

Mechanical cues in cellular signalling and communication

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Abstract Multicellular organisms comprise an organized array of individual cells surrounded by a meshwork of biomolecules and fluids. Cells have evolved various ways to communicate with each other, so that they can exchange information and thus fulfil their specified and unique functions. At the same time, cells are also physical entities that are subjected to a variety of local and global mechanical cues arising in the microenvironment. Cells are equipped with several different mechanisms to sense the physical properties of the microenvironment and the mechanical forces arising within it. These mechanical cues can elicit a variety of responses that have been shown to play a crucial role *in vivo*. In this review, we discuss the current views and understanding of cell mechanics and demonstrate the emerging evidence of the interplay between physiological mechanical cues and cell-cell communication pathways.

Keywords Mechanotransduction · Mechanosensitivity · Microenvironment · Force · Mechanics · Cells

Introduction

The richness and diversity of the cellular microenvironment has a profound impact on the physiology and function of living cells and organisms. In recent years, it has become

increasingly evident that cells must integrate and respond to a complex interplay of biochemical and physical signalling during many normal and disease processes (Baker and Chen 2012; Brown and Discher 2009; Butcher et al. 2009; Buxboim et al. 2010; Janmey and Miller 2011; Mammoto et al. 2012; Schwarz and Gardel 2012; Sukharev and Sachs 2012; Vogel and Sheetz 2006). The cellular microenvironment is well-known as possessing many biochemical cues, which regulate many cellular processes. However, the importance of the mechanical properties of the microenvironment and of the mechanical forces arising within it has only recently begun to be fully appreciated. The mechanical characteristics of the microenvironment have important impacts on a diverse number of cellular processes ranging from proliferation to transcription to organogenesis (Brown and Discher 2009; Kunda et al. 2008; Mammoto et al. 2012; Stewart et al. 2011). Although many advances have occurred in the past decade alone, the inherently physical nature of the cell was recognized soon after the invention of the microscope (Pelling and Horton 2008). Indeed, technological advancements at the micro- and nano-scale have led to many of the recent developments in this field. Quantitative microscopy, super-resolution imaging, local probe microscopy, microfabrication, microfluidics and the development of mechanically tuneable hydrogels have all played key roles in the elucidation of the function of mechanical forces and of the physical properties of the microenvironment during key physiological processes (Engler et al. 2006; Kim et al. 2009; Kurth et al. 2012).

Nevertheless, the mechanisms through which mechanical information is communicated between cells and the conversion of this information into biochemical signalling (and vice versa) are still under intense investigation. Moreover, not only are these pathways likely to be cell-type-specific but they are also likely to be highly dependent on the type of microenvironment in which a cell exists. For example, skeletal muscle precursor cells (myoblasts) are found within the

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surface of skeletal muscle fibres that comprise a dynamic microenvironment constantly undergoing mechanical stretch and compression (McCullen et al. 2010) and the complex process of myogenesis also occurs optimally in a manner dependent on the elasticity of the surrounding tissue (Carmignac and Durbeej 2012; Engler et al. 2004). Notably, most of the mutations that result in muscular dystrophy specifically affect the ability of myoblasts to maintain proper biochemical and physical contact with the microenvironment (Carmignac and Durbeej 2012). Conversely, endothelial cells in blood vessels are exposed to stretch and compression during pulsatile blood flow but are also exposed to high amounts of fluid shear stress, which, among other effects, directs cellular alignment and remodelling processes that can become disrupted during the progression of disease (Shi and Tarbell 2011). This microenvironment (Fig. 1) is highly distinct from the muscle tissue microenvironment of myoblasts: myoblasts are found embedded in a three-dimensional tissue, surrounded by skeletal muscle fibres, whereas endothelial cells exist at an effectively two-dimensional interface with fluid. These two examples illustrate the diversity of cellular microenvironments that has led to numerous adaptations and strategies by which cells can sense and respond to mechanical cues: mechanosensation and mechanotransduction, respectively. The biomedical and biomechanics communities have generated a large body of knowledge on the manner in which mechanical forces are developed by and affect tissues, organs and the body at the macroscopic level. More recently, researchers have begun to examine the effect of mechanical forces on single cells, at the molecular level. These studies have been facilitated by the advent of more accessible tools in the Life Sciences and by the interdisciplinary exchange between cell biologists, physicists, material scientists and engineers. In this review, we begin by discussing physical microenvironmental parameters that are relevant to cells in vivo. We will then describe the way that these parameters vary in distinct types of cellular microenvironments. Following this, we will discuss the known mechanisms by which cells are able to sense mechanical cues and to integrate this information into a cellular and multi-cellular response. Finally, we will examine the influence that mechanical forces and changes in the mechanical properties of the microenvironment ultimately have on cell behaviour and explore whether cell-cell communication pathways are mechanosensitive. Our goal is to provide a relatively broad glimpse of the available literature on this emerging interdisciplinary field in order to present details and examples that are relevant in cell and tissue research.

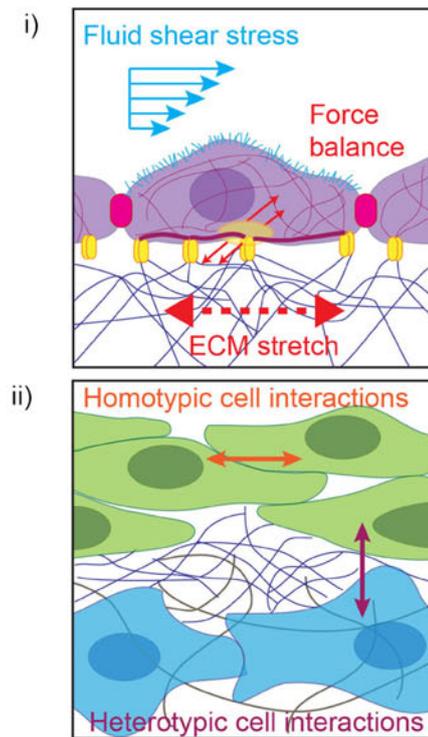
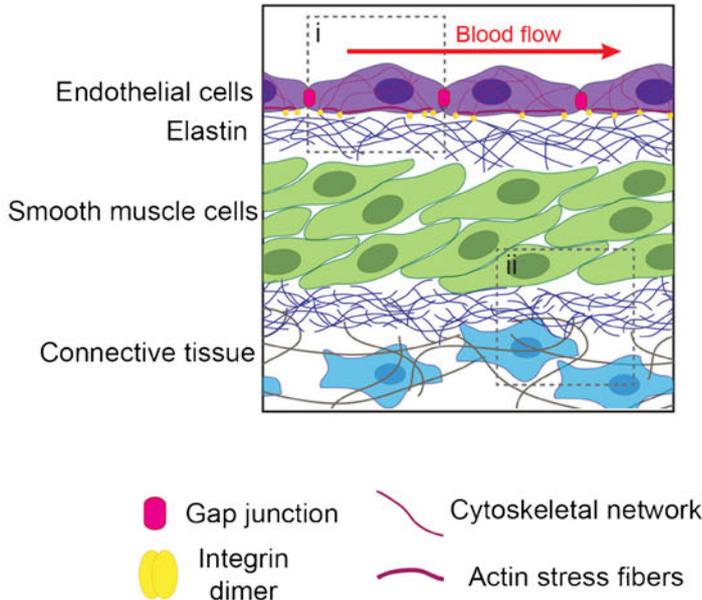
Physical parameters of the cellular microenvironment

Before diving into the biology of mechanotransduction and mechanosensitivity, we need to distinguish and define the

Fig. 1 The cellular microenvironment and its physical properties. **a** Cross-section of a typical blood vessel. Endothelial cells (EC) line the vessel and are constantly exposed to pulsatile blood flow. Smooth muscle cells (SMCs) are situated below the EC monolayer and are responsible for the generation of contractions and for regulating the diameter of the blood vessel. The outermost layer of the blood vessel is populated by fibroblasts, which are responsible for modulating the extracellular matrix (ECM) of this heterogeneous tissue. The various cell populations are separated by unique layers of deposited ECM proteins of diverse and variable composition. Note that cells in this example experience various mechanical microenvironments, e.g., endothelial cells experience shear stress from blood flow and the rigidity of the underlying elastin-dominated ECM and cell-cell contacts. The SMC and the fibroblasts are situated in the interstitium of the blood vessel and, therefore, the shear stress that they experience is only that of the interstitial flow, which is much lower than that of the blood flow (based on Shi and Tarbell 2011). **b** Representation of uni-axial tension applied to a flexible substrate (*i*). Uni-axial (along one direction) stress (force/unit area) can be applied to stretch a substrate (PDMS polydimethylsiloxane) upon which adherent cells are cultured. This tensile stress causes the substrate and cells upon it to experience strain (i.e., an elongation in the direction of the load). In an attempt to balance the pulling force of the substrate, the cells also respond by increasing what are known as traction forces, in the opposite direction of the substrate pull (see *inset*). The amount to which both the substrate and cell stretch is dependent on their respective Young's moduli (a material property that determines how much the materials deform in response to a known stress). Representation of shear flow induced on an adherent cell by using a microfluidic device (*ii*). By applying pressure, the fluid (media, for example) is forced to flow in the microfluidic channel causing shear stress to act on the cell. No fluid motion occurs where the fluid contacts the sides of the microfluidic device, thus causing a velocity gradient with maximum flow velocity at the centre of the channel. The magnitude of shear stress acting on the cell is dependent on both the region in which it lies within the velocity gradient and the viscosity of the fluid. Representation of an atomic force microscope (AFM) cantilever applying a force above a cell nucleus (*iii*). By applying a known force to the cell, the deflection of the cantilever can be measured in order to determine the resistance of the cell to deformation. This is a commonly employed method to determine Young's modulus of a cell and it requires the use of fitting this force-deformation data to the Hertz model as shown. Parameters of the cantilever, such as its stiffness, k (a geometric parameter related to the material properties E and the moment of inertia I [$k=EI/L$]) and Poisson's ratio ν , must be known. Poisson's ratio is a material property (the ratio of the strain in the direction transverse to the loading axis and along the axis of applied stress). This material parameter must be estimated for the cell and is usually set as $\nu=0$ being related to an incompressible material

