Adhesion-mediated mechanosensitivity: a time to experiment, and a time to theorize

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Adhesion-mediated signaling provides cells with information about multiple parameters of their microenvironment, including mechanical characteristics. Often, such signaling is based on a unique feature of adhesion structures: their ability to grow and strengthen when force is applied to them, either from within the cell or from the outside. Such adhesion reinforcement is characteristic of integrin-mediated cell–matrix adhesions, but may also operate in other types of adhesion structures. Though the amount of knowledge about adhesion-mediated signaling is growing rapidly, the mechanisms underlying force-dependent regulation of junction assembly are largely unknown. Experiments have been carried out that have started to uncover the major signaling pathways involved in the response of adhesion sites to force. Theoretical models have also been used to address the physical mechanisms underlying adhesion-mediated mechanosensing.

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Introduction: why are cell adhesions mechanosensitive?

The adhesion machinery of cells consists of specialized subcellular contact sites of distinct structure and molecular composition, formed by transmembrane receptors associated with the cytoskeleton. These sites serve as local anchors, linking cells to the extracellular matrix (ECM) or to their neighbors, in a way that supports the long-range assembly of tissues with specific structural and mechanical properties. The mechanistic inter-relationships between the formation and maintenance of local adhesions, which have a length scale of a few micrometers, and tissue scaffolds measuring centimeters or more are rather intriguing. One attractive possibility is that trans-tissue forces acting on individual adhesion sites

can affect their fate. Indeed, recent studies have established that at these sites, cells can not only sense the chemical properties of external surfaces (e.g. the presence of diverse ECM ligands and cell–cell adhesion molecules), but also a variety of mechanical cues, such as mechanical forces (stresses) and deformations (strains) [1–7]. These include global tension generated within the tissue, external mechanical perturbation, shear stress and intracellular forces applied to the adhesion sites by the cell's own contractile system.

The physiological significance of the adhesion response to mechanical stimuli requires additional thought. Intuitively, it is widely accepted that a primary function of adhesion sites is to ensure the correct and stable positioning of cells within tissues. Given that external perturbations are often unpredictable in terms of their extent and timing, a cell should develop a 'just in time' mechanism, whereby the strength of the adhesion adjusts itself dynamically to the amount of stress (and/or strain) applied to the adhesion site. Such a mechanism provides necessary versatility in terms of cellular dynamics, yet it depends on the existence of a mechanosensitive assembly of adhesions.

Strong mechanical forces (including those developed in some cases by cells themselves) can, obviously, disrupt cell–cell and cell–matrix adhesions. However, recent experiments also demonstrated the existence of force-dependent adhesion growth (rather than dissociation). These surprising results stimulated biologists, chemists and physicists to study this intriguing phenomenon extensively, and to attempt to model it theoretically. In this article we examine some of the recent studies that explore the issue of adhesion mechanosensitivity. We highlight some of its major characteristics, and address the possible mechanisms that might be involved in the adhesion-mediated detection of mechanical stimuli, and in the cellular response to them.

Key experimental evidence on the mechanosensitivity of focal adhesions

We focus here on the mechanosensing behavior of distinctive adhesion structures, focal adhesions (FAs), formed by different cell types (e.g. fibroblasts or epithelial cells) attached to a rigid substrate [8,9]. FAs are large (several microns long) and structurally polarized adhesion sites associated at their proximal ends with contractile bundles of actin filaments (called 'stress fibers'). Their formation depends either on 'natural' matrix proteins, or on RGD (arginine-glycine-aspartate) peptides present at high

densities (an inter-ligand spacing of ∼50 nm or less) on the surface [10•,11•]. Their interactions with the ECM are mediated by different integrins, mainly $\alpha_5\beta_1$ and $\alpha_v\beta_3$.

Along with the integrins and some other transmembrane molecules (e.g. syndecans), >100 additional adaptor and signaling proteins comprise the cytoplasmic domain of FAs [8,12]. These 'plaque proteins' form numerous links, both direct and indirect, between integrin molecules and actin filaments, and regulate the assembly, turnover and signaling activities triggered by the adhesive interactions. This complex and versatile organization renders the FA an unusual sensory organ, capable of responding to diverse external features and signals such as ECM protein density [10•,11•], topography [13,14•], rigidity [15,16,17••], and mechanical perturbation [18,19,20•].

