Single cell rigidity sensing: A complex relationship between focal adhesion dynamics and large-scale actin cytoskeleton remodeling

Mukund Gupta, Bryant Doss, Chwee Teck Lim, Raphael Voituriez, and Benoit Ladoux

Mechanobiology Institute (MBI), National University of Singapore, Singapore; Department of Biomedical Engineering, Faculty of Engineering, National University of Singapore, Singapore; CNRS UMR 7600, Université Pierre et Marie Curie, Paris, France; Institut Jacques Monod (IJM), CNRS UMR 7592 & University Paris Diderot, Paris, France

ABSTRACT
Many physiological and pathological processes involve tissue cells sensing the rigidity of their environment. In general, tissue cells have been shown to react to the stiffness of their environment by regulating their level of contractility, and in turn applying traction forces on their environment to probe it. This mechanosensitive process can direct early cell adhesion, cell migration and even cell differentiation. These processes require the integration of signals over time and multiple length scales. Multiple strategies have been developed to understand force- and rigidity-sensing mechanisms and much effort has been concentrated on the study of cell adhesion complexes, such as focal adhesions, and cell cytoskeletons. Here, we review the major biophysical methods used for measuring cell-traction forces as well as the mechanosensitive processes that drive cellular responses to matrix rigidity on 2-dimensional substrates.

INTRODUCTION
All tissues comprise an extracellular matrix (ECM) that surrounds the cells inside the tissues, and it is the major constituent of connective tissues. The matrix is a dynamic structure that provides physical support to the cells and is required to maintain tissue homeostasis. It provides various mechanical/physical cues—confinement, topography, stiffness—to the cells that are important for cellular function. In particular, matrix stiffness is critical for many cellular processes such as cell migration, differentiation, proliferation, apoptosis, and plays an important role in tissue development. The stiffness of the ECM is a measure of the resistance it can provide to applied forces, and can vary over orders of magnitude, from a few Pa in brain tissues to over 100 kPa for pre-calcified bones. Deficiencies in cell’s ability to sense and respond to matrix stiffness, or alterations in matrix mechanical properties, have been implicated in various diseases such as cancer and fibrosis. An understanding of how cells sense and respond to substrate stiffness can help in identifying treatments for these diseases, as well as lead to better designs of functional tissue scaffolds for tissue transplantsations. Presently, our understanding of the mechanisms of matrix stiffness sensing is limited.

Various mechanosensory mechanisms, spanning different spatial scales, have been proposed but a complete picture is yet to emerge. These range from mechanisms mediated by local proteins or protein assemblies (stretch-sensitive ion channels and adhesion complexes), to those mediated at a more global or cellular scale by large protein networks such as actin cytoskeleton. The relative roles of these different mechanisms remain essentially undetermined and their potential coordination at the cell scale uncertain. Perhaps these mechanisms, which act at different spatial scales, could be probing the matrix stiffness at different scales as well, and the response of the cell could result from their global integration. Here, we review the different techniques that have been used to study substrate stiffness sensing, and where we currently stand in our understanding of the underlying mechanisms.

TECHNICAL APPROACHES TO STUDY CELL TRACTION FORCES AND RIGIDITY SENSING
The extra-cellular matrix provides a fibrillar 3-dimensional (3D) environment to the cells, that comprises...
varying compositions of over 300 proteins, 200 glycoproteins, and 30 proteoglycans, which provide tissue specific chemical and mechanical environment to the cells. Its stiffness is mainly determined by the constituent elastic fibers, fibrillar collagens, and glycosaminoglycans. In order to study the effect of matrix stiffness on cells in a controlled and reproducible manner, various experimental approaches have been used to mimic the matrix in vitro: cells have either been embedded within 3D polymer gels, or adhered to 2D surfaces. ECM proteins such as collagen type I, fibrin, and Matrigel have been used to generate 3D gels for such studies, but currently it is difficult to precisely control their mechanical properties in a reproducible manner. Also, among other problems, it is difficult to quantitatively image cells in such systems. Thus, most of the studies involve adhering cells on top of 2-dimensional (2D) substrates, although recently 3D fibrillar matrices have been produced in a more controlled manner. Although natural ECM proteins such as collagen have been used for making 2D gels, it is difficult to clearly isolate the effect of stiffness from other structural changes in these gels. Fully synthetic hydrogels - made of materials such as poly(ethylene glycol) or PEG, PolyAcrylAmide (PAA), and sugars such as hyaluronan, dextran or alginate – have helped overcome most of the shortcomings of 2D gels made of natural ECM proteins. Of these synthetic gels, PAA gels have been used most extensively for studying the effect of substrate stiffness on cell behavior, where the elasticity of the gels is set by the concentration of bis-acrylamide cross-linking. Besides PAA gels, the other commonly used 2D substrate for such studies is microfabricated micropillar substrates. These substrates consist of dense arrays of vertical columnar structures on whose tops the cells can adhere. The key difference between the PAA gels and micropillar substrates is that while PAA gels provide a continuous surface to cells, the micropillar substrates provide a discontinuous surface since they are an array of discrete columns. This difference in topography also leads to different stiffness definitions for the 2 substrates: stiffness of PAA gels is defined in terms of Young’s modulus, and stiffness of micropillars is defined in terms of a spring constant. Young’s modulus is a measure of the amount of stress required to produce a certain strain in a material, and is an intrinsic property of a material that is independent of geometry—the elasticity or Young’s modulus for PAA gels can be changed by adjusting the ratio of cross-linkers to base polymers in the gel (lower crosslinking leads to lower stiffness). Moduli ranging from 0.1 kPa to 100 kPa have been obtained for PAA gels, with moduli below 10 kPa considered soft, and above 10 kPa considered stiff. This definition of soft and stiff is based on the stiffness of tissues and substrate stiffness dependent differentiation of stem cells, and may change with the cell type. Spring constant is a measure of the amount of force required to deform a structure by a certain magnitude, and is dependent on both the material property and geometry of the structure – the stiffness of micropillars can be tuned by changing Young’s modulus of the material that they are made of (polymers such as PDMS), or by changing their diameter and height. Spring constants ranging from 1 nN/μm to >100 nN/μm have been obtained for micropillars, with substrates below 10 nN/μm considered as soft. The stiffness of micropillar substrates can be defined at 2 different scales—a local scale, given by Young’s modulus of PDMS corresponding to the top surface of the pillar, and a global scale, given by the spring constant of the micropillar as a whole. One advantage of micropillars over PAA gels is that change in the stiffness of PAA gels also leads to a change in the pore size of the gel and hence its surface chemistry, while that of micropillars can be changed independently of surface chemistry by changing their geometry. However, recent evidence shows that pore size of the gel does not significantly alter protein tethering and hence surface chemistry. Micropillars have been adapted to apply forces on cells, study cell migration in 3D, integrated into microfluidic channels, and mounted on stretchable membranes. Micropillars with diameter as low as half a micron have also helped to detect local mechanosensing events during cell spreading, with deflections of a few nanometers being measured.

Both the PAA gels and micropillar substrates are linearly elastic substrates, whereas in vivo extracellular matrix is non-linear. The effects of non-linear elasticity on cell behavior have only recently been given some attention, and more work needs to be done.

