Mechanotransduction – a field pulling together?

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Summary

Mechanical stresses are ever present in the cellular environment, whether through external forces that are applied to tissues or endogenous forces that are generated within the active cytoskeleton. Despite the wide array of studies demonstrating that such forces affect cellular signaling and function, it remains unclear whether mechanotransduction in different contexts shares common mechanisms. Here, I discuss possible mechanisms by which applied forces, cell-generated forces and changes in substrate mechanics could exert changes in cell function through common mechanotransduction machinery. I draw from examples that are primarily focused on the role of adhesions in transducing mechanical forces. Based on this discussion, emerging themes arise that connect these different areas of inquiry and suggest multiple avenues for future studies.

Key words: Mechanical force, Traction force, Mechanobiology, cell adhesion, Stiffness, Rigidity

Introduction

Mechanical forces have long been implicated in regulating many physiologic and pathologic processes. Mechanical loading induces hypertrophy and strengthening of skeletal muscles, tendons, ligaments and bones, whereas prolonged exposure to weightlessness left early astronauts prone to fractures (Burkholder, 2007; Duncan and Turner, 1995; Hattner and McMillan, 1968). Similar hypertrophic thickening occurs in the heart with unchecked hypertension, although in this case resulting in potentially dangerous consequences (Weber et al., 1989; Westerhof and O'Rourke, 1995). More subtly, differences in flow-induced shear stress in veins versus arteries specify the endothelium in part to take on a venous versus arterial phenotype, and the distribution of shear stresses within the arterial tree renders certain regions susceptible to inflammation, explaining the observed distribution of atherosclerotic plaques (Davies et al., 1995; Garcia-Cardena et al., 2001). Such examples span a wide range of mechanical settings (see Box 1 for terminology) and traditionally have provided the primary motivation for the study of mechanotransduction, or how forces are transduced into biochemical and functional responses.

Recent studies also implicate forces that are generated by the contractile activity – or contractility – of cells in regulating cell function, and suggest a much broader role for mechanotransduction in biology. It has long been suggested that mechanical forces that are generated by the contractile activity of cells contribute to the physical folding, extension and cavitation events that are associated with morphogenesis (Odell et al., 1981; Keller et al., 2003; His, 1874; Kiehart et al., 2000; Moore et al., 2005). The existence of such forces was first demonstrated by the ability of adherent cells to wrinkle thin films of silicone elastomers (Harris et al., 1980), as well as to drive matrix reorganization (Stopak and Harris, 1982), and have since been quantified using a variety of more advanced approaches (Oliver et al., 1995; Dembo and Wang, 1999; Galbraith and Sheetz, 1997; Balaban et al., 2001; Tan et al., 2003). Interestingly, these forces appear not only to drive physical changes in the developing embryo, but also are transduced to affect cellular signaling, gene expression and cell function, which are crucial to developmental programming (Lee et al., 2006; Somogyi and Rorth, 2004; Farge, 2003). Blocking such cell-generated forces appears to alter many basic cellular functions, such as proliferation, differentiation, sorting and migration (Huang et al., 1998; McBeath et al., 2004; Sordella et al., 2003; Krieg et al., 2008; Lo et al., 2004).

Recent efforts to modulate the stress that is generated by these cellular forces, by simply altering the mechanical stiffness of the substrate (Box 1) against which cells pull, mirror the effects that are caused by directly altering cellular contractility (Pelham and Wang, 1997; Paszek and Weaver, 2004; Engler et al., 2006). Thus, even when not externally applied, cells experience endogenous mechanical forces that are generated by their internal cytoskeletal machinery, and these forces can be modulated by numerous factors. As such, mechanotransduction may have a more pervasive role in regulating cellular function than previously appreciated.

Do different mechanical contexts – applied forces, cell-generated forces and stiffness sensing – exert changes in cell function through common mechanotransduction mechanisms? What is known about how forces are generated and experienced by cells? What are some of the mechanisms of mechanotransduction that are being examined? Here, these issues will be discussed. This article is not an exhaustive compilation of the literature, which has been provided by several excellent reviews (Ingber, 2003; Orr et al., 2006) but, rather, a discussion of common emerging themes that connect different areas of inquiry (applied forces, contractile forces and matrix mechanical properties) in the hope of providing some context for the future of this field.