FAs usually evolve from another type of cell–matrix adhesion, namely 'focal complexes' (FXs), small (less than a micron in size) dot-like adhesions that are continuously formed and turned over under the protruding lamellipodia [21,22]. Some focal complexes undergo 'maturation', transforming into FAs which grow and extend centripetally, concomitantly with the formation of actin stress fibers [22,23].

Several compelling lines of evidence suggest that the FX-to-FA transition, as well as FA growth and maintenance, depends upon the continuous application of local force pulling on these structures by means of the cell's contractile machinery. First, inhibition of myosin-II-driven contractility leads to the accumulation of FXs and the disappearance of FAs [24–28], while activation of myosin II induces FA assembly [29–31]. Second, application of external force to FAs stimulates their growth in the direction of the force even when myosin II activity is suppressed [18]. Third, the size of FAs and the forces applied to them are usually proportional, with coefficients of proportionality (∼5 nN/μm^2) that are remarkably similar among different cell types [32,33]. Fourth, on a soft matrix, where forces strong enough to pull on the adhesions cannot be generated [34•], large FAs are not formed [34•,35,36], and fifth, mechanical manipulation of fibronectin-coated beads on the cell's dorsal surface 'reinforces' the indirect interactions of these beads with the actin cytoskeleton [37,38]. Thus, FAs act as bona fide 'just-in-time' mechanosensing devices, responding to local force by increased assembly, and to the relaxation of force by disassembly.

Beyond focal adhesions: similarities and differences in adhesion-mediated mechanosensory responses

It is noteworthy that the mechanosensitivity of FAs and other types of integrin-mediated adhesions is involved in a variety of complex cellular responses to environmental features; these include substrate stretching [19,39];

substrate rigidity variations, leading to migration in the direction of increasing rigidity ('durotaxis') [17••,40••,41]; and fluid shear stress [20•,42,43]. A detailed description of the cell responses explored by these experimental systems is beyond the scope of this review (for a more comprehensive discussion, see [1–3,6,44]).

Though other types of integrin-mediated adhesions besides focal adhesions demonstrate apparent mechanosensitivity, the mode of response and perhaps even the underlying molecular mechanisms are not necessarily identical. For example, 'fibrillar adhesions' associated with ECM fibrils [45] evolve from FAs in a force-dependent manner, although unlike FAs they do not disassemble when the force is relaxed [46]. Podosomes, another type of integrin-mediated contact, respond to substrate rigidity differently from FAs [47], so that not their shape, but their lifespan depends on substrate flexibility. There is even a substantial difference between the response of an individual cell to the integrin-mediated contacts formed on its ventral and dorsal aspects [48].

On the other hand, recent studies suggest that cadherin-mediated cell–cell adherens junctions (AJs) depend on myosin II-driven contractility, somewhat akin to integrin-mediated FAs. Thus, it was shown that inhibition of myosin II function by chemical inhibitors leads to reduced accumulation of AJ proteins at the cell–cell interface [49••]. Similar results were obtained when cell contractility was inhibited by overexpression of caldesmon, a regulatory protein inhibiting myosin-II ATPase [24]. Moreover, another type of cell–cell junction receptor (from the immunoglobulin family), platelet endothelial cell adhesion molecule (PECAM)-1, was shown to function as a major mechanosensing molecule [50], upstream of the $\alpha_v\beta_3$ integrin, in the response of confluent endothelial cells to fluid shear stress [51••]. The last example suggests that cell response to mechanical stimuli can depend on a hierarchy of mechanosensing modules. Thus, mechanosensitivity (manifested by force-dependent reinforcement of adhesion strength) may not be unique to adhesion mediated by particular integrin complexes, but may instead be characteristic of various types of cell adhesions.

Complexity: struggling to see the forest for the trees

Even such seemingly simple processes as the force-mediated assembly of focal adhesions are often dauntingly complex. Thus, while the molecular repertoire of FA components has been mostly uncovered [8,12,52], the detailed functional interplay of these proteins and their spatial organization within the adhesion site are largely unknown. Consequently, the precise mechanisms whereby cells can sense the physical properties of their environment — surface rigidity, ligand density, and local or global mechanical perturbations — remain elusive.

