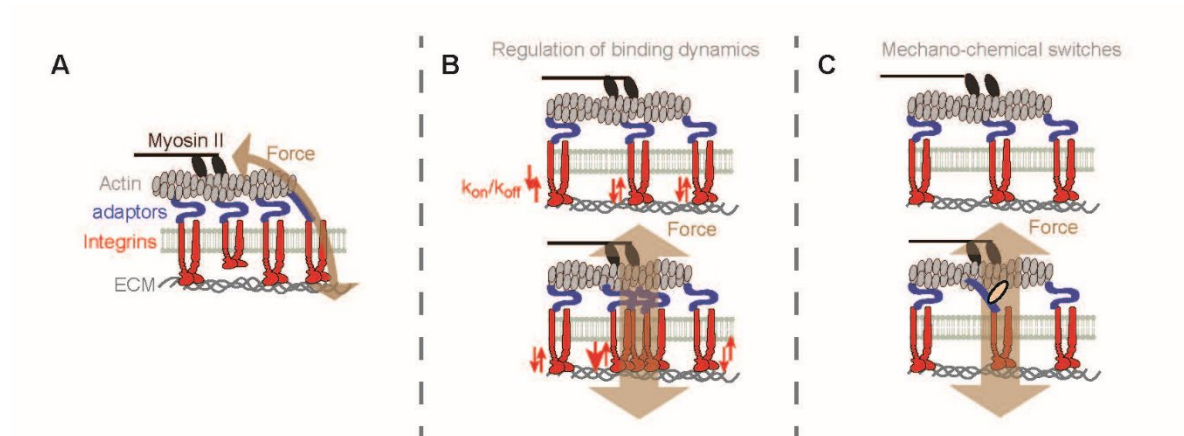


Figure 1. Molecular mechanosensing mechanisms. **A)** Cell-matrix adhesions link the ECM to actin through integrins and adaptor proteins. As myosin exerts force on actin, this is transmitted through adaptor proteins and integrins. **B)** Force application increases the lifetime of catch bonds between integrins and the ECM, potentially increasing clustering. **C)** Force application induces conformational changes in adaptor proteins (such as protein unfolding), leading to downstream biochemical events such as protein binding to newly exposed unfolded domains in adaptor proteins.

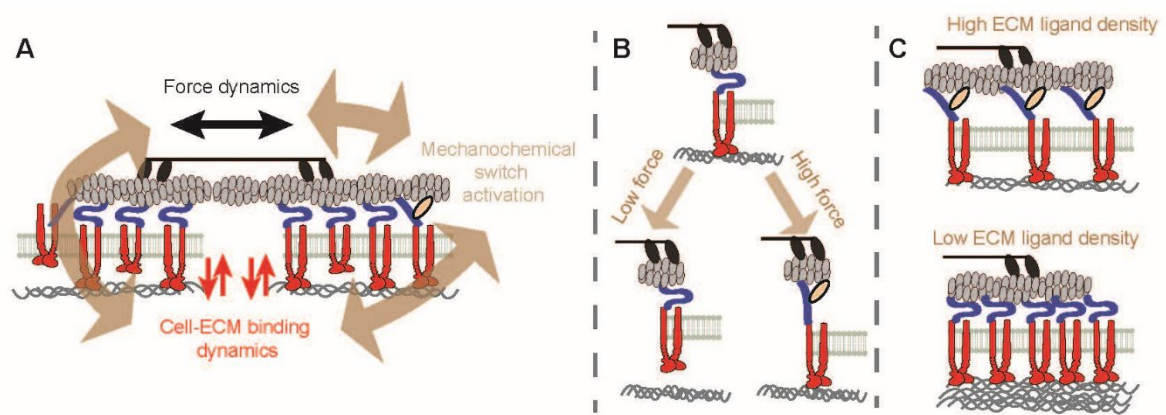


Figure 2. Integration of mechanosensing through contractility and ECM properties. **A)** The regulation of force dynamics, both at the nano-scale and at the cell level, both affects and is impacted by cell-ECM binding dynamics, and the activation of mechanochemical switches. This leads to a feedback mechanism integrating cell response. **B)** If force is transmitted through an adaptor protein (talin) and an integrin-ECM bond, the different properties under force of talin and the integrin-ECM bond lead to integrin unbinding if the force is low, and talin unfolding (and subsequent vinculin binding) if the force is high. **C)** If ECM coating density is low, contractility is distributed among few ligands, leading to a high force per molecule that triggers talin unfolding. If ECM density is high, more integrins bind, distributing the force and preventing talin unfolding.

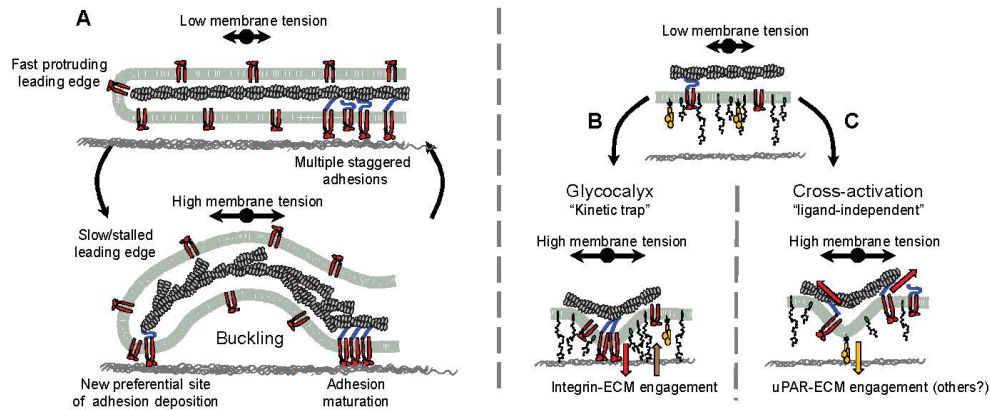


Figure 3. Integration of mechanosensing through membrane tension. **A)** Membrane tension promotes adhesion positioning and lamellipodium buckling at the cell leading edge. The leading edge during cell migration constantly cycles between the two situations depicted, promoting the deposition of multiple adhesion rows. Those adhesion rows will later either disassemble or mature into focal adhesions upon myosin mediated contractility. **B)** The length of the glycocalyx impairs integrin binding. However, at the few sites where integrins are bound, the counteracting forces between the glycocalyx (brown arrow) and integrins (red arrow) generate membrane deformation and a “kinetic trap” favoring clustering and activation. **C)** ECM binding of non-integrins ligands such as uPAR can increase membrane tension and potentially deform the membrane, inducing “ligand-independent” integrin activation and triggering downstream signals.