United we stand – integrating the actin cytoskeleton and cell–matrix adhesions in cellular mechanotransduction

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Summary

Many essential cellular functions in health and disease are closely linked to the ability of cells to respond to mechanical forces. In the context of cell adhesion to the extracellular matrix, the forces that are generated within the actin cytoskeleton and transmitted through integrin-based focal adhesions are essential for the cellular response to environmental clues, such as the spatial distribution of adhesive ligands or matrix stiffness. Whereas substantial progress has been made in identifying mechanosensitive molecules that can transduce mechanical force into biochemical signals, much less is known about the nature of cytoskeletal force generation and transmission that regulates the magnitude, duration and spatial distribution of forces imposed on these mechanosensitive complexes. By focusing on cell-matrix adhesion to flat elastic substrates, on which traction forces can be measured with high temporal and spatial resolution, we discuss our current understanding of the physical mechanisms that integrate a large range of molecular mechanotransduction events on cellular scales. Physical limits of stability emerge as one important element of the cellular response that complements the structural changes affected by regulatory systems in response to mechanical processes.

This article is part of a Minifocus on Mechanotransduction. For further reading, please see related articles: 'Deconstructing the third dimension – how three-dimensional culture microenvironments alter cellular cues' by Brendon M. Baker and Christopher S. Chen (*J. Cell Sci.* **125**, [079509]). 'Finding the weakest link – exploring integrin-mediated mechanical molecular pathways' by Pere Roca-Cusachs et al. (*J. Cell Sci.* **125**, [095794]). 'Signalling through mechanical inputs: a coordinated process' by Huimin Zhang and Michel Labouesse (*J. Cell Sci.* **125**, [093666]). 'Mechanosensitive mechanisms in transcriptional regulation' by Akiko Mammoto et al. (*J. Cell Sci.* **125**, [093005]). 'Molecular force transduction by ion channels: diversity and unifying principles' by Sergei Sukharev and Frederick Sachs (*J. Cell Sci.* **125**, [092353]).

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Introduction

Mechanical processes have emerged as important regulators in the complex interplay between cells and their environment (Discher et al., 2005; Vogel and Sheetz, 2006). The forces generated by cells are not only essential for the morphological changes that take place during cell spreading, migration and division, but also underlie morphological tissue changes during development (Lecuit and Lenne, 2007; Wozniak and Chen, 2009). In the adult organism, cells and tissues maintain a preferred level of mechanical stress (Brown et al., 1998) and diseases such as cancer or atherosclerosis are closely related to the disruption of tensional homeostasis (DuFort et al., 2011). It is now also known that mechanics influence downstream biochemical and genetic signaling pathways to alter cell proliferation and fate (Hoffmann et al., 2011). In the context of adherent cells, mechanotransduction is closely related to sites of adhesion and the coupling of these sites to force-generating processes in the actin cytoskeleton (Geiger et al., 2009).

Motivated, in part, by these observations, substantial progress has been made in identifying and characterizing the molecular basis of mechanosensitive cell adhesion to the extracellular matrix (ECM) (Zaidel-Bar et al., 2007; Kuo et al., 2011; Prager-Khoutorsky et al., 2011). In particular, several proteins with mechanosensitive functions have been identified, and it has been shown that their integration into mature cell–matrix adhesions, such as focal adhesions, depends on the presence of force. These proteins include the adhesion-related proteins zyxin (Yoshigi et al., 2005), p130 Cas (Sawada et al., 2006), talin, vinculin (del Rio et al., 2009), and filamin A (Ehrlicher et al., 2011). However, in order to understand how cellular mechanotransduction pathways function, it is also necessary to understand how force is generated and transmitted to the site at which it is transduced into a biochemical response (Hoffman et al., 2011). For instance, forces applied to a mobile integrin receptor will result in a motion that is determined by the effective viscosity of the plasma membrane, whereas forces applied to an integrin receptor bound to an immobilized ligand within the ECM will result in the build-up of tension that is modulated by stiffness of the matrix. Thus, the nature of force transmission within the local environment is as important as the mechanosensitive molecules themselves.

In terms of cellular force generation during cell–matrix adhesion, the actin cytoskeleton has the most prominent role (Fletcher and Mullins, 2010; Gardel et al., 2010). Actin binding proteins mediate the assembly of actin filaments into a diverse assortment of networks and bundles that are interconnected to form a mechanically coherent actin cytoskeleton. Forces generated by actin polymerization or by myosin II motor activity are transmitted across the cell and, eventually, to the adhesion

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sites. The organization of the actin cytoskeleton determines the nature of force transmission from the molecular to cellular length scales. Distinct functional modules of the actin cytoskeleton exist and drive diverse physiological processes (Small et al., 1998). In particular, this is true for migrating cells, in which two types of actin networks, the lamellipodium and the lamella, have distinct roles in the protrusion of the leading edge, adhesion assembly and the generation of tension on focal adhesion sites (Ponti et al., 2004; Giannone et al., 2007; Gardel et al., 2010). Each of these modules is characterized by a specific composition and architecture as well as precise dynamics and, thus, leads to different characteristics of force transmission towards mechanosensitive molecules.

In this Commentary, we discuss the mechanisms of force transmission within the actin cytoskeleton and at focal adhesions as essential prerequisites and modulators of mechanotransduction events. We focus on force transmission within the lamellipodium and lamella as the two most prominent actin modules involved in regulating adhesion assembly and maturation to allow cell adhesion to flat substrates. From a technical point of view, this system is ideal for detailed quantitative studies, including advanced methods for fluorescence microscopy and traction-force measurements. Whereas we focus on cell adhesion to two-dimensional substrates, the challenge of continuing these studies in three dimensions will be discussed in an accompanying article by Brendon M. Baker and Christopher S. Chen (*J. Cell Sci.* **125**, [079509]). Here, we discuss different molecular mechanisms that make the actin and adhesion structures sensitive to force, and how these structures are integrated on the cellular scale. Finally, we conclude with evidence for the relevance of physical instabilities that might have an important function in the spatiotemporal regulation of cytoskeletal organization.

