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Review

Stretchy Proteins on Stretchy Substrates: The Important Elements of Integrin-Mediated Rigidity Sensing

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Matrix and tissue rigidity guides many cellular processes, including the differentiation of stem cells and the migration of cells in health and disease. Cells actively and transiently test rigidity using mechanisms limited by inherent physical parameters that include the strength of extracellular attachments, the pulling capacity on these attachments, and the sensitivity of the mechanotransduction system. Here, we focus on rigidity sensing mediated through the integrin family of extracellular matrix receptors and linked proteins and discuss the evidence supporting these proteins as mechanosensors.

What Is Rigidity and Why Is It Important?

Local rigidities form a landscape within an organism. During development, this landscape acts in concert with chemical cues to shape the organism (Keller et al., 2003). Conversely, recovery from injury can require cells to sense and reshape this landscape, as seen in healing of bones and wounds (Chao et al., 1989; Grinnell, 1994; Hinz, 2006). Numerous pathologies are characterized by disturbance of the rigidity landscape, including atherosclerosis, arthritis, osteoporosis, and fibrosis of the heart, lung, kidney, and liver (Ingber, 2003). There are also a growing number of medical uses for pharmacological drugs that affect the rigidity of tissues, including Botulinum toxin (relaxes smooth muscles) and Rho kinase inhibitors (relaxes cytoskeleton) (Felber, 2006; Hahmann and Schroeter, 2010). Recently, an appreciation for the altered rigidity responses of cancer cells has emerged (Kostic et al., 2009; Wang et al., 2000b). However, rigidity sensing defects are likely to have an important role in classical "anchorage independence" studies (Stoker et al., 1968) that examined the ability of cancerous, but not normal, cells to proliferate on each other when in suspension.

In a broad sense, rigidity is a measure of the relationship between applied forces and the resulting stretch of a material. To quantify it, the elastic (or Young's) modulus and the shear modulus are commonly employed. Both are defined as the ratio of the stress (applied force per unit area) to the resulting strain. For the elastic modulus (***E***), the force is applied perpendicular to the material's surface, whereas for shear modulus (***G***), the force is applied parallel to the surface (Figure 1A). Because strain is a dimensionless value, the units for rigidity are force per area, with the SI unit being the Pascal (Burdun et al., 1972). However, to ease interpretation of the values, this review will use the proposed "natural" SI units for pressure of nN/μm^2 (equivalent to kPa, Hochmuth, 2000). There are two useful observations that assist the interpretation of elastic and shear moduli values. The first is that they represent the amount of force per unit area required to double the length of a material (elastic modulus) or deflect it by a distance equal to its height (shear modulus). The second is that elastic and shear moduli are related by the following function: $\boldsymbol{E} = 2\boldsymbol{G}(\boldsymbol{1}+\boldsymbol{\nu})$, where $\boldsymbol{\nu}$ is the Poisson ratio. For materials that do not change volume under stretch, the Poisson ratio equals 0.5. As a consequence, the elastic modulus will be three times its shear modulus.

There are important limitations to shear and elastic moduli values of biological materials. In particular, they assume a linear relationship between stress and strain, which is not the case for either cells or extracellular matrix (ECM) components (Storm et al., 2005). Rather biological material has a viscoelastic behavior where the relationship between stress and strain depends not only on the magnitude of the force applied, but also on the rate of application (Fabry et al., 2001; Puxkandl et al., 2002). This implies that stiffness values reported in the literature will depend on the range of forces applied and on the time scale of force application. This review will examine the reported physical and motility parameters of tissues, cells, and proteins and project those constraints onto how cells sense rigidities through integrins.

The Rigidity Landscape

The rigidity landscape of a tissue arises from its constituent cells and ECM. The elastic moduli of tissues range from 0.1 to 30,000,000 nN/μm^2 (Table 1). The typical hierarchy of tissue rigidity is that brain (0.1–10 nN/μm^2) is softer than muscle (10–100 nN/μm^2), which is softer than areolar connective tissue and arteries (100–1,000 nN/μm^2), which are softer than bone (15,000,000–30,000,000 nN/μm^2).

The rigidity of a tissue is neither static nor uniform. For instance, the stiffness of brain decreases with age (Gefen et al., 2003). Local rigidity differences have also been observed in the kidney cortex and hippocampus (Elkin et al., 2007; Kallel et al., 1998). Further, there are local rigidity differences within individual cells (Roca-Cusachs et al., 2008; Yamada et al., 2000).

The Rigidity Preferences of Cells

The rigidity preferences of cells generally reflect their native environments. Neutrophils exist in a broad range of rigidities

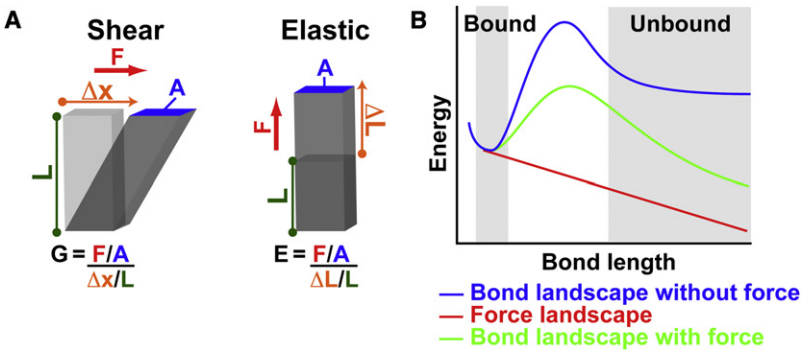

Figure 1. Rigidity Moduli and the Energy Landscapes of a Slip Bond

(A) Stress is the amount of force applied per area (F/A) and strain is the displacement in the direction of applied force relative to initial length (Δx/L or ΔL/L). While both elastic and shear moduli are the ratio of stress over strain, there is a difference in the direction of the applied force.

(B) The energy landscapes of a slip bond with and without applied force.

from the blood to a variety of tissues during inflammation; these cells attach and have similar shapes on glass as on 0.002 nN/μm^2 gels (Yeung et al., 2005). From a soft tissue like the brain, neurons are more branched on substrates of 0.05 nN/μm^2 compared to 0.55 nN/μm^2 and they extend faster on substrates of 0.002 nN/μm^2 compared to 0.130 nN/μm^2 (Balgude et al., 2001; Flanagan et al., 2002). Fibroblasts and endothelial cells, originating from tissues of "midrange" stiffnesses, do not spread or display actin stress fibers on surfaces softer than 3 nN/μm^2 (Pelham and Wang, 1997; Yeung et al., 2005). From the stiff environment of cartilage and bone, chondrocytes do not spread well on substrates softer than 7.4 nN/μm^2 (Subramanian and Lin, 2005), and the degree of adhesion protein clustering in preosteoblasts is maximized at 60 nN/μm^2 (Kong et al., 2005). Cells can also be guided by differences in rigidity, a phenomenon known as durotaxis. Both fibroblasts and leukocytes have been reported to move toward rigid substrates (Lo et al., 2000; Mandeville et al., 1997). Another related cellular process is endothelial cell remodeling to shear stress. Although this process differs from rigidity sensing in that it does not appear to require cells to actively pull on their environment, both processes involve sensing mechanical force in a time- and magnitude-sensitive manner and may therefore share similar mechanosensory mechanisms (Ando and Yamamoto, 2009).

Bond Strengths

Throughout the rigidity sensing process, the key molecular mechanism by which force is detected is through its effect on the binding and conformation of proteins. Two interacting proteins can be described as having a "bound" and an "unbound" state, with an energy barrier necessary to switch between the two. Analogously, the switch between two conformations in a single protein (e.g., folded/unfolded or open/closed channel) can be characterized in the same way. A higher barrier will make it more difficult to change configurations. As first envisioned by Bell (1978), the force required to break a bond can be understood as an input of energy into the system. Force reduces the activation barrier between the two, thereby promoting the transition to the unbound state (Figure 1B). This type of behavior, common to most protein interactions, is called a slip bond. Catch bonds are exceptions to this rule. In response to applied force, the energy landscape of catch bonds changes in such a way as to increase their strength (energy barrier) under certain force regimes (Prezhdo and Pereverzev, 2009; Thomas et al., 2008). The possibility that these bonds have an important role in rigidity sensing will be discussed below.

Quantifying the energy barrier between bound and unbound states is the optimal way to characterize a protein bond. However, this value is difficult to obtain in live cells, which are strongly out of thermodynamic equilibrium (having large local and temporal variations in energy levels) (Collin et al., 2005). For this reason, studies usually report the force needed to break a bond in a particular assay. For these assays, the rate at which force is loaded will affect the force measurement (Evans, 2001). This important caveat must be taken into account when comparing force values, and that is why in this review, we specify the loading rate whenever reported.

Determinants of What Cells Can Sense through Integrins

Going from the ECM to the inside of the cell, the matrix-integrin bonds are the primary links. Integrins are transmembrane heterodimers composed of an α and a β chain. In vertebrates, there are 18 α and 8 β subunits that assemble into 24 different heterodimers (Takada et al., 2007). Integrins mediate attachment to a variety of ECM components, including fibronectin, collagen, laminin, and vitronectin (Barczyk et al., 2010). In general, the α subunit determines the substrate specificity,

Table 1. Elasticity of Cells and Tissues

Tissue Type	Elastic Modulus (nN/μm^2)	Key References
Brain	0.1–10	(Elkin et al., 2007; Gefen et al., 2003; Hirakawa et al., 1981; Metz et al., 1970)
Muscle	12–100	(Collinsworth et al., 2002; Engler et al., 2006; Mathur et al., 2001)
Fat	20	(Ophir et al., 1999)
Artery	100–3,800	(Bank and Kaiser, 1998; Intengan et al., 1999)
Areolar connective tissue *(fibroblasts in collagen)*	600–1,000	(Chapuis and Agache, 1992; Wakatsuki et al., 2000)
Bone	17,100,000–28,900,000	(Reilly et al., 1974; Schaffler and Burr, 1988)