Focal adhesions are highly dynamic multi-protein arrays that undergo continuous turnover, which is precisely regulated in time and space (e.g. by interaction with microtubules [53]). Thus, it is reasonable to suggest that, at any given moment and in every subcellular location, the properties of FAs are constantly being fine-tuned by a variety of environmental cues. We are not yet certain which of the molecular interactions within FAs are regulated by force. An attractive possibility is that the force-induced changes in the states of some FA components ultimately affect the intricate network of phosphorylation and dephosphorylation events within FA.

On the basis of data from several experimental systems, protein tyrosine kinases such as FAK [13,54,55], Src [37,56,57**] or Fyn [15,58] are thought to be involved in integrin-dependent mechanosensitivity. Recent studies also suggest that the tyrosine phosphatases RPTP-α [40**,58] and SHP-2 [50,59] could play a potentially important role in these processes. The underlying mechanisms may involve the modulation of phosphorylation-dependent protein–protein interactions that are highly prominent in focal adhesions [60•].

Adaptor proteins such as paxillin and p130Cas [15,20•], as well as zyxin [22,39•,61], are probably also involved in the cellular response to mechanical stress. In particular, zyxin recruitment to nascent focal complexes accompanies their maturation into FAs [22]. Moreover, zyxin translocates from FAs to stress fibers following substrate stretching, when force is applied [39•]. The adhesion-dependent pathways regulating the activities of small Rho family GTPases [20•,55,62,63] and Rap1 [19,64] are also potential targets for the force-dependent regulation of FA assembly. All these and other signaling events (thoroughly reviewed elsewhere [1,2,7,44,65,66]) have yet to be integrated into a unified molecular scheme. However, in the absence of such a model, it seems useful to formulate some possible 'ground rules', based on basic physical principles, that might drive FA mechanosensitivity and could assist in the integration of relevant molecular pathways into a coherent whole.

Physical modeling: from ground rules to the focal adhesion assembly line

Physical models developed during the past several years have attempted to address several aspects of adhesion-mediated mechanosensitivity: the primary physical mechanisms governing FA mechanosensing and dynamics [67•–69•,70]; the shear-stress profile along individual FAs [71]; the spatial control of FA distribution [72]; the interplay between FAs, the small GTPases Rac and Rho, and actin stress fibers, which guide a directional reorganization of the actin cytoskeleton [73]; and the effects of substrate elasticity on FA formation [74–76]. Below, we overview and compare three specific models of the mechanism of FA mechanosensing [67•–69•,70]. The

models differ in their assumptions concerning the physical factors underlying mechanosensing and the dynamic behavior of FAs in the course of their growth and shrinkage.

Principles of mechanosensing

The major distinction between the proposed models lies in their assumptions concerning the physical principles underlying mechanosensing. One assumption is that focal adhesions, like many other cellular mechanosensing devices, contain special molecular switches represented by multi-modular proteins (thoroughly discussed recently in [77]), which react to the application of force by changing their state from inactive to active, or vice versa. In the force-induced active conformations, these proteins are believed to support FA self-assembly. Most models propose that these protein switches sense stress, and switch to an active conformation when the stress exceeds a critical value (see, for example, [68•] and Figure 1). Such a mechanism could be based, for instance, on the stress-induced conformational transition of some of the FA-associated proteins, or on the modulation of activity of specific enzymes (e.g. kinases, phosphatases), which could turn on or turn off 'phosphorylation switches'. Indeed, various adhesion-related molecules — ranging from extracellular fibronectin [78–80], via integrins themselves [81,82], to adaptor proteins such as talin [83,84] and vinculin [85,86] and signaling enzymes such as Src [87] and possibly FAK [88] — were shown to be regulated by such large-scale conformational reorganization (i.e., a transition from a 'closed' to an 'open' conformation). However, apart perhaps from fibronectin [77], the direct role of mechanical stress in triggering such conformational transitions remains largely hypothetical.