Applied and cell-generated forces

Cells and subcellular structures can experience stresses from many sources. External forces range from whole-body forces such as gravity and exercise-induced stresses on the musculoskeletal system, to tissue-specific forces such as the shear stress of blood flowing across endothelium, to the stretch of vessels owing to blood pressure, and to microscopic forces that occur when contracting cells pull on surrounding extracellular matrix (ECM) and on each other (Fig. 1A). There are many different cellular responses to such forces, and many different mechanisms by which such forces are transduced to mediate such responses – too many to enumerate here

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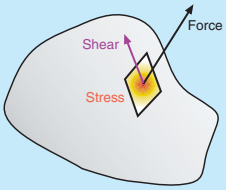
Box 1. Force, stress, contractility, tension, compliance, shear and all that: a simple description of mechanotransduction terminology and usage

Force and stress

Force is a vector (with magnitude and direction) that is classically defined with accelerating a mass and, in studies of cells, is either applied to elicit a response or is measured to determine a mechanical reaction from cells. *Stress*, by contrast, speaks of a force per unit area (see Figure). Whereas force and stress often increase together and, therefore, can be used interchangeably in limited settings, stress is more difficult to calculate when a force is applied because the area across which a force is applied can change during an experiment and must be measured. Similarly, when stress is applied (e.g. shear stress of fluid flowing over a cell), force is more difficult to report. As such, it is best not to mix these two terms.



Tension, compression, pressure and shear

These terms refer to different types of forces and stresses. A cell in *tension* experiences a tensile force (or stress) from outside that pulls to elongate the cell. A cell can attempt to contract using myosin motors (like a muscle) but be unable to do so because it is attached to an inflexible substrate. In this case, the cell is in tension because, by convention, the external force from outside (i.e. the substrate) is counteracting the cell by acting to lengthen the cell. Thus, although seemingly paradoxical, this state is often described as contractile tension. Conversely, a *compressive* force (or stress) would act to decrease the length of the cell. By convention, unless otherwise specified, tensile and compressive forces generally refer to forces that are applied in one dimension. 2D forces are often referred to as 'biaxial' (such as when inflating a balloon on which a cell is attached). *Pressure* is a 3D compressive stress (not force) but, unlike other forms of stress, is isotropic. *Shear*-forces or -stresses refer to those that act in-plane to the area experiencing the force (as opposed to tension or compression which act perpendicular to that plane). Because shear forces are almost always applied to cells through a fluid medium, and nearly all of the mathematics for fluidic mechanics are expressed in stresses, studies involving shear generally refer to shear stress.

Stiffness, compliance, elasticity and rigidity

These terms refer to properties of a material. *Stiffness* describes either spring stiffness or material stiffness. Both reflect resistance to deformation. Spring stiffness is calculated by dividing applied force by the movement of the point where the force was applied. Note that this spring stiffness is specific to the dimensions and geometry of the experiment and, as such, cannot be translated and compared with other studies. Material stiffness (sometimes referred to as the modulus or Young's modulus) is the geometry-independent equivalent and refers to an inherent property of the material itself. *Compliance* is the inverse of stiffness (a material that is less stiff is more compliant). *Elasticity* is often used synonymously with compliance, although its strict usage refers to the degree to which a material is energy storing (elastic, like a spring) versus dissipating (like a viscous fluid). *Rigidity* is now used synonymously with stiffness, although it classically refers to a spring (geometry-specific) stiffness.

Strength and hardness

These quantities are unlikely to be commonly used in the context of mechanotransduction, as they generally refer to experiments that injure a material. Strength refers to the stress at which a material permanently deforms or fractures, and hardness or softness often refer to an experiment-specific degree to which a material scratches, deforms or fractures when indented by a sharp object. Thus, strength, hardness and stiffness should never be used synonymously.

but they are reviewed in more depth by Orr et al. (Orr et al., 2006). Therefore, just as in every transduction system, there is no one molecular or anatomical structure that drives all mechanotransduction responses. Perhaps the most well-described (sub)cellular sites for sensing mechanical forces are primary cilia, stretch-modulated ion channels and focal adhesions. Other putative sites for mechanosensing include the nuclear lamina and nucleus itself, the cytoskeleton and the cortical membrane (Lammerding et al., 2005; Han et al., 2004; Tavernarakis and Driscoll, 2001; Sukharev and Corey, 2004; Resnick and Hopfer, 2007). For the purposes of this discussion, I make two gross oversimplifications: first, I consider forces that are categorized simply as those that arise outside of the cell ('applied forces') and those that are generated inside the cell by the actin-myosin cytoskeleton; and second, I focus on how these two types of forces might be related with respect to how they are transduced.