Coordination of adhesion and actin dynamics

We begin our discussion with a review of experimental observations regarding the organization of adhesions and the actin cytoskeleton in cells that have been grown on flat culture substrates. In both quiescent and migrating cells, the assembly of cell–matrix adhesions is initiated at protruding cell edges. Adhesion to the ECM is initiated within lamellipodia or filopodia at the cell periphery (Ridley, 2011). The lamellipodium consists of an Arp2/3-dependent sheet-like array of dendritic actin that is assembled close to the cell membrane, thus, driving a rapid retrograde flow into the cell. The actin monomers that are required at the membrane are provided by disassembly of actin in regions proximal to the leading edge (Pollard and Borisy, 2003). Within this dynamic, 'treadmilling' actin array, microclusters of activated integrins with enhanced affinity towards the ECM move retrogradely with F-actin until they bind to the ECM (Wiseman et al., 2004; Choi et al., 2008) (see steps 1 and 2 in Fig. 1). During integrin–ECM engagement, the adapter proteins talin and paxillin are recruited to integrin clusters to form nascent adhesions. The nascent adhesions are dependent on actin polymerization, and in the absence of myosin-mediated cues, disassemble at the base of the lamellipodium where a majority of the lamellipodial actin depolymerizes.

Closer to the cell body, the lamellipodium is replaced by the lamella, a network of contractile actin networks and bundles that is enriched with tropomyosin and myosin II. A small fraction of the nascent adhesions formed in the lamellipodium undergo a myosin-dependent maturation process at the lamellipodium–lamella boundary, wherein focal adhesions become more stably associated with the ECM, increase in size and become elongated (Choi et al., 2008; Alexandrova et al., 2008) (see steps 2 and 3, Fig. 1). In addition to changes in focal adhesion morphology, myosin-mediated maturation involves changes in composition that alter both the structural properties and signaling activity of the adhesions (Zamir et al., 1999; Zaidel-Bar et al., 2003). In this way, adhesions directly regulate pathways for, for instance, growth, differentiation and apoptosis. Focal adhesion maturation culminates in either the disassembly of the focal adhesion plaque after approximately 20–40 minutes – which is possibly regulated by the targeting of microtubules into focal adhesions (Kaverina et al., 1999) – or the formation of longer-lived fibrillar adhesions, which function in the remodeling of the ECM.

In situations where strong adhesion to an external surface is absent, for example in fast-moving cells, such as keratocytes or cells plated on poly-L-lysine substrates, the lamellipodium–lamella boundary is not well defined and the lamellipodium and lamella are thought to gradually fade into each other (Alexandrova et al., 2008). In the presence of sufficiently strong adhesive cues, however, adhesion growth and elongation at the lamellipodium–lamella boundary occurs concomitantly with the formation of contractile actin bundles (Fig. 1), which can be further differentiated by their orientation to the leading cell edge (Small et al., 1998; Pellegrin and Mellor, 2007; Gardel et al., 2010). Whereas radial stress fibers are oriented perpendicular to the leading edge and are anchored at one end to a focal adhesion, transverse arcs run parallel to the leading edge and are not necessarily anchored in focal adhesions (Hotulainen and

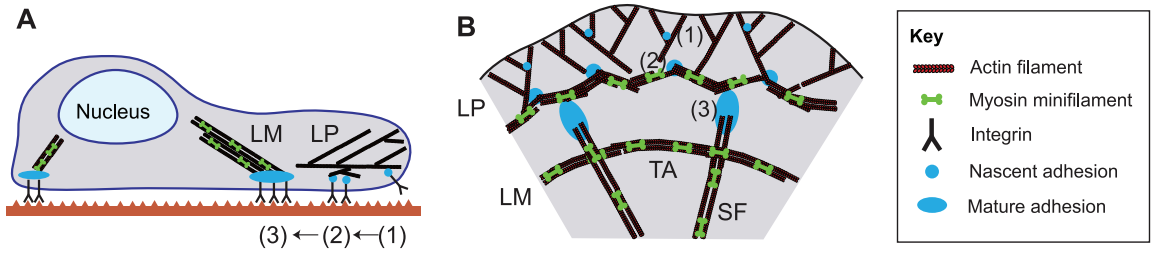

Fig. 1. Adhesion and actin organization at different stages of adhesion assembly. (**A**) Side view schematic. (1) The lamellipodium (LP) drives nascent adhesions assembly at the leading edge of the cell. (2) Nascent adhesions recruit different cytoplasmic proteins and strengthen their linkage to the actin cytoskeleton. (3) Focal-adhesion maturation occurs within the lamella (LM). (**B**) Top view schematic. The LP–LM boundary is associated with the generation of different contractile actomyosin bundles. Transverse arcs (TA) are parallel to the cell edge and the LP–LM boundary. Radial stress fibers (SF) are anchored in focal adhesions and oriented perpendicular to the cell edge. The locations of the types of focal adhesion described in (A) are indicated by (1) to (3).

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Lappalainen, 2006; Burnette et al., 2011) (Fig. 1). Radial stress fibers can be further classified into dorsal or ventral, depending on whether they extend from a focal adhesion to the dorsal side or are located on the ventral side and attach to a second focal adhesion (Hotulainen and Lappalainen, 2006). Whereas radial stress fibers are usually straight, peripheral bundles lining the cell contour are strongly bent inwards (typically with a circular-arc shape) between anchoring focal adhesions as a result of the combined pull of the plasma membrane and contractile cell body (Zand and Albrecht-Buehler, 1989; Small et al., 1998; Bischofs et al., 2008; Rossier et al., 2010). Ventral stress fibers, transverse arcs and peripheral bundles display alternating patterns of α-actinin and myosin II decoration along short (~1–5 μm) F-actin filaments (Hotulainen and Lappalainen, 2006). Owing to this resemblance to sarcomeres, these actin structures are expected to be contractile.