An alternative view is that mechanosensor switch is triggered by local elastic strain [67•], which does not result from protein conformational change and may have a character of extension or compression. In a series of studies [67,70,75], focal adhesions were modeled as two-layered structures, the lower layer of which is attached to the substrate, contains integrins, and possesses mechanosensitive properties (Figure 2). When force is applied to the FA's upper layer, the front edge of the mechanosensitive layer undergoes compression, resulting in an increased affinity for the plaque proteins (e.g. talin), and leading to FA enlargement (see below).

The underlying properties of both stress switches and strain switches can be formulated on the basis of simple estimates of their energy requirements. Stress can promote the conformational transition of a protein if the thermodynamic work produced by the stress significantly decreases the activation energy of the transition, or compensates for the related energy penalty. This is possible if the conformational transition is accompanied by a considerable change in the protein's dimensions. A paradigm

Figure 1
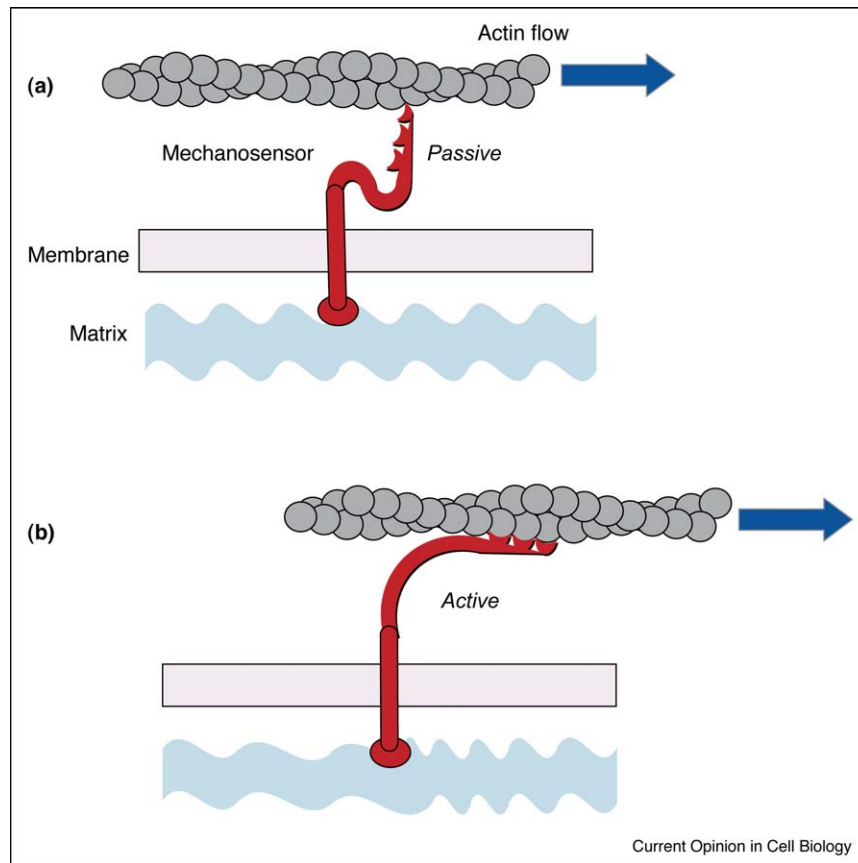
Stress-driven model for adhesion site mechanosensitivity [68•]. The mechanosensitive protein unit (depicted by red symbol) is connected to an actin filament moving with retrograde actin flow. This results in a dragging force acting on the protein unit from the filament. The mechanosensitive unit can be in one of two states that differ in the strength of link to the actin filament. **(a)** Passive state corresponds to a weak slip link. **(b)** Active state is characterized by a reinforced link. Transition from the passive to the active state occurs when the dragging force overcomes a critical value and involves a conformational change of the mechanosensitive protein. The model suggests a mechanism for maturation of focal complexes controlled by elasticity of the extracellular substrate, which undergoes deformation upon the force transmitted by the focal complex proteins.