Are external and cell-generated forces similar? One can characterize forces by their magnitude, direction and dynamics (Box 1). The threshold magnitude of external forces that trigger cellular responses appears to be in the pN to nN range (Choquet et al., 1997; Jiang et al., 2003; Goldschmidt et al., 2001). Interestingly, most measurements of cell-generated forces have reported magnitudes from 1-100 nN per focal adhesion (Tan et al., 2003; Dembo and Wang, 1999; Balaban et al., 2001). Endogenous, myosin-generated forces appear to be almost always contractile, such that the cell is under tension and forces that are applied to substrates are directed inward, towards the centroid of the cell (Tan et al., 2003; Dembo and Wang, 1999). Although there are instances in which this directionality is not true, such as when cells extend filopodia or lamellipodia (Prass et al., 2006), the generalization remains true for most measurements of cellular forces. By contrast, many types of external forces might not have such a radial direction. Moreover, most experimentally applied forces are step changes or those that follow a physiologic frequency (for example, 1 Hz to model the cardiac cycle), whereas live recordings of cellular forces appear to suggest that most non-muscle cells exhibit complex force dynamics with a much wider frequency spectrum of activity; intracellular signaling cycles may have their own circadian rhythms that lead to cycles of cytoskeletal activation and deactivation (Galbraith and Sheetz, 1997; Giannone et al., 2004). These differences might lead one to conclude that cells transduce external and internal forces with entirely different mechanisms. However, recent studies increasingly suggest that these two forces are coupled.

Perhaps the first indication of coupling between applied and cell-generated forces comes from numerous reports demonstrating that mechanotransduction of applied forces is often lost when myosin-based contractility is inhibited (Zhao et al., 2007; Sarasa-Renedo et al., 2006; Torsoni et al., 2005). One explanation for this finding is that cell-generated forces and applied forces must act together on a mechanosensor to generate a response. Consider for a moment how force could be transduced. The application of force to an object can result in two basic responses – translation or stress. That is, if the object is not fixed in place by other forces, it will accelerate