The exact organization of the actin and adhesion systems depends strongly on the properties of the ECM. Micropatterned substrates have been used to control the spatial distribution of ECM ligands, and, by using appropriately designed patterns, the propensity for different actin modules can be altered (Parker et al., 2002; Lehnert et al., 2004; Théry et al., 2006; Rape et al., 2011; Tseng et al., 2012). Cell adhesion and organization also depends strongly on matrix stiffness. Most cell types cultured on very soft elastic substrates fail to form stress fibers or elongated adhesions, whereas cells cultured on stiff plastic or glass substrates tend to develop large focal adhesions and stress fibers (Pelham and Wang, 1997). During the last decades, many essential cellular processes have been shown to strongly depend on adhesive geometry and matrix stiffness, including cell survival (Chen et al., 1997) and stem cell differentiation (Engler et al., 2006; Kilian et al., 2010).

Strikingly, the ability of cells to sense these environmental determinants seems to be strongly coupled to myosin-dependent effects on adhesions and the actin cytoskeleton, because inhibiting myosin activity abrogates substrate-dependent phenotypes. Adhesion plaques change size in response to either myosin-generated or externally applied forces (Choquet et al., 1997; Chrzanowska-Wodnicka and Burridge, 1996; Riveline et al., 2001), concomitantly with the formation of a stress fiber at the adhesion plaque (Choi et al., 2008) and recruitment of focal adhesion proteins (Zamir et al., 1999; Zaidel-Bar et al., 2003). Similarly, changes in the mechanical loading of stress fibers leads to structural changes and the redistribution of mechanosensitive proteins, such as zyxin, both in the stress fibers and the adhesions they are anchored to (Yoshigi et al., 2005; Lele et al., 2006; Colombelli et al., 2009; Smith et al., 2010).

In summary, a large body of evidence suggests that force is one of the main factors that mediate the close coupling between the actin cytoskeleton and the assembly of focal adhesions. To investigate this relationship further, it is, therefore, essential to develop methods that allow us to measure cellular forces and to correlate them with the organization and dynamics of the adhesion and actin systems. We will discuss some of these, as well as the knowledge gained from such studies in the following sections.

Measuring cellular force

In general, force measurements rely on measuring the displacement of a calibrated material in response to being subjected to the force of interest. In the context of cell–matrix adhesion, the standard approach that has been established over the last two decades is traction-force microscopy on soft elastic substrates. To carry out this procedure, synthetic substrates are prepared from well characterized polymeric materials, such as polyacrylamide or polydimethylsiloxane. Tracking substrate deformation with fluorescent marker beads and applying the elasticity theory enables the displacement data to be used to estimate the traction-force pattern (Dembo and Wang, 1999; Schwarz et al., 2002; Butler et al., 2002) (Fig. 2). By establishing an integrated workflow of cell experiments, image processing and force reconstruction, cellular traction forces can be measured with micrometer resolution (Sabass et al., 2008) (Fig. 2D). Such studies have revealed a very close correlation between adhesion structure, actin organization and traction-force pattern. Recently, the principles of traction-force microscopy have been extended to measure non-planar traction forces on flat substrates (Hur et al., 2009; Maskarinec et al., 2009; Delanoë-Ayari et al., 2010) and traction forces of cells encapsulated in three-dimensional hydrogels (Legant et al., 2010).

Cellular traction forces can also be measured by adhering cells to microfabricated elastic pillar arrays (Tan et al., 2003). Here, adhesions interact with an individual pillar, and the force that is required to deflect the pillar is determined by the pillar radius and height (Box 1). Pillars have the advantage of being able to isolate forces from individual adhesions and measure force without the need for computational force reconstruction. By creating oval-shaped pillars, it is possible to explore the effects of an anisotropic mechanical environment (Saez et al., 2007). Furthermore, three-dimensional elastic scaffolds have recently been created by direct laser writing to measure cellular forces (Klein et al., 2010).

It is instructive to consider the magnitude of cell-generated forces. In traction-force measurements, cells are plated on ligand-coated soft elastic polymer gels with an elastic modulus (Young's modulus, see Box 1 for an explanation of the technical terms) ranging from 100 Pa to 50 kPa. This range covers the diversity of stiffness values encountered by cells in their physiological environments, which can vary from 100 Pa in the brain to 10 kPa in connective tissue and 50 kPa in bone (Discher et al., 2005; Janmey et al., 2009). The traction stresses measured for migrating 3T3 fibroblasts are $\sigma = 100$ kdyn/cm^2 $= 10$ kPa (Dembo and Wang, 1999). Thus, the cell-generated stresses are of a similar magnitude to the stiffness of the surrounding microenvironment. Because single focal adhesions typically have a lateral dimension of $d = 1$ μm, the typical force at focal adhesions is expected to be in the order of $F = \sigma d^2 = 10$ nN. Indeed, force measurements have shown that the force transmitted by a single adhesion lies within this magnitude (Balaban et al., 2001; Tan et al., 2003). Interestingly, because a single myosin II motor generates force on the piconewton scale (Finer et al., 1994; Veigel et al., 2003), a force of 10 nN at focal adhesions must correspond to the activity of tens of thousands of myosin II motors within the actin cytoskeleton.