Figure 2
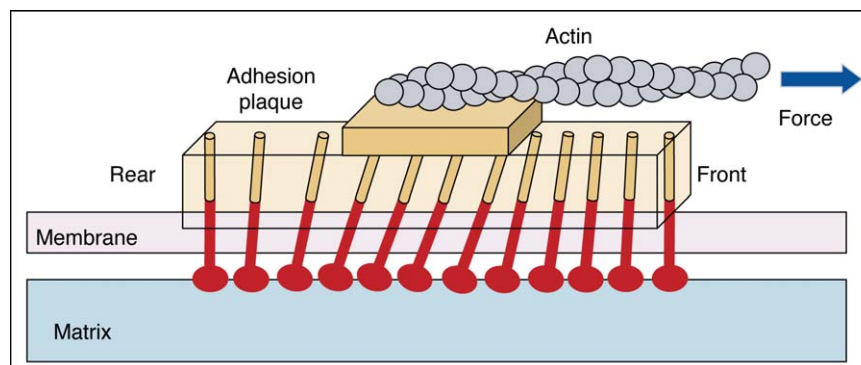
Strain-driven mechanism for focal adhesion mechanosensitivity [67•,70,75]. The focal adhesion (FA) consists of two layers: the lower layer, containing the mechanosensitive proteins, and the upper layer, which is directly connected to the actin filaments and transmits the force to the lower layer. Activation of the mechanosensitive proteins driving the FA assembly is suggested to be triggered by compression of the top of the lower layer relatively to its bottom. This strain is generated at the 'front' edge of the FA. The model explains the FA growth in the direction of the pulling force.

for the sensing of molecular stress is stretch-activated ion channels, which open when two-dimensional membrane stress — lateral tension γ — is applied [2,89]. The area occupied by a stress-sensing channel in the membrane plane changes by ΔA, so that the stress-related energy change, $\Delta F = -\gamma \cdot \Delta A$, is sufficiently large to drive the transition [2,89].

In the case of focal adhesions, the stress induced by actin filaments points in the direction of the pulling (contractile) force. For sensing such directed stress, the conformational transition must result in the protein stretching, ΔL, in the stress direction. The energy produced by the stress in this case can be estimated as $\Delta F = -\gamma \cdot L_{per} \cdot \Delta L$, where L_{per} is the protein's linear dimension, measured in the direction perpendicular to that of the stress. The required elongation of the protein (ΔL) can be estimated from the absolute value of the energy required $|\Delta F|$, which must at least exceed the characteristic thermal energy $|\Delta F| > k_B T \approx 0.6$ kcal/mol ($k_B T$ is the product of the Boltzmann constant and the absolute temperature). Taking into account that the stress-induced force acting on a single protein in the FA is $\gamma \cdot L_{per} \approx 1$ pN [32], one obtains $\Delta L > 4$ nm. Hence, the necessary protein elongation is of the order of the dimensions of the protein itself, a rather strong limitation that should be taken into account in assigning the ability to sense stress to the aforementioned protein candidates.

The essence of strain sensing lies in the dependence of plaque protein affinity on the deformation of the mechanosensing layer, mentioned above [67[•],70]. The specific mechanism underlying strain sensing can be readily understood if the attachment of a plaque protein to the sensor layer is coupled to the deformation of the latter. In the case that the sensor layer is not subjected to any strain prior to the binding of a plaque protein, the energy of the required deformation is 'paid' at the expense of the binding energy. However, if the sensor layer does undergo deformation prior to binding, the related strain energy is 'pre-paid', and the effective affinity of the plaque proteins increases. Within this context, strain sensing can be effective — i.e., the binding constant of the plaque proteins can be considerably changed by straining the sensor layer — if the deformation energy coupled to the binding of one plaque protein is larger than the thermal energy $k_B T$. This imposes a requirement on the sensor layer's stretching-compression rigidity, κ, whose value must be larger than $\kappa > (k_B T)/a^2$, where a is the binding-coupled deformation, with an order of magnitude of the dimension of a plaque protein $a \approx 5$ nm. nm. The estimation gives $\kappa > 0.15$ mN/m; this value is feasible, as it does not exceed the stretching rigidities of other biological materials such as actin filaments, which can be readily determined on the basis of the measured values of filament flexural and bending rigidities [90]. It should be emphasized that strain sensing may also be based on more complex deformations, which can result in such phenomena as the dependence of FA dynamics on the substrate elasticity [75].