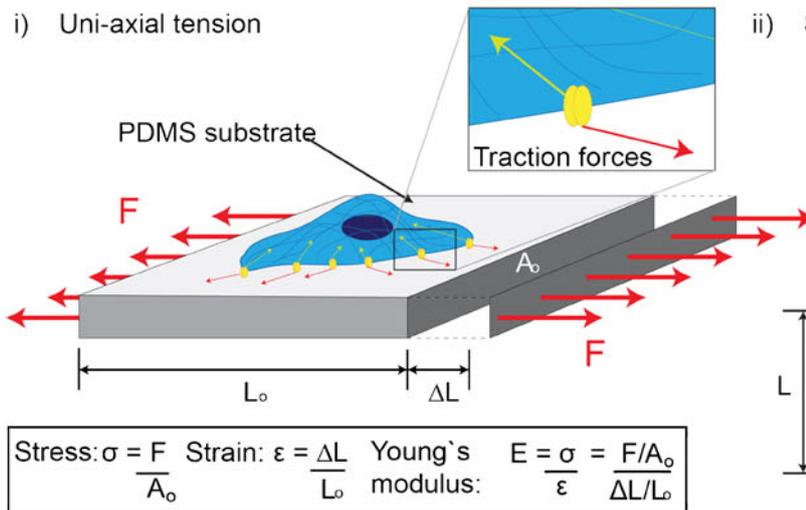
critical mechanical properties and forces that are relevant to cells in vivo and in vitro at the macro- and micro-scale. At the tissue and cellular levels, these forces can persist as stresses and strains and, more likely, as complex combinations of the two. Stress is defined as the ratio of an applied force to the area upon which it is acting. In turn, a stress causes material deformation (strain). Strain refers to the relative elongation or displacement of a material in response to an applied force (Fig. 1). Most materials behave in this manner and are known as elastic materials, i.e., they tend to elongate linearly, proportional to the magnitude of applied force, until they reach a certain yielding threshold. Cells typically respond to stress via strain. For example, the stretching of the extra-cellular matrix (ECM) can result in

a Physico-mechanics of cells and tissues

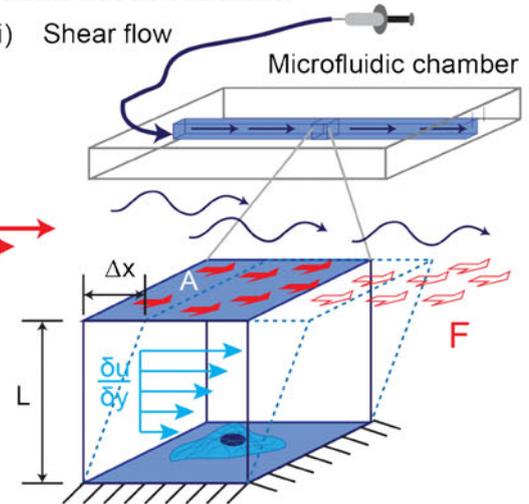


b Methods and mechanical parameters relevant to cell and tissue research

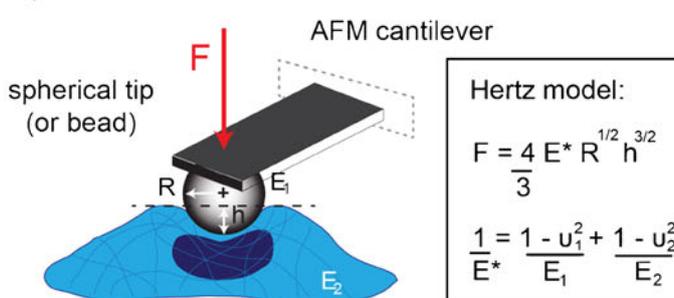
i) Uni-axial tension



ii) Shear flow



iii) AFM measurement of E



Shear stress: $\tau = \frac{F}{A} = \mu \frac{\delta u}{\delta y}$
 (↑ Viscosity, ← velocity gradient)

Shear strain: $\gamma = \frac{\Delta x}{L}$

Shear modulus: $G = \frac{\tau}{\gamma}$

an elongation of actin stress fibres, the tension of which can be recovered following the cessation of the substrate stretch (Mizutani et al. 2004). Subcellular strains, such as deformations of the plasma membrane (Farsad and De Camilli 2003), or the bending of primary cilia (Jensen et al. 2004) have also been observed.

At the macro-scale, tissues can withstand various amounts of force. Bone, one of the strongest materials of the body, can withstand high compressive forces (~170 MPa) and relatively weaker tensile forces (~100 MPa; Cowin 1998; Rho et al. 1998) as networks of cells are aligned in the direction of the recurrent force. Soft tissues, on the other hand, are much more extensible than bone, e.g., muscle tissue can withstand a large range of compressive and particularly tensile forces. At the cellular level, however, much smaller forces are at play, i.e., those in the pico- to nano-Newton range. Since most cells are typically on the order of about 10 microns, these forces are more acquiescent to their size. For example, the single stroke of a myosin II motor has been determined by optical tweezer measurements to be ~5 nm (Ruegg et al. 2002).

As will be discussed in more detail later, cells can transmit forces in order to adapt to or change their local microenvironment. Traction forces generated by cells are transmitted along stress fibres and are directed through focal adhesion sites to the ECM. They have been shown to be in the order of 10^2 to 10^5 dyn/cm² (~10 to 1,000 Pa). For example, the magnitude of cell traction forces have been shown to be sufficient enough to cause substrate wrinkling and fluorescent bead displacement, as measured during in vitro experiments (Beningo and Wang 2002; Fig. 1).

Although subcellular components drive many intracellular forces, one aim of mechanobiology is to elucidate the mechanical properties of a cell as a singular unit. At the macroscale, this coarse-graining approach can be sufficient for determining overall mechanical properties; however, we should note here that it is often more difficult to gain an idea of the physical properties of a cell, as cells are spatially and temporally heterogeneous (Pelling and Horton 2008; Pelling et al. 2009). The most common mechanical properties measured by modern biophysics tools include: Young's (elastic) modulus (E), stiffness (k), shear (or rigidity) modulus (G) and viscosity (η). Although other parameters are used to describe the behaviour of a material, usually only two are required to describe its elastic properties: typically E and G are measured experimentally (Fig. 1). The most common measurement, Young's modulus, is a measure of the elasticity of a material (Fig. 1) and relates the stress (N/m² or Pascals (Pa) units of pressure) applied to a material to the amount of strain (dimensionless ratio of relative deformation) that it experiences. The Young's modulus simply reveals the extent to which a material is deformable. Elasticity depends on the stress applied and should not be confused with material stiffness, which is a measure of rigidity. Rather than depending on

stress, stiffness is a ratio of the force applied and the resulting displacement/deformation in that same direction. The shear modulus, similar to E , denotes the ratio of stress applied to the resulting strain; however, this case applies in a shearing velocity gradient. i.e., under fluid flow (Fig. 1). When shear forces are involved, materials undergo shear strain. For example, when an adherent cell experiences fluid flow on its apical surface, the cells experience a shear stress and can undergo shearing deformations resulting in their reorganization (Ng et al. 2005).

Distinct cellular microenvironments

Cells are found in vastly different two-dimensional and three-dimensional (3D) microenvironments depending on the tissue type (Baker and Chen 2012). As noted above, the cellular context of myoblasts and endothelial cells is highly distinct. Endothelial cells experience high shear stress when examined from their apical side, whereas the same endothelial cells contact other cells within the underlying interstitium when pictured from their basal side (Fig. 1). The "interstitium" is a term used to describe the physical and chemical features of the intercellular space in tissue (Wiig and Swartz 2012) and consists in two components at the cellular interface: the ECM, which can be viewed as a meshwork of proteins and glycosaminoglycans and the interstitial fluid, which permeates the porous ECM and cellular network (Wiig and Swartz 2012). The ECM gives rise to several mechanical parameters that play a critical role in cell biology, including matrix elasticity, mechanical stress (cell-induced stretch and compression) and mechanical strain (deformation of cells). Conversely, the interstitial fluid also plays an important role in providing physical cues to which cells are sensitive, including shear stress and mechanical pressure driven by local fluid dynamics. Finally, the cell-cell interface forms a further distinct feature of the local microenvironment. These three particular interfaces broadly define the general physical and biochemical characteristics of the cellular microenvironment. Here, we will focus on the general description of the microenvironment by describing these three important cellular interfaces.