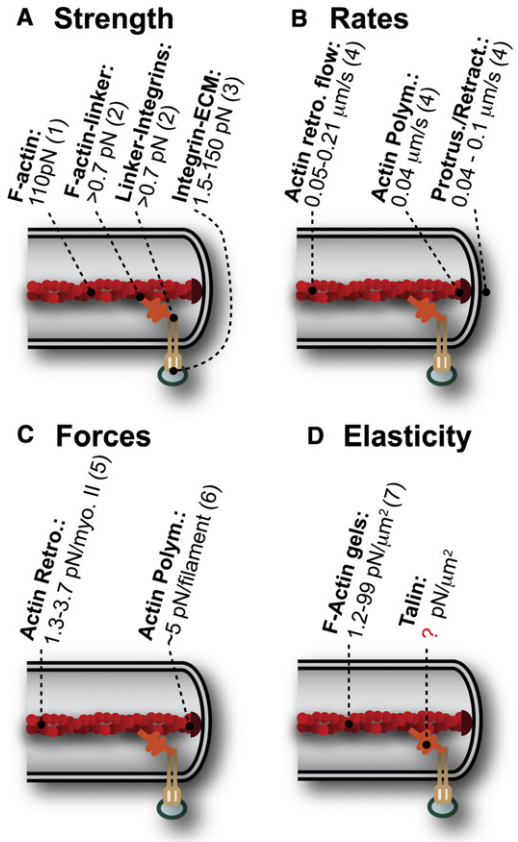

Figure 2. Important Parameters of Rigidity Sensing

Reported values of the strength (A), rates (B), forces (C), and elasticity (D) of components involved in the coupling of the ECM to the cytoskeleton through integrins. References: (1) (Kishino and Yanagida, 1988), (2) (Jiang et al., 2003), (3) Table 2, (4) see text, (5) Table 1, (6) (Kovar and Pollard, 2004; Mogilner and Oster, 1996; Peskin et al., 1993), (7) (Tseng et al., 2005).

while the β subunit attaches to actin through intermediary proteins (Vicente-Manzanares et al., 2009). Integrins undergo a mechanical cycle in forming adhesive contacts (Puklin-Faucher and Sheetz, 2009), and there are important functional differences between integrin complexes. For instance, less stable αvβ3 integrins initiate signal transduction, while higher matrix forces are supported by α5β1 integrins (Roca-Cusachs et al., 2009).

Four key parameters determine what rigidities cells can sense through integrins: (1) the strength of integrin binding to extracellular ligands, (2) the force and (3) the speed of cell retractions, and (4) the sensitivity of associated mechanosensors.

Strength of the Integrin Link to the ECM

Matrix attaches to integrins and integrins attach to actin filaments through several direct and indirect routes. However, talin, filamin, α-actinin, and tensin are integrin binding proteins that also bind directly to actin (Delon and Brown, 2007). Of these proteins, talin appears to be the one involved in the initial adhesion events, as it is located closer to the leading edge than α-actinin (Izzard, 1988; Zaidel-Bar et al., 2003), while filamins appear to be involved in later adhesion events (Calderwood et al., 2001). As the adhesion matures, additional proteins are recruited to reinforce the linkage, including vinculin, which binds actin and talin (Burridge and Mangeat, 1984). In the absence of talin, vinculin staining is diffuse and traction forces are disrupted (Jiang et al., 2003; Zhang et al., 2008).

Thus, there are five noncovalent interactions in series that are needed to develop the forces on early extracellular linkage: (1) myosin and actin interaction, (2) actin monomer assembly into filaments, (3) actin filament binding to linker protein (e.g., talin), (4) linker binding to integrin, and (5) integrin binding to the ECM component. The strengths of many of these connections are either unknown or poorly characterized (Figure 2A). Actin filaments can withstand forces up to 110 pN (Kishino and Yanagida, 1988). In some cases, tropomyosin and cross-linking proteins strengthen actin filaments (Liu and Bretscher, 1989; Tseng et al., 2005). For instance, the elastic modulus of actin filament gels increases from 1.2 to 99 pN/μm^2 in the presence of fascin and α-actinin (Tseng et al., 2005). The specific strength of the linker/actin or linker/integrin bond is unclear, but there is an initial talin-mediated slip bond that releases at about 0.7 pN per fibronectin (2 pN per fibronectin trimer, Jiang et al., 2003). This likely represents the strength of talin's initial bond to actin filaments that is subsequently reinforced by additional actin binding proteins like vinculin.

A number of reports suggest that the weakest link is not within the cell, but rather in the integrin bond to the ECM. The bond strength between $\alpha_5\beta_1$ integrin and fibronectin has been by far the most studied. Reports have placed its strength as low as 0.1 pN or as high as 69 pN (Table 2). Higher estimates correspond to single molecule measurements, while lower values are average forces per molecule in measurements involving adhesions with multiple molecules (compare values labeled "S" and "A" in Table 2). The large discrepancy between both types of measurements suggests that most integrins in adhesions are not bound and that only a small fraction are sustaining forces in the tens of piconewtons.

Table 2. Integrin-ECM Bond Strength

Integrin/Ligand	Method	Loading Rate (pN/s)	Strength (pN)*	Reference
$\alpha_5\beta_1$ / fibronectin	Magnetic tweezers	-	0.1–0.65 (A)	(Roca-Cusachs et al., 2009)
	Spinning disk	-	1.5–2 (A)	(Friedland et al., 2009)
	Laser tweezers	~40	13–28 (S)	(Thoumine et al., 2000)
	AFM	~10,000	39 (S)	(Sun et al., 2005)
	AFM	100 10,000	~50 (S) ~100 (S)	(Li et al., 2003a)
$\alpha_{IIb}\beta_3$ / fibrinogen	Laser tweezers	20,000	60–150 (S)	(Litvinov et al., 2002)

* S, single molecule measurement; A, average value calculated per molecule from a measurement on multimolecular adhesions.

Table 3. Molecular Motor Forces

Motor	Strength (pN)	Key References
Myosin II	1.3–3.7	(Finer et al., 1994; Guilford et al., 1997; Ishijima et al., 1994; Molloy et al., 1995; Tyska et al., 1999)
Myosin V	3–5	(Clemen et al., 2005; Mehta et al., 1999; Uemura et al., 2004)
Myosin VI	2.8	(Rock et al., 2001)
Kinesin	1.9–6	(Kuo and Sheetz, 1993; Meyhofer and Howard, 1995; Svoboda and Block, 1994)

Cellular Forces

Cells can exert protrusion and retraction forces. Protrusive forces by both filopodia and lamellipodia have been observed (Prass et al., 2006; Sheetz et al., 1992). Recently, it has been reported that the protrusion force of lamellipodial is between 20 and 80 pN/μm^2 (Shahapure et al., 2010). However, protrusion forces are unlikely to mediate rigidity sensing, since integrins at the leading edge are not normally attached to ECM ligands (Galbraith et al., 2007; Schmidt et al., 1993). Conversely, retraction forces appear to be the relevant forces, since ligand binding causes integrin association to the retrogradely flowing actin (Duband et al., 1988; Felsenfeld et al., 1996; Schmidt et al., 1993). Because microtubules are not involved in retraction forces, the relevant cellular motors are myosin and not kinesin nor dynein (Cai et al., 2010). This agrees with findings that nonmuscle myosin II is required for the rigidity sensing of stem cells (Engler et al., 2006).

Contractile forces depend upon bipolar myosin filaments and a single myosin head generates between 1.3 and 5 pN of force (Table 3). During the retraction of cellular structures, the individual forces of myosins are additive and can reach up to 2,000,000 pN for a whole cell (Table 4). However, these larger forces are distributed over numerous integrin-matrix adhesion sites, begging the question of the amount of force felt on individual proteins. Patterned substrates of RGD have demonstrated that a spacing of ~60 nm is sufficient for strong binding (Arnold et al., 2004; Cavalcanti-Adam et al., 2007; Koo et al., 2002), with smaller spacing having little additional effect. Thus, the 60 nm spacing (corresponding to a density of approximately 300 integrin receptors per μm^2) can be regarded as characterizing the density of bound integrins in cells. The retraction forces on a known integrin substrate can reach up to 10,000 pN/μm^2 (Table 4). If we divide this number by the integrin density, this gives an estimate of approximately 30 pN per integrin bond. Complexes of three to five integrins are required in order to couple to the cytoskeleton (Coussen et al., 2002) and the adhesive force per matrix ligand can be 7-fold higher with pentameric complexes (Roca-Cusachs et al., 2009). Thus, an early adhesion complex may sustain on the order of 100–165 pN per adhesion unit. Others have used the maximum density of integrins and thus obtained a much lower force of 1 pN per integrin bond (Balaban et al., 2001). Our estimate is based on the idea that

Table 4. Pulling Strengths of Cells

Cell Type	Method	Substrate	Region	Force (pN)	Force per Contact Area (pN/μm^2)	Maximum Loading Rate per Contact Area (pN/s·μm^2)	Reference
Macrophage	Optical trap	IgG	Filopodium	1–16	1–10[b]	-	(Kress et al., 2007)
Spinal neuron	Pillars and optical trap	Polylysine or netrin-1	Filopodium	2–60	1–30[a,b]	-	(Moore et al., 2009)
DRG neuron	Micropipette	Polylysine, collagen IV & laminin	Growth cone	500	-	-	(Lamoureux et al., 1989)
SCG neuron	Compliant substrate	Laminin	Filopidium	970	-	-	(Bridgman et al., 2001)
Epithelial	Pillars	Fibronectin	Edge	5,000	1,500	~2 [a]	(du Roure et al., 2005)
Keratocyte	Compliant substrate	Silicone	Whole cell	16,000	-	-	(Lee et al., 1994)
Keratocyte	Compliant substrate	Silicone	Whole cell	45,000	27–59	-	(Oliver et al., 1995)
Smooth muscle	Pillars	Fibronectin	Edge Center	60,000 15,000	8,500[a] 2,100[a]	24[a]	(Tan et al., 2003)
Fibroblast	Microcantilever	Laminin	Leading edge Center Trailing edge	7,000 30,000 100,000	870 1,500 3,910	-	(Galbraith and Sheetz, 1997)
Keratocytes	Compliant substrate	Silicone	Edge (flank)	680,000	130,000	1,250	(Burton et al., 1999)
Fibroblast	Compliant substrate	Collagen	Whole cell	2,000,000	10,000	-	(Dembo and Wang, 1999)
Smooth muscle	Micropipettes	Anionic bead	Whole cell	2,100,000	26,750[b]	-	(Warshaw and Fay, 1983)

[a] Assuming contact is the top surface area of pillar.
[b] Assuming contact is 1/2 of bead area.

only a fraction of integrins are engaged with the ECM, and it is consistent with the in vitro single molecule measurements of integrin-ECM strength (Table 2).