Cellular traction forces that are measured on soft elastic substrates or using pillars are expected to be smaller than the forces that are at work inside cells, because force vectors pointing into different directions cancel each other out in the vectorial sum that is transmitted to the substrate (Bischofs et al., 2009). However, measuring forces inside cells has proven to be quite challenging. Examining the recoil of cytoskeletal elements in response to laser ablation in combination with appropriate

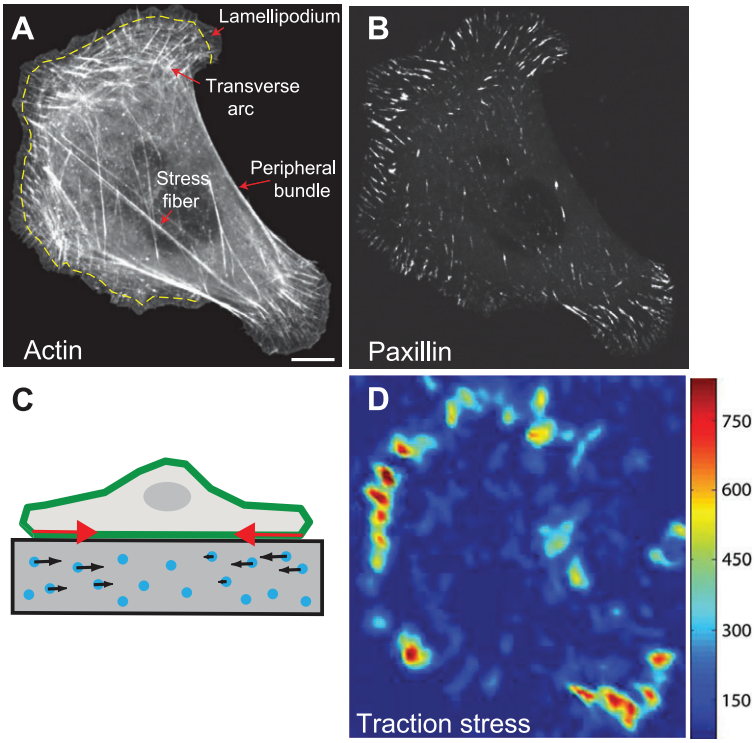

Fig. 2. Traction-force microscopy. (**A**) Actin and (**B**) focal adhesions in a U2OS cell visualized by GFP–actin and mApple–paxillin, respectively. The yellow dashed line in the actin image indicates the boundary between the lamellipodium and lamella, which contains transverse arcs and stress fibers. Whereas stress fibers are straight, peripheral bundles are invaginated as a result of the contractility within the cell body. Scale bar, 10 μm. (**C**) Forces exerted during traction-force microscopy. During traction-force microscopy, traction forces are reconstructed on the basis of substrate displacements that are tracked with fluorescent beads embedded in a soft elastic substrate. (**D**) Reconstructed traction stresses exerted on the underlying fibronectin-coated polyacrylamide substrate. Clearly, actin organization, the localization of adhesions marked by GFP–paxillin and the traction-force pattern show strong correlations with each other. In particular, regions of high forces correlate with the presence of focal adhesions.

mechanical models can provide an estimate of the forces acting on the molecules residing in the actin and adhesion complexes. Laser ablation has proven to be useful in the assessment of spatial variation in stress fiber tension (Kumar et al., 2006; Tanner et al., 2010) and in demonstrating that the mechanosensitive protein zyxin localizes to regions of large stress between stress fibers and the extracellular substrate (Colombelli et al., 2009). An alternative and less invasive approach is the development of fluorescent biosensors whose fluorescence intensity depends on the level of tension. This approach has recently been successfully implemented for a vinculin biosensor to measure the force acting in focal adhesions (Grashoff et al., 2010).

One challenge for the future will be to develop novel assays to measure cellular forces with high spatial and temporal resolution, both between cells and their environment as well as inside cells. Traction-force microscopy on flat elastic substrates has the great advantage that it can be easily combined with fluorescence microscopy and, therefore, is especially suited for quantitative correlation between cellular forces and the organization of cell–matrix adhesions and the actin cytoskeleton. As we will discuss in the following sections, this approach has revealed that different mechanisms of force transmission are at work at the lamellipodium and the lamella, respectively.

Force transmission at the lamellipodium

The nature and magnitude of force transmission differs greatly between the lamellipodium and the lamella (Fig. 3A). Force transmission between the cell and the ECM is initiated by nascent adhesions within the lamellipodium. Here, forces generated by actin polymerization drive a rapid retrograde flow of actin and integrin complexes. By quantitatively assessing the retrograde flow dynamics of lamellipodial actin with fluorescent speckle microscopy in addition to measuring cell traction force, correlations between actin retrograde-flow dynamics and the magnitude of the traction force can be obtained (Gardel et al., 2008). As integrins become engaged to the ECM, traction forces increase from ~2 to 150 pN over an area of 0.1 μm^2. This causes a reduction in the retrograde flow, which, in PtK1 cells, slows down from 25 to 2 nm/s (Gardel et al., 2008). For the fast flow in the lamellipodium, an inverse relationship between flow and force is observed (Fig. 3B). The low magnitude of traction force generated within the lamellipodium is likely to reflect the limits of force generation by actin polymerization against the leading cell membrane as well as the limits of establishing strong contact under fast flow. The slowing of retrograde flow during adhesion assembly has resulted in the idea that matrix adhesions serve as a molecular clutch that engages retrograde flow to the underlying ECM and, thus, converts retrograde flow into cell protrusion. Indeed, an inverse correlation between the speed of retrograde flow and forward protrusion has been observed (Lin et al., 1996; Jurado et al., 2005; Giannone et al., 2009). In many cell types, adhesion assembly does not completely stall retrograde flow and, in such situations, protrusion occurs only if filament assembly is faster than retrograde flow (Lin et al., 1996; Ponti et al., 2004; Giannone et al., 2007).