ECM interface

All cells in tissues are surrounded by a meshwork of proteins (e.g., collagen, fibronectin, laminin) and glycosaminoglycans (e.g., heparan sulfate, syndecan, glypican) collectively referred to as the ECM (Hynes 2009). The ECM has two key roles in the maintenance of multicellular organisms. First, it acts as a physical scaffold providing a structural framework in which tissue-specific cells are interspersed and, as such, the

ECM is a defining feature of the physical properties of a given tissue (Cox and Epler 2011). Cells have evolved various mechanisms to sense the physical characteristics of the ECM (stiffness, strain, rigidity; Geiger et al. 2009). Cellular mechanosensation is therefore central in various cell-fate decisions: development, homeostasis and cell migration (Farge 2011; Ghajar and Bissell 2008). Second, cells are influenced by the biochemical nature of the ECM. The different types of constituent proteins in the ECM will bind to different cell surface receptors (integrins) and thus selectively influence only the cell types that express those specific receptors (Kass et al. 2007). In addition, the ECM serves as an integrative extracellular signalling station. For example, the binding of fibroblast growth factor 2 to heparan sulfate (constituent of the ECM) is necessary for the fibroblast-to-myofibroblast transition and the activation of endothelial cells during wound healing (Rapraeger et al. 1991), as reviewed in Schultz and Wysocki (2009). Notably, a bidirectional interdependence exists between the physico-biochemical properties of the ECM and the cell phenotype: cells secrete and remodel the ECM, whereas the latter in turn signals back to the cells and influences their response and fate (Cox and Epler 2011).

The importance of the physical properties of the ECM has been illustrated in many studies. For example, Engler et al. (2006) have demonstrated that mesenchymal stem cells can assume different cell lineages, solely dependent on the rigidity of the substrate on which they are cultured. Soft matrices (0.1 and 1 kPa) favour neurogenic differentiation, harder matrices (11 kPa) myogenic differentiation and rigid matrices (34 kPa) osteogenic cell differentiation (Engler et al. 2006). Following this study, many publications have aimed to understand the molecular details of the interactions between the ECM physical properties and stem cell differentiation with the aim of generating tissues *ex vivo*, a major goal in regenerative medicine (Ciapetti et al. 2012). Moreover, time-dependent changes in matrix elasticity have also been demonstrated to have a key role in directing stem cell fate (Guvendiren and Burdick 2012; Young and Engler 2011). On the other hand, Weaver and colleagues have demonstrated that the culturing of a non-malignant cell line model (MCF10a) on ECMs with stiffness moduli mimicking those of breast cancer (approximately 1,200 Pa) lead to a change of the cell phenotype to resemble malignancy (Paszek et al. 2005). Importantly, this ECM-based change of the phenotype is linked to an increase in focal adhesion size, a higher expression of vinculin, a higher phosphorylation by focal adhesion kinase, more prominent stress fibres and an elevated extracellular-signal-regulated kinase (ERK) activation (Paszek et al. 2005).

More recently, ECM stiffness has been shown to be crucial for determining cell behaviour in 3D. For example, fibroblasts display a lamellipodia-based migration when cultured in 3D collagen matrices, whereas cells grown in

cell-derived matrices migrate via lobopodia, cellular protrusions that are associated with a mesenchymal cell phenotype (Petrie et al. 2012). Importantly, whether cells migrate via lamellipodia or lobopodia is dependent on the Young's modulus and the strain-hardening properties of the 3D matrix. This demonstrates the way in which the physical properties of the microenvironment of the cell can "instruct" the cell towards different modes of motility. Moreover, this study demonstrates that pathologies such as cancer metastasis are crucially dependent on the simple physical properties of the cellular microenvironment (Petrie et al. 2012).

Importantly, apoptosis also seems to be regulated by matrix stiffness (Pelling et al. 2009). During early stages of apoptosis (after 120 min of staurosporine treatment), a clear mechanical breakdown in cell elasticity was observed; the Young's modulus of the cell decreased from 5.1 kPa to 1.6 kPa after the initiation of apoptosis (Pelling et al. 2009). Importantly, this breakdown of cells stiffness was rescued on soft substrates (18 kPa and 35 kPa), a result that was speculated to be attributable to a matrix-dependent modulation of caspase activity (Pelling et al. 2009).

The biomedical importance of the stiffness of the ECM matrix is also exemplified in a broad range of diseases collectively described as "fibrotic" diseases, i.e., pathologies in which there is a misregulated ECM homeostasis. Such diseases include pulmonary fibrosis (Suki and Bates 2008) and cardiovascular disease (Berk et al. 2007). A unifying feature of all these diseases is the progressive stiffening and hence loss of elasticity of the fibrotic tissue, both of which are detrimental for tissue function. For example, cardiovascular disease is associated with the progressive stiffening of blood vessels attributable to an altered balance of the main constituent of the ECM of the blood vessel wall (collagen and elastin, Fig. 1; Zieman et al. 2005). A well-studied tissue with a drastic change in mechanical properties under pathological conditions is the ascending aorta (Choudhury et al. 2009). Several studies involving passive and active stretch experiments indicate a progressive stiffening of diseased aortas accompanied by an increased production of collagen and decreased production of elastin (Choudhury et al. 2009; Rouleau et al. 2012; Tremblay et al. 2010).

Interstitial fluid interface

The interstitial fluid consists of water and various solutes necessary for tissue homeostasis. Interstitial fluid flow results from the filtering of blood from the capillaries and its passage through tissue and drainage into the lymphatic system. The interstitial fluid is the physical medium in which secreted signalling molecules are transported between secreting cells and target cells (paracrine cell-cell signalling) and also serves as a transport pathway of nutrients, antigens and cytokines from blood to tissue (Wiig and Swartz 2012).

Importantly, the composition of the ECM is of crucial importance in determining the effective hydrostatic pressure exerted by interstitial fluid flow; for example, certain constituents, such as collagen, can bind water more efficiently and thus influence this pressure. Thus, interstitial fluid flow is not well-understood, mainly because the shear stress that cells are actually exposed to is the result of the complex interaction between the interstitial fluid flow and the non-uniform ECM structure (Swartz and Fleury 2007). Simulation experiments examining physiologically relevant fluid flows through materials of various porosity predict that cells are exposed to a much higher local shear stress by the interstitial fluid than expected, especially because of the variation of the ECM composition and the complexity of the intimate interaction between cells and the ECM (Pedersen et al. 2007). Estimated rates of interstitial fluid flow in the wall of blood vessels are 10^{-5} to 10^{-6} cm/s and the resulting shear stress is predicted to be in the order of 1 dyn/cm² (Shi and Tarbell 2011; Swartz and Fleury 2007). On the other hand, in non-vascular and lymphatic tissue, cells experience much lower interstitial-fluid-flow-induced shear: <0.1 dyn/cm² (Swartz et al. 1996; Wiig and Swartz 2012).

How do cells respond to shear stress experienced from fluid flow? Endothelial cells, for example, respond to higher shear stresses by losing their cell-cell adhesions and becoming more contractile (Ting et al. 2012). This is important both in normal physiological homeostasis (neutrophil transmigration from blood to tissue) and in disease (tumour invasiveness). Simulated superphysiological flow rates (mimicking interstitial flow in aberrant situations) have also been demonstrated to induce a fibroblast-to-myofibroblast transition (Ng et al. 2005). Importantly, these studies reveal a remodelling of the ECM and also a realignment of the myofibroblast-phenotype cells along the direction of flow (Ng et al. 2005).

Cell-cell interface

In addition to forces stemming from the ECM or interstitial fluid, cells also experience forces from neighbouring cells through their direct contact (Johnson 2011). Desmosomes and adherens junctions are based on cell-surface proteins (cadherins) that are packed in a cis-orientation between connected cells, whereas tight junctions and gap junctions, on the other hand, consist of more complex arrays of proteins (claudins, occludins and connexins) that form juxtaposed assemblies between neighbouring cells and form channels for the intercellular transfer of small molecules (Franke 2009; Green et al. 2010). These physical connections between cells play an important role in force transmission and in defining the local material properties of the cells (Johnson 2011). Cell contractility is a major mechanism through which cells interact and is mainly driven by the actomyosin component of the

cytoskeleton (Levayer and Lecuit 2012). Importantly, synchronous multicellular contractility is often employed to drive tissue morphogenesis. For example, actin cables constitute supracellular actomyosin contractile structures formed by epidermal cells. During the embryogenesis of both *Drosophila* and *Caenorhabditis elegans*, these supracellular actin cables are regarded as the main driving force for dorsal closure (Kiehart et al. 2000; Simske and Hardin 2001). In addition to the synchronous large-scale contractility of actin cables, cells adjacent to the leading edge of the closing epidermis (amnioserosa cells) have been demonstrated to produce a further contractile force critical for dorsal closure (Solon et al. 2009). Another unexpected source of the physiological mechanical force produced during development has been demonstrated in vivo during *Drosophila* embryogenesis by Toyama et al. (2008): apoptosis of a selected set of cells contributes mechanical forces necessary for the process of dorsal closure. Thus, apoptosis serves not only as a means to eliminate unnecessary cells during development but also provides mechanical cues for the further development of the organism (Toyama et al. 2008).