Retraction Speeds and Loading Rates

Cell retraction determines the rate at which force is applied to bonds and that affects how much force is required to break a bond either between or within proteins. Thus, the in vivo retraction rates are important. The reported velocities of nonmuscle myosin IIa and IIb are 0.29 and 0.092 μm/s, respectively (Pato et al., 1996; Wang et al., 2000a). In living cells, actin is transported rearward at rates between 0.05 and 0.21 μm/s (Fisher et al., 1988; Forscher and Smith, 1988; Theriot and Mitchison, 1991). These are very consistent with the reported velocities of integrin rearward movement, 0.08–0.23 μm/s (Choquet et al., 1997). In a variety of cases, retraction rates can be as high as 0.6 μm/s when there are no or weak adhesions but drop to approximately 0.05 μm/s when adhesions occur (Giannone et al., 2004; Kress et al., 2007; Zhang et al., 2008). In cells with adhesions that sense rigidity, the typical values for the loading rate then are in the range of 0.05 μm/s or less.

Although loading rates of force over time have not been addressed directly, rough estimates based on published plots of the traction force over time indicate loading rates between 2 and 1,250 pN/(s·μm^2) (Table 4; Burton et al., 1999; du Roure et al., 2005; Tan et al., 2003). Given that there could be up to 300 integrins per square micron (see above), this translates to a loading rate of 0.007 to 4 pN/s on individual integrins.

Mechanosensors

How can the generation of force on the matrix be sensed? There are 5 basic mechanisms that have been suggested for mechanosensing through integrins: (1) catch bond formation, (2) channel opening, (3) enzyme regulation, (4) exposure of phosphorylation sites, or (5) exposure of binding sites. All could play significant roles in adhesion-related processes.

Catch Bonds

As mentioned above, the lifetime of a bond generally shortens (slips) with applied force; however, a subset of interactions "catch" (strengthen) under certain regimes of applied force. Two catch bonds have been described in the ECM connection to the cytoskeleton through integrins: one involving the binding of integrins with the ECM, the other between actin and myosin. Not much is known about the catch bond between myosin II and actin filaments, but it has been reported to have a maximum lifetime at 6 pN (Guo and Guilford, 2006). The catch bond between integrins and the ECM is far more studied. The idea of integrin involvement in the mechanosensing process was proposed originally by Ingber (1991), and several reports have shown that extracellular rigidity causes strengthening of the integrin linkage (Balaban et al., 2001; Choquet et al., 1997; Riveline et al., 2001). Computer simulations have outlined the atomic-level mechanism of how mechanical force could increase the affinity of integrins for their ligand (Jin et al., 2004; Puklin-Faucher et al., 2006). Lateral forces from the cytoskeleton onto integrins may induce structural changes that activate the catch bond (Zhu et al., 2008). However, early work failed to observe a catch bond between α5β1 integrin and fibronectin (Li et al., 2003a). Recently, using a more sensitive atomic force microscopy (AFM) setup that maintained the force constant at values as low as 4 pN and a retraction rate of 0.2 μm/s (close to physiological rates of ~0.05 μm/s), a catch bond between integrin and fibronectin was observed at forces of 10–30 pN (Kong et al., 2009). Interestingly, this catch bond did not require straightening of the integrin tail region, as previously suspected (Chigaev et al., 2003). The α5β1 integrins bind to fibronectin through two distinct sites: an RGD sequence and a "synergy" site. This catch bond functions, at least in part, through recruitment of the synergy binding site (Friedland et al., 2009). Being the primary bond in the rigidity sensing process, the catch bond between the ECM and integrins has an elegant efficiency. However, as discussed below, additional components may be recruited to sense rigidity.

Channel Opening

In hearing, touch, and other mechanical senses, ion channels convert mechanical force into electrical and chemical signals (Sukharev and Corey, 2004). The classical case of force-dependent channel opening is in the hair cells of the auditory system. In such systems, force perpendicular to the membrane opens the channel. Similarly, cytoskeletal forces could pull on a channel associated with the early adhesion complex through lateral associations with the integrins. In endothelial cells, mechanical stress on integrin-bound beads causes calcium entry into cells within 2–5 s (Matthews et al., 2006). Stretch activated ion-channels have been reported to be important for traction forces at the leading edge, as well as vinculin and tyrosine phosphorylation accumulation at adhesion sites (Munevar et al., 2004). However, the stretch-activated channels were not involved in the initial strengthening of the integrin-mediated link to the ECM, but rather in the later stages of cell repositioning (>60 s, Matthews et al., 2006). This is consistent with mechanosensitive ion channels requiring filamin recruitment (Glogauer et al., 1998).

Enzyme Regulation

A number of enzymes are known to change their kinetics in response to mechanical stimulation; these include kinases, phosphatases, adenylate cyclases, and GTPases (Table 5). A key question that has yet to be conclusively answered is whether mechanical force exerted on these proteins alters their function or whether altered kinetics reflects mechanical activation of upstream or downstream effectors. A case for direct activation by mechanical force has been made for the kinase domain in titin (Grater et al., 2005). However, its relevance to integrin-mediated rigidity is unclear. The strongest case for an enzyme regulated by force related to integrin rigidity sensing is focal adhesion kinase (FAK). FAK's amino FERM domain has been proposed to inhibit its kinase activity (Figure 3A; Cooper et al., 2003). FAK does not bind to integrins directly, but its tyrosine kinase activity increases with mechanical force (Figure 3A and Table 5; Tomar and Schlaepfer, 2009). This is particularly relevant because loss of FAK inhibits the ability of the cell to sense collagen rigidity (Li et al., 2002a; Wang et al., 2001b).

A local but indirect mechanical activation has been proposed for the receptor-like protein tyrosine phosphatase-α (RPTPα). This transmembrane phosphatase forms an autoinhibited dimer in which each of their helix-turn-helix wedges insert into the other's catalytic cleft (Jiang et al., 2000; Tabernero et al., 2008) that associates with αvβ3 integrins and becomes activated by restrained fibronectin (von Wichert et al., 2003). Moreover, it is known to activate Src family kinases (SFKs), which are themselves activated within approximately 300 ms after force

Table 5. Mechanical Activation of Enzymes through Integrins

Enzyme	Type	Function	Regulation by Mechanical Stimuli	Key References
Adenylate cyclase	Adenylate cyclase	Generates cAMP	Activated by twisting RGD coated beads	(Meyer et al., 2000)
FAK	Tyrosine kinase	Focal adhesion turnover and integrin activation	Activated by externally applied force and cell contraction	(Domingos et al., 2002; Michael et al., 2009; Wang et al., 2001a)
Fyn	Tyrosine kinase	Regulates signal transduction	Activated within ~300 ms of force on fibronectin beads	(Kostic and Sheetz, 2006; Na et al., 2008; Wang et al., 2005)
MAPKs	Serine/threonine kinases	Gene expression	Activated by force on α1 or β3 integrin	(Schmidt et al., 1998)
Rap1	GTPase	Mitogenic. Activates MAP kinases	Activated when cell is stretched. cAMP is well established activator of Rap1.	(Sawada et al., 2001)
Rho	Rho GTPase	Increases actomyosin contraction	Activated by fluid shear	(Shiu et al., 2004)
Rac	Rho GTPase	Promotes lamellipodial and adhesion formation	Inactivated by myosin contraction or cell stretching	(Katsumi et al., 2002)
RPTPα	Tyrosine phosphatase	Activates Src family kinases	Activated by restrained fibronectin beads	(von Wichert et al., 2003)
Src	Tyrosine kinase	Regulates signal transduction.	Activated by vitronectin	(Felsenfeld et al., 1999)

application on fibronectin beads (den Hertog et al., 1993; Na et al., 2008; Zheng et al., 2000), and to cause localization of the Src-family kinase fyn near the leading edge (Kostic and Sheetz, 2006).

Exposing Phosphorylation Sites

There is considerable evidence that cell stretching and matrix rigidity increases tyrosine phosphorylation of important proteins (Glogauer et al., 1997; Li et al., 2002b; Pelham and Wang, 1997; Schmidt et al., 1998; Wang et al., 2005). An interesting observation can be made when comparing mechanical responses of intact cells versus when their plasma membranes have been stripped away—intact cells (but not stripped cells) will respond to mechanical stimulus by activating Src family kinases (Na et al., 2008; Sawada et al., 2006; Wang et al., 2005). Importantly, mechanical stimulus in both cases leads to increased tyrosine phosphorylation. This not only reinforces the idea that the transmembrane phosphatase RPTPα is required for Src activation, but also suggests that there is increased availability of phosphorylation sites upon stretching. To date, only the Cas protein family has been proposed to undergo conformational changes that expose phosphorylation sites (Sawada et al., 2006). This protein family contains a substrate domain with multiple Src family kinase tyrosine phosphorylation sites flanked by domains capable of binding a variety of proteins (Figure 3B; Geiger, 2006). When incubated with active Src kinase, increased tyrosine phosphorylation of the substrate domain of p130Cas is observed upon stretch (Sawada et al., 2006). Upon phosphorylation, the substrate domain of Cas becomes a docking site for a variety of proteins including Crk, Nck, and Ship2 (Mayer et al., 1995; Prasad et al., 2001; Schlaepfer et al., 1997) that then activate GTP exchange factors to locally form active small G-proteins such as Rap1 (Tamada et al., 2004). More recent studies are supportive of a role for p130Cas in fibronectin, but not collagen, rigidity sensing (Kostic and Sheetz, 2006).

Exposing Protein Interaction Sites

The other major mechanism for mechanotransduction is the exposure of protein-protein binding sites by stretch. In cells that have been stripped of their plasma membrane, stretching leads to the increased cytoskeleton binding of paxillin, focal adhesion kinase (FAK), p130Cas, PKB/Akt, C3G and CrkII (Sawada and Sheetz, 2002; Tamada et al., 2004). Interestingly, myosin II contractility is required for the recruitment of FAK, zyxin, vinculin, and α-actinin, but not for the recruitment of paxillin, talin, and α1 integrin (Pasapera et al., 2010). Unfortunately, most of the direct protein-protein interactions are unclear. The only case where stretch-dependent binding of one protein to another has been documented is during stretching of talin, which leads to binding of vinculin (del Rio et al., 2009). Talin has 11 potential vinculin binding sites, as well as binding sites for actin filaments, integrins, and a number of other proteins (Figure 3C). Stretching of talin by mechanical forces therefore appears to play an important role in the reinforcement of early adhesions through the recruitment of additional actin binding proteins. Fibronectin is a major ECM protein that has been shown to expose protein interaction sites under mechanical strain (Vogel, 2006). Cellular contraction can expose cryptic sites in fibronectin that are important for its assembly into a matrix (Zhong et al., 1998). Thus, both intracellular and extracellular protein stretching may have important consequences.