It is not immediately clear how matrix adhesions can simultaneously transmit forces to the ECM and maintain the connection to the flowing actin cytoskeleton. To understand this, we must consider the finite lifetime of individual biomolecular bonds within the adhesion site, which undergo repeated bond dissociation and association (Bell, 1978). While one bond breaks and reforms, the other bonds can still carry the force and, thus, transmit it to the substrate. In the context of two objects sliding past each other, this allows transmission of tension even in the presence of movement. Such mechanisms have been used to understand, for example, how force builds up in muscle fibers (Huxley, 1957; Duke, 1999) or how multiple motors transport cargo (Holzbaur and Goldman, 2010; Guérin et al., 2010). On a

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Box 1. Physical concepts relating to cellular forces

Elastic forces

Force is usually quantified by displacement of a calibrated elastic system. The perfectly elastic system is a linear spring with force $F=kx$, where k is the spring constant and x is the displacement. In traction-force microscopy on pillar arrays, the effective spring constant is $k=\frac{3\pi E R^4}{4L^3}$, where E is the Young's modulus of the pillar material, and R and L are pillar radius and height, respectively. By varying the dimensions of R and L, the spring constant of the pillars can be tuned in the range of 1 to 100 nN/μm.

Viscous forces

If a plate is moved over a surface that is lubricated with a fluid, the viscous force $F=\xi v$ grows linearly with velocity v. The friction coefficient is determined by $\xi=\eta A/h$, where η is the viscosity of the lubricating fluid, A is the surface area of the moving plate and h is the thickness of the fluid layer. A linear relationship between force and velocity is also observed for the retrograde flow of actin across mature focal adhesions, indicating an ideal viscous coupling.

Stress and strain

In spatially extended systems, such as soft elastic substrates, force F is distributed over a finite surface area A. Therefore, it is appropriate to consider stress $\sigma=F/A$ instead of force F itself. The physical dimension of stress is $Pa=N/m^2$. In contrast to pillar assays, which measure traction force, traction-force microscopy on soft elastic substrates measures traction stress. Like force, stress leads to displacement. For spatially extended systems, this is usually measured as dimensionless strain ε. For a rod or slab of elastic material and length L elongated by ΔL, strain is simply relative displacement, $\varepsilon=\Delta L/L$.

Young's modulus

The Young's modulus is the ratio between the stress required to deform a soft object and the effected strain, $E=\sigma/\varepsilon$. The larger the Young's modulus, the stiffer the material and the greater the stress required to achieve the same strain. Because Young's modulus and stress have the same physical dimension ($Pa=N/m^2$), they have to be of a similar magnitude ($E\approx\sigma$) to achieve appreciable levels of strain ($\varepsilon\approx1$). Therefore, it is expected that connective tissue cells, whose physiological environment has a typical Young's modulus of 10 kPa, typically exert a stress in the order of 10 kPa on their substrate. Traction-force microscopy with soft elastic substrates is often implemented using polyacrylamide gels, whose Young's modulus can be easily tuned over the physiologically relevant range from 100 Pa to 50 kPa.

Material failure and fracture

If stress and strain become sufficiently large, solid materials start to change their internal structure. If elastic materials are loaded beyond the yield stress they become plastic, and after unloading, do not return to their initial state. Failure occurs if the material loses its ability to transmit forces. The most catastrophic mode of failure is fracture. Each failure process will lead to local stress relief, and, therefore, has the potential to bring the system back to a stable situation. However, in many cases, failure of one part of the system increases force on another part. This mechanism might lead to cascading failure, with a catastrophic loss of stability. Recently, it has been suggested that the actin network in the lamellipodium disintegrates under stresses as small as 40 Pa.

macroscopic level, this is analogous to the friction between two surfaces sliding past each other, for example, in a ball bearing. Therefore, mathematical models for the clutch at nascent adhesions combine elements of sliding friction with the specific nature of ligand–receptor bonds (Chan and Odde, 2008; Srinivasan and Walcott, 2009; Sabass and Schwarz, 2010; Li et al., 2010). These models explain the observed inverse relationship between flow and traction if flow is sufficiently fast. Additionally, they predict the existence of a 'stick–slip' regime, where the adhesions fail intermittently. In this regime, a stable solution to the dynamic equations does not exist for intermediate velocity, and therefore, the system switches between phases with low and high velocity. Such stick–slip behavior has been observed experimentally in filopodial adhesions (Chan and Odde, 2008). This phenomenon provides an example of how oscillatory phenomena can arise by purely mechanical mechanisms.

Force transmission at the lamella

Myosin II activity within the lamella stabilizes the attachment of nascent adhesions to the ECM and mediates a maturation process by which the size of adhesions is increased and their composition modified (Choi et al., 2008). Adhesion maturation under force has first been reported for focal adhesions, which grow under both externally applied or internally generated force (Riveline et al., 2001). Forces generated by clusters or 'ribbons' of bipolar myosin II minifilaments embedded in a cross-linked lamellar F-actin network or transverse arcs drive myosin IIA-dependent retrograde flow within the lamella (Verkhovsky et al., 1995; Svitkina et al., 1997; Alexandrova et al., 2008). Myosin II activity generates tension on nascent adhesions, resulting in a transient sliding of the nascent adhesions before they stabilize to the ECM (Aratyn-Schaus and Gardel, 2010) (Fig. 3A). After focal adhesions stabilize, their elongation occurs concomitantly with the assembly of stress fibers at the focal adhesion plaque. During this growth, the stress at the focal adhesion plaque increases (Fig. 3C) (Balaban et al., 2001; Tan et al., 2003; Stricker et al., 2011). Tension is important for stabilizing focal adhesion plaques, and focal adhesions experience, on average, a stress of 5.5 $nN/\mu m^2=5.5$ kPa (Balaban et al., 2001; Tan et al., 2003). Recent evidence suggests that the correlation between adhesion size and traction is strongest during the assembly process (Stricker et al., 2011) and requires an intact signaling system (Prager-Khoutorsky et al., 2011). Mature focal adhesions are typically elongated in the direction of the attached stress fibers, an effect that might be a direct result of the force transmitted through the stress fibers (Nicolas et al., 2004; Shemesh et al., 2005; Besser and Safran, 2006; Walcott et al., 2011). Mature focal adhesions provide stable adhesive interactions between the cell and the ECM over 10–20-minute time scales. The relatively stable state of the combined system of focal adhesions and stress fibers allows the cell to probe its mechanical environment over large temporal and spatial scales and in specific directions. Therefore, mature focal adhesions are believed to be important for the direction-dependent rigidity response of adherent cells (Zemel et al., 2010; Walcott and Sun, 2010; Besser and Schwarz, 2010).