A separate principle of mechanosensing, which does not rely on any hypothetical protein switch, has been suggested in [69[•]] (Figure 3). This model shows that the elastic stress generated within the plaque in the direction parallel to the plasma membrane by the attached stress fibers can, in and of itself, cause FA self-assembly and growth in the direction of the pulling force, whereas reducing that force results in FA disassembly. This mechanism of mechanosensing is based purely on thermodynamic principles, according to which the stretching stress decreases the protein's chemical potential within the plaque, thereby enhancing self-assembly via the introduction of new plaque molecules from the cytoplasm. This phenomenon can also be understood in more intuitive terms, in that the addition of new molecules to

Figure 3

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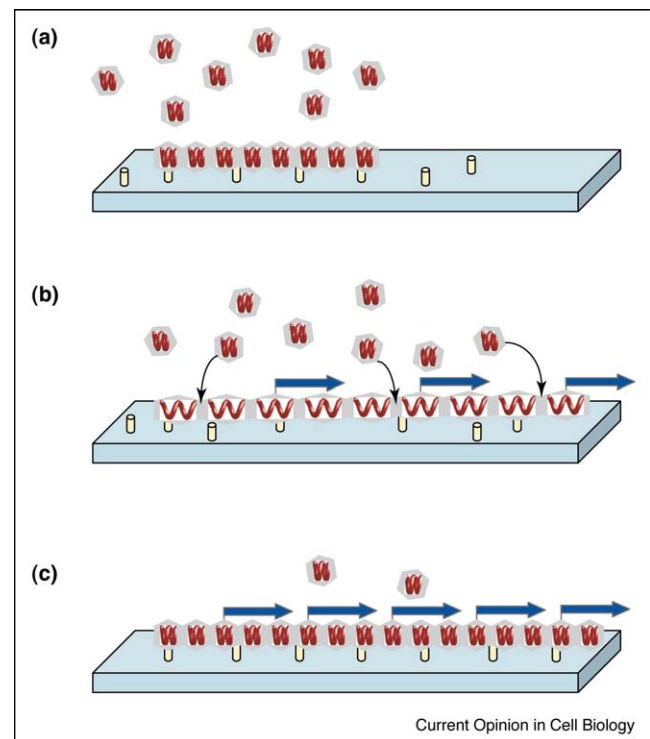
Thermodynamic model for focal adhesion mechanosensitivity [69[•]]. **(a)** The focal adhesion (FA) is modeled as an aggregate of elastic building blocks illustrated by hexagons containing short springs. The FA is connected to the substrate by links represented by small rods distributed along the aggregate surface. Free building blocks and free links enabling the FA assembly are distributed outside the focal adhesion. **(b)** Application of the pulling forces results in aggregate stretching and related accumulation of the elastic energy. The points of force application are distributed along the FA upper surface. **(c)** Insertion of new proteins into the aggregate results in energy relaxation, which drives FA assembly.

the stretched plaque would reduce the stresses, and decrease the corresponding elastic energy.

While this model does not entail any special conformational changes on the part of proteins, it implies that the FA plaque is elastic in nature and capable of accommodating itself to mechanical stress. Moreover, the plaque must possess a mechanism which enables it to acquire new FA proteins without undergoing stretch-induced rupture. This ability may require the presence of delicate molecular devices with properties similar to those of the members of the formin protein family [91,92], which are able to maintain a stable connection to an associated protein complex (the barbed ends of actin filaments in the particular case of formin) and, at the same time, enable insertion of new protein monomers into the complex, and the consequent stabilization of the growing structure.

There is increasing evidence that formins and, in particular, the *Diaphanous*-related formin mDia1, could conceivably mediate the force-dependent growth of focal adhesions [18,23]. Recent experimental studies indicate that formins are involved in actin polymerization at focal adhesion sites [93[••],94[•]]. Theoretical considerations further suggest that actin polymerization, upon capping by formins, can be enhanced by pulling forces [95[•]]. Further modeling and experimentation are needed to understand the specific mechanism by which formins can control FA mechanosensing.

The 'thermodynamic model' described in [69[•]] accounts for all the FA mechanosensing behavior experimentally observed thus far.