Is Protein Stretching Physiological?

A fundamental question in examining protein stretching as a mechanism of mechanotransduction is whether adhesion proteins can be stretched with physiological force. As outlined in the above sections, an estimate of the pulling force on individual integrins is on the order of 30 pN leading to between 100 and 165 pN on a minimal integrin adhesion complex of 3–5 integrins. Although stretching of titin, spectrin, and ankyrin have been studied extensively both in vitro and in silico

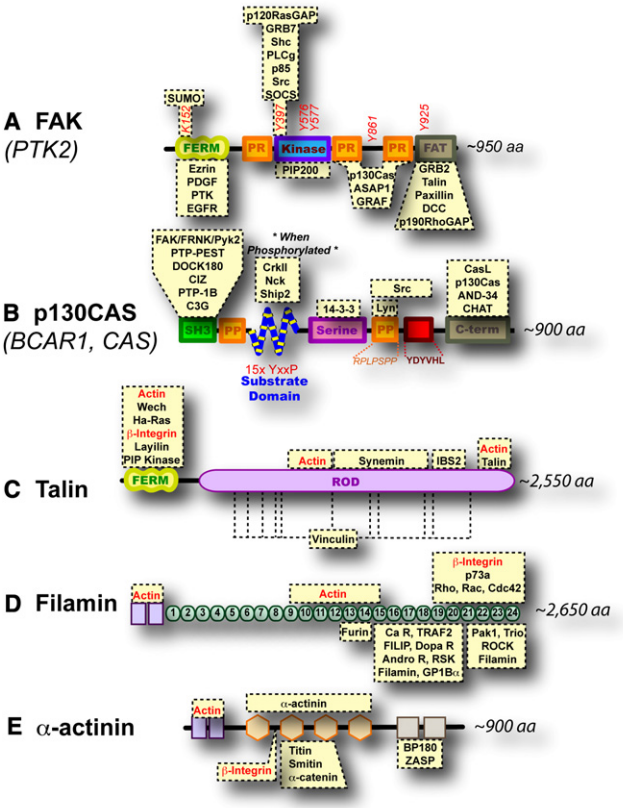

Figure 3. Mechanosensory Proteins in Integrin-Mediated Rigidity Sensing

Proteins that bind directly to the depicted domains are highlighted in yellow boxes.

(A) FAK does not bind integrins or actin directly, but its kinase activity is regulated by mechanical force, and it has been hypothesized that removal of the FERM domain from the kinase could play a role (Cooper et al., 2003).

(B) The substrate domain of p130Cas contains 15 tyrosine residues that become exposed upon stretching (Sawada et al., 2006).

(C) Stretching of talin's rod domain exposes vinculin binding sites (del Rio et al., 2009).

(D) Extension of filamin immunoglobulin repeats (labeled 1–24) has been shown by AFM (Furuike et al., 2001) and could regulate the binding of proteins.

(E) α-actinin forms antiparallel dimers; mechanical force could regulate this dimerization or its association with other proteins.

(Sotomayor and Schulten, 2007), their relevance to integrin-mediated rigidity sensing is unclear. On the other hand, stretching of talin to expose vinculin binding sites was initially estimated based on computer simulations to require 13 pN (Lee et al., 2007). When AFM was used to measure this value directly, it took only 0.2 s for talin to unfold under a constant force of 20 pN (del Rio et al., 2009). Another protein family that bridges integrins to actin is filamin (Zhou et al., 2010). Filamins have an actin-binding domain followed by 24 immunoglobulin repeats and bind integrins through their C-terminal domain (Figure 3D). Using AFM, the immunoglobulin repeats of filamin have been extended with 50 pN of force at a pulling speed of 0.37 μm/s (Furuike et al., 2001). Focal adhesion kinase (FAK) is another integrin-associated protein whose elasticity has been addressed. The FAT domain is responsible for targeting FAK to focal adhesions (Hildebrand et al., 1993). Computer simulations have predicted that the FAT domain of FAK will extend with under 75 pN of force (Mofrad et al., 2004).

From these data, it seems that the stretching of talin's vinculin binding domain, filamin's immunoglobulin domains, and potentially FAK's FAT domain do occur at physiologically relevant forces. Moreover, direct support has been obtained by looking at the number of exposed cysteines in proteins (Johnson et al., 2007). This technique has shown that in stretched live cells, normally buried cysteines in vimentin and myosin IIa are exposed.

Mechanisms of Rigidity Sensing

Substrate rigidity influences a number of cellular processes including cell adhesion, actin flow, retraction forces, gene expression, and cell lineage (Bard and Hay, 1975; Choquet et al., 1997; Engler et al., 2006; Friedl and Brocker, 2000; Giannone et al., 2004; Lo et al., 2000; Opas and Dziak, 1990; Pelham and Wang, 1997; Peyton and Putnam, 2005; Saez et al., 2005; Yeung et al., 2005). However, in its most basic form, integrin-mediated rigidity sensing can be taken as the decision to couple and reinforce the link between an extracellular ligand and the cytoskeleton. For instance, fibroblasts presented with fibronectin-coated beads under restraining forces of 0.02 or 0.18 pN/nm will preferentially couple stiff beads to their cytoskeleton (Choquet et al., 1997; Jiang et al., 2006). Whether integrin-cytoskeleton linkages become reinforced depends upon the mechanical properties of the microenvironment and the intracellular components that make up this link. Thus, rigidity responses depend upon both the nature of the matrix and the cell-type specific components involved in the responses.

An early event after ligand binding to integrins involves the activation of Src family kinases (potentially through mechanical effects on integrins that activate RPTPα, as mentioned above). This is supported by their rapid activation (within 300 ms) after applied force and the observation that the Src family kinases Fyn and Src are required for rigidity sensing on fibronectin and vitronectin, respectively (Felsenfeld et al., 1999; Kostic and Sheetz, 2006; Na et al., 2008). Although the mechanism is unclear, the activation of Src family kinases leads to the bridging of integrins to the cytoskeleton through talin. This is supported by the observations that ligand binding couples integrins to the cytoskeleton and that talin is not required for SFK activation (Duband et al., 1988; Felsenfeld et al., 1996; Schmidt et al., 1993; Zhang et al., 2008). Once coupled to retrograde flowing actin, mechanical force on integrins could engage the integrin/ECM catch bond. Force on talin could then expose vinculin binding sites that stabilize and recruit additional links to actin (del Rio et al., 2009). Consistent with this possibility, myosin II contractility is required for vinculin but not for talin recruitment (Pasapera et al., 2010). Finally, activation of FAK could reverse adhesions and restart the process. This is supported by observed roles of FAK in adhesion turnover and the requirement of talin in the activation of FAK (Ilic et al., 1995; Zhang et al., 2008). Thus, rigidity sensing is an active process that is transient and multiple steps could be sensitive to the matrix rigidity (Figure 4A).

In such a model, substrate rigidity determines the loading rate felt on the integrin-ECM catch bond. Just as catch bonds have a force providing maximum lifetime in a scenario of constant force application, they will have a corresponding optimal loading

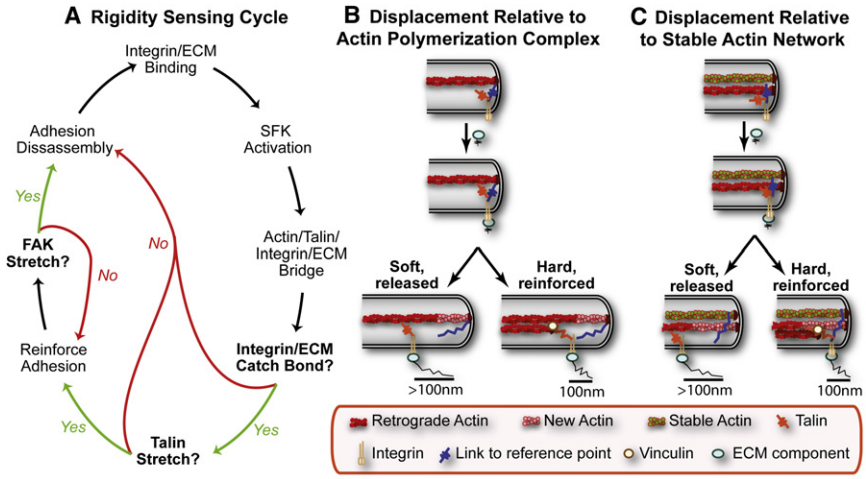

Figure 4. The Rigidity Sensing Cycle and Models for Uniform Displacements

(A) A possible rigidity sensing cycle involves three mechanosensory events: (1) integrin/ECM catch bond formation, (2) stretching of talin that reinforces the adhesion by recruiting vinculin, and (3) stretching of FAK that activates its kinase domain leading to the disassembly and recycling of the adhesion.

(B and C) Two models to explain the uniform displacements of approximately 100 nm, one in which the reference structure is the polymerization complex (B), and the other in which a stable actin network provides the reference structure (C). In both models, the key decision is based on whether the extension of the link to retrograde flowing actin (e.g., talin) occurs before the link to the reference structure is broken.

rate in scenarios where force is loaded progressively. Thus, at the optimal rigidity, the loading rate will maximize bond lifetime, providing the opportunity for the applied force to trigger subsequent mechanotransduction events. Regardless of the particular model for mechanotransduction, coupling between rearward flowing actin and the substrate is key for rigidity sensing. Detailing the temporal series of mechanosensory events in rigidity sensing will require clever experimental setups that merge high-resolution imaging and sensitive force-sensing techniques.

Rigidity Sensing Based on 100 nm Displacement Events?

When integrins bind to matrix, there is evidence that rigidity sensing involves uniform displacements of the substrate. If epithelial cells are grown on pillars with spring constants ranging from 1 to 100 pN/nm, they displace the pillars by ~130 nm regardless of their stiffness (Saez et al., 2005). Similarly, when fibroblasts are presented with beads in a laser tweezers force ramp of 0.02 or 0.18 pN/nm, the beads in the softer tweezers are more likely to be released from the cytoskeleton (Jiang et al., 2006). If, however, the softer beads were moved in the tweezers to produce 10–20 pN of force within 100 nm of the initial position, reinforcement occurred as with the stiffer beads. Therefore, cells appear to sense rigidity based on whether a threshold force is obtained within a given displacement of approximately 100–150 nm.