**Cell traction force measurements**

Cellular traction forces are one of the key measurements used to quantify cellular response to ECM stiffness. Adherent cells exert forces on the surrounding ECM, generated by their internal actomyosin networks, which helps them in adhering to the matrix, probe its stiffness, and even remodel it. One of the first demonstration of cellular traction was performed by using compliant sili-con-rubber substrate, on which adherent cells could produce wrinkling patterns. These wrinkling patterns clearly showed for the first time that non-muscle cells could exert forces on their substrate. More quantitative techniques were developed, based on the principle that traction forces can be extrapolated from substrate deformations. Of these cellular traction force measurement techniques, the ones that are based on using...
PAA gels and micropillars are the most commonly used. The one using PAA gels is conventionally called Traction Force Microscopy (TFM). Since PAA gels undergo linear elastic deformations, the forces exerted on them can be computed from their surface deformation using models from continuum mechanics.\textsuperscript{1,3,34} To quantify the substrate deformation, fluorescent beads are embedded into the PAA gel and used as fiducial markers. The beads are tracked using microscopy to produce a displacement map for the substrate, which is then used to solve the ‘inverse’ problem using the Boussinesq’s formulation: an equation for the displacement data in infinite half-space is derived into a convolution integral using Green’s tensor, discretized and then inverted to determine traction forces.\textsuperscript{50} The inversion step is computationally very intensive and several regularization steps are required to account for the noise in displacement data. The algorithms for this are still being actively improved.\textsuperscript{51} Traction force measurement technique using micropillars overcomes these limitations of the PAA gel-based TFM since it is a discontinuous substrate where there is no strain propagation. However, there could be some strain propagation due to substrate warping for micropillars with small aspect ratios,\textsuperscript{52} but it is negligible at the micron scale distance between micropillars. Thus, each of the micropillar behaves as a linear elastic spring, force on which can be calculated independently from other micropillars. The micropillar tops act as fiducial markers that can be used to obtain a displacement map of the substrate, which can then directly be converted into traction force map by scaling using the micropillar spring constant.\textsuperscript{35-37,53} Although, both PAA gels and micropillars have mostly been used to measure traction forces in 2D (xy plane) only, some recent studies using PAA gels have shown that cells adherent on 2D substrates can also exert forces normal to the substrate (z direction).\textsuperscript{54,55} These normal forces were of a similar order of magnitude as the forces applied tangentially to the substrate (xy plane), thus indicating that adherent cells probe their 2D substrates in all 3 spatial dimensions. Traction forces ranging from <1 nN to tens of nN, and dependent on substrate stiffness, have been observed at FAs, using both PAA gels and micropillars.\textsuperscript{13,21,35} Also, measurements using micropillars showed that the stress at the FAs increases from \(~1\) kPa to \(~10\) kPa with increasing substrate stiffness.\textsuperscript{21} Stresses with similar order of magnitude were also measured using PAA gels of comparable stiffnesses.\textsuperscript{13,56} Together, both PAA gels and micropillars, provide a means to simultaneously modulate substrate stiffness and measure traction forces, and this has led to invaluable insights into mechanisms of mechanosensing.

Until recently, it was not possible to reliably measure traction forces exerted by cells embedded in a 3D gel. Most of the 3D traction force measurement methods developed recently use some fiducial marker embedded in the gel to measure the strain in the gel—either fluorescent beads,\textsuperscript{57,58} or the structural component of the gel itself.\textsuperscript{59} The fiducial markers are used to obtain the 3D strain field in the gel, using confocal microscopy, which can then be used to computationally infer the traction forces exerted by the cells embedded inside the gel. The method to calculate cell traction forces from the 3D gel strain field is similar to that used for 2D gels, but is computationally more complex due to the additional dimension. A numerical technique called Finite Element Method is usually used to solve the 3D partial differential equations involved.\textsuperscript{57-59} Legant et al. found that fibroblast cells embedded in PEG gels—which are linearly elastic—exerted traction stresses ranging from 0.1 to 5 kPa, with the largest forces localized at the tips of cell protrusions.\textsuperscript{57} It should be noted that the highest traction stresses reported by Legant et al. are of the same order of magnitude as those observed at focal adhesions in cells adhered to 2D substrates.\textsuperscript{12,21,35} Recently, 3D traction force measurements have also been performed for cells embedded in gels that are viscoelastic and non-linear, and hence closer in their mechanical properties to in-vivo matrix.\textsuperscript{58,59}

Besides measurement of forces exerted by cells on their environment, measurement of tension across proteins inside and outside cells has also provided key insights into mechanosensing. Recent development of FRET-based molecular tension sensors have enabled intra-cellular and extra-cellular tension measurements at molecular scale.\textsuperscript{60-64} These sensors employ Förster (fluorescence) resonance energy transfer (FRET) between a donor fluorophore and an acceptor fluorophore, where the energy transfer depends on the distance between the 2 fluorophores — increasing distance implies stretching of the protein and hence a tension on it. The sensors can detect forces in the range of a few pN/molecule. For example, using a vinculin FRET based sensor, Grashoff et al. showed that the average force per vinculin molecule in stable FAs is \sim 2.5 pN.\textsuperscript{50} Using FRET-based sensors, Morimitsu et al. showed that tension is distributed heterogeneously across integrin molecules in focal adhesions,\textsuperscript{62} and Kubow et al.\textsuperscript{63} showed that force across fibronectin fibrils tunes their interaction with collagen fibers in the ECM. Also, Wang et al. developed a novel DNA-based force sensor to measure forces exerted by single integrin molecules on their substrate, and found that they apply a peak tension of 40 pN.\textsuperscript{64}

**Cellular response to mechanical cues**

One of the earliest qualitative studies on the effect of substrate stiffness, using freely-floating and surface
adhered collagen gels, had shown that mammary epithelial cells proliferate on stiff substrates (adhered collagen gels), and differentiate on soft substrates (freely-floating collagen gels). Later, Choquet et al. showed unequivocally that cells respond to the stiffness of their substrate and applied force, by reinforcing their focal adhesions (FAs). This suggested that the cell-substrate adhesions, or FAs, could be involved in substrate stiffness sensing. Most of the subsequent studies have characterized the response of cells to their substrate's stiffness by measuring cell spreading or area, cell migration, FA area and dynamics, actin cytoskeleton polarization, and cellular traction forces. These studies show that cells are unable to spread on soft substrates while they can spread completely on stiffer ones, cells prefer to migrate toward stiffer substrates, FAs are larger on stiffer substrates, actin cytoskeleton is more polarized on stiffer substrates with stress fibers aligning in the same direction, and cells exert higher traction forces on the stiffer substrates. Many types of cells—stem cells, muscle cells, neurons, fibroblasts, epithelial cells, and neutrophils—have shown an ability to sense substrate stiffness and exert forces on their substrate. One of the most interesting responses of cells to substrate stiffness was found for mesenchymal stem cells, which differentiated into different tissue lineages depending on the substrate stiffness—neuron-like on soft and bone-like on stiff. Actomyosin contractility was found to play a major role in these responses. It is to be noted that these mechanoresponses could occur at different time-scales—from a few minutes during cell spreading to a few days during cell differentiation—and may require the integration of multiple mechanosensing events over time. More recently, matrix stiffness has also been found to modulate the level of laminA/laminB in the nucleus, and activity of downstream effectors of Hippo pathway (YAP and TAZ). Although several mechanisms have been suggested by which cells could sense the substrate stiffness, and respond as shown by all these studies, a clear picture has still not emerged to explain how mechanical signals are transmitted from the extracellular matrix to the nucleus. The different cellular structures that have been proposed as possible mechanosensors act at different spatial scales - FAs and ion channels act at the molecular scale, compared to the actin cytoskeleton that acts typically at the micron scale. Of these, the small-scale mechanosensors, FAs have been thought to be the primary mechanosensors. However, recent studies show that FAs in themselves cannot explain mechanosensing completely, and the actin cytoskeleton could be playing an important role as well at a larger scale.