The details of force transmission at focal adhesions are not clear. Forces at mature focal adhesions can result from polymerization-dependent retrograde flow in the lamellipodium, actin polymerization and network contraction in the lamella, or contraction through stress fibers, with the relative importance of these processes being dependent on the type of cell and environmental conditions. In both keratocytes and PtK1 cells, the rate of lamellar actin retrograde flow varies from 5 to

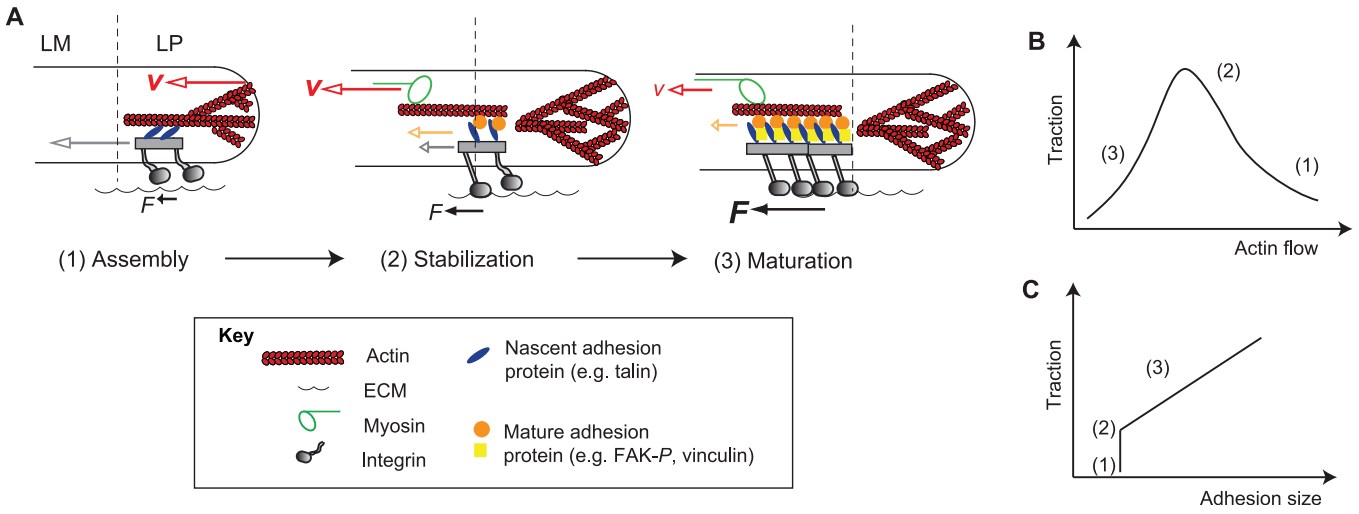

Fig. 3. Correlations between retrograde flow, adhesion size and traction forces during adhesion assembly. (**A**) Different kinds of traction force are generated in different actin modules. (1) During adhesion assembly, polymerization forces drive rapid retrograde flow of actin (red arrow) and associated adhesion proteins, and weakly ligated integrins (gray arrow). Low traction forces (black arrow, F) are exerted. (2) During adhesion stabilization, myosin stresses drive the flow of actin; retrograde movement of adhesions is reduced as they become engaged to the ECM and traction forces on the ECM build. (3) During adhesion maturation, actin retrograde flow is continuous as a stress fiber assembles at the adhesion plaque, focal adhesion proteins accumulate, adhesion elongates and traction forces increase. (**B**) The typical correlation between retrograde flow speed and traction force shows a biphasic form, with the peak separating two different types of friction regimes. (**C**) The typical correlation between focal adhesion size and traction force shows different regimes for small and large adhesions. In B and C, different regimes of the correlations are related to the different stages [(1), (2) and (3)] of the assembly process as shown in A. *F*, force; *v*, velocity.

30 nm/s, and a direct relationship between traction stress and actin retrograde flow has been observed (Gardel et al., 2008; Fournier et al., 2010; Barnhart et al., 2011) (Fig. 3B). Indeed, such a direct proportionality is predicted by theories of friction in the limit of very slow flow (Chan and Odde, 2008; Srinivasan and Walcott, 2009; Sabass and Schwarz, 2010; Li et al., 2010). Together with the inverse relationship between flow and traction force in the limit of very fast flow, this leads to a biphasic relationship, as observed experimentally (Fig. 3B). The molecular determinants of this frictional coupling are not known, but likely candidates include vinculin and zyxin, because they have been shown to localize to regions of large shear (Hu et al., 2007; Shtengel et al., 2009).

In cells containing stress fibers that are anchored at focal adhesions, traction forces are not well correlated to changes in retrograde flow dynamics. Owing to their sarcomeric structure, it is expected that stress fibers are important for force generation in this context, and several models that predict the corresponding forces have been suggested (Besser and Schwarz, 2007; Stachowiak and O'Shaughnessy, 2008). Such models can also predict that, after stress fiber stimulation, peripheral regions tend to contract whereas central regions tend to expand (Peterson et al., 2004). However, a recent report has indicated that the formation of stress fibers only accounts for a moderate amount (30%) of the force generated by the cell (Aratyn-Schaus et al., 2011), indicating that contractile networks also have an important function. This is consistent with earlier accounts stating that substantial traction is achieved before stress-fiber formation during cell spreading (Reinhart-King et al., 2005; Cai et al., 2010), and with theoretical models that predict adhesion forces through network contraction (Deshpande et al., 2006; Bischofs et al., 2008; Rubinstein et al., 2009; Guthardt Torres et al., 2012). In the future, this issue might be further clarified, for example, by combining high-resolution microscopy with theoretical modeling.