This raises the question of what limits the distance of the sensing event. If we consider a fibronectin-$\alpha_5\beta_1$ catch bond acting at the force that provides maximum lifetime (20 pN), the substrate rigidity needed to cause a displacement of 100 nm is on the order of 10 nN/μm^2 and therefore in the rigidity range of several different tissues (Box 1 and Table 1). However, as the substrate rigidity is changed, so will the displacement events, which are not what has been observed experimentally (Saez et al., 2005). Therefore, a mechanism involving more than a catch bond is presumably necessary.

One possibility is that this distance represents the stretching of a mechanosensor linking the integrin complex to a reference structure within the cell. Proteins are typically folded into domains of 2.5 nm containing 200 amino acids (Bao, 2009). If we assume that the maximum extension per amino acid is 0.4 nm (Ainavarapu et al., 2007), a domain of only 200 amino acids could be stretched to 80 nm—in others words, by 32 times its original length and close to the observed displacement of 100 nm. A portion of 407 amino acids from talin's rod domain (containing 5 of its 11 potential vinculin binding sites) increases its length by 140 nm upon the application of force (del Rio et al., 2009). Thus extension of intracellular proteins is within the range of the observed displacements of approximately 100 nm.

A final point that emerges if protein stretching underlies the uniform displacement events is that it requires attachment to a reference structure. Potential reference structures within the cell include the actin polymerization complex or a rigid actin network (Figures 4B and 4C). In regard to the latter possibility, two overlapping actin networks are seen at the periphery of cells (Giannone et al., 2007; Ponti et al., 2004). For simplicity, these rigidity-sensing models are based on only single integrin

Box 1. Estimating Optimal Rigidity for an Integrin-ECM Catch Bond Engagement with an Optimal Loading of 20 pN

To evaluate the order of magnitude of the rigidity that would likely result in a displacement of ~100 nm, we did the following calculation. We considered a minimal adhesion complex with three integrins spaced 60 nm from one another, each exerting a force of 20 pN. We thus assumed a total force (***F***) of 60 pN, which for simplicity we modeled as a single point force exerted in the middle of three integrins located at a distance (***x***) of 30 nm from the source. The displacement ***d*** in a material of shear modulus ***G*** caused by such a point force as a function of the distance to the source ***x*** (along the direction of force application) is $\boldsymbol{d} = \boldsymbol{F}/(2\pi \boldsymbol{G x})$ (Landau et al., 1986). If we consider a displacement (***d***) of 100 nm, we can calculate the corresponding shear modulus as $\boldsymbol{G} = \boldsymbol{F}/(2\pi \boldsymbol{d x})$. By assuming an incompressible material with a Poisson ratio of 0.5 where the elastic modulus is 3× the shear modulus, then $\boldsymbol{E} = 3\boldsymbol{G} = 3\boldsymbol{F}/(2\pi \boldsymbol{d x}) \approx 10$ nN/μm^2. Given the assumptions and approximations, this value should be taken only as an estimate of the order of magnitude involved, but it does provide a useful guide to the range of rigidities for which such a configuration would be tuned.

receptors. As discussed above, complexes of three to five integrins act synergistically (Coussen et al., 2002). Integrin multimerization may initiate downstream signaling and be an integral part of the rigidity sensing machinery (Li et al., 2003b; Paszek et al., 2005). Thus, more complicated models involving integrin multimerizaiton and potentially mechanosensory proteins that bridge integrins may be necessary.

Conclusion

In summary, the mechanism of integrin-mediated rigidity sensing is constrained by physical characteristics of the cytoskeleton-integrin-matrix link. Relevant factors include the force and loading rate that cells employ to probe the substrate, the sensitivity of their mechanotransduction system, and the strength of their attachment to the ECM. Current measurements indicate that the liganded integrins move rearward at about 50 nm/s and can support on the order of 30 pN per bond. Although integrin-matrix catch bonds can explain reduced adhesion to soft surfaces and cell rounding, they cannot explain the observed uniform displacements over a wide range of rigidity. If displacements of approximately 100 nm are indeed important elements of the rigidity-sensing process of cells, a key challenge will be to identify the structure that provides the reference point.

We focused here on rigidity sensing through integrins. However, tissue rigidity can influence cellular behavior through cadherin-mediated adhesions (Tsai and Kam, 2009). Little is known of the rigidity sensing mediated through cell-cell or other ECM receptors; however, recently it has been shown that mechanical forces regulate adherens' cell-cell junction size (Liu et al., 2010). Because other transmembrane receptors are bridged to the cytoskeleton through many of the same intracellular proteins, it is likely that similar rigidity-sensing mechanisms exist.

Finally, an important gap in our knowledge is how the short-term rigidity sensing described here is translated into the long-term rigidity sensing involved in cell differentiation. Although much may be shared between the two processes, they may have radically different time constants and frequencies. In fibroblasts, there are periodic pulls on the matrix that produce early adhesions every 24 s (Giannone et al., 2004). However, it is not evident that stem cells test the rigidity of the environment in the same way and with the same frequency over the full period of differentiation that can take 7 days or more. Thus, it remains a key challenge to link the early rigidity mechanisms described here to the long-term processes involved in cellular differentiation.

REFERENCES

Ainavarapu, S.R., Brujic, J., Huang, H.H., Wiita, A.P., Lu, H., Li, L., Walther, K.A., Carrion-Vazquez, M., Li, H., and Fernandez, J.M. (2007). Contour length and refolding rate of a small protein controlled by engineered disulfide bonds. Biophys. J. *92*, 225–233.

Ando, J., and Yamamoto, K. (2009). Vascular mechanobiology: endothelial cell responses to fluid shear stress. Circ. J. *73*, 1983–1992.

Arnold, M., Cavalcanti-Adam, E.A., Glass, R., Blummel, J., Eck, W., Kantlehner, M., Kessler, H., and Spatz, J.P. (2004). Activation of integrin function by nanopatterned adhesive interfaces. ChemPhysChem *5*, 383–388.

Balaban, N.Q., Schwarz, U.S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L., and Geiger, B. (2001). Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat. Cell Biol. *3*, 466–472.

Balgude, A.P., Yu, X., Szymanski, A., and Bellamkonda, R.V. (2001). Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures. Biomaterials *22*, 1077–1084.

Bank, A.J., and Kaiser, D.R. (1998). Smooth muscle relaxation: effects on arterial compliance, distensibility, elastic modulus, and pulse wave velocity. Hypertension *32*, 356–359.

Bao, G. (2009). Protein Mechanics: A New Frontier in Biomechanics. Exp. Mech. *49*, 153–164.

Barczyk, M., Carracedo, S., and Gullberg, D. (2010). Integrins. Cell Tissue Res. *339*, 269–280.

Bard, J.B., and Hay, E.D. (1975). The behavior of fibroblasts from the developing avian cornea. Morphology and movement in situ and in vitro. J. Cell Biol. *67*, 400–418.

Bell, G.I. (1978). Models for the specific adhesion of cells to cells. Science *200*, 618–627.

Bridgman, P.C., Dave, S., Asnes, C.F., Tullio, A.N., and Adelstein, R.S. (2001). Myosin IIB is required for growth cone motility. J. Neurosci. *21*, 6159–6169.

Burdun, G.D., Kolchin, O.M., Kogan, A.I., and Gorbunkov, A.V. (1972). Application of the International (SI) System of Units to mechanics, strength of materials, and mechanical engineering. Meas. Tech. *15*, 860–864.

Burridge, K., and Mangeat, P. (1984). An interaction between vinculin and talin. Nature *308*, 744–746.

Burton, K., Park, J.H., and Taylor, D.L. (1999). Keratocytes generate traction forces in two phases. Mol. Biol. Cell *10*, 3745–3769.

Cai, Y., Rossier, O., Gauthier, N.C., Biais, N., Fardin, M.A., Zhang, X., Miller, L.W., Ladoux, B., Cornish, V.W., and Sheetz, M.P. (2010). Cytoskeletal coherence requires myosin-IIA contractility. J Cell Sci.

Calderwood, D.A., Huttenlocher, A., Kiosses, W.B., Rose, D.M., Woodside, D.G., Schwartz, M.A., and Ginsberg, M.H. (2001). Increased filamin binding to beta-integrin cytoplasmic domains inhibits cell migration. Nat. Cell Biol. *3*, 1060–1068.

Cavalcanti-Adam, E.A., Volberg, T., Micoulet, A., Kessler, H., Geiger, B., and Spatz, J.P. (2007). Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. Biophys. J. *92*, 2964–2974.

Chao, E.Y., Aro, H.T., Lewallen, D.G., and Kelly, P.J. (1989). The effect of rigidity on fracture healing in external fixation. Clin. Orthop. Relat. Res., 24–35.

Chapuis, J.F., and Agache, P. (1992). A new technique to study the mechanical properties of collagen lattices. J. Biomech. *25*, 115–120.

Chigaev, A., Buranda, T., Dwyer, D.C., Prossnitz, E.R., and Sklar, L.A. (2003). FRET detection of cellular alpha4-integrin conformational activation. Biophys. J. *85*, 3951–3962.

Choquet, D., Felsenfeld, D.P., and Sheetz, M.P. (1997). Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell *88*, 39–48.

Clemen, A.E., Vilfan, M., Jaud, J., Zhang, J., Barmann, M., and Rief, M. (2005). Force-dependent stepping kinetics of myosin-V. Biophys. J. *88*, 4402–4410.

Collin, D., Ritort, F., Jarzynski, C., Smith, S.B., Tinoco, I., Jr., and Bustamante, C. (2005). Verification of the Crooks fluctuation theorem and recovery of RNA folding free energies. Nature *437*, 231–234.

Collinsworth, A.M., Zhang, S., Kraus, W.E., and Truskey, G.A. (2002). Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. Am. J. Physiol. Cell Physiol. *283*, C1219–C1227.

Cooper, L.A., Shen, T.L., and Guan, J.L. (2003). Regulation of focal adhesion kinase by its amino-terminal domain through an autoinhibitory interaction. Mol. Cell. Biol. *23*, 8030–8041.

Coussen, F., Choquet, D., Sheetz, M.P., and Erickson, H.P. (2002). Trimers of the fibronectin cell adhesion domain localize to actin filament bundles and undergo rearward translocation. J. Cell Sci. *115*, 2581–2590.

del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J.M., and Sheetz, M.P. (2009). Stretching single talin rod molecules activates vinculin binding. Science *323*, 638–641.