**Role of focal adhesions in mechanosensing and rigidity sensing**

Focal adhesions (FAs) are intra-cellular protein complexes that are the contact points between the cell and its surrounding matrix, and were first observed by electron-microscopy. They transmit the internal forces, generated by cellular actomyosin contractile networks, to the substrate on which the cell adheres and hence could help in probing its stiffness. In experiments done using PAA gels, it was observed that the size of FAs increases as the stiffness of the substrate increases. Balaban et al. measured forces exerted by cells at single FAs and found that it is proportional to the FA area, implying a constant stress of 5.5 nN/μm² at the FA. However, this was not found to be true for the smaller nascent FAs, which were observed to apply large forces. Also, Riveline et al. used micropipettes to directly apply force to the FAs and found that they elongated in the direction of the applied forces. These observations—increasing area of FA with substrate stiffness, constant stress at FA sites, and anisotropic growth of FAs—led to the hypothesis that FAs could be directly involved in sensing the substrate stiffness.

It is believed that the underlying mechanism involves a force-dependent stretching of some proteins inside FAs, leading to unfolding of cryptic binding sites, which in turn can recruit other signaling molecules. This would lead to further reinforcement of the FA and initiate a signaling cascade leading to a cellular response. Since the actomyosin network is connected to the matrix through the FAs, which comprise several molecules, each of the constituent molecules at the FA could be a potential candidate for the mechanical to chemical signal transduction for sensing substrate stiffness. Of these proteins, the adaptor protein talin, which connects integrin molecules to actin filaments, has been shown to unfold under cellular level forces leading to exposure of cryptic binding sites for the protein vinculin. Using magnetic tweezers to apply pN level forces on single talin molecules, del Rio et al. showed that vinculin binding sites, buried under bundles of α-helices, get exposed due to stretching of talin molecule. Vinculin recruitment then helps in reinforcing the integrin-actin linkage. Also, Margadant et al. showed that inside cells as well talin molecules get stretched and then recruit vinculin molecules to the FA. Vinculin, in turn, has also been shown to stretch and change its configuration under force. Using a FRET-base tension sensor, Grashoff et al. had shown that vinculin molecules are under a force of ~2.5 pN in stable adhesions, and are in a stretched configuration in assembling FAs, and non-stretched configuration in disassembling FAs. This suggests an important role for vinculin in FA maturation.
and force transmission to the actin cytoskeleton. Also, force dependent stretching of other proteins in the FAs can tune their activity: the scaffold protein p130Cas exposes cryptic phosphorylation sites, and the actin binding protein filamin exposes sites for binding cytoplasmic tail of integrin molecules. Hence, force-mediated changes in protein conformation—either through mechanical stretching, or force-dependent enzyme modifications—help in transducing the mechanical signals into chemical signals at the FAs. Also, recently, Plotnikov et al showed, based on traction force data, that each FA can act as an autonomous local substrate stiffness sensor by applying a ‘tugging’ force on the substrate. Using high resolution TFM on PAA gels, they observed that the traction forces could fluctuate within a single FA, depending on the substrate stiffness—the center of the peak force within a FA fluctuated from its distal end to its proximal end on a soft substrate, while it remained stable at the center of the FA on stiffer substrates. The origin of these local fluctuations in force remains unclear but may be attributed to fluctuations in the actomyosin contractility itself, or to transient force-dependent interactions between actin filaments and FA proteins, also called the ‘clutch’ model. The force-dependent modulation of protein activity in FAs and tugging forces at these sites indicate that they mediate a local mechanosensory mechanism that occurs at the nanometer scale.

Several theoretical studies have been conducted to understand the force dependent growth of FAs. Shemesh et al. proposed a general mechanism, independent of the molecular details, by adapting the theory of surfactant adsorption at an interface. They proposed that in the presence of heterogeneous and anisotropic stress on the FA, the adhesion will be distorted; this distortion, leading to a variation in its density, would cause adsorption of additional proteins at the low density regions so as to minimize the energy of adsorbed proteins. However, this model does not take molecular details into account, and predicts larger adhesions on softer substrates which is contrary to experimental results. Nicolas et al. proposed a model for adsorption of proteins into an existing adhesion plaque that takes into account the conformation-induced change in protein affinity caused by the applied forces. Assuming that the stress at the FAs remains constant and treating the adhesions as elastic plaques, they proposed that the condensation of molecules at the adhesion plaque could be driven by the minimization of the actomyosin contractile energy that the cell expends at the FA. This could predict the substrate stiffness dependent limitation of FA size because of the increasing elastic energy required on soft substrates to maintain a constant stress at the adhesion.

However, the proposed models and other mechanisms of sensing by FAs such as tugging, remain essentially very localized, whereby a FA can only probe the substrate stiffness at a spatial scale local to itself. For example, it is not clear how the ‘tugging’ force mechanism could lead FAs to sense the stiffness of micropillar substrates. In studies performed with micropillars, the small-scale stiffness (Young’s modulus) was kept constant, while the large-scale stiffness (spring constant) was changed by tuning the height of the micropillars. Thus, the micropillars provided a high small-scale stiffness (2 MPa) and a lower large-scale stiffness (5–100 nN/μm or 3–70 kPa). The ‘tugging’ force from FAs would be able to probe only the local stiffness of the micropillar tips at the scale of a single FA. However, cells were found to still respond differently to micropillars of different heights, implying that they could also sense the large-scale stiffness of the micropillars. This indicates that there should be other mechanisms at a scale larger than a single FA that could sense the large-scale stiffness of the micropillar. On continuous substrates such as PAA gels, as in the case of the study by Plotnikov et al. where tugging forces were observed, the stiffness does not depend on length-scale and hence does not allow for the identification of mechanisms at a scale larger than a FA.

Increasing evidence shows that mechanosensory mechanisms mediated only by FAs are not sufficient to explain substrate stiffness sensing. It is not clear how these local sensors could lead to a response at the cellular scale, and it seems that they play a prominent role at a local scale while other mechanisms would also be involved at larger scales. Recent experiments show that the earlier observations of constant stress at FAs might be dependent on the state of the FAs. It was observed that the FA grows proportionally to the applied force only during its growth phase; once it matures, the force can either decrease or increase with time while the FA area remains constant. Not only is the stress applied by FAs time-dependent, it also depends on the stiffness of the substrate: the same FA area can sustain higher stresses on substrates with higher stiffness. Interestingly, Oakes et al. showed that although force is required for FA growth, it in itself is not sufficient: a template of actin stress fibers at FAs is necessary for the FA to grow and mature. Together, these studies indicate that the previous results, on which the hypothesis that FAs are the primary mechanosensors was based, do not provide a complete picture.