In summary, for both lamellipodium and lamella, the organization of the adhesion system strongly depends on the organization of the actin system and vice versa, thus, these elements cannot be considered in isolation. However, because adhesions and actin are organized differently in lamellipodium and lamella, different quantitative correlations arise between traction force and cell organization. The forces measured in this context can only be understood if theoretical concepts are used that incorporate the underlying physical mechanisms, such as friction between retrograde flow and substrate, or contractility in networks and bundles anchored at focal adhesions. Moreover, each module can only be understood when its coupling to the cellular context is also considered. For example, the functioning of the lamella cannot be understood without also considering how it is made possible through the spatial proximity to the lamellipodium. With a growing body of experimental data and theoretical models available for the exact magnitude and time dependence of the forces acting in the different modules, we can now address the question of how these forces couple into mechanosensitive processes on the molecular level.

Mechanosensitivity based on force transmission

As discussed above, changes in tension on the actin fibers influences the stability, size and composition of adhesions. Over the last few years, a number of different mechanisms by which mechanosensitive proteins can sense such changes in tension and react to them have been discovered. One way in which increased tension could alter the composition of a cytoskeletal module is to increase the affinity of existing binding sites or to expose cryptic binding sites, which could result in the accumulation of, for example, scaffolding proteins or post-translational modifications

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(Fig. 4A). For example, stretching talin has been shown to reveal a vinculin binding site, and subsequently, leads to the interaction of these two proteins (del Rio et al., 2009). Stretching has also been found to enhance the phosphorylation status of p130Cas (also known as BCAR1), leading, in turn, to the activation of several G-proteins (Sawada et al., 2006). Whereas these mechanisms increase the association rate of proteins with the adhesion, a force-mediated decrease in the dissociation rate could provide another possible mechanism for effective protein recruitment (Fig. 4B). In the traditional Bell model of binding interactions, applied force accelerates the kinetics of unbinding (Bell, 1978). However, several cytoskeletal proteins, including myosin and integrin, have been found to exhibit an increased affinity to their ligands under applied load ('catch bonding') (Veigel et al., 2003; Kong et al., 2009). Such a mechanism might assist in the recruitment and/or maintenance of focal adhesions and myosin filaments to sites of high cortical tension (Effler et al., 2006).

Tension could also induce localization of proteins to specific sites by changing the spatial organization of binding partners ('templating') (Fig. 4C). For instance, the accumulation of α-actinin into actin bundles during assembly of stress fibers occurs concomitantly with the formation of bundled actin, suggesting that the α-actinin localization is enhanced because the filament bundle acts as a binding template (Aratyn-Schaus et al., 2011; Oakes et al., 2012). An extreme, but by no means unlikely, case of structural reorganization under force would be material failure under stress. For instance, the rupture of actin filaments exposes barbed ends and, thus, results in the recruitment of barbed-end binding proteins (Fig. 4D). Such a mechanism is thought to function in the repair of stress fibers. Stress fibers tend to break under their own stress, with a rate of 0.03 breaks/minute/cell in mouse fibroblasts. Furthermore, zyxin has been found to localize to points of acute strain within stress fibers, initiating the recruitment of vasodilator-stimulated phosphoprotein (VASP) and α-actinin, which facilitates F-actin polymerization and cross-linking and consequently mediates local stress fiber repair (Smith et al., 2010).

Given the growing list of mechanosensitive molecules and mechanisms, a picture emerges in which the cellular response is determined not by a single mechanosensor that is spatially localized to specific cellular structures, but by a network of integrated mechanosensory elements. Thus, the mechanosensitive response of adherent cells, although not only based on biochemical mechanisms, might exhibit similar network characteristics to signaling or gene expression networks, including feedback loops that give rise to, for example, multi-stability and oscillations (Tyson et al., 2003). Spatial coordination and mechanics are increasingly recognized as key elements for biochemical networks (Kholodenko et al., 2010), and a similar theme is emerging for the mechanosensitive network of molecules involved in mediating adhesion of cells to the extracellular matrix. As our quantitative understanding of the different cytoskeletal modules and their interplay with the adhesion structure improves, we must also understand how these cytoskeletal modules are coupled on the cellular scale. Whereas regulation through the localization of biochemical signals is certainly very important (Ridley, 2011), signal coordination might also be affected by differences in the physical stability of various modules and their mechanical coordination, as we discuss in the next section.

Limits of physical stability and mechanosensitive responses

The example of severing of actin filaments under mechanical stress (Fig. 4D) shows that the limits of physical stability of proteins and their assemblies might be an important element of the mechanosensitive response on the molecular scale. Similar mechanisms might also take place at the cellular level. For instance, it has been suggested that coupling to the adhesion sites induces sufficient mechanical stress in the lamellipodium as to lead to its disintegration (Shemesh et al., 2009). The predicted fracture line is consistent with the observed lamellipodium–lamella boundary and is thought to be the origin of transverse arc assembly. The repeated occurrence of stress build-up and release within the lamellipodium might explain the cycles of protrusion and pausing that are observed in cell migration (Giannone et al., 2007; Shemesh et al., 2009). Such mechanical processes might complement the known biochemical mechanisms that regulate