Delon, I., and Brown, N.H. (2007). Integrins and the actin cytoskeleton. Curr. Opin. Cell Biol. *19*, 43–50.

Dembo, M., and Wang, Y.L. (1999). Stresses at the cell-to-substrate interface during locomotion of fibroblasts. Biophys. J. *76*, 2307–2316.

den Hertog, J., Pals, C.E., Peppelenbosch, M.P., Tertoolen, L.G., de Laat, S.W., and Kruijer, W. (1993). Receptor protein tyrosine phosphatase alpha activates pp60c-src and is involved in neuronal differentiation. EMBO J. *12*, 3789–3798.

Domingos, P.P., Fonseca, P.M., Nadruz, W., Jr., and Franchini, K.G. (2002). Load-induced focal adhesion kinase activation in the myocardium: role of stretch and contractile activity. Am. J. Physiol. Heart Circ. Physiol. *282*, H556–H564.

du Roure, O., Saez, A., Buguin, A., Austin, R.H., Chavrier, P., Silberzan, P., and Ladoux, B. (2005). Force mapping in epithelial cell migration. Proc. Natl. Acad. Sci. USA *102*, 2390–2395.

Duband, J.L., Nuckolls, G.H., Ishihara, A., Hasegawa, T., Yamada, K.M., Thiery, J.P., and Jacobson, K. (1988). Fibronectin receptor exhibits high lateral mobility in embryonic locomoting cells but is immobile in focal contacts and fibrillar streaks in stationary cells. J. Cell Biol. *107*, 1385–1396.

Elkin, B.S., Azeloglu, E.U., Costa, K.D., and Morrison, B., 3rd. (2007). Mechanical heterogeneity of the rat hippocampus measured by atomic force microscope indentation. J. Neurotrauma *24*, 812–822.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell *126*, 677–689.

Evans, E. (2001). Probing the relation between force–lifetime–and chemistry in single molecular bonds. Annu. Rev. Biophys. Biomol. Struct. *30*, 105–128.

Fabry, B., Maksym, G.N., Butler, J.P., Glogauer, M., Navajas, D., and Fredberg, J.J. (2001). Scaling the microrheology of living cells. Phys. Rev. Lett. *87*, 148102.

Felber, E.S. (2006). Botulinum toxin in primary care medicine. J. Am. Osteopath. Assoc. *106*, 609–614.

Felsenfeld, D.P., Choquet, D., and Sheetz, M.P. (1996). Ligand binding regulates the directed movement of beta1 integrins on fibroblasts. Nature *383*, 438–440.

Felsenfeld, D.P., Schwartzberg, P.L., Venegas, A., Tse, R., and Sheetz, M.P. (1999). Selective regulation of integrin–cytoskeleton interactions by the tyrosine kinase Src. Nat. Cell Biol. *1*, 200–206.

Finer, J.T., Simmons, R.M., and Spudich, J.A. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. Nature *368*, 113–119.

Fisher, G.W., Conrad, P.A., DeBiasio, R.L., and Taylor, D.L. (1988). Centripetal transport of cytoplasm, actin, and the cell surface in lamellipodia of fibroblasts. Cell Motil. Cytoskeleton *11*, 235–247.

Flanagan, L.A., Ju, Y.E., Marg, B., Osterfield, M., and Janmey, P.A. (2002). Neurite branching on deformable substrates. Neuroreport *13*, 2411–2415.

Forscher, P., and Smith, S.J. (1988). Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J. Cell Biol. *107*, 1505–1516.

Friedl, P., and Brocker, E.B. (2000). T cell migration in three-dimensional extracellular matrix: guidance by polarity and sensations. Dev. Immunol. *7*, 249–266.

Friedland, J.C., Lee, M.H., and Boettiger, D. (2009). Mechanically activated integrin switch controls alpha5beta1 function. Science *323*, 642–644.

Furuike, S., Ito, T., and Yamazaki, M. (2001). Mechanical unfolding of single filamin A (ABP-280) molecules detected by atomic force microscopy. FEBS Lett. *498*, 72–75.

Galbraith, C.G., and Sheetz, M.P. (1997). A micromachined device provides a new bend on fibroblast traction forces. Proc. Natl. Acad. Sci. USA *94*, 9114–9118.

Galbraith, C.G., Yamada, K.M., and Galbraith, J.A. (2007). Polymerizing actin fibers position integrins primed to probe for adhesion sites. Science *315*, 992–995.

Gefen, A., Gefen, N., Zhu, Q., Raghupathi, R., and Margulies, S.S. (2003). Age-dependent changes in material properties of the brain and braincase of the rat. J. Neurotrauma *20*, 1163–1177.

Geiger, B. (2006). A role for p130Cas in mechanotransduction. Cell *127*, 879–881.

Giannone, G., Dubin-Thaler, B.J., Dobereiner, H.G., Kieffer, N., Bresnick, A.R., and Sheetz, M.P. (2004). Periodic lamellipodial contractions correlate with rearward actin waves. Cell *116*, 431–443.

Giannone, G., Dubin-Thaler, B.J., Rossier, O., Cai, Y., Chaga, O., Jiang, G., Beaver, W., Dobereiner, H.G., Freund, Y., Borisy, G., and Sheetz, M.P. (2007). Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell *128*, 561–575.

Glogauer, M., Arora, P., Chou, D., Janmey, P.A., Downey, G.P., and McCulloch, C.A. (1998). The role of actin-binding protein 280 in integrin-dependent mechanoprotection. J. Biol. Chem. *273*, 1689–1698.

Glogauer, M., Arora, P., Yao, G., Sokholov, I., Ferrier, J., and McCulloch, C.A. (1997). Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching. J. Cell Sci. *110*, 11–21.

Grater, F., Shen, J., Jiang, H., Gautel, M., and Grubmuller, H. (2005). Mechanically induced titin kinase activation studied by force-probe molecular dynamics simulations. Biophys. J. *88*, 790–804.

Grinnell, F. (1994). Fibroblasts, myofibroblasts, and wound contraction. J. Cell Biol. *124*, 401–404.

Guilford, W.H., Dupuis, D.E., Kennedy, G., Wu, J., Patlak, J.B., and Warshaw, D.M. (1997). Smooth muscle and skeletal muscle myosins produce similar unitary forces and displacements in the laser trap. Biophys. J. *72*, 1006–1021.

Guo, B., and Guilford, W.H. (2006). Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. Proc. Natl. Acad. Sci. USA *103*, 9844–9849.

Hahmann, C., and Schroeter, T. (2010). Rho-kinase inhibitors as therapeutics: from pan inhibition to isoform selectivity. Cell. Mol. Life Sci. *67*, 171–177.

Hildebrand, J.D., Schaller, M.D., and Parsons, J.T. (1993). Identification of sequences required for the efficient localization of the focal adhesion kinase, pp125FAK, to cellular focal adhesions. J. Cell Biol. *123*, 993–1005.

Hinz, B. (2006). Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. Eur. J. Cell Biol. *85*, 175–181.

Hirakawa, K., Hashizume, K., and Hayashi, T. (1981). No To Shinkei *33*, 1057–1065.

Hochmuth, R.M. (2000). Micropipette aspiration of living cells. J. Biomech. *33*, 15–22.

Ilic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995). Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature *377*, 539–544.

Ingber, D. (1991). Integrins as mechanochemical transducers. Curr. Opin. Cell Biol. *3*, 841–848.

Ingber, D.E. (2003). Mechanobiology and diseases of mechanotransduction. Ann. Med. *35*, 564–577.

Intengan, H.D., Thibault, G., Li, J.S., and Schiffrin, E.L. (1999). Resistance artery mechanics, structure, and extracellular components in spontaneously hypertensive rats: effects of angiotensin receptor antagonism and converting enzyme inhibition. Circulation *100*, 2267–2275.

Ishijima, A., Harada, Y., Kojima, H., Funatsu, T., Higuchi, H., and Yanagida, T. (1994). Single-molecule analysis of the actomyosin motor using nano-manipulation. Biochem. Biophys. Res. Commun. *199*, 1057–1063.

Izzard, C.S. (1988). A precursor of the focal contact in cultured fibroblasts. Cell Motil. Cytoskeleton *10*, 137–142.

Jiang, G., den Hertog, J., and Hunter, T. (2000). Receptor-like protein tyrosine phosphatase alpha homodimerizes on the cell surface. Mol. Cell. Biol. *20*, 5917–5929.

Jiang, G., Giannone, G., Critchley, D.R., Fukumoto, E., and Sheetz, M.P. (2003). Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. Nature *424*, 334–337.

Jiang, G., Huang, A.H., Cai, Y., Tanase, M., and Sheetz, M.P. (2006). Rigidity sensing at the leading edge through alphavbeta3 integrins and RPTPalpha. Biophys. J. *90*, 1804–1809.

Jin, M., Andricioaei, I., and Springer, T.A. (2004). Conversion between three conformational states of integrin I domains with a C-terminal pull spring studied with molecular dynamics. Structure *12*, 2137–2147.

Johnson, C.P., Tang, H.Y., Carag, C., Speicher, D.W., and Discher, D.E. (2007). Forced unfolding of proteins within cells. Science *317*, 663–666.

Kallel, F., Ophir, J., Magee, K., and Krouskop, T. (1998). Elastographic imaging of low-contrast elastic modulus distributions in tissue. Ultrasound Med. Biol. *24*, 409–425.

Katsumi, A., Milanini, J., Kiosses, W.B., del Pozo, M.A., Kaunas, R., Chien, S., Hahn, K.M., and Schwartz, M.A. (2002). Effects of cell tension on the small GTPase Rac. J. Cell Biol. *158*, 153–164.

Keller, R., Davidson, L.A., and Shook, D.R. (2003). How we are shaped: the biomechanics of gastrulation. Differentiation *71*, 171–205.

Kishino, A., and Yanagida, T. (1988). Force measurements by micromanipulation of a single actin filament by glass needles. Nature *334*, 74–76.

Kong, F., Garcia, A.J., Mould, A.P., Humphries, M.J., and Zhu, C. (2009). Demonstration of catch bonds between an integrin and its ligand. J. Cell Biol. *185*, 1275–1284.

Kong, H.J., Polte, T.R., Alsberg, E., and Mooney, D.J. (2005). FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness. Proc. Natl. Acad. Sci. USA *102*, 4300–4305.

Koo, L.Y., Irvine, D.J., Mayes, A.M., Lauffenburger, D.A., and Griffith, L.G. (2002). Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. J. Cell Sci. *115*, 1423–1433.