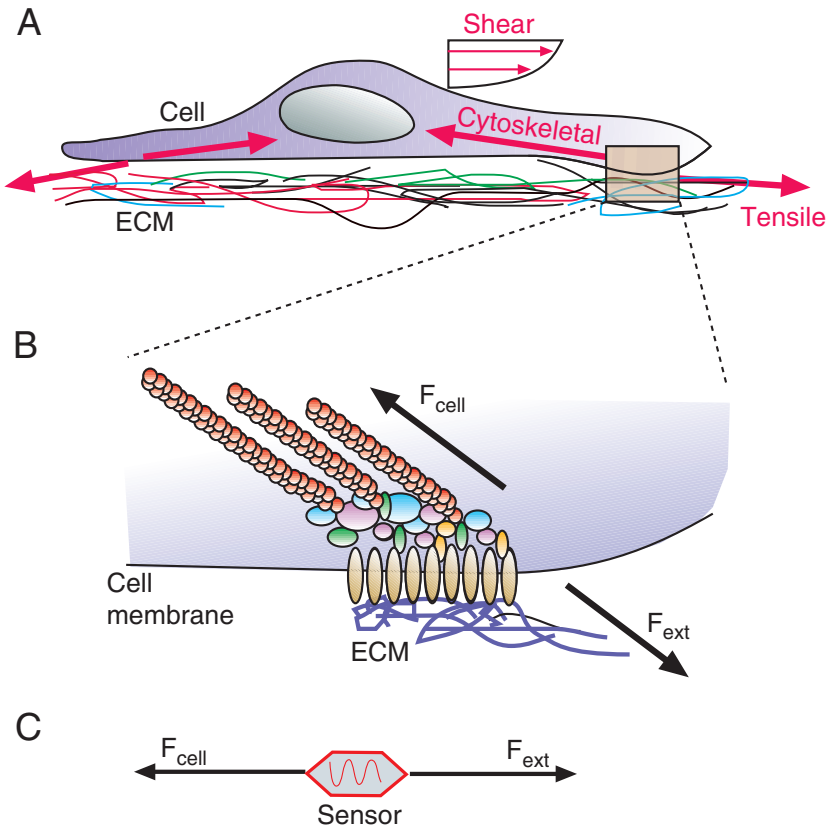


Fig. 1. Application of force to a cell. (A) Cells can be exposed to multiple types of forces, such as shear forces through fluid flow over the cell, tensile forces acting through the ECM, and cytoskeletally generated contractile forces. Depicted is a single cell attached to a complex ECM (illustrated as a multicolored fabric). (B) Close-up of a focal adhesion showing the balance of external and internal forces (F_{ext} and F_{cell}, respectively) in driving stress at a mechanosensor. Depicted are actin stress fibers (red) anchored into focal adhesions (multicolored array of proteins) that bind to the ECM (blue) through integrins (brown). (C) This balance of forces provides the stress necessary for mechanical sensing.

(causing the object to translate). If, however, net forces are zero, then the object will experience stress. The object can then respond (mechanically) to this stress by deforming reversibly (elastically) or irreversibly (inelastically).

In the context of cells, there are very few states of force disequilibrium that lead to whole-body acceleration (a 1 pN disequilibrium, less than the magnitude required to break most single-receptor bonds, would accelerate a cell at ~1 m/second^2). Thus, applied forces must necessarily be sensed by increased stress and resultant deformation of a sensor. The focal adhesion provides an excellent illustration of this: these adhesions are connected on the cytoplasmic face by the actin cytoskeleton and on the extracellular face by the ECM (Fig. 1B,C). A cytoskeletally generated force leads to stress in the focal adhesion because an equal and opposite reactive force arises in the ECM (which can be thought of as a passive spring). Studies demonstrate that when the ECM is not rigid – for example, when cells attach to small, submicrometer-diameter beads coated with ECM ligands – cytoskeletal tension and stress at adhesions do not develop and focal adhesions fail to mature (Galbraith et al., 2002). Conversely, an externally applied force through the ECM results in stress at the focal adhesion only when the actin cytoskeleton provides an opposite, reactive force that balances the applied force (Wang et al., 1993; Choquet et al., 1997). In this case, the additional factor is that the actin cytoskeleton may give rise to this force through both passive deformation (similar to a spring) and through changes in myosin motor activity. Thus, one can explain how the inhibition of myosin-generated contractility could effectively prevent the development of stress in the focal adhesion, as any external force would simply stretch the cell (and the adhesion site). Although there are some distinctions, similar arguments can be made based on the dependence of stretch-activated ion-channel activity on cytoskeletal integrity, applied forces and cell-generated forces (Pellegrini et al., 2001; Glogauer et al., 1995).

A second possible interpretation for external-internal force coupling also exists. External forces trigger active changes in cytoskeletal structure and cellular force generation, which do more than merely balance the external force. For example, using optical traps, Choquet et al. demonstrated that, when cells pulled on adhesions and the substrate bound to that adhesion resisted this pull (allowing stress to develop at the adhesion), adhesions would mature and be reinforced, and cells would subsequently pull with substantially higher forces on that same adhesion (Choquet et al., 1997). Applying forces globally to cells – for example, by stretching underlying flexible substrates – leads to rapid and sustained activation of RhoA (a Rho-family GTPase) and myosin, and to the development of stress fibers (Torsoni et al., 2005; Zhao et al., 2007; Liu et al., 2007).

A recent study by Trepat and colleagues suggests that, in addition to such specific signaling mechanisms, certain types of change in cytoskeletal mechanics in response to applied forces – such as adaptive deformation to large applied forces through cytoskeletal depolymerization and repolymerization – are an inherent property of dense sol-gel networks (in which monomers in solution and polymers forming gel coexist in rapid equilibrium) and are shared by all cells that have cytoskeletons (Trepat et al., 2007). That is, this property only requires having a phase transition that allows the cytoskeleton to shift through different physical forms, and not a specific biochemical mechanism. Interestingly, when applying minute forces to single adhesion sites and subsequently measuring cellular traction forces, one observes massive changes in cellular contractility that involve a relaxation followed by recovery of