Role of actin cytoskeleton in response to mechanical cues

There is increasing evidence now for stiffness sensing mechanisms at spatial scales larger than FAs, which could be mediated by the actin cytoskeleton. In order to measure the cell response to substrate stiffness at the
cellular scale, Mitrossilis et al. suspended cells between 2 microplates; they could tune the stiffness of one of the microplates in real-time, and measure the force exerted by cells simultaneously. They found that the time-scale at which cells can respond to changes in substrate stiffness is very fast (less than a second), and therefore could not be due to minutes-long biochemical signaling at FAs. This was explained by considering the actin cytoskeleton as a large-scale stiffness sensor dependent on the interaction between myosin II molecular motors and actin filaments that could respond to substrate stiffness in milliseconds. Recent reports suggest that actomyosin-based sarcomeric contractile units are also directly involved in stiffness sensing during cell spreading. Using micropillars of diameter less than a micron, Wolfenson et al. found that the micropillars deflected in steps of 2 nm, with the number of steps being different for different substrate stiffnesses, thus applying different level of forces. Tropomyosin was found to restrict the size and number of steps on soft substrates. Also, recent studies have shown that as the stiffness of the substrate increases, the number of actin stress fibers in the cell increases and they tend to align parallel to each other, thus polarizing the cell. This reorganization could be due to tension dependent regulation of different molecular players involved in actin cytoskeleton reorganization. It has been shown that tension across actin stress fibers can affect their dynamics by regulating binding activity of proteins such as zyxin and phosphoERK. Also, the activity of actin filament cross-linkers such as α-actinin, the actin filament nucleating protein formin, and the actin filament depolymerizing factor coflin can be modulated by tension, which could lead to tension-dependent reorganization of the actin cytoskeleton.

The reorganization of the actin cytoskeleton could lead it to directly sense the substrate stiffness at a large scale. Zemel et al. showed that the actin stress fibers in stem cells become aligned as the substrate stiffness is increased; the increase in stress fiber alignment, measured as an order parameter, was non-monotonic, and peaked at a certain stiffness of the substrate. To understand how this alignment could play a role in mechanosensing, they modeled the cell as an active elastic inclusion on a deformable substrate, with both passive forces—arising from the elasticity of the cell and the substrate—and the active forces due to actomyosin contractility. Actomyosin forces were modeled as local ‘force dipoles’ that would be formed by the forces at the 2 ends of a stress fiber. A phenomenological coupling between the local stress in the cell and the orientation of force dipole was introduced which helped in explaining the experimentally observed correlation between actin order parameter, cell shape anisotropy, and substrate stiffness. This model could explain the non-monotonic actin cytoskeleton polarization with substrate stiffness for stem cells.

On the other hand, studies on fibroblast cells have shown a monotonic increase of actin polarization with substrate stiffness. Compared to the non-monotonic increase in stem cells, this monotonic increase in fibroblasts could be due more mature stress fibers in them that can withstand higher stresses at high substrate stiffnesses. Stress fibers in stem cells are nascent and not as thick as those in fibroblasts, and might collapse due to higher stresses on stiffer substrates, thus leading to a non-monotonic increase in actin polarization. Friedrich et al. had developed a theoretical framework, wherein they showed that weak elastic interactions between contractile actin filaments would lead to a non-monotonic increase in actin ordering as a function of substrate stiffness, while a strong interaction would lead to a monotonic increase. Thus, the difference in increase of actin polarization in the 2 cell types could also be explained by weaker interaction between stress fibers in stem cells, and stronger interactions in fibroblasts. Also, the studies with fibroblasts were performed on micropillar substrates where mechanical interactions between FAs are negligible through the substrate, as opposed to the work of Zemel et al. that was performed on PAA gels.

The experimental data obtained for fibroblast cells have been used to develop theoretical models in the framework of the active gel theory which suggest that actin cytoskeleton acts as a large-scale mechanosensor. This approach assumes that the cytoskeleton behaves as an elastic nematic gel, and provides a mechanism of the emergence of order at the cellular scale. In this case, the coupling between substrate stiffness and order induced by the gel activity is responsible for a substrate stiffness-dependent transition from isotropic to nematic order at the cellular scale (Fig. 1A). This phase transition may be governed by the density of actin filaments (f-actin) within the cell (Fig. 1B): as substrate rigidity increases, f-actin density is increased reaching a critical value over which nematic order appears and the stress becomes anisotropic. Critically, higher stiffness substrates lead to lower compressive strains in the cytoskeleton and increased f-actin density in the cells, which is a property of active systems only. On soft substrates, the contractile force of molecular motors is dissipated by the sliding of actin filaments because the soft substrates cannot provide enough resistance. Hence the filaments are under lower tension on soft substrates with higher strains compared to the case when cells are on stiff substrates which can resist the sliding of the filaments and produce smaller strains. The strain-dependent tensile state of the actin cytoskeleton could change f-actin density by regulating actin filament...
dynamics at the molecular scale—for higher stiffness, strains will be lower and hence tension higher, which would lead to higher polymerization rates and lower depolymerization rates.\textsuperscript{16,17,88-90} Thus, the coupling between the actin filament polarization and substrate stiffness predicts an isotropic to nematic transition of actin cytoskeleton organization: below a critical stiffness the actin cytoskeleton would remain disorganized and isotropic, and above it, the cytoskeleton would become increasingly organized as the substrate stiffness in increased.

In addition, a substrate stiffness-dependent change in rheology of the actin cytoskeleton was also observed.\textsuperscript{24} On very soft substrates the lifetime of the actin filaments is shorter than the case of cells on the stiffer substrates. Circular cables of actin filaments were observed around the nucleus, which flowed centripetally toward the nucleus. This indicated that the actin filaments experience low friction on a soft substrate and are not strongly anchored to the substrate. This is in contrast to the behavior of the actin cytoskeleton on stiff substrates where the stress fibers are firmly anchored to the substrate and are stable over time scales similar to those for actin flow on soft substrates. Consequently, the cell shape appears to be also affected by substrate stiffness resulting in circular shapes.

\textbf{Figure 1.} Cells sense substrate stiffness by adapting their rheology and actin organization. (A) Actin cytoskeleton transitions from an isotropic phase on soft substrates to a nematic phase on stiff substrates. Reproduced from Gupta \textit{et al.}, with permission from Nature Publishing Group.\textsuperscript{24} (B) This isotropic-nematic phase transition is governed by the density of actin filaments (F-actin) within the cell. At any particular F-actin density, there will be an optimal F-actin organization with minimal free energy. As the alignment of actin filaments increases, the myosin motors within the actin gel will produce higher anisotropic active stress. This active stress will lead to a compressive strain within the actin gel, which critically depends on the substrate stiffness since the contractile actin filaments are anchored to the substrate—higher substrate stiffness will lead to lower compressive strain in the actin gel. The cellular strain will in turn regulate the actin filament density by tuning F-actin polymerization and depolymerization, which would then again regulate the F-actin organization. This feedback loop would lead to an isotropic to nematic transition in F-actin organization at a critical actin filament density dependent on the substrate stiffness.
on soft substrates and more polarized cell shapes (with higher aspect ratio) as stiffness increases. Importantly, it shows that substrate stiffness not only induces a large scale isotropic-nematic transition but also a change of the cell rheology from viscous to elastic behavior. The change in the rheology of the cells could also be driven by the tuning of binding affinity of actin filament crosslinker α-actinin, as shown recently by Ehrlicher et al.93 Previously, Solon et al. had shown that the elasticity of fibroblast cells increases as the stiffness of the substrate is increased, suggesting that the cells tune their internal stiffness to match that of the substrate.94 Interestingly, cancer cells have been observed to have lower elasticity on stiffer matrices, suggesting that they tune their elasticity to be different than their surrounding tumor environment which is stiff.109,95 However, the observation that not only the elasticity but also the viscosity of the cytoskeleton changes, needs to be taken into account to understand the large-scale mechanisms by actin cytoskeleton in further detail. Thus, adaptation of both the rheology and organization of the actin cytoskeleton could be the mechanisms that cells employ to sense the substrate stiffness at large scales.