Kostic, A., Lynch, C.D., and Sheetz, M.P. (2009). Differential matrix rigidity response in breast cancer cell lines correlates with the tissue tropism. PLoS ONE *4*, e6361.

Kostic, A., and Sheetz, M.P. (2006). Fibronectin rigidity response through Fyn and p130Cas recruitment to the leading edge. Mol. Biol. Cell *17*, 2684–2695.

Kovar, D.R., and Pollard, T.D. (2004). Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces. Proc. Natl. Acad. Sci. USA *101*, 14725–14730.

Kress, H., Stelzer, E.H., Holzer, D., Buss, F., Griffiths, G., and Rohrbach, A. (2007). Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. Proc. Natl. Acad. Sci. USA *104*, 11633–11638.

Kuo, S.C., and Sheetz, M.P. (1993). Force of single kinesin molecules measured with optical tweezers. Science *260*, 232–234.

Lamoureux, P., Buxbaum, R.E., and Heidemann, S.R. (1989). Direct evidence that growth cones pull. Nature *340*, 159–162.

Landau, L.D., Pitaevskii, L.P., Lifshitz, E.M., and Kosevich, A.M. (1986). Theory of Elasticity, Volume 7 (Oxford: Butterworth-Heinemann).

Lee, J., Leonard, M., Oliver, T., Ishihara, A., and Jacobson, K. (1994). Traction forces generated by locomoting keratocytes. J. Cell Biol. *127*, 1957–1964.

Lee, S.E., Kamm, R.D., and Mofrad, M.R. (2007). Force-induced activation of talin and its possible role in focal adhesion mechanotransduction. J. Biomech. *40*, 2096–2106.

Li, F., Redick, S.D., Erickson, H.P., and Moy, V.T. (2003a). Force measurements of the alpha5beta1 integrin-fibronectin interaction. Biophys. J. *84*, 1252–1262.

Li, L., Okura, M., and Imamoto, A. (2002a). Focal adhesions require catalytic activity of Src family kinases to mediate integrin-matrix adhesion. Mol. Cell. Biol. *22*, 1203–1217.

Li, R., Mitra, N., Gratkowski, H., Vilaire, G., Litvinov, R., Nagasami, C., Weisel, J.W., Lear, J.D., DeGrado, W.F., and Bennett, J.S. (2003b). Activation of integrin alphaIIbbeta3 by modulation of transmembrane helix associations. Science *300*, 795–798.

Li, S., Butler, P., Wang, Y., Hu, Y., Han, D.C., Usami, S., Guan, J.L., and Chien, S. (2002b). The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. Proc. Natl. Acad. Sci. USA *99*, 3546–3551.

Litvinov, R.I., Shuman, H., Bennett, J.S., and Weisel, J.W. (2002). Binding strength and activation state of single fibrinogen-integrin pairs on living cells. Proc. Natl. Acad. Sci. USA *99*, 7426–7431.

Liu, H.P., and Bretscher, A. (1989). Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. Cell *57*, 233–242.

Liu, Z., Tan, J.L., Cohen, D.M., Yang, M.T., Sniadecki, N.J., Ruiz, S.A., Nelson, C.M., and Chen, C.S. (2010). Mechanical tugging force regulates the size of cell-cell junctions. Proc. Natl. Acad. Sci. USA *107*, 9944–9949.

Lo, C.M., Wang, H.B., Dembo, M., and Wang, Y.L. (2000). Cell movement is guided by the rigidity of the substrate. Biophys. J. *79*, 144–152.

Mandeville, J.T., Lawson, M.A., and Maxfield, F.R. (1997). Dynamic imaging of neutrophil migration in three dimensions: mechanical interactions between cells and matrix. J. Leukoc. Biol. *61*, 188–200.

Mathur, A.B., Collinsworth, A.M., Reichert, W.M., Kraus, W.E., and Truskey, G.A. (2001). Endothelial, cardiac muscle and skeletal muscle exhibit different viscous and elastic properties as determined by atomic force microscopy. J. Biomech. *34*, 1545–1553.

Matthews, B.D., Overby, D.R., Mannix, R., and Ingber, D.E. (2006). Cellular adaptation to mechanical stress: role of integrins, Rho, cytoskeletal tension and mechanosensitive ion channels. J. Cell Sci. *119*, 508–518.

Mayer, B.J., Hirai, H., and Sakai, R. (1995). Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. Curr. Biol. *5*, 296–305.

Mehta, A.D., Rock, R.S., Rief, M., Spudich, J.A., Mooseker, M.S., and Cheney, R.E. (1999). Myosin-V is a processive actin-based motor. Nature *400*, 590–593.

Metz, H., McElhaney, J., and Ommaya, A.K. (1970). A comparison of the elasticity of live, dead, and fixed brain tissue. J. Biomech. *3*, 453–458.

Meyer, C.J., Alenghat, F.J., Rim, P., Fong, J.H., Fabry, B., and Ingber, D.E. (2000). Mechanical control of cyclic AMP signalling and gene transcription through integrins. Nat. Cell Biol. *2*, 666–668.

Meyhofer, E., and Howard, J. (1995). The force generated by a single kinesin molecule against an elastic load. Proc. Natl. Acad. Sci. USA *92*, 574–578.

Michael, K.E., Dumbauld, D.W., Burns, K.L., Hanks, S.K., and Garcia, A.J. (2009). Focal adhesion kinase modulates cell adhesion strengthening via integrin activation. Mol. Biol. Cell *20*, 2508–2519.

Mofrad, M.R., Golji, J., Abdul Rahim, N.A., and Kamm, R.D. (2004). Force-induced unfolding of the focal adhesion targeting domain and the influence of paxillin binding. Mech. Chem. Biosyst. *1*, 253–265.

Mogilner, A., and Oster, G. (1996). Cell motility driven by actin polymerization. Biophys. J. *71*, 3030–3045.

Molloy, J.E., Burns, J.E., Sparrow, J.C., Tregear, R.T., Kendrick-Jones, J., and White, D.C. (1995). Single-molecule mechanics of heavy meromyosin and S1 interacting with rabbit or Drosophila actins using optical tweezers. Biophys. J. *68*, 298S–305S.

Moore, S.W., Biais, N., and Sheetz, M.P. (2009). Traction on immobilized netrin-1 is sufficient to reorient axons. Science *325*, 166.

Munevar, S., Wang, Y.L., and Dembo, M. (2004). Regulation of mechanical interactions between fibroblasts and the substratum by stretch-activated Ca2+ entry. J. Cell Sci. *117*, 85–92.

Na, S., Collin, O., Chowdhury, F., Tay, B., Ouyang, M., Wang, Y., and Wang, N. (2008). Rapid signal transduction in living cells is a unique feature of mechanotransduction. Proc. Natl. Acad. Sci. USA *105*, 6626–6631.

Oliver, T., Dembo, M., and Jacobson, K. (1995). Traction forces in locomoting cells. Cell Motil. Cytoskeleton *31*, 225–240.

Opas, M., and Dziak, E. (1990). Effects of a tumour promoter, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), on expression of differentiated phenotype in the chick retinal pigmented epithelial cells and on their interactions with the native basement membrane and with artificial substrata. Differentiation *43*, 20–28.

Ophir, J., Alam, S.K., Garra, B., Kallel, F., Konofagou, E., Krouskop, T., and Varghese, T. (1999). Elastography: ultrasonic estimation and imaging of the elastic properties of tissues. Proc. Inst. Mech. Eng. [H] *213*, 203–233.

Pasapera, A.M., Schneider, I.C., Rericha, E., Schlaepfer, D.D., and Waterman, C.M. (2010). Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. J. Cell Biol. *188*, 877–890.

Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., et al. (2005). Tensional homeostasis and the malignant phenotype. Cancer Cell *8*, 241–254.

Pato, M.D., Sellers, J.R., Preston, Y.A., Harvey, E.V., and Adelstein, R.S. (1996). Baculovirus expression of chicken nonmuscle heavy meromyosin II-B. Characterization of alternatively spliced isoforms. J. Biol. Chem. *271*, 2689–2695.

Pelham, R.J., Jr., and Wang, Y. (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc. Natl. Acad. Sci. USA *94*, 13661–13665.

Peskin, C.S., Odell, G.M., and Oster, G.F. (1993). Cellular motions and thermal fluctuations: the Brownian ratchet. Biophys. J. *65*, 316–324.

Peyton, S.R., and Putnam, A.J. (2005). Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. J. Cell. Physiol. *204*, 198–209.

Ponti, A., Machacek, M., Gupton, S.L., Waterman-Storer, C.M., and Danuser, G. (2004). Two distinct actin networks drive the protrusion of migrating cells. Science *305*, 1782–1786.

Prasad, N., Topping, R.S., and Decker, S.J. (2001). SH2-containing inositol 5′-phosphatase SHIP2 associates with the p130(Cas) adapter protein and regulates cellular adhesion and spreading. Mol. Cell. Biol. *21*, 1416–1428.

Prass, M., Jacobson, K., Mogilner, A., and Radmacher, M. (2006). Direct measurement of the lamellipodial protrusive force in a migrating cell. J. Cell Biol. *174*, 767–772.

Prezhdo, O.V., and Pereverzev, Y.V. (2009). Theoretical aspects of the biological catch bond. Acc. Chem. Res. *42*, 693–703.

Puklin-Faucher, E., Gao, M., Schulten, K., and Vogel, V. (2006). How the headpiece hinge angle is opened: New insights into the dynamics of integrin activation. J. Cell Biol. *175*, 349–360.

Puklin-Faucher, E., and Sheetz, M.P. (2009). The mechanical integrin cycle. J. Cell Sci. *122*, 179–186.

Puxkandl, R., Zizak, I., Paris, O., Keckes, J., Tesch, W., Bernstorff, S., Purslow, P., and Fratzl, P. (2002). Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. Philos. Trans. R. Soc. Lond. B Biol. Sci. *357*, 191–197.

Reilly, D.T., Burstein, A.H., and Frankel, V.H. (1974). The elastic modulus for bone. J. Biomech. *7*, 271–275.

Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A.D. (2001). 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. *153*, 1175–1186.

Roca-Cusachs, P., Alcaraz, J., Sunyer, R., Samitier, J., Farre, R., and Navajas, D. (2008). Micropatterning of single endothelial cell shape reveals a tight coupling between nuclear volume in G1 and proliferation. Biophys. J. *94*, 4984–4995.