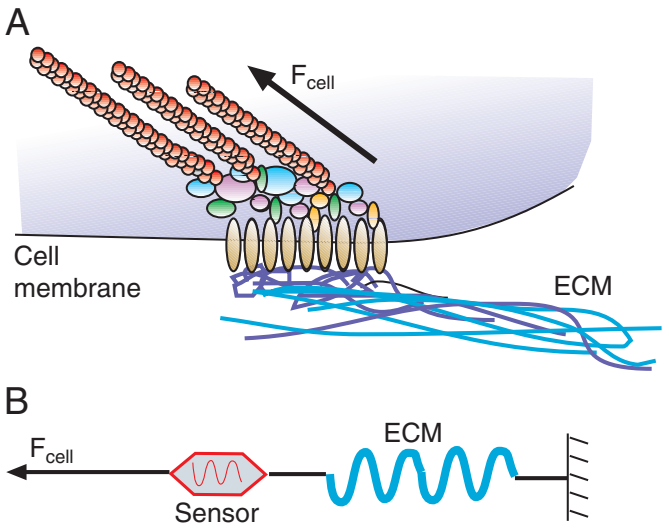


Fig. 2. Effects of stress on a cell. (A) Focal adhesion that experiences stress from a cell-generated contractile force (F_{cell}) pulling against an ECM (blue). (B) Stress generated in the focal adhesion (sensor) depends on both the cell-generated force (F_{cell}) and the stiffness of the ECM.

contractility, the timing of which persists beyond the time of external force application (Sniadecki et al., 2007). Such observations naturally suggest that the transduction of external forces is not simply a force-signaling-response transduction sequence, and not even an external-internal force balance leading to a stress-signaling-response transduction sequence, but rather a dynamic feedback system involving external force, signaling, internal force, more signaling and then response. This distinction of including the activation of cell-generated forces in response to an external force is not merely semantic. Recall that external forces probably differ in direction, distribution across the cell and dynamics from those that are developed through the biochemical regulation of cytoskeletal tension. As such, the next section discusses these cell-generated forces and their contribution to mechanotransduction in greater depth.

Cell-generated forces and their regulation

The physical process of generating contractile force in non-muscle cells essentially involves the activation of non-muscle myosin-II motors, which act to crosslink, organize and affect sliding between actin microfilaments (Landsverk and Epstein, 2005). In the presence of strong adhesion against an underlying substrate, these filaments tend to coalesce into stress fibers that are tethered at sites of adhesion (Chrzanowska-Wodnicka and Burridge, 1996). Although I will focus exclusively on myosin-regulated contractile forces, it is possible that other signals that alter actin polymerization and organization also alter force, and that other cytoskeletal elements such as microtubules also contribute to net forces (Dogterom and Yurke, 1997; Stamenovic et al., 2002; Reinhart-King et al., 2005; Prass et al., 2006; Heidemann et al., 1990).

Whereas there are numerous mechanisms to modulate myosin activity, the best-described involves increased phosphorylation of non-muscle myosin II regulatory light chain (MLC) by myosin-light-chain kinase (MLCK) or the Rho effector p160ROCK, which additionally inactivates myosin phosphatase (Amano et al., 1996; Ishizaki et al., 1996; Kimura et al., 1996). Not surprisingly, many different stimuli can modulate these signaling pathways and affect downstream traction forces. For example, many growth factors, cytokines and integrin ligands can activate these pathways. Interestingly, many of the downstream effects of growth-factor stimulation and integrin-mediated adhesion appear to require changes in cell contractility. For example, in the context of proliferative stimulation, pharmacologic inhibition of MLCK or disruption of the actin cytoskeleton arrests proliferation (Huang et al., 1998). Inhibiting RhoA activity with C3 exoenzyme or dominant-negative mutants of RhoA blocks cell proliferation, whereas microinjection of constitutively active RhoA promotes DNA synthesis (Pirone et al., 2006; Olson et al., 1995; Yamamoto et al., 1993; Welsh et al., 2001; Sahai et al., 2001). Inhibition of ROCK with Y-27632, or dominant-negative ROCK suppresses mitogen-induced DNA synthesis of cells in vitro and in vivo (Pirone et al., 2006; Sawada et al., 2000; Uchida et al., 2000; Uehata et al., 1997). Similar findings that suggest a role for cell-generated forces in modulating cell differentiation, migration and gene expression have also been reported (McBeath et al., 2004; Hoang et al., 2004; Liu and Senger, 2004).