Sensing of mechanical cues (mechanosensation)

Research has led to the understanding that cells sense physical forces and the mechanical properties of the microenvironment via several distinct mechanisms and structures (Eyckmans et al. 2011; Vogel and Sheetz 2006). Although not exhaustive, we will describe several major cellular structures involved in mechanosensation and mechanotransduction processes, namely, focal adhesions and integrins, the cytoskeleton, the nucleus, mechanosensitive ion channels and the primary cilium.

Integrins and focal adhesions

Integrins are evolutionary conserved cell-adhesion receptors that mediate the interaction of cells with the ECM (Barczyk et al. 2010). There are 24 heterodimers consisting in one α and several types of β chains. The α chain is extracellular and is able to bind selectively to distinct motifs of the ECM (RGD sequences [fibronectin], laminin and collagen-specific sequences; Barczyk et al. 2010). The second constituent of integrin receptors are the β subunits, which translate a conformational change of the activated α -subunit across the membrane and communicate this to the actomyosin cytoskeleton (Geiger et al. 2009). Thus, upon binding to the ECM, the integrin heterodimer experiences a conformational change leading to an active state of the intracellular tail of the β chain. The activation is also dependent on the binding of two types of proteins: talins and kindlins (Geiger et al. 2009). In their activated state, integrins have an improved binding affinity

to the F-actin cytoskeleton (Geiger et al. 2009) and act as a positive feedback loop by activating more integrins and forming clusters of activated integrin moieties. This cascade effect is followed by the further recruitment of proteins, such as vinculin and focal adhesion kinase (FAK), which, in turn, leads to the formation of F-actin-rich stress fibres (Geiger et al. 2009). This complex array of proteins (activated integrins, talins, vinculin, FAK, etc.) is referred to as a focal adhesion and, in its mature form, represents the main link between the ECM and the contractile acto-myosin system inside the cell (Geiger et al. 2009). Indeed, most of the mechanical forces from the ECM are transmitted to the cell via integrin-based focal adhesions. The reverse is also valid: the inherent contractility of cells (both muscle and non-muscle) is transmitted to the ECM via focal adhesion complexes (Kirmse et al. 2011).

The cytoskeleton

The cytoskeleton consists in three major components: filamentous actin (F-actin; 7 nm wide), microtubules (MTs; 25 nm wide) and intermediate filaments (IFs; 8–12 nm wide; Fletcher and Mullins 2010). All three components are dynamic filamentous biopolymers composed of arrays of monomers with specific polymerization rates. F-actin and MTs are assembled in a head-to-tail fashion by repeating monomer subunits, thereby conferring a strict polarity on the resulting filament. This polarity is also reflected in the dissimilar polymerization rates of both ends of the filament: the fast growing end is referred to as the plus end and the more slowly growing end as the minus end.

In contrast to F-actin and MTs, IFs are assembled in a head-to-head order and therefore lack polarity. The latter probably explains the surprising lack of interest of the cell biological community to study IFs. Only recently have IFs been shown to be able to participate in signalling, e.g., by binding to 14-3-3 proteins (Kim et al. 2006). Nevertheless, IFs serve important mechanobiological functions. For example, *in vitro* and *in vivo* studies demonstrate that IFs have a comparatively low initial stiffness but extremely high elasticity (Fudge et al. 2003; Kreplak et al. 2008) and have emerged as crucial players in mechanotransduction. Vimentin IFs have been shown to associate with focal adhesions via β -3 integrin and this β -3-mediated adhesion requires intact vimentin IFs (Bhattacharya et al. 2009). Importantly, lamins are a class of IFs that provide structural support of the nucleus and participate in DNA replication, transcription and repair (Dahl et al. 2008). The mechanobiology of MTs, on the other hand, is better understood than that for IFs, mainly because of the availability of specific drugs that disrupt the MT cytoskeleton and also because of the critical importance of the MT cytoskeleton in cell division. For example, MTs play a crucial role in the mechanosensing of ECM stiffness via its interaction

with focal adhesions and the actomyosin cytoskeleton (Kaverina et al. 2002; Myers et al. 2011). In addition, changes of stress fibre dynamics after local mechanical force application has been shown to be dependent on an intact MT cytoskeleton (Guolla et al. 2012).

F-actin has been recognized as the major regulator and transducer of mechanical stimuli to cells (Schwarz and Gardel 2012). It can be organized in several different ultrastructures, of which filopodia and lamellipodia represent primary organelles of cell motility (Mejillano et al. 2004). Stress fibres, on the other hand, consist of anti-parallel-oriented actinin-cross-linked F-actin bundles between which arrays of myosin II bundles are bound (Tojkander et al. 2012). Myosin II is an F-actin-based motor able to exert force (tension) towards the plus end of actin filaments upon ATP hydrolysis and the resulting tension allows for the translocation of F-actin bundles in stress fibres in an anti-parallel fashion (Ciobanasu et al. 2012). The net result is a shortening of the stress fibre, ultimately leading to contractility, which plays a major role in the response of the cell to changes in its microenvironment (Tojkander et al. 2012). Cells cultured on ECM substrates of variable elastic moduli display different organizations of their underlying stress fibres (Rodriguez et al. 2004; Zemel et al. 2010) leading to the modulation of the amount of force that a cell can exert on the matrix. For example, cells are generally observed to exert stronger traction forces on stiffer substrates and weaker traction forces on softer substrates (Wang 2009). The initial force-sensing mechanism is thought to lie in the interaction between integrin receptors in the lamellipodium/filopodium and the substrate (Geiger et al. 2009). Integrin receptors are bound to the F-actin cytoskeleton via talin (Geiger et al. 2009). As F-actin in filopodia and lamellipodia experience a constitutive retrograde flow, integrin receptors experience a surface retrograde backwards movement. In the event of an attachment to a component of the ECM by the α -chain subunit, the integrin dimer experiences a pulling tension between the F-actin network (via talin) at one end and the ECM at the other (Geiger et al. 2009). This leads to a conformational change in the β -chain subunit of the integrin dimer, subsequently resulting in an activated state of the receptor and recruitment of paxilin and FAK (Geiger et al. 2009). This transient molecular assembly is referred to as “nascent adhesion” (Ciobanasu et al. 2012). The downstream signalling pathway that follows nascent adhesion involves the further recruitment of adhesion-specific proteins, such as vinculin and zyxin, which function to strengthen the interaction between the integrin receptor and the F-actin cytoskeleton. Importantly, this process is dependent on new F-actin polymerization as demonstrated by the necessity of the F-actin-nucleating formin mDia1, even though the presence of mDia at the focal complex still remains to be shown (Tojkander et al. 2012).

Transition from this transient adhesion into a mature focal adhesion depends on myosin II contractility (Tojkander et al. 2012). Importantly, a recent proteomic study of purified focal adhesions by Kuo et al. (2011) has shown that the specific inhibition of myosin II activity results in the loss of half of the focal-adhesion-specific proteins and a concomitant enrichment of molecular players involved in lamellipodia regulation.

Mechanosensitive integral membrane proteins

Since the plasma membrane is the outermost interface between the cell and its physical microenvironment, mechanical stimulation of the cell is inevitably first sensed at the plasma membrane. Therefore, cells have evolved several ways to sense and transduce local changes in the mechanical properties of the plasma membrane (Sukharev and Sachs 2012). In the following, we discuss three classes of mechanosensitive integral membrane proteins positioned at the forefront of local mechanosensitivity: mechanosensitive ion channels, transient receptor potential cation channels and mechanosensitive G-protein-coupled receptors (GPCRs).

Mechanosensitive (also “stretch-activated”) ion channels represent transmembrane protein assemblies, which are able to sense changes in plasma membrane tension by changing their permeability to ions (Fig. 2; Haswell et al. 2011; Sachs and Morris 1998). Importantly, these channels are omnipresent in all species and all cell types. A large amount of knowledge about the structure, function and regulation of mechanosensitive ion channels has been gained by the characterization of the MscL and MscS channels of *Escherichia coli* (Haswell et al. 2011). For eukaryotic cells, potassium and sodium voltage-gated channels have been the lead examples of mechanosensitive ion channels (Hamill 2006). In the case of potassium voltage-gated ion channels, the solving of their crystal structures (Long et al. 2005) has revealed that the so-called S4 transmembrane domain is the main gating sensor, which is both voltage- and membrane-tension-sensitive (Krepkiy et al. 2009).

Transient receptor potential (TRP) channels represent a large family of cation-permeable membrane channels described within the past decade (Nilius et al. 2007). Several of its members display mechanosensitive gating, e.g., TRPC1, TRPV2, TRPV4, TRPP2 and TRPA1 (Nilius et al. 2007). A direct example of the means by which mechanical force induces the opening of TRP channels was recently provided by Matthews et al. (2010), whereby β 1-integrin-coated magnetic beads and magnetic field gradients were used to apply brief (500 ms) pulses of tensional force on cells resulting in a fast Ca^{2+} intracellular influx attributable to the activation of TRPV4.