Roca-Cusachs, P., Gauthier, N.C., Del Rio, A., and Sheetz, M.P. (2009). Clustering of alpha(5)beta(1) integrins determines adhesion strength whereas alpha(v)beta(3) and talin enable mechanotransduction. Proc. Natl. Acad. Sci. USA *106*, 16245–16250.

Rock, R.S., Rice, S.E., Wells, A.L., Purcell, T.J., Spudich, J.A., and Sweeney, H.L. (2001). Myosin VI is a processive motor with a large step size. Proc. Natl. Acad. Sci. USA *98*, 13655–13659.

Saez, A., Buguin, A., Silberzan, P., and Ladoux, B. (2005). Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophys. J. *89*, L52–L54.

Sawada, Y., Nakamura, K., Doi, K., Takeda, K., Tobiume, K., Saitoh, M., Morita, K., Komuro, I., De Vos, K., Sheetz, M., and Ichijo, H. (2001). Rap1 is involved in cell stretching modulation of p38 but not ERK or JNK MAP kinase. J. Cell Sci. *114*, 1221–1227.

Sawada, Y., and Sheetz, M.P. (2002). Force transduction by Triton cytoskeletons. J. Cell Biol. *156*, 609–615.

Sawada, Y., Tamada, M., Dubin-Thaler, B.J., Cherniavskaya, O., Sakai, R., Tanaka, S., and Sheetz, M.P. (2006). Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell *127*, 1015–1026.

Schaffler, M.B., and Burr, D.B. (1988). Stiffness of compact bone: effects of porosity and density. J. Biomech. *21*, 13–16.

Schlaepfer, D.D., Broome, M.A., and Hunter, T. (1997). Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, p130cas, and Nck adaptor proteins. Mol. Cell. Biol. *17*, 1702–1713.

Schmidt, C., Pommerenke, H., Durr, F., Nebe, B., and Rychly, J. (1998). Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins. J. Biol. Chem. *273*, 5081–5085.

Schmidt, C.E., Horwitz, A.F., Lauffenburger, D.A., and Sheetz, M.P. (1993). Integrin-cytoskeletal interactions in migrating fibroblasts are dynamic, asymmetric, and regulated. J. Cell Biol. *123*, 977–991.

Shahapure, R., Difato, F., Laio, A., Bisson, G., Ercolini, E., Amin, L., Ferrari, E., and Torre, V. (2010). Force generation in lamellipodia is a probabilistic process with fast growth and retraction events. Biophys. J. *98*, 979–988.

Sheetz, M.P., Wayne, D.B., and Pearlman, A.L. (1992). Extension of filopodia by motor-dependent actin assembly. Cell Motil. Cytoskeleton *22*, 160–169.

Shiu, Y.T., Li, S., Marganski, W.A., Usami, S., Schwartz, M.A., Wang, Y.L., Dembo, M., and Chien, S. (2004). Rho mediates the shear-enhancement of endothelial cell migration and traction force generation. Biophys. J. *86*, 2558–2565.

Sotomayor, M., and Schulten, K. (2007). Single-molecule experiments in vitro and in silico. Science *316*, 1144–1148.

Stoker, M., O'Neill, C., Berryman, S., and Waxman, V. (1968). Anchorage and growth regulation in normal and virus-transformed cells. Int. J. Cancer *3*, 683–693.

Storm, C., Pastore, J.J., MacKintosh, F.C., Lubensky, T.C., and Janmey, P.A. (2005). Nonlinear elasticity in biological gels. Nature *435*, 191–194.

Subramanian, A., and Lin, H.Y. (2005). Crosslinked chitosan: its physical properties and the effects of matrix stiffness on chondrocyte cell morphology and proliferation. J. Biomed. Mater. Res. A *75*, 742–753.

Sukharev, S., and Corey, D.P. (2004). Mechanosensitive channels: multiplicity of families and gating paradigms. Sci. STKE *2004*, re4.

Sun, Z., Martinez-Lemus, L.A., Trache, A., Trzeciakowski, J.P., Davis, G.E., Pohl, U., and Meininger, G.A. (2005). Mechanical properties of the interaction between fibronectin and alpha5beta1-integrin on vascular smooth muscle cells studied using atomic force microscopy. Am. J. Physiol. Heart Circ. Physiol. *289*, H2526–H2535.

Svoboda, K., and Block, S.M. (1994). Force and velocity measured for single kinesin molecules. Cell *77*, 773–784.

Tabernero, L., Aricescu, A.R., Jones, E.Y., and Szedlacsek, S.E. (2008). Protein tyrosine phosphatases: structure-function relationships. FEBS J. *275*, 867–882.

Takada, Y., Ye, X., and Simon, S. (2007). The integrins. Genome Biol. *8*, 215.

Tamada, M., Sheetz, M.P., and Sawada, Y. (2004). Activation of a signaling cascade by cytoskeleton stretch. Dev. Cell *7*, 709–718.

Tan, J.L., Tien, J., Pirone, D.M., Gray, D.S., Bhadriraju, K., and Chen, C.S. (2003). Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc. Natl. Acad. Sci. USA *100*, 1484–1489.

Theriot, J.A., and Mitchison, T.J. (1991). Actin microfilament dynamics in locomoting cells. Nature *352*, 126–131.

Thomas, W.E., Vogel, V., and Sokurenko, E. (2008). Biophysics of catch bonds. Annu. Rev. Biophys. *37*, 399–416.

Thoumine, O., Kocian, P., Kottelat, A., and Meister, J.J. (2000). Short-term binding of fibroblasts to fibronectin: optical tweezers experiments and probabilistic analysis. Eur. Biophys. J. *29*, 398–408.

Tomar, A., and Schlaepfer, D.D. (2009). Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. Curr. Opin. Cell Biol. *21*, 676–683.

Tsai, J., and Kam, L. (2009). Rigidity-dependent cross talk between integrin and cadherin signaling. Biophys. J. *96*, L39–L41.

Tseng, Y., Kole, T.P., Lee, J.S., Fedorov, E., Almo, S.C., Schafer, B.W., and Wirtz, D. (2005). How actin crosslinking and bundling proteins cooperate to generate an enhanced cell mechanical response. Biochem. Biophys. Res. Commun. *334*, 183–192.

Tyska, M.J., Dupuis, D.E., Guilford, W.H., Patlak, J.B., Waller, G.S., Trybus, K.M., Warshaw, D.M., and Lowey, S. (1999). Two heads of myosin are better than one for generating force and motion. Proc. Natl. Acad. Sci. USA *96*, 4402–4407.

Uemura, S., Higuchi, H., Olivares, A.O., De La Cruz, E.M., and Ishiwata, S. (2004). Mechanochemical coupling of two substeps in a single myosin V motor. Nat. Struct. Mol. Biol. *11*, 877–883.

Vicente-Manzanares, M., Choi, C.K., and Horwitz, A.R. (2009). Integrins in cell migration–the actin connection. J. Cell Sci. *122*, 199–206.

Vogel, V. (2006). Mechanotransduction involving multimodular proteins: converting force into biochemical signals. Annu. Rev. Biophys. Biomol. Struct. *35*, 459–488.

von Wichert, G., Jiang, G., Kostic, A., De Vos, K., Sap, J., and Sheetz, M.P. (2003). RPTP-alpha acts as a transducer of mechanical force on alphav/beta3-integrin-cytoskeleton linkages. J. Cell Biol. *161*, 143–153.

Wakatsuki, T., Kolodney, M.S., Zahalak, G.I., and Elson, E.L. (2000). Cell mechanics studied by a reconstituted model tissue. Biophys. J. *79*, 2353–2368.

Wang, F., Harvey, E.V., Conti, M.A., Wei, D., and Sellers, J.R. (2000a). A conserved negatively charged amino acid modulates function in human nonmuscle myosin IIA. Biochemistry *39*, 5555–5560.

Wang, H.B., Dembo, M., and Wang, Y.L. (2000b). Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. Am. J. Physiol. Cell Physiol. *279*, C1345–C1350.

Wang, H.B., Dembo, M., Hanks, S.K., and Wang, Y. (2001a). Focal adhesion kinase is involved in mechanosensing during fibroblast migration. Proc. Natl. Acad. Sci. USA *98*, 11295–11300.

Wang, S.C., Makino, K., Xia, W., Kim, J.S., Im, S.A., Peng, H., Mok, S.C., Singletary, S.E., and Hung, M.C. (2001b). DOC-2/hDab-2 inhibits ILK activity and induces anoikis in breast cancer cells through an Akt-independent pathway. Oncogene *20*, 6960–6964.

Wang, Y., Botvinick, E.L., Zhao, Y., Berns, M.W., Usami, S., Tsien, R.Y., and Chien, S. (2005). Visualizing the mechanical activation of Src. Nature *434*, 1040–1045.

Warshaw, D.M., and Fay, F.S. (1983). Tension transients in single isolated smooth muscle cells. Science *219*, 1438–1441.

Yamada, S., Wirtz, D., and Kuo, S.C. (2000). Mechanics of living cells measured by laser tracking microrheology. Biophys. J. *78*, 1736–1747.

Yeung, T., Georges, P.C., Flanagan, L.A., Marg, B., Ortiz, M., Funaki, M., Zahir, N., Ming, W., Weaver, V., and Janmey, P.A. (2005). Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil. Cytoskeleton *60*, 24–34.

Zaidel-Bar, R., Ballestrem, C., Kam, Z., and Geiger, B. (2003). Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. J. Cell Sci. *116*, 4605–4613.

Zhang, X., Jiang, G., Cai, Y., Monkley, S.J., Critchley, D.R., and Sheetz, M.P. (2008). Talin depletion reveals independence of initial cell spreading from integrin activation and traction. Nat. Cell Biol. *10*, 1062–1068.

Zheng, X.M., Resnick, R.J., and Shalloway, D. (2000). A phosphotyrosine displacement mechanism for activation of Src by PTPalpha. EMBO J. *19*, 964–978.

Zhong, C., Chrzanowska-Wodnicka, M., Brown, J., Shaub, A., Belkin, A.M., and Burridge, K. (1998). Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. J. Cell Biol. *141*, 539–551.

Zhou, A.X., Hartwig, J.H., and Akyurek, L.M. (2010). Filamins in cell signaling, transcription and organ development. Trends Cell Biol. *20*, 113–123.

Zhu, J., Luo, B.H., Xiao, T., Zhang, C., Nishida, N., and Springer, T.A. (2008). Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces. Mol. Cell *32*, 849–861.