In general, these studies clearly suggest a ubiquitous role for cell-generated forces in regulating cell signaling and function, and prompt additional questions. What initiates this force generation? Do many different stimuli modulate cellular forces? Do they modulate the magnitude and spatiotemporal dynamics in common or unique ways? Are these effects all mediated through a common set of pathways, such as the RhoA and MLCK pathways, or do different signaling pathways modulate contractility through as yet uncharacterized means? Surprisingly little has been reported that sheds light on these questions, probably because there is limited access to approaches that measure cellular forces, and tools have not yet been developed to address questions that require a systematic comparison of different stimuli, signals and cell types with respect to their effects on force generation.

Once generated by different stimuli, how are these cellular forces transduced into biochemical effects? At focal adhesions, numerous proteins are phosphorylated and recruited to sites of adhesion in a stress-dependent manner, and inhibition of contractility results in rapid disassembly of such structures, suggesting that adhesions are one possible site for mechanotransduction (Chrzanowska-Wodnicka and Burridge, 1996; Galbraith et al., 2002; Wang et al., 1993). Such studies have pointed to focal adhesion kinase (FAK), Src and other associated molecules within focal adhesions in transducing force to responses such as proliferation and differentiation (von Wichert et al., 2008; Wang et al., 2001). However, because direct manipulations of such pathways can feed back to modulate force generation itself (Pirone et al., 2006; Palazzo et al., 2004; Lim et al., 2008; Arthur et al., 2000), it has been difficult to separate their roles in force generation versus force transduction. One alternative approach to identifying putative sensors is to provide better spatial and temporal descriptions of endogenous signaling following stimulation with force. Observing the activation of Src spatiotemporally with a FRET probe has demonstrated activation at adhesion sites following stress application, and rapid diffusion of active Src into the cell (Wang et al., 2005). Nonetheless, the challenge of numerous feedback loops between adhesion, signaling and cellular contractility remains a barrier to identifying the contribution of each player to the overall mechanotransduction response.

Thus, one is still faced with the question: what is the actual event that converts mechanical stress to a biochemical event? One of the most plausible proposed mechanisms involves protein unfolding.

In this model, stresses in the cytoskeleton, focal adhesions and/or ECM cause conformational changes in specific proteins. Fibronectin has been shown to reveal normally cryptic regions when experiencing stresses that are comparable to those generated by cells against ECM (Erickson, 1994; Abu-Lail et al., 2006; Smith et al., 2007). Some controversy remains as to the exact nature of the unfolding process – whether it primarily involves inter- or intra-domain rearrangements – but the demonstration of new binding sites on fibronectin is incontrovertible. Similarly, it has now been shown that p130cas, an important scaffolding protein within focal adhesions, alters its structure under stress to render it more susceptible to Src-mediated phosphorylation, thereby promoting force-mediated adhesion assembly (Sawada et al., 2006). The application of a classic biochemical method – shotgun labeling of cysteine residues – to cells under tension has revealed numerous other proteins (including vimentin, filamin, myosin and spectrin) that either unmask cryptic sequences or alter their assembly under stress (Johnson et al., 2007). Such studies provide an important experimental validation of much theoretical and computational power that can be and has been applied to the prediction of force-mediated effects on proteins (Pabon and Amzel, 2006; Hytonen and Vogel, 2008). These exciting developments now set a challenge for the near future in defining more explicitly how such structural changes exert their individual effects and how signaling is modulated as a whole.

Stiffness sensing

A case has been made for the necessary development of mechanical stress within the cytoskeleton for mechanotransduction. One way of alleviating or preventing such stress is if cells are either detached from a physical substrate; another is if the substrate itself provides no resistance to deformation when cells pull on them. The extent to which a substrate resists deformation is described by its stiffness. Different tissues and organs span a wide range of mechanical properties, one of which is material stiffness (Discher et al., 2005). Moreover, such properties change during tissue development as well as during pathological processes such as fibrosis or tumorigenesis (Georges et al., 2007; Paszek and Weaver, 2004; Wozniak et al., 2003; Davidson et al., 1999).