Dynamics of FA proteins
Another important difference between the suggested models of mechanosensing lies in the assumed position of the mechanosensor within the focal adhesion and the related mode of molecular exchange between the focal adhesion and the cytoplasm. The model suggested by [68[•]] assumes that the stress sensor(s) is located at the interface between the plaque and the stress fiber. In that case, the stress-mediated transition of the molecular switches is proposed to stabilize and reinforce the connection between the stress fibers and the plaque, resulting in the transformation of focal complexes into mature FAs.

The strain sensors, on the other hand, were proposed to be located in the integrin layer interacting with the ECM [67[•],70]. This model requires the sensor-containing layer to be more extended than the plaque, so that actin forces transmitted through the plaque compress this layer ahead of and extend it behind the plaque [67[•],70]. The compressed molecular switches generate the binding of new plaque proteins in proximity to the FA–stress-fiber junction, while the extended molecular switches favor plaque disassembly at the rear of the FA, together resulting in FA treadmilling. Importantly, the bulk of the FA does not participate in this protein exchange.

Finally, in the thermodynamic model [69[•]], the elastic stresses of the plaque itself stimulate FA self-assembly, so that the effective mechanosensors can be seen as located within the plaque. It is noteworthy that, in contrast to the strain-sensing model, the molecular exchange with the cytoplasm is suggested to involve the entire plaque volume, rather than the limited areas at the front and rear of the focal adhesion.

The assumptions of the strain-sensing model [67[•],70] and the 'thermodynamic model' [69[•]] result in some qualitatively different predictions concerning protein dynamics in the course of FA self-assembly. According to the former model [67[•],70], the mechanosensitive addition and detachment of plaque proteins take place only near the FA edges proximal to and distant from the stress fibers, respectively. This proposition implies that FAs can grow without moving with respect to the substrate or, alternatively, crawl along the substrate by treadmilling. No strain-driven internal motion of proteins within the FA is envisaged in this scenario.

In the thermodynamic model [69[•]], it is suggested that the proteins either join or leave the plaque along its entire area according to the relationship between the chemical potential in the cytoplasm and the local chemical potentials at each point within the FA. As a result, the concentration of the newly recruited proteins is expected to be distributed diffusively, throughout the plaque. Moreover, this model predicts internal treadmilling-like motion of proteins within the plaque, whether or not the latter grows, shrinks or maintains constant, steady-state dimensions. This movement can progress in different directions, depending on the regime of FA assembly (or disassembly), and should contribute to the internal intermixing of FA proteins. Experimental testing of these predictions is crucial if we are to clarify the validity of these theories and advance our understanding of the mechanisms underlying the regulation of focal adhesion dynamics.

Conclusions and unanswered questions
As discussed above, investigations of adhesion-mediated mechanosensitivity have reached a rather peculiar stage. On the one hand, the phenomenon has been described and validated in many experimental systems, and its phenotypic manifestations are well-documented; yet the underlying molecular mechanisms are still elusive. We know that a cell can sense quite accurately and respond to deviations from the characteristic stress of $5 \text{ nN}/\mu\text{m}^2$. We also know that force induces the directional assembly of adhesions; moreover, this process is regulated by Rho GTPases and modulated by protein phosphorylation or dephosphorylation. However, we are still not certain whether mechanosensitivity is governed

by a single protein or by large multi-protein complexes, nor have we identified the proteins involved.

In a similar vein, it is not yet clear in which subcellular site the force-induced effect is sensed. Is the mechanosensor associated with the cytoskeleton? With the scaffolding plaque or the integrin-containing layer? With the plasma membrane, perhaps? Is it, in fact, part of the extracellular matrix? The molecular perturbations induced by the mechanical force are enigmatic, too. Does this force directly 'switch on' or 'switch off' enzymatic activities? Might it affect the conformation of specific proteins? Could it perturb protein–protein interactions in large multi-protein complexes?

While definitive answers to most of these questions are not yet available, there are new techniques (e.g., the knock-down of specific molecules using an siRNA approach) that enable systematic elimination of individual proteins from living cells, and analysis of the resulting phenotypic changes. The development of theoretical models of adhesion-mediated mechanosensitivity, coupled with a deeper understanding of the underlying physical principles, are essential pre-requisites for the design of effective experimental strategies aimed at deciphering the molecular underpinnings of this intriguing phenomenon.

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