Along this line, the coupling between FAs and actin cytoskeleton may explain this viscoelastic transition since softer substrates induce short-lived FAs, and, hence, a low friction.19 Since FAs serve as mechanical links between the actin cytoskeleton and the underlying substrate, this low friction favors actin flows in response to contractile stress which, in turn, contribute to destabilize FAs. At the cellular scale on soft substrates, low friction forces and radial actin flow favor circular cellular shapes, and consequently, non-motile behavior of cells. In contrast, as stiffness increases, the assembly of stable stress fibers and their large-scale polarization induce cell shape changes and could promote a front-back polarity axis. Consequently, such behavior may explain the ability of cells to move toward stiffer environments.2

Interestingly, this behavior is reminiscent of other apparently different situations including cell-cell contact dynamics and early cell spreading (Fig. 2).22,96 Cell spreading and actin organization of mesenchymal and epithelial cells on recombinant cadherin-coated glass surfaces,97 mimicking the formation of actual cadherin-mediated cell-cell contacts,96 revealed a behavior similar to cells seeded on fibronectin-coated soft substrates.24 On cadherin-coated substrates, cells remain round and isotropic with no sign of polarization as observed at early stages of spreading on ECM-coated stiff substrates or on soft ECM-coated surfaces.24,99 As on soft surfaces, these cells never polarize and present an isotropic organization of the actin filament network with tangential actomyosin arc sitting at the rear of a circular lamellipodium. It is noteworthy that these cells present a polarized phenotype on fibronectin-coated surfaces and migrate, which could be due to myosin II-driven remodeling of the actin cytoskeleton.96,99 Moreover, the amplitude of forces transduced at cadherin-mediated adhesions is lower than those transduced at FAs,100 and consequently it may favor a viscous-like behavior of the cells with lower friction and higher flows of actin. Another example deals with cell spreading. At early times, fibroblasts spreading on fibronectin-coated plastic surfaces have been described as initially round, isotropic with no sign of polarization of the actin cytoskeleton.101 However, cells polarize over time and initiate migration. Again, this process is accompanied by a switch of the organization of the actin filament network from an isotropic organization characterized by the formation of a tangential contracting actomyosin arc sitting at the rear of the circular lamellipodium as described on a soft substrate to a polarized distribution of stress fibers mostly oriented in the direction of the lamellipodium-uropod axis.101 Tee et al. modeled this isotropic to polarized transition in cell organization to understand what causes the break in symmetry.101 They modeled the actin cytoskeleton as being composed of non-contractile radial filaments (RFs) and contractile transverse filaments (TFs), initially in a symmetric/isotropic configuration. The TFs could slide over the RFs due to alternate binding and unbinding events. The contractility of the TFs introduces a centripetal force that pulls the RFs inwards, and a tangential force that applies rotational moment on RFs about their anchoring FAs. Interestingly, the model predicts that the 2 forces on RFs would lead to spontaneous unidirectional rotation of the RFs, which would break the symmetry of the actin filaments, finally leading to a polarized state. This model proposed by Tee et al. provides a good example of a mechanism for actin reorganization in different situations.

**Conclusion and perspectives**

Cell responses to physical cues appear as important regulators of various biological functions. After taking a back seat in favor of genetics, it becomes increasingly evident that force sensing is crucial during the different steps of the cellular life. Transduction of mechanical inputs into biochemical signals as cells probe substrate elasticity or respond to external forces involve various mechanosensitive components that act at various length-scales and time-scales. However, the interplay between these different mechanosensitive units remains unclear. Particular attention should be made to focus on the coupling between these different length-scale processes in order to unravel the different sequential steps leading to appropriate cellular responses.

Here, we focus on the role of mechanosensitive units such as actin and FAs within the cytoplasm. They are indeed crucial to transmit the mechanical
information but intensive research is also directed toward biosensors that mediate communication between the cytosolic and the nuclear compartments.\textsuperscript{102} Indeed, since substrate elasticity and mechanics can govern cell fate and transcriptional activity,\textsuperscript{3,71} it remains crucial to understand how mechanotransduction pathways are coupled to gene expression.\textsuperscript{72,103}

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.
Acknowledgments

The authors thank Samuel Safran, group members from Mechanobiology Institute (MBI) and Institut Jacques Monod. The authors would also like to thank MBI Microfabrication core (Gianluca Grenci and Mohammed Ashraf), MBI Science Communication core (Andrew Wong and Steven Wolf) and MBI Microscopy core (Felix Margadant) for continuous support.

Funding

Financial supports from the Human Frontier Science Program (grant RGP0040/2012), the European Research Council under the European Union’s Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement n° 617233 (to B.L.), Agence Nationale pour la Recherche project “PillarCell” (ANR 13-NANO-0011) and the Mechanobiology Institute are gratefully acknowledged.

References

[1] Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009; 326:1216-9; PMID:19965464; http://dx.doi.org/10.1126/science.1176009
[2] Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophys J 2000; 79:144-52; PMID:10866943; http://dx.doi.org/10.1016/S0006-3495(00)76279-5
[3] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126:677-89; PMID:16923388; http://dx.doi.org/10.1016/j.cell.2006.06.044
[4] McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 2004; 6:483-95; PMID:15068789; http://dx.doi.org/10.1016/S1534-5807(04)00075-9
[5] Ulrich TA, de Juan Pardo EM, Kumar S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. Cancer Res 2009; 69:4167-74; PMID:19435897; http://dx.doi.org/10.1158/0008-5472.CAN-08-4859
[6] Discher DE, Janmey P, Wang Y-L. Tissue cells feel and respond to the stiffness of their substrate. Science 2005; 310:1139-43; PMID:16293750; http://dx.doi.org/10.1126/science.1116995
[7] Wozniak MA, Chen CS. Mechanotransduction in development: a growing role for contractility. Nat Rev Mol Cell Biol 2009; 10:34-43; PMID:19197330; http://dx.doi.org/10.1038/nrm2592
[8] Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science 2009; 324:1673-7; PMID:19556500; http://dx.doi.org/10.1126/science.1171643
[9] Jaaoulou DE, Lammerding J. Mechanotransduction gone awry. Nat Rev Mol Cell Biol 2009; 10:63-73; PMID:19197333; http://dx.doi.org/10.1038/nrn2597
[10] Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, et al. Tensional homeostasis and the malignant phenotype. Cancer Cell 2005; 8:241-54; PMID:16169468; http://dx.doi.org/10.1016/j.ccr.2005.08.010
[11] Ladoux B, Nicolas A. Physically based principles of cell adhesion mechanosensitivity in tissues. Rep Prog Phys Phys Soc G B 2012; 75:116601; http://dx.doi.org/10.1088/0034-4885/75/11/116601
[12] Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, Sabanay I, Mahalu D, Safran S, Bershadsky A, Addadi L, et al. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat Cell Biol 2001; 3:466-72; PMID:11331874; http://dx.doi.org/10.1038/35074532
[13] Plotnikov SV, Pasapera AM, Sabass B, Waterman CM. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. Cell 2012; 151:1513-27; PMID:23260139; http://dx.doi.org/10.1016/j.cell.2012.11.034
[14] Hayakawa K, Tatsumi H, Sokabe M. Actin stress fibers transmit and focus force to activate mechanosensitive channels. J Cell Sci 2008; 121:496-503; PMID:18230647; http://dx.doi.org/10.1242/jcs.022053
[15] Mitrossilis D, Foucher J, Guiroy A, Desprat N, Rodriguez N, Fabry B, Asnacios A. Single-cell response to stiffness exhibits muscle-like behavior. Proc Natl Acad Sci U S A 2009; 106:18243-8; PMID:19805036; http://dx.doi.org/10.1073/pnas.0903994106
[16] Colombelli J, Besser A, Kress H, Reynaud EG, Girard P, Calpe A. Mechanosensing in actin stress fibers revealed by a close correlation between force and protein localization. J Cell Sci 2009; 122:1665-79; PMID:19401336; http://dx.doi.org/10.1242/jcs.042986
[17] Hayakawa K, Tatsumi H, Sokabe M. Actin filaments function as a tension sensor by tension-dependent binding of coflin to the filament. J Cell Biol 2011; 195:721-7; PMID:22123860; http://dx.doi.org/10.1083/jcb.201102039
[18] del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, Sheetz MP. Stretching single talin rod molecules activates vinculin binding. Science 2009; 323:638-41; PMID:19179532; http://dx.doi.org/10.1126/science.1162912
[19] Elosegui-Artola A, Bazellières E, Allen MD, Andreu I, Oria R, Sunyer R, Gomm JJ, Marshall JF, Jones JL, Trepat X, et al. Rigidity sensing and adaptation through regulation of integrin types. Nat Mater 2014; 13:631-7; PMID:24793358; http://dx.doi.org/10.1038/nmat3960
[20] Stricker J, Aratyn-Schaus Y, Oakes PW, Gardel ML. Spatio-temporal constraints on the force-dependent growth of focal adhesions. Biophys J 2011; 100:1809-17; PMID:21689521; http://dx.doi.org/10.1016/j.bpj.2010.12.087
[21] Trichet L, Le Digabel J, Hawkins RJ, Vedula SRK, Gupta M, Ribraut C, Hersen P, Voituriez R, Ladoux B. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. Proc Natl Acad Sci U S A 2012; 109:6933-8; PMID:22509005; http://dx.doi.org/10.1073/pnas.1117810109
[22] Prager-Khoutorsky M, Lichtenstein A, Krishnan R, Rajendran K, Mayo A, Kam Z, Geiger B, Bershadsky AD. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. Nat Cell Biol 2011; 13:1457-65; PMID:22081092; http://dx.doi.org/10.1038/ncb2370
[23] Zemel A, Rehfeldt F, Brown AEX, Discher DE, Safran SA. Optimal matrix rigidity for stress fiber polarization in stem cells. Nat Phys 2010; 6:648-73; PMID:20563235; http://dx.doi.org/10.1038/nphys1613