GPCRs are a class of integral membrane proteins that are of central importance in canonical signal transduction. The

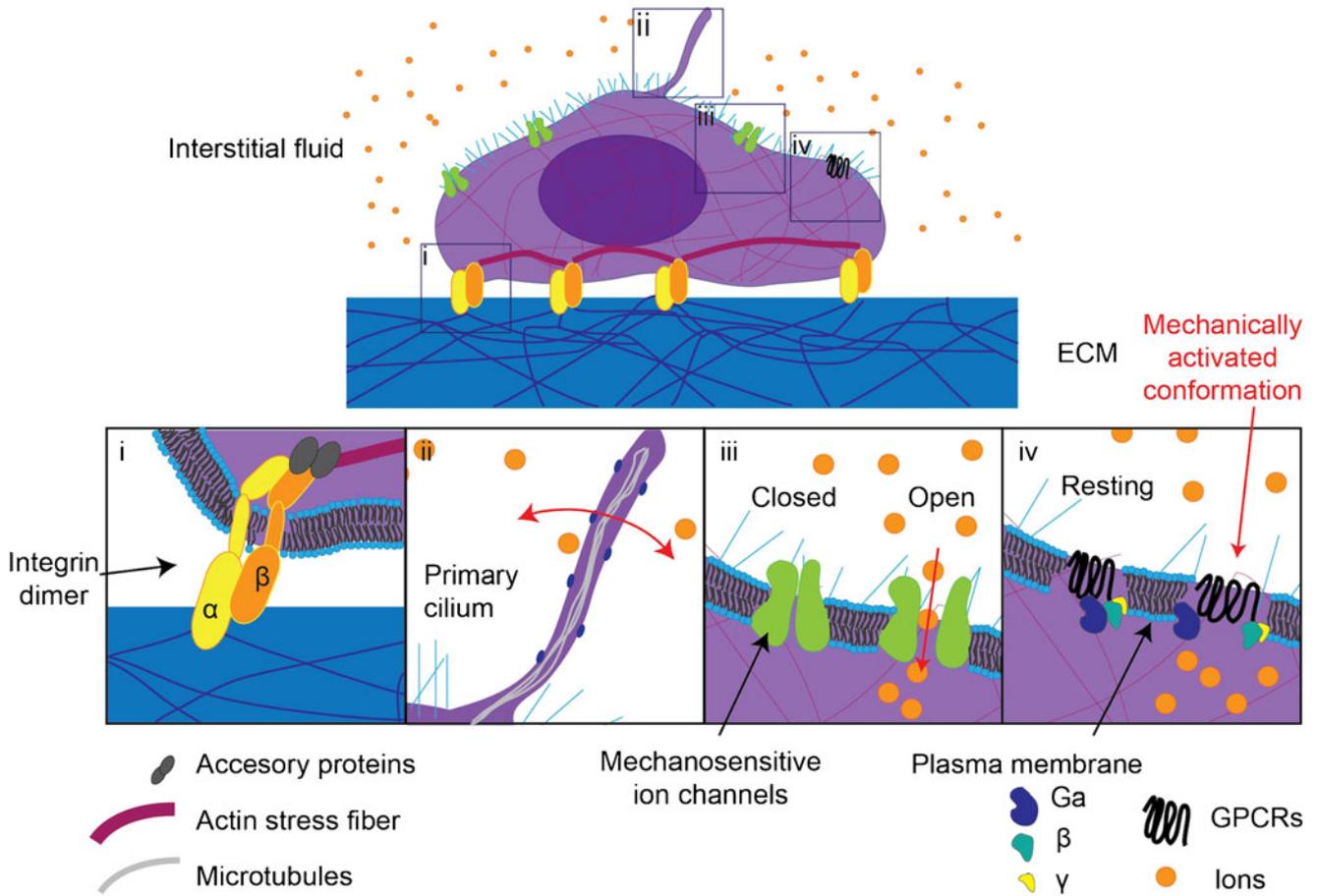
Fig. 2 Mechanosensation and mechanotransduction pathways. **a** The main primary mechanosensing pathways. *i* Focal adhesions are complex multi-protein complexes, which connect the internal cytoskeleton via the F-actin to the extracellular matrix (ECM). *ii* The primary cilium is a singular protrusive organelle situated at the apical surface of most cells in the body. *iii* Cilia are microtubule-based and are equipped with mechanosensitive ion channels. *iv* Mechanosensitive integral membrane proteins are the primary moieties that are able to detect changes in plasma membrane tension. Among the best characterized are mechanosensitive ion channels, which change their ion permeability upon membrane tension changes and mechanosensitive G-protein-coupled receptors (GPCRs), which can assume a mechanically-induced active conformation in addition to agonist-bound activation. **b** The main mechanotransduction intracellular pathways are summarized (*Src* a proto-oncogene tyrosine-protein kinase). Note that the immediate response to applied force is usually a modulation of the two primary second messengers: Ca^{2+} and cAMP. Downstream of this immediate response are changes in kinase activity (protein kinases A and C, extracellular-signal-regulated kinase, etc.) and the activation of transcription factors (myocardin-related transcription factor-A, cAMP response element-binding protein). Ultimately, these intracellular genetic and biochemical signals result in an integrated cell behavior response

accepted model of their function is that a GPCR-specific agonist binds to the extracellular part of the receptor leading to a conformational change in the protein complex. This results in the uncoupling of the G_{α} and $G_{\delta\gamma}$ subunits, which then activate diverse intracellular signalling cascades (Oldham and Hamm 2008). During the past decade, GPCRs have also been shown to be mechanosensitive, i.e., membrane stretch can lead to an active GPCR conformation without agonist binding (Fig. 2). This was first shown for the angiotensin II type 1 receptor (AT_1), which was activated in the absence of an agonist by a 20% stretch of cardiomyocytes and AT_1 -expressing HEK cells (Zou et al. 2004). There are many similar examples in the literature of GPCRs that display mechanosensitive activation (for a recent review, see Storch et al. 2012). The downstream effect of mechanical activation of GPCRs can be broad, depending on the type of GPCR and the G-proteins that are activated intracellularly (e.g., $G_{q/11}$, G_s). The mechanical activation of GPCRs and the subsequent activation of G_s alpha protein is also the most likely upstream mechanism that leads to altered cAMP levels after mechanical stimulation (Meyer et al. 2000). Thus, GPCRs seem to be a polymodal integration station for both biochemical and mechanical signal transduction. We are only just starting to understand the details and the broad flexibility endowed to cells by GPCRs.

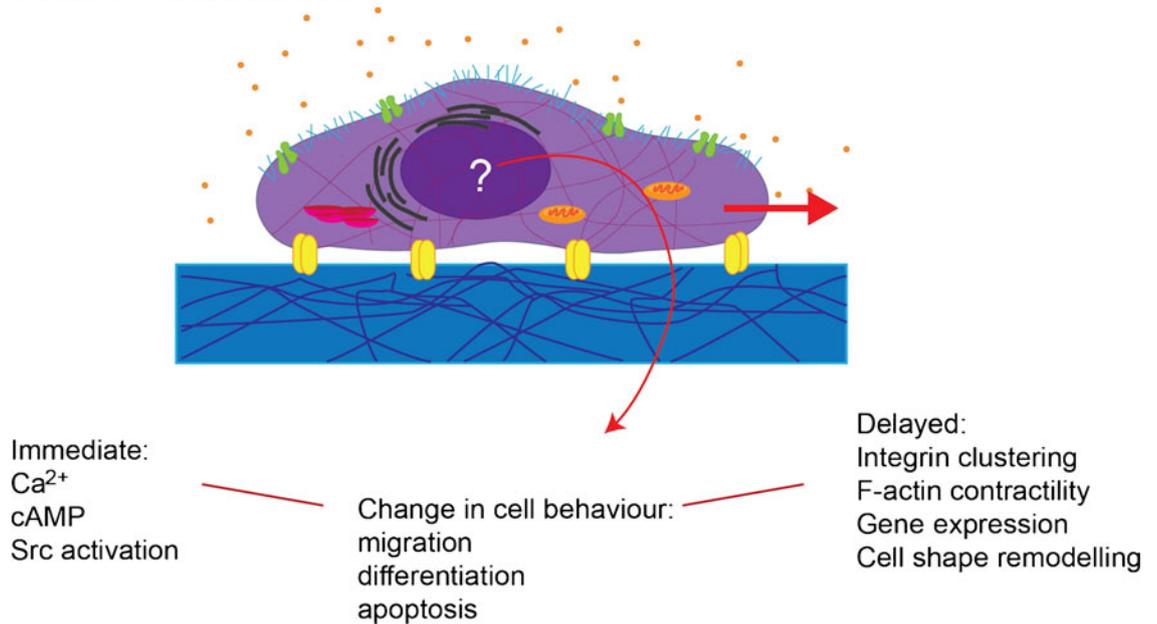
Primary cilia

Primary cilia are MT-based singular protruding extensions present on the apical surface of all cells in the body (Hoey et al. 2012). They have just recently received the attention due to them and, as is now clear, these organelles serve as a major cellular mechanosensor (Hoey et al. 2012). The primary cilium acts as a flexible “antenna” that can deform upon fluid flow shear stress (Fig. 2; Praetorius and Spring

a Mechanosensing pathways



b Mechanotransduction pathways



2003). One way in which signal transduction proceeds at the primary cilium might be via mechanosensitive ion channels

(polycystin-based channels) and an influx of regulatory cations (Fliegauf et al. 2007). The ciliary membrane is a

continuation of the plasma membrane (Rohatgi and Snell 2010) but there are indications of physiological differences between the two. Electron microscopy studies have revealed a unique structure known as the ciliary “necklace” at the base of the cilium (Gilula and Satir 1972). The molecular identity of the necklace remains a matter of research but, based on immunostaining studies, it displays an enrichment of ion channels and receptors (Zhang et al. 2004). Importantly, the disruption of the MT network in epithelial cells while maintaining the primary cilium results in a decrease in the induction of the shear response marker KLF2 after an applied fluid flow (Hierck et al. 2008). The mechanisms involved in the homeostasis and mechanosensing of the primary cilium are just beginning to emerge (Hoey et al. 2012). For example, the primary cilium length is regulated by the balance between the two basic second messengers, namely, Ca^{2+} and cAMP (Besschetnova et al. 2010). Thus, despite the rapidly accumulating literature on the subject, the role of the primary cilium in mechanotransduction and mechanosensation pathways is yet to be fully elucidated.