As these changes in mechanical properties are tightly coupled in vivo to numerous changes in ECM composition, crosslinking and density, it has until recently been impossible to decouple the relative impact of mechanical from biochemical changes in matrix, with regard to cellular responses. However, developments in the use of matrix-functionalized synthetic substrates – most notably polyacrylamide gels – have enabled studies that suggested that substrate stiffness itself can alter numerous cellular functions including migration, proliferation and differentiation (Li et al., 2007; Peyton and Putnam, 2005; Leach et al., 2007; Lo et al., 2000). Some cell types appear to migrate preferentially towards stiffer regions of a matrix, a phenomenon now termed ‘durotaxis’ (Lo et al., 2000; Jiang et al., 2006; Gray et al., 2003; Wong et al., 2003). Increasing stiffness also appears to enhance proliferation in numerous normal as well as transformed cell types, and facilitates tumor progression (Paszek and Weaver, 2004; Wozniak et al., 2003; Georges and Janmey, 2005). Interestingly, mesenchymal stem cells will differentiate into different lineage fates as a function of material stiffness, and appear to do so in a way that would promote tissue-specific healing (Engler et al., 2006). For example, on brain-tissue-like stiffness cells undergo neuronal differentiation, whereas muscle-equivalent stiffness promotes myogenesis.

The mechanism by which cells sense stiffness remains poorly defined, but early evidence suggests that the sensing system involves modulation of the same players as in other mechanotransduction pathways – integrins, focal adhesions and myosin-based contractility. The simplest explanation would be that a compliant substrate directly reduces the stress at cell-matrix adhesions in an actively contracting cell (Fig. 2). In turn, this decreased stress suppresses the normal maturation of focal adhesions and provides evidence that compliant substrates probably suppress certain types of signals while promoting others. Supporting this notion is the finding that focal adhesions are smaller or absent in cells that are cultured on compliant substrates, and that FAK phosphorylation is decreased (Paszek and Weaver, 2004). Such an effect could occur through increased activation of these signaling pathways with increased stress or, indirectly, through increased phosphatase activity for settings of decreased stress. Indeed, the receptor-like protein tyrosine phosphatase RPTPα has been shown to be crucial for force sensing at adhesions (von Wichert et al., 2003). However, RhoA activity also appears to be suppressed in compliant settings, which suggests that stiffness feeds back to alter directly the degree of myosin activation and cellular contractility itself (Engler et al., 2006; Wozniak et al., 2003). Because RhoA and integrin signaling appear to have numerous coupled feedforward and feedback loops (the very topologies of which could be altered by mechanics), at this point we cannot conclude at what level(s) substrate stiffness impinges on this mechanochemical sensing system. Indeed, mutant integrins that constitutively cluster and the modulation of RhoA can induce cells on compliant substrates to revert to a phenotype that is characteristic of stiff substrates (Paszek and Weaver, 2004; Wozniak et al., 2003). As such, many questions remain.

Perhaps most fundamental is whether the transduction of stiffness to a response actually involves sensing stress. The existing data are certainly consistent with the possibility that focal adhesions (or other sensors) alter their structure and function simply and primarily as a result of changes in stress when cells are attached to substrates of different stiffnesses. For example, Engler et al. used pure collagen-I gels as well as polyacrylamide gels (two polymers that form gels through very different mechanisms) to show that cellular responses were equivalent on gels of equivalent stiffness (Engler et al., 2004). Although these data support a strong case for a mechanical basis for sensing, they do not exclude several other plausible mechanisms.

As all of these studies use polymers with varying degrees of crosslinking to alter stiffness, it is impossible to decouple the nanomolecular changes in polymer-chain mobility, flexibility and hydration. In fact, for such gels, bulk stiffness changes as a direct result of these molecular changes. Although decoupling these molecular from bulk effects may be more a matter of semantics and practical insight, such changes could also alter the accessibility, shielding and effective ability of cellular receptors to engage and cluster matrix ligands that are immobilized to these polymers. Using other model systems, it has been demonstrated that such polymer shielding can alter cell adhesion and effective integrin-binding affinity (Houseman and Mrksich, 2001; Keselowsky et al., 2005). Thus, changes in substrate stiffness might simply be a coincident marker of the ability of the substrate to affect integrin ligation, in which case the observed changes in RhoA signaling and mechanical tension might be downstream effects of a primary modulation of integrin signaling.