[24] Gupta M, Sarangi BR, Deschamps J, Nematbakhsh Y, Zemel A, Rehfeldt F, Brown AEX, Discher DE, Safran SA. Overview of the matrisome into nonadhesive hydrogels useful for tissue restorations are regulated by substrate deformations. Adv Mater 2011; 23:H41-56; PMID:26461445; http://dx.doi.org/10.1038/nmat4444

[25] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 2014; 15:802-12; PMID:25355505; http://dx.doi.org/10.1038/nrm3896

[26] Callan-Jones A, Margadant F, Masure P, Gueritte N, Breder J, Herve R-M, Lim CT, Voit M, Jaggy S, Ladoux B. Adaptive rheology and ordering of cell cytoskeleton govern matrix rigidity sensing. Nat Comm 2015; 6:7525; PMID:26109233; http://dx.doi.org/10.1038/ncomms8525

[27] Hynes RO, Naba A. Overview of the matrisome: an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol 2012; 4:a004903; PMID:21937732; http://dx.doi.org/10.1101/cshperspect.a004903

[28] Baker BM, Trappmann B, Wang WY, Sakar MS, Kim IL, Shenoy VB, Burdick JA, Chen CS. Cell-mediated fibre recruitment drives extracellular matrix mechanosensing in engineered fibriolar microenvironments. Nat Mater 2015; 14:1262-8; PMID:26461445; http://dx.doi.org/10.1038/nmat4444

[29] Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material’s perspective. Curr Opin Biotechnol 2013; 24:948-53; PMID:23611564; http://dx.doi.org/10.1016/j.copbio.2013.03.020

[30] Herr DL, Hubbell JA. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J Biomed Mater Res 1998; 39:266-76; PMID:9457557; http://dx.doi.org/10.1002/(SICI)1097-4636(199802)39:2<266::AID-JBM14-3.0.CO;2-B

[31] Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. Adv Mater 2011; 23:H41-56; PMID:21394792; http://dx.doi.org/10.1002/adma.201003963

[32] Lévesque SG, Shoichet MS. Synthesis of enzyme-degradable, peptide-cross-linked dextran hydrogels. Bioconjug Chem 2007; 18:874-85; http://dx.doi.org/10.1021/bc0602127

[33] Nolan TJ, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc Natl Acad Sci U S A 2003; 100:1484-9; PMID:12552122; http://dx.doi.org/10.1038/ncomms8525

[34] du Roure O, Saez A, Bugui A, Austin RH, Chavrier P, Silberzan P, Silberzan P, Ladoux B. Force mapping in epithelial cell migration. Proc Natl Acad Sci U S A 2005; 102:2390-5; PMID:15695588; http://dx.doi.org/10.1073/pnas.0408482102

[35] Yang MT, Fu J, Wang Y-K, Desai RA, Chen CS. Assessing stem cell mechanobiology on microfabricated elastomeric substrates with geometrically modulated rigidity. Nat Protoc 2011; 6:187-213; PMID:21293460; http://dx.doi.org/10.1038/nprot.2010.189

[36] Ghassemi S, Meacci G, Liu S, Gondarenko AA, Mathur A, Roca-Cusachs P, Sheetz MP, Hone J. Cells test substrate rigidity by local contractions on submicrometer pillars. Proc Natl Acad Sci 2012; 109:5328-33; PMID:22431603; http://dx.doi.org/10.1073/pnas.1119886109

[37] Trappmann B, Gautrot JE, Connelly JT, Strange DGT, Li Y, Oyen ML, Cohen Stuart MA, Boehm H, Li B, Vogel V, et al. Extracellular-matrix tethering regulates stem-cell fate. Nat Mater 2012; 11:642-9; PMID:22635042; http://dx.doi.org/10.1038/nmat3339

[38] Wener JH, Vincent LG, Fuhrmann A, Choi YS, Hribar KC, Taylor-Weiner H, Chen S, Engler AJ. Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 2014; 13:979-87; PMID:25108614; http://dx.doi.org/10.1038/nmat4051

[39] le Digabel J, Bias N, Fresnais J, Berret J-F, Hersen P, Ladoux B. Magnetic micropillars as a tool to govern substrate deformations. Lab Chip 2011; 11:2630-6; PMID:21674117; http://dx.doi.org/10.1039/c1lc20263d

[40] Ghibaudo M, Meglio J-MD, Hersen P, Ladoux B. Mechanics of cell spreading within 3D-micropatterned environments. Lab Chip 2011; 11:805-12; PMID:21132213; http://dx.doi.org/10.1039/C0LC00221F

[41] Mann JM, Lam RHW, Weng S, Sun Y, Fu J. A silicone-based stretchable micropost array membrane for monitoring live-cell subcellular cytoskeletal response. Lab Chip 2012; 12:731-40; PMID:22193351; http://dx.doi.org/10.1039/C2LC020896B

[42] Lam RHW, Sun Y, Chen W, Fu J. Elastic micropost integrations into microfluidics for flow-mediated endothelial mechanotransduction analysis. Lab Chip 2012; 12:1865-73; PMID:22437210; http://dx.doi.org/10.1039/c2lc21146g