Responding to mechanical cues (mechanotransduction)

Cells respond to changes in the physical properties of the microenvironment or the influence of physical force in several ways, including changes in second messenger signaling (intracellular Ca^{2+} , cAMP), internal remodelling of the cytoarchitecture and changes of gene expression (Eyckmans et al. 2011). Importantly, cellular responses to mechanical signals are likely to be cell-type- and stimulus-type-specific. Indeed, these pathways form a mechanotransduction response phenotype. In the following sections, we discuss several cellular response scenarios, as viewed from the perspective of internal cellular changes, and the overall response in cell behaviour.

Secondary messenger signalling

A multitude of studies has demonstrated an increase of intracellular levels of Ca^{2+} after mechanical stimulation by shear flow in several cell types: mouse L cells (Grierson and Meldolesi 1995), medial collateral ligament and anterior cruciate ligament fibroblast (Hung et al. 1997) and rabbit tendon cells (Archambault et al. 2002). These modulations of intracellular Ca^{2+} are most probably attributable to the activation of mechanosensitive ion channels in the plasma membrane, as caused by the experienced shear flow. Intracellular increase of Ca^{2+} can also be accomplished by mechanically stimulating osteoblast (Charras and Horton 2002) and myoblast (Formigli et al. 2005) cells by means of an atomic force microscope (AFM).

Experiments with collagen-coated magnetic beads and magnetic fields have shown that up to 80% of fibroblasts also respond with an elevation of intracellular Ca^{2+} (Glogauer et al. 1995). Importantly, the F-actin cytoskeleton seems to play a cell-type-specific role in mechanically induced Ca^{2+} homeostasis: Cyt D pretreatment of cells before mechanical stimulation leads to an increase in Ca^{2+} amplitude in coated-bead stimulation (Glogauer et al. 1995), whereas mechanical stimulation via AFM-cantilever-attached microbeads shows no dependence on CytD treatment (Charras and Horton 2002). In addition to Ca^{2+} changes, cells are able to respond by modulating cAMP levels (Meyer et al. 2000). Thus, local mechanical stimulations of HT1080 cells leads to an increase of cAMP and a subsequent activation and recruitment of protein kinase A (PKA) regulatory subunits from the cytoplasm to sites of activated integrins (Whittard and Akiyama 2001). The latter indicates that cAMP and PKA activation are involved in both cell-cell and cell-substrate adhesion (Whittard and Akiyama 2001).

Remodelling of cytoarchitecture

As previously mentioned, the acto-myosin cytoskeleton reorganizes itself based on the stiffness of the underlying substrate (Hoffman et al. 2011). Presumably, this effect is attributable to the more efficient clustering of ECM-bound integrin receptors, which in turn leads to the recruitment of more focal adhesion proteins and a more potent downstream effect on their assembly and contractility (Geiger et al. 2009; Hoffman et al. 2011). Notably, no one-to-one correlation has been found between substrate stiffness, focal adhesion size and stress fibre organization (Bershadsky et al. 2006). More recently, Trichet et al. (2012) have demonstrated that, even though a linear relationship exists between the traction force exerted by cells and the focal adhesion area at a given substrate rigidity, focal adhesions of the same size could exert stronger traction forces on stiffer substrates. Based on the latter observation, the authors have speculated that the response of cells to rigidity probably involves a large-scale global mechanosensing mechanism, rather than a localized mechanism dependent on a single focal adhesion (Trichet et al. 2012).

In addition to changes in the physical properties of the microenvironment, the cytoskeleton also displays localized remodelling over short timescales (seconds to minutes) in response to localized mechanical stimulation. In a recent study, the F-actin cytoskeleton underwent highly anisotropic and localized deformation throughout the cell body following localized nano-Newton forces applied directly above the nucleus (Guolla et al. 2012). Moreover, the application of physical force caused an increase in acto-myosin contractility, which was quantified by measuring stretch and compression strain dynamics along actin stress fibres (Guolla et al. 2012).

Importantly, these deformation and strain dynamics were not observed in the absence of an intact MT cytoskeleton. Although the F-actin network is perceived as the main force-sensing component of the cytoskeleton, MTs and IFs are also influenced by mechanical stimuli: the localized application of nano-Newton forces above the cell nucleus also results in anisotropic viscous relaxation of the MT and IF networks (Na et al. 2008; Wang and Pelling 2012). These processes occur within seconds and are highly dependent on the physiological state of the cell (Pelling et al. 2009).

Response of the nucleus

The above description of the mechanics of the cytoskeletal network makes it evident that externally applied mechanical forces are dissipated via a complex and highly dynamic mechanical framework. In the following, we will describe some details of what is known about nuclear mechanics and responses to mechanical cues in the microenvironment. As previously mentioned, the inner membrane of the nucleus is lined with a class of IFs (lamins) that give it its structural stiffness (Dechat et al. 2008). Importantly, lamins mediate the binding of the nucleus to the cytoskeleton via an array of accessory proteins collectively referred to as the LINC complex (linker of nucleoskeleton and cytoskeleton; Crisp et al. 2006). Interestingly, the disruption of cytoskeletal elements has a variable effect on the nuclear shape and size. Depolymerization of F-actin leads to a decrease in nuclear size, whereas the inhibition of MT polymerization leads to an increase in nuclear size (Mazumder and Shivashankar 2010). This and other studies demonstrate that the shape of the cell nucleus in eukaryotic cells is dependent on actomyosin tension, whereas the MT cytoskeleton and chromatin exert compressive forces on and inside the nucleus, respectively.

Importantly, changes in nuclear morphology have been linked to both normal stem cell differentiation and several pathological conditions. For example, the nuclei of human adult stem cells are stiffer than the nuclei of human embryonic stem cells and the nuclei of human embryonic stem cells become six-fold stiffer as they terminally differentiate (Pajeroski et al. 2007). On the other hand, changes in nuclear shape, such as indentations, folds, undulations and dispersed heterochromatin, are routinely used as a marker for cancer detection (for a review, see Zink et al. 2004). In addition, a class of diseases caused by mutations in lamin genes (laminopathies) are characterized by a highly aberrant nuclear shape and ultimately changed nuclear mechanics (Dahl et al. 2008; Jaalouk and Lammerding 2009; Zwerger et al. 2011). Whether the resulting pathologies are the result of compromised and weakened nuclear mechanics, which would more easily be damaged under external stress, or of a purely gene regulatory defect is a matter of debate. As noted

recently, these two hypotheses are most probably not mutually exclusive (Zwerger et al. 2011).

Mechanical signal transduction to the nucleus via the cytoskeleton can be much faster than the conventionally described ligand-stimulated second-messenger-based pathways. This was demonstrated with the use of a proto-oncogene tyrosine-protein kinase (Src)-fluorescence resonance energy transfer reporter, which provided a real-time observation of Src activation (Na et al. 2008). When cells were stimulated via magnetic beads coated with RGD peptide (applied load was 17.5 Pa), Src activation was achieved in less than 0.3 s, whereas the stimulation of cells with endothelial growth factor took 12 s to result in active Src (Na et al. 2008). In this line of thought, it is noteworthy that cells respond faster to change in substrate stiffness than to applied load. A study by Mitrossilis et al. (2010) involved the use of a parallel plate setup with a double-output feedback loop in order to control the substrate stiffness and the applied force independently; by measuring cell traction forces and altering the substrate stiffness or the applied force, they showed that the earliest response (0.1 s) of cells was attributable to a change in substrate stiffness and not to a change in applied load. Arguing that mechanochemical signal transduction takes longer, the authors concluded that the initial response of cells to changes in substrate stiffness is purely mechanical, i.e., mechanical deformation of the cytoskeleton (Mitrossilis et al. 2010). The latter two examples demonstrate that mechanical force propagation along the cytoskeleton can be utilized as an ultra-fast intracellular second messenger, as an alternative to canonical mechanochemical transduction.