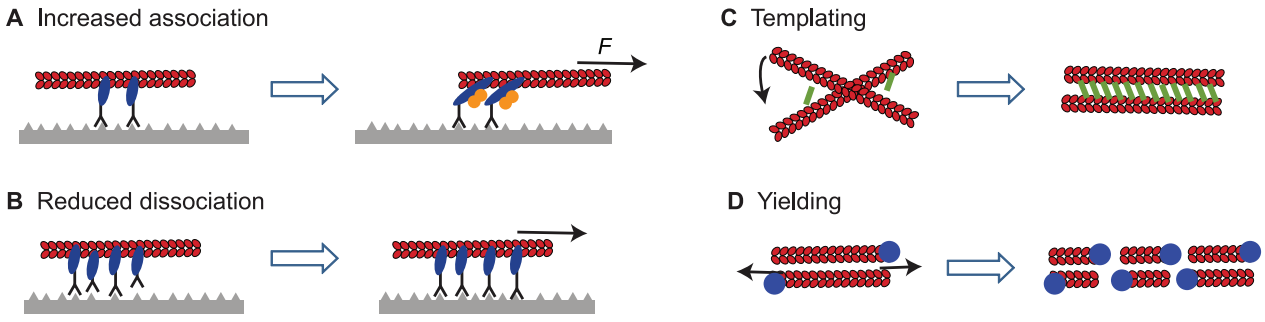

Fig. 4. Mechanisms of force-dependent protein recruitment to actin filaments. (**A**) Force-induced stretching of proteins (blue ellipse) results in the exposure of cryptic binding sites or increased affinity, thus, increasing the association of binding partners (orange). In this schematic, force is applied through F-actin (red filament) that is bound to rigid ECM (gray) through different binding partners. Examples include binding sites in talin and p130Cas. (**B**) Force might also decrease dissociation kinetics, for example for integrins and myosin, which have been shown to act as catch bonds. Here, this effect is shown schematically for integrins under tension. (**C**) The re-organization of structures under force might facilitate the recruitment of binding partners. In this example, actin filaments that are cross-linked at a large angle can only facilitate a small number of cross-links (green) between both filaments. Parallel filaments, however, allow the formation of a much higher number of cross-links to be formed between both filaments. (**D**) Under tension, actin filaments rupture and expose barbed ends, a process that leads to the recruitment of barbed-end binding proteins (blue), such as VASP, mDia or capping protein.

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lamellipodium size through Rho GTPase signaling at adhesions (Delorme et al., 2007; Iwasa and Mullins, 2007; Ridley, 2011).

Myosin-generated stresses within the lamella might also probe the limits of lamellar stability. Myosin-generated lamellar contractions are also sites of actin disassembly (Gupton and Waterman-Storer, 2006; Wilson et al., 2010), suggesting that myosin can drive mechanically mediated severing of actin filaments or enhance cofilin-mediated severing of actin filaments. Such coupling between contraction and network disassembly provides a means to maintaining a uniform density of actin filaments across the cell. Understanding the consequences of how the feedback between internal stresses and network disintegration impacts the limits of force generation by contractile lamellar networks will provide additional insight into the mechanical regulation of cytoskeletal networks.

The limits of physical stability might also be central to the regulation of focal adhesions. Force is crucial to the assembly of focal adhesions, as they build under tension and disintegrate under low force (Riveline et al., 2001). This counterintuitive phenomenon makes focal adhesions truly mechanosensitive and has been explained with different mechanisms of force-mediated growth (Nicolas et al., 2004; Shemesh et al., 2005; Besser and Safran, 2006; Walcott et al., 2011). The adhesion strength has been shown experimentally to be determined by adhesion size, integrin clustering and adhesion composition (Goffin et al., 2006; Selhuber-Unkel et al., 2010). Although it is difficult to demonstrate experimentally owing to limited spatial resolution, force distribution in an elastic system is unlikely to be homogeneous. Theoretical models predict that, as adhesion size increases or substrate stiffness decreases, force localizes more and more strongly to the adhesion rim, eventually leading to a crack-like failure of the adhesion site (Qian et al., 2009; Gao et al., 2011). Such a mechanism would also work for apparent catch bonds, as they always become slip bonds at sufficiently high forces. Thus, limits of physical stability would not only provide a means to switch the state of actin modules, but also of adhesion structures. Even more importantly, their close integration leads to a feedback cycle, where failure in adhesions leads to destabilization of actin modules and vice versa. Therefore, eventually, only those combinations that mutually stabilize each other will survive. Although the molecular details might differ for different cell types and environmental conditions, such a positive feedback cycle is crucial for a definitive cell response upon adhesion to different extracellular environments, such as, for example, for the establishment of cell polarity as a function of matrix stiffness (Zemel et al., 2010; Besser and Schwarz, 2010; Prager-Khoutorsky et al., 2011).

Concluding remarks and perspectives

In recent years, much progress has been made in identifying the molecular composition of adhesions and the actin cytoskeleton as well as mechanisms of mechanotransduction at the molecular level. However, the emerging complexity of the mechanosensitive system of adherent cells might be similar to the complexity encountered in signaling or gene expression networks. In order to bring this molecular understanding to the cellular level, we need to understand how adhesion and actin architectures, which integrate the effect of a network of spatially distributed mechanosensitive complexes, are built. By focusing on the instructive cases of the two actin modules, lamellipodium and lamella, we have argued that the combination of quantitative experiments and theoretical modeling can help to understand the physical principles underlying the transmission of force to the sites of cellular mechanotransduction. In particular, we suggest that limited physical stability might emerge as one of the main mechanisms used by cells to switch the state of their functional modules.

The main challenge for the future is to combine these mechanical insights with detailed studies of the protein dynamics in adherent cells. For example, high-resolution imaging might help to further our understanding of how focal adhesions and the corresponding actin structures are organized, but it also needs to be complemented by high-resolution measurements of cellular forces as well as theoretical models in order to understand how this organization is determined by force. For cases like the lamella, where different force-generating processes (such as contractile bundles and networks) contribute to the traction force measured on the substrate, it is essential to clarify their relative importance and how this ratio is dynamically regulated by the cell as a function of environmental conditions (including stiffness and dimensionality). Such insights might then be ideal starting points to understand in a more general way how different cell types (e.g. fibroblasts and macrophages, which both migrate through connective tissue) regulate the different organization and dynamics of their actin and adhesion systems, and how this relates to the way they accomplish their physiological functions in vivo.

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