[43] Wolfenson H, Meacci G, Liu S, Stachowiak MR, Iskratsch T, Ghassemi S, Roca-Cusachs P, O’Shaughnessy B, Hone J, Sheetz MP. Tropomyosin controls sarcomere-like contractions for rigidity sensing and suppressing growth on soft matrices. Nat Cell Biol 2016; 18:33-42; PMID:26619148; http://dx.doi.org/10.1038/ncli03277

[44] Chaudhuri O, Gu L, Darnell M, Klumpers D, Bencherif SA, Weaver JC, Huebsch N, Mooney DJ. Substrate stress relaxation regulates cell spreading. Nat Comm 2015; 6:6364; PMID:25695512; http://dx.doi.org/10.1038/ncomms7365

[45] Harris AK, Wild P, Stopak D. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science 1980; 208:177-9; PMID:6987736; http://dx.doi.org/10.1126/science.6987736

[46] Galbraith CG, Sheetz MP. A micromachined device provides a new bend on fibroblast traction forces. Proc Natl Acad Sci U S A 1997; 94:9114-8; PMID:9256444; http://dx.doi.org/10.1073/pnas.94.17.9114
[49] Dembo M, Wang YL. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. Biophys J 1999; 76:2307-16; PMID:10096925; http://dx.doi.org/10.1016/S0006-3495(99)77386-8

[50] Schwarz US, Balaban NQ, Riveline D, Bershadsky A, Geiger B, Safran SA. Calculation of forces at focal adhesions from elastic substrate data: the effect of localized force and the need for regularization. Biophys J 2002; 83:1380-94; PMID:12203264; http://dx.doi.org/10.1016/S0006-3495(02)73909-X

[51] Soiné JRD, Brand CA, Stricker J, Oakes PW, Garidel ML, Schwarz US. Model-based traction force microscopy reveals differential tension in cellular actin bundles. PLoS Comput Biol 2015; 11:e1004076; http://dx.doi.org/10.1371/journal.pcbi.1004076

[52] Schoen I, Hu W, Klotzsch E, Vogel V. Probing cellular traction forces by micropillar arrays: contribution of substrate warping to pillar deflection. Nano Lett 2010; 10:1823-30; PMID:20387859; http://dx.doi.org/10.1021/nl100533c

[53] Gupta M, Kocgozlu L, Sarangi BR, Margadant F, Ashraf M, Ladoux B. Micropillar substrates: a tool for studying cell mechanobiology. Methods Cell Biol 2015; 125:289-308; PMID:25640435; http://dx.doi.org/10.1016/bs.mcb.2014.10.009

[54] Maskarinec SA, Franck C, Tirrell DA, Ravichandran G. Quantifying cellular traction forces in three dimensions. Proc Natl Acad Sci 2009; 106:22108-13; PMID:200018765; http://dx.doi.org/10.1073/pnas.0904565106

[55] Delanoë-Ayari H, Rieu JP, Sano M. 4D traction force microscopy reveals asymmetrical cortical forces in migrating Dicytostelium cells. Phys Rev Lett 2010; 105:248103; http://dx.doi.org/10.1103/PhysRevLett.105.248103

[56] Ghibaudo M, Saez A, Trichet L, Xayaphoummine A, Browaey J, Silberzan P, Buguin A, Ladoux B. Traction forces and rigidity sensing regulate cell functions. Soft Matter 2008; 4:1836-43; PMID:18040992; http://dx.doi.org/10.1039/b804103b

[57] Legant WR, Miller JS, Blakely BL, Cohen DM, Genin GM, Chen CS. Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nat Methods 2010; 7:969-71; PMID:21076420; http://dx.doi.org/10.1038/nmeth.1531

[58] Toyjanoova J, Hannen E, Bar-Kochba E, Darling EM, Henann DL, Franck C. 3D viscoelastic traction force microscopy. Soft Matter 2014; 10:8095-106; PMID:25170569; http://dx.doi.org/10.1039/C4SM01271B

[59] Steinwachs J, Metzner C, Skodzek K, Lang N, Thievesen I, Mark C, Münster S, Aifantis KE, Fabry B. Three-dimensional force microscopy of cells in biopolymer networks. Nat Methods 2016; 13:171-6; PMID:26641311; http://dx.doi.org/10.1038/nmeth.3685

[60] Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, et al. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature 2010; 466:263-6; PMID:20613844; http://dx.doi.org/10.1038/nature09198

[61] Gayraud C, Borghi N. FRET-based molecular tension microscopy. Methods 2016; 94:33-42; PMID:26210398; http://dx.doi.org/10.1016/j.jymeth.2015.07.010

[62] Morimatsu M, Mekhdjian AH, Adhikari AS, Dunn AR. Molecular tension sensors report forces generated by single integrin molecules in living cells. Nano Lett 2013; 13:3985-9; PMID:23859772; http://dx.doi.org/10.1021/nl4005145

[63] Kubow KE, Vukmirovic R, Zhe L, Klotzsch E, Smith ML, Gourdon D, Luna S, Vogel V. Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix. Nat Commun 2015; 6:6806; PMID:26272817; http://dx.doi.org/10.1038/ncomms9026

[64] Wang X, Ha T. Defining Single Molecular Forces Required to Activate Integrin and Notch Signaling. Science 2013; 340:991-4; PMID:23704575; http://dx.doi.org/10.1126/science.1231041

[65] Emerman JT, Pitelka DR. Maintenance and induction of morphological differentiation in dissociated mammmary epithelium on floating collagen membranes. In Vitro 1977; 13:316-28; PMID:559643; http://dx.doi.org/10.1007/BF02616178

[66] Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin–cytoskeleton linkages. Cell 1997; 88:39-48; PMID:9019403; http://dx.doi.org/10.1016/S0006-3495(00)81856-5

[67] Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V, Janmey PA. Effects of substrate stiffness on cell morphology, cytoskeleton structure, and adhesion. Cell Motil Cytoskeleton 2005; 60:24-34; PMID:15573414; http://dx.doi.org/10.1002/cm.20041

[68] Rabodzey A, Alcaide P, Luscinskas FW, Ladoux B. Mechanical forces induced by the transendothelial migration of human neutrophils. Biophys J 2008; 95:1428-38; PMID:18390614; http://dx.doi.org/10.1529/biophysj.107.119156

[69] Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D. Substrate compliance versus ligand density in cell on gel responses. Biophys J 2004; 86:617-28; PMID:14695306; http://dx.doi.org/10.1016/S0006-3495(04)74140-5

[70] Swift J, Ivanovska IL, Buxboim A, Harada T, Dingal PCDP, Pinter J, Pajerowski JD, Spinler KR, Shin J-W, Tewari M, et al. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science 2013; 341:1240104; PMID:23990565; http://dx.doi.org/10.1126/science.1240104

[71] Dupont S, Morsut L, Aragona M, Enzo E, Giuliani S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicchianti S, et al. Role of YAP/TAZ in mechanotransduction. Nature 2011; 474:179-83; PMID:21654799; http://dx.doi.org/10.1038/nature10137

[72] Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol 2009; 10:75-82; PMID:19197334; http://dx.doi.org/10.1038/nrm2594

[73] Bodinger KA, Dembo M, Kaverina I, Small JV, Wang YL. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts.
[75] Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, Kam Z, Geiger B, Bershadsky AD. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 2001; 153:1175-86; PMID:11402062; http://dx.doi.org/10.1083/jcb.153.6.1175