Mechanically induced changes in gene expression

The types of cell response pathways described so far (second messengers, cytoskeleton, nuclear mechanics) are immediate and transient in nature. On the other hand, cells are able to respond to mechanical cues in their microenvironment by altering their cell fate and behaviour, a response that is mediated by long-term changes in gene expression profiles. An example of gene regulation following local mechanical stimulus is the upregulation of alpha smooth muscle actin (SMA), which is a hallmark of increased cell contractility in the process of fibroblast-to-myofibroblast transition (Hinz 2010). By using coated magnetic beads to apply a tensile mechanical stimulus, McCulloch and colleagues detected the upregulation of SMA within 5 min of force application (Wang et al. 2002). Force application induced RhoA kinase activity (10 min), the phosphorylation of LIM kinase (5–10 min) and the de-phosphorylation of cofilin (5 min; Wang et al. 2002). Importantly, the authors showed a nuclear translocation of the transcriptional co-activator myocardin-related transcription factor-A (MRTF-A) and demonstrated a 3.5-fold increase of

SMA promoter activation dependent on MRTF-A (Wang et al. 2002). Notably, a study by the same group demonstrated that shear stress, from interstitial flow (0.1–0.3 dyn/cm²), can also lead to the expression of SMA and the secretion of transforming growth factor- β 1 (TGF β 1) from fibroblasts, i.e., to fibroblast-to-myofibroblast transition (Ng et al. 2005). Other examples of mechanically modulated genes include: collagen isoforms, fibronectin and laminin (Yasuda et al. 1996b), matrix metalloproteinase 2, tissue inhibitors of metalloproteinase 2 and TGF β (Yasuda et al. 1996a) and FAK, p44/p42 mitogen-activated protein (MAP) kinase, ERK1/2 and p38 MAP kinase (Seko et al. 1999a, b). The examples given above represent just a snapshot of the available literature on mechanically induced gene regulation (for more details, the reader is referred to the following excellent reviews: Shivashankar 2011; Wang et al. 2007). A central example for the intensive research in the field of mechanically induced gene expression is the recent finding that the downstream effectors of the Hippo pathway, namely, the nuclear transcription factors YAP/TAZ, are tightly regulated by mechanical cues from the microenvironment, such as substrate stiffness, stress fibres and cytoskeleton tension (Dupont et al. 2011). Importantly, the regulation of yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) by mechanical cues seems to be independent of the classical upstream sequential phosphorylation cascade via Hippo and the large tumour suppressor kinases, suggesting that mechanical force transduction can be viewed as a separate signalling pathway (Dupont et al. 2011).

Even though a large body of literature exists on the topic, we still lack an understanding of the integration of immediate and long-lasting responses established usually in single-cell models or in isolation *in vivo* (focusing on one component or one cell type) in the context of tissues in which heterotypic interactions occur between dissimilar cells and between cells and the ECM. How are single-cell (or single-cell-type) responses communicated to neighbouring and distantly situated cells within tissues? In the following section, we discuss specific examples of the manner in which this can be achieved.

Role of mechanical cues in cell-cell communication

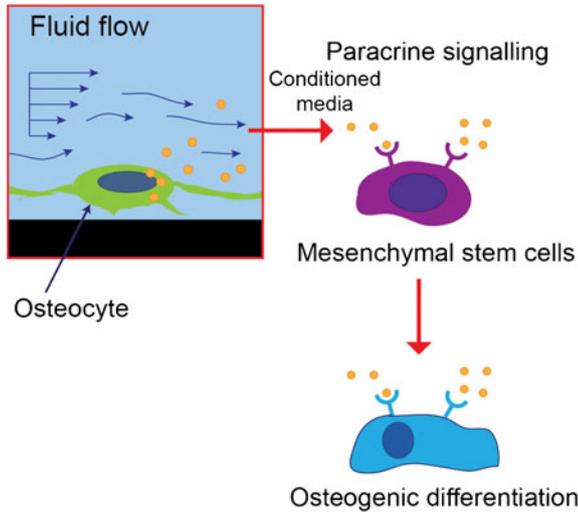
Cell-cell communication is of central importance for normal homeostasis of multicellular organisms and any misregulation of communication pathways leads to pathologies. Pathways of cell-cell communication include: the secretion of signalling molecules and transport across the interstitium to target cells, gap junctions, neurotransmission and intercellular nanotubes (see other articles in this special issue). A paramount example of the misregulation of communication is cancer, which is a broad definition of pathologies whereby (usually) single cells

Fig. 3 Examples of the interplay between mechanical forces and cell-cell communication pathways. **a** An example of shear-induced cell-cell communication based on the study by Hoey et al. (2011). Osteocytes were stimulated by shear flow. The conditioned medium was transferred to cultures of mesenchymal stem cells (MSCs) leading to the induction of osteogenic genes in the MSCs. **b** Examples of mechanically stimulated cell-cell communication between gap-junctionally connected cells. *Top* Typical arrangement of closely apposed cells interconnected by gap junctions. Mechanical stimulation via AFM of one cell (*middle*) resulted in elevation of intracellular Ca²⁺ (Charras and Horton 2002). Neighbouring cells also display an elevated Ca²⁺ signal. *Bottom* In the case of tunneling nanotube-connected (TNT) cells, mechanical stimulation of one cell leads to membrane depolarization, which is communicated to the nanotube-connected cell via gap junctions (PBS phosphate-buffered saline). The activation of voltage gated ion channels then leads to influx of Ca²⁺ (Wang et al. 2010). **c** *In vivo* example of contractility-dependent coupling of cell populations (EP epidermal tissue, AC actin cable, AS amnioserosa). Note the antiphase coupling between the pulsations (fluctuations of cell area) of neighbouring cells (Solon et al. 2009). **d** Example of the mechanical modulation of neurotransmission based on Boucher et al. (2012). A model of a node of Ranvier in which the mechanosensitivity of the voltage-dependent sodium and potassium ion channels is simulated to demonstrate the dependence of neurotransmission on mechanical force via the coupled left-shift (CLS) of voltage-gated ion channels

fail to respond to communication cues from their microenvironment and start proliferating in an uncontrolled manner, incompatible with the proper functioning of the organism as a whole. In the preceding sections, we described key points regarding the response of single cells (or isolated single-type cell populations) to mechanical cues at the molecular and single-cell level. In this section, we will discuss examples of the interplay between mechanical cues from the microenvironment and a possible communication of the resulting response to cells that do not experience the initial mechanical stimulus.

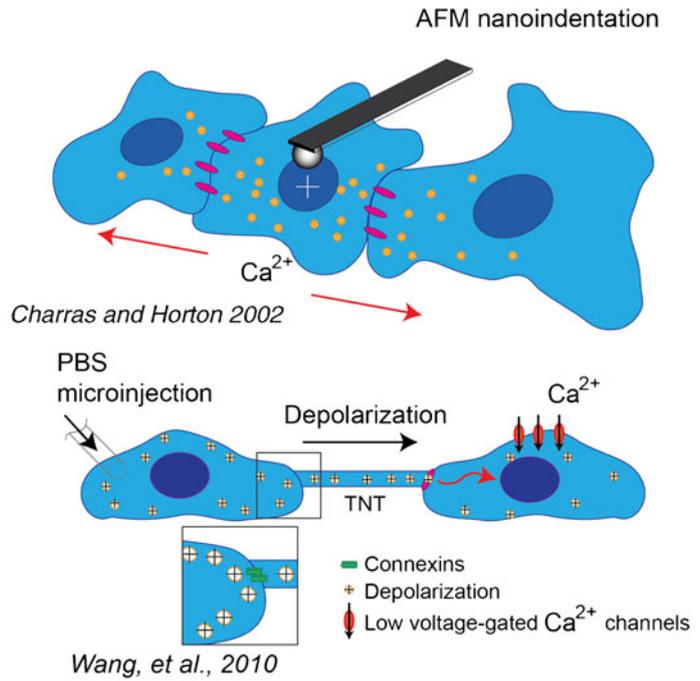
One clear example of a force applied to one cell having a response in neighbouring cells comes from the previously mentioned study of Charras and Horton (2002) (Fig. 3). In this study, single osteoblasts were stimulated by a controlled application of force via AFM-cantilever-bound microbeads; this treatment resulted in elevations of intracellular calcium. Interestingly, increases in Ca²⁺ could be also observed in neighbouring non-stimulated cells, a result that was demonstrated to be attributable to gap junction communication (Charras and Horton 2002). Whether cells *in vivo* exert such local mechanical forces and whether the same type of response is observed in adjacent cells remain to be shown. A similar phenomenon was reported for nanotube-connected normal rat kidney cells. Wang et al. (2010) have demonstrated that nanotube-connected cells can communicate microinjection-induced mechanical changes in membrane potential along nanotubes (Wang et al. 2010). Furthermore, a large enough depolarization of the nanotube-connected non-stimulated cell results in an influx of Ca²⁺, most likely via L-type voltage-gated Ca²⁺ channels. Importantly, this type of