It has been reported that changes in stiffness cause a change in cell shape, in which cells retain a more rounded morphology on compliant substrates and take on a more flattened shape on stiff

substrates (classically associated with cells that are cultured on hard plastic). How stiffness changes cell shape is not known and could involve the mechanical or integrin-mediated mechanisms just described. But because changes in cell shape are also associated with changes in RhoA-mediated contractility and focal adhesion formation, shape changes could themselves be embedded within the control loop that involves RhoA, contractility, focal adhesions and changes in cell function. However, one early study suggests that even when cell shape is constrained, focal adhesion dynamics are still different on compliant versus stiff substrates, suggesting that stiffness-induced changes in cell shape are not likely to explain stiffness sensing (Guo and Wang, 2007). Thus, although at its core stiffness sensing probably involves transducing a stress into biological signals, multiple mechanisms might be at play.

Concluding remarks

Although the themes raised in this discussion of mechanotransduction necessarily draw more heavily from one example (focal adhesions), the overall conclusions can be applied more broadly. One is reminded that numerous additional sites have been implicated in transducing mechanical stresses, including cell-cell junctions (Liu et al., 2007; Tzima et al., 2005), stretch-activated ion channels (Pellegrini et al., 2001; Sukharev and Corey, 2004; Tavernarakis and Driscoll, 2001), primary cilia (Resnick and Hopfer, 2007), the cytoskeleton itself (Han et al., 2004) and the nucleus (Maniotis et al., 1997; Lammerding et al., 2005). As in focal adhesions, mechanotransduction at these sites probably also depends on the development of stresses that are generated by a balance of external forces and cell-generated forces, and involves molecular conformational changes that result in signaling. Importantly, evidence clearly points to multiple pathways for crosstalk between different transduction sites, such as between cell-cell junctions and focal adhesions: in endothelium, platelet endothelial-cell adhesion molecule 1 (PECAM1) appears to be crucial for transducing shear stresses in a manner that involves modulating integrin binding (Tzima et al., 2005). Cadherin engagement can modulate RhoA signaling and contractility, as well as mechanically alter how stresses are distributed across cells (Nelson et al., 2004; Nelson et al., 2005). As such, mechanotransduction for cells in isolation might be quite different from how forces are transduced in different multicellular contexts. One crucial focus of future studies will involve the further characterization of such cooperative mechanisms, as well as clearly defining the similarities and differences between different mechanosensory systems.

One question that remains to be answered is how specificity may arise in mechanotransduction responses. That is, it has been clearly demonstrated that different magnitudes and types of forces (e.g. laminar versus turbulent shear flow, uniaxial versus biaxial stretch, stretch versus shear) lead to different responses, but how such differences are produced remains unexplored. Certainly, one can reason that the spatiotemporal signatures in mechanical stimuli could result in spatiotemporal signatures in cellular signaling from a common set of distributed sensors (e.g. focal adhesions). One can also imagine that different sensors (e.g. protein unfolding events) occur at different thresholds, or that different sites (e.g. cell-cell junctions versus nucleus) are primed to sense certain forces better than others. Going forward, the field will transition from identifying the contexts in which forces are important to elucidating how such forces exact specific effects.

Another challenge is to link mechanotransduction studies across different length scales in order to provide a more global view of its mechanisms. From identifying stress-induced molecular deformations and their functional effects, to mapping system-wide changes in cell signaling networks, to understanding forces in complex, multicellular processes in whole organisms, we are still unable to cross the boundaries between these length scales. A great deal of this challenge arises from two crucial features of mechanotransduction: that cell and tissue structure both modulate and are sculpted by mechanical stresses, and that there are numerous feedback loops between cell signaling and force generation. Thus, we are faced with mapping the spatial distribution of forces at the subcellular level through to stress gradients at the tissue level, linking these distributions to structural and functional effects, and tracking the evolution of these mechanical-functional effects through time. Theoretical constructs are in the early stages to develop computational models of various aspects of this problem, from focal adhesion assembly (Shemesh et al., 2005; Nicolas et al., 2008), to cytoskeletal contractility (Pathak et al., 2008; Deshpande et al., 2006) to physical models of morphogenesis (Odell et al., 1980; Keller et al., 2003). The experimental barriers are even more daunting. Undoubtedly, the challenge will call upon many disciplines to make both theoretical and experimental innovations, in order to provide a path towards understanding how biological systems operate in a physical world.

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