[76] Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 2009; 10:21-33; PMID:19197329; http://dx.doi.org/10.1038/nrm2593

[77] Hytönen VP, Vogel V. How force might activate talin’s vinculin binding sites: SMD reveals a structural mechanism. PLoS Comput Biol 2008; 4:e24; PMID:18282082; http://dx.doi.org/10.1371/journal.pcbi.0040024

[78] Margadant F, Chew LL, Hu X, Yu H, Bate N, Zhang X, Sheetz M. Mechanotransduction in vivo by repeated talin stretch-relaxation events depends upon vinculin. PLoS Biol 2011; 9:e1001223; PMID:22205879; http://dx.doi.org/10.1371/journal.pbio.1001223

[79] Sawada Y, Tamada M, Dubin-Thaler BJ, Cherniavskaya O, Sakai R, Tanaka S, Sheetz MP. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 2006; 127:1015-26; PMID:17129785; http://dx.doi.org/10.1016/j.cell.2006.09.044

[80] Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. Mechanical strain in actin networks regulates FilGAP and integrin binding to filament A. Nature 2011; 478:260-3; PMID:21926999; http://dx.doi.org/10.1038/nature10430

[81] Case LB, Waterman CM. Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. Nat Cell Biol 2015; 17:955-63; PMID:26121555; http://dx.doi.org/10.1038/nccb3180

[82] Chan CE, Odde DJ. Traction dynamics of filopodia on compliant substrates. Science 2008; 322:1687-91; PMID:19074349; http://dx.doi.org/10.1126/science.1163595

[83] Shemesh T, Geiger B, Bershadsky AD, Kozlov MM. Focal adhesions as mechanosensors: a physical mechanism. Proc Natl Acad Sci U S A 2005; 102:12383-8; PMID:16113084; http://dx.doi.org/10.1073/pnas.0500254102

[84] Nicolas A, Geiger B, Safran SA. Cell mechanosensitivity controls the anisotropy of focal adhesions. Proc Natl Acad Sci U S A 2004; 101:12520-5; PMID:15314229; http://dx.doi.org/10.1073/pnas.0403539101

[85] Nicolas A, Safran SA. Limitation of cell adhesion by the elasticity of the extracellular matrix. Biophys J 2006; 91:61-73; PMID:16581840; http://dx.doi.org/10.1529/biophysj.105.077115

[86] Oakes PW, Beckham Y, Stricker J, Gardel ML. Tension is required but not sufficient for focal adhesion maturation without a stress fiber template. J Cell Biol 2012; 196:363-74; PMID:22291038; http://dx.doi.org/10.1083/jcb.201107042

[87] Mitrossilis D, Fouchard J, Pereira D, Postic F, Richert A, Saint-Jean M, Asnacios A. Real-time single-cell response to stiffness. Proc Natl Acad Sci U S A 2010; 107:16518-23; PMID:20823257; http://dx.doi.org/10.1073/pnas.1007940107

[88] Hirata H, Gupta M, Vedula SRK, Lim CT, Ladoux B, Sokabe M. Actomyosin bundles serve as a tension sensor and a platform for ERK activation. EMBO Rep 2015; 16:250-7; PMID:25550404; http://dx.doi.org/10.15252/embr.201439140

[89] Courttemanche N, Lee JY, Pollard TD, Greene EC. Tension modulates actin filament polymerization mediated by formin and profilin. Proc Natl Acad Sci U S A 2013; 110:9752-7; PMID:23716666; http://dx.doi.org/10.1073/pnas.1308257110

[90] Lieleg O, Claessens MMAE, Bausch AR. Structure and dynamics of cross-linked actin networks. Soft Matter 2010; 6:218-25; http://dx.doi.org/10.1039/B912163N

[91] Friedrich BM, Safran SA. Nematic order by elastic interactions and cellular rigidity sensing. EPL Europhys Lett 2011; 93:28007; http://dx.doi.org/10.1209/0295-5075/93/28007

[92] Ehrlicher AJ, Krishnan R, Guo M, Bidan CM, Weitz DA, Pollak MR. Alpha-actinin binding kinetics modulate cellular dynamics and force generation. Proc Natl Acad Sci U S A 2015; 112:6619-24; PMID:25918384; http://dx.doi.org/10.1073/pnas.1505652112

[93] Friedrich BM, Safran SA. Nematic order by elastic interactions and cellular rigidity sensing. EPL Europhys Lett 2011; 93:28007; http://dx.doi.org/10.1209/0295-5075/93/28007

[94] Sengupta K, Georges PC, Janmey PA. Fibroblast adaption and stiffness matching to soft elastic substrates. Biophys J 2007; 93:456-61; PMID:18045965; http://dx.doi.org/10.10152/biophysj.106.10138

[95] Baker EL, Bonnecaze RT, Zaman MH. Extracellular matrix stiffness and architecture govern intracellular rheology in cancer. Biophys J 2009; 97:1013-21; PMID:19686648; http://dx.doi.org/10.1016/j.bpj.2009.05.054

[96] Plestant C, Strale P-O, Seddiki R, Nguyen E, Ladoux B, Mege R-M. Adhesive interactions of N-cadherin limit the recruitment of microtubules to cell–cell contacts through organization of actomyosin. J Cell Sci 2014; 127:1660-71; PMID:24522194; http://dx.doi.org/10.1242/jcs.131284

[97] Gavard J, Lambert M, Grosheva I, Marthiens V, Iriponoulou T, Riou J-F, Bershadsky A, Mege R-M. Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. J Cell Sci 2004; 117:257-70; PMID:14657280; http://dx.doi.org/10.1242/jcs.008857

[98] Gauthier NC, Fardin MA, Roca-Cusachs P, Sheetz MP. Temporary increase in plasma membrane tension coordinates the activation of exocytosis and contraction during cell spreading. Proc Natl Acad Sci U S A 2011; 108:14467-72; PMID:21808040; http://dx.doi.org/10.1073/pnas.1105845108

[99] Kolmarik AJ, Lee K-M, Han SJ, Bui DA, Davidson M, Mogilner A, Danuser G. Competition for actin between two distinct F-actin networks defines a bistable switch for cell polarization. Nat Cell Biol 2005; 7:1595-66; PMI:20823257; http://dx.doi.org/10.1073/pnas.1007940107

[100] Ladoux B, Anon E, Lambert M, Rabozdey A, Hersen P, Buguin A, Silberman P, Mege R-M. Strength dependence of cadherin-mediated adhesions. Biophys J 2010; 98:534-42; PMID:20519949; http://dx.doi.org/10.1016/j.bpj.2009.10.044

[101] Tee YH, Shemesh T, Thiagarajan V, Hariadi RF, Anderson KL, Page C, Volkmann N, Hanein D, Sivaramakrishnan S,
Kozlov MM, et al. Cellular chirality arising from the self-organization of the actin cytoskeleton. Nat Cell Biol 2015; 17:445-57; PMID:25799062; http://dx.doi.org/10.1038/ncb3137

[102] Isermann P, Lammerding J. Nuclear mechanics and mechanotransduction in health and disease. Curr Biol 2013; 23:R1113-21; PMID:24355792; http://dx.doi.org/10.1016/j.cub.2013.11.009

[103] Shivashankar GV. Mechanosignaling to the cell nucleus and gene regulation. Annu Rev Biophys 2011; 40:361-78; PMID:21391812; http://dx.doi.org/10.1146/annurev-biophys-042910-155319