a Mechanical forces and paracrine signalling

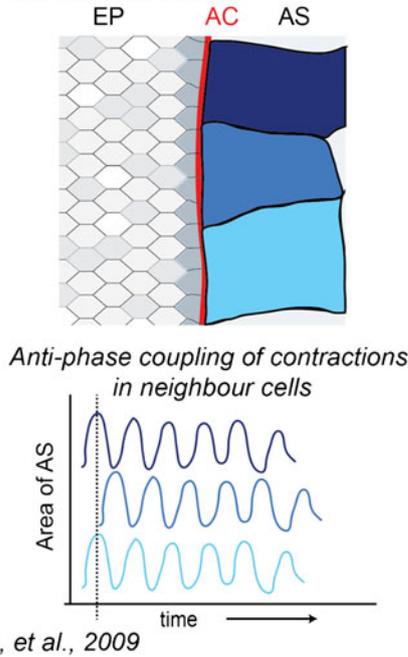


Hoey, et al., 2011

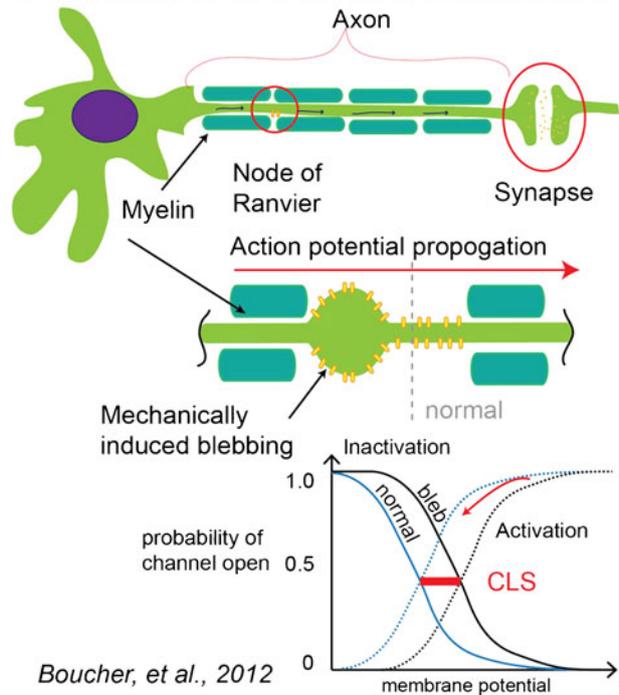
b Mechanical forces and gap junctional signalling



c Mechanical forces and contact dependent signalling



d Mechanical forces and neurotransmission



intercellular signalling is dependent on the presence of gap junction(s) along the nanotube (Wang et al. 2010; Fig. 3).

Gap junctions have been shown to be mechanically upregulated in at least two studies (Buschmann et al. 2010; Zhuang et al. 2000). In one case, upregulation of connexin

40 was induced via fluid flow stimulation of the vascular endothelium (Buschmann et al. 2010). On the other hand, exposing monolayers of neonatal rat myocytes to pulsatile stretch for 6 h (similar to physiological heart beating) results in an upregulation of connexin43 and N-cadherin (Zhuang

et al. 2000). More importantly, the propagation of action potentials across the cell monolayer was more efficient after stretch. These results demonstrate that the application of mechanical force on a model for heart muscle tissue leads to downstream biochemical (and possibly epigenetic) signalling, which in turn alters physiological intercellular communication via gap junctions. Another example of a physiological relationship between mechanical force and paracrine cell-cell communication has been demonstrated in mesenchymal stem cell (MSCs) differentiation (Hoey et al. 2011). A rocking table was employed to mechanically stimulate osteocytes for 24 h. Subsequently, the osteocyte culture media was transferred to a population of MSCs; this led to a significant upregulation of osteogenic genes (OPN and COX-2) and subsequently to MSCs differentiating into osteoclasts.

In vivo, cells are often mechanically stimulated as a localized group, as opposed to mechanically activating/perturbing single cells, as is often carried out in vitro. Collective mechanical action between groups of cells in *Drosophila* embryos has been shown to drive the process of dorsal closure (Solon et al. 2009; Fig. 3). Dorsal closure was shown to be dependent on pulsatile mechanical contractions of the cells in the underlying epithelium. The source of these pulses was observed to come from a ratchet-like mechanism, i.e., neighbouring cells communicate the pulsations between each other and are dependent on intercellular tension (Solon et al. 2009). Importantly, when Solon et al. (2009) artificially induced a cell-autonomous contractility in one cell, the coupling of pulsations that was observed under normal conditions was eliminated.

Similarly, intercellular mechanical force propagation is increasingly becoming clear as playing a key role in collective cell migration. Traction force and Fourier transform microscopy of migrating cell monolayers have revealed a wave-like propagation of mechanical stresses between cells across the migrating cell sheath; this seems central for coordinated cell motility (Serra-Picamal et al. 2012; Trepatt et al. 2009). Even though the involvement of intercellular mechanical force in regulating cell migration is evident from many studies, a molecular explanation for this relationship has only recently been described (Weber et al. 2012). Pulling on isolated mesendoderm cells from *Xenopus* embryos via cadherin-coated beads promotes cell motility in the opposite direction and reorganization of the cytoskeleton to induce directed cell motility (Weber et al. 2012). Thus, the latter finding provided the first evidence for a molecular link between cell-to-cell mechanotransduction and collective cell migration. The propagation of mechanical forces between cells in vivo has also been implicated in governing tissue morphogenesis and organ size regulation (Shraiman 2005). Allowing cell division, cell growth and regulated cell death to be dependent on the mechanical stress experienced

by single cells within a tissue can account for the observed tissue growth rates in model systems such as the imaginal wing disc of *Drosophila* (Shraiman 2005). Importantly, despite the lack of a full grasp on the molecular explanation of the latter idea, cell division rates of cultured epithelial cells have recently been demonstrated to be dependent on mechanical interaction and constraint (Puliafito et al. 2012).

Mechanical forces also have an important role to play in neurotransmission. Recently, primary rat cortical neurons were cultured as two separate populations connected by axons formed along a deformable substrate (Monnerie et al. 2010). The response of the dendritic tree between the two neuronal cell populations to mechanical stretch was studied. After a transient application of 80% strain to the axons, the dendrites responded by forming periodic swellings along the dendritic shaft. Furthermore, the size of these swellings decreased over time and their formation was shown to be sodium- and N-methyl-D-aspartate-receptor-dependent. The consequences of mechanical perturbation at the nodes of Ranvier have also been recently investigated (Boucher et al. 2012; Fig. 3). At present, the experimental analysis of the effect of mechanical force on neurotransmission at the nodes of Ranvier is impossible. Therefore, the authors (Boucher et al. 2012) have employed a theoretical approach based on known parameters of voltage-gated ions channels, ion pumps and experimental data from the mechanosensitivity of the gating of these channels. The authors demonstrate that mechanical perturbation at one node of Ranvier leads to dramatic changes in the way that an action potential propagates between adjacent nodes. The authors integrate a well-described experimentally observed effect of stretching voltage-dependent Na^+ ion channels, namely, the so-called coupled-left shift, which renders Na channels ion-permeable at lower membrane potential after the application of stress. Importantly, mechanical perturbation of only one node of Ranvier can lead to a dampening of action potential transmission to other downstream nodes of Ranvier. Thus, the slightest imbalance and perturbation of a neural network can have enormous consequences in neurotransmission.

Concluding remarks and outlook

A common theme in modern cell sciences is the elucidation of the molecular basis of intracellular and intercellular signalling. Genetic regulation, interactions between signalling cascades, intracellular transport and cell migration are all active areas of study supplying the biomedical community with a rich resource for the understanding of basic biological processes. In addition, as is becoming increasingly clear, the cellular micro-environment plays a key role in determining cell function and cell fate. Apart from the strictly biochemical signature that is presented to single cells by the ECM and solutes in the

interstitial fluid, the physical properties of the interstitium are also able to define and direct cell behaviour: the varying of substrate rigidity can direct stem cells into totally different cell lineages, whereas changes of tissue rigidity is a hallmark of many pathological conditions, such as cancer and fibrotic diseases. However, many questions about the manifestation of the link between substrate rigidity and cell fate decisions *in vivo* remain, although ongoing interdisciplinary efforts should certainly fill this gap in our knowledge.

In this review, we have attempted to provide an overview of a rapidly growing field of interdisciplinary research. The work highlighted here is certainly not exhaustive and many examples of the importance of mechanical cues exist in cell biology, as discussed in several recent reviews (Eyckmans et al. 2011; Farge 2011; Janmey and Miller 2011; Mammoto et al. 2012; Zwerger et al. 2011). Here, we have also focussed on the use of mechanical cues in mammalian cell communication and physiology. However, mechanical cues play an equally important role in other kingdoms. Although beyond the scope of this review, bacteria and fungi also display a clear dependence on mechanical cues. The physical interactions between cells and the mechanical properties of these cells are required in many fundamental processes, including biofilm formation, motility, division, signalling and communication (Dufrene 2002, 2008; Dupres et al. 2010; Muller and Dufrene 2011). Interestingly, these cell types rely on vastly different mechanisms in order to generate mechanical forces and to respond to mechanical cues in the microenvironment. Therefore, mechanical cues have a ubiquitous role in all biology, across kingdoms.

Many details about the ability of mammalian cells to detect and respond to mechanical cues from the microenvironment have been elucidated and certain common themes appear to recur. For example, cells can “feel” matrix rigidity and the local application of force, through integrins and signalling via focal adhesions and the actomyosin cytoskeleton. We should state that, even though actomyosin contractility appears to be a principle manifestation of the cell response, MTs and IF components of the cytoskeleton play equally important roles and are under investigation. Moreover, the primary cilium appears to play an important role *in vivo* and our knowledge regarding this important structure has experienced a tremendous leap in the last decade. Importantly, one cannot ignore or over-generalize cellular context, as the variety of mechanosensation and mechanotransduction pathways also appears to be cell-type- and cell-state-dependent.

With our current knowledge of mechanobiology at the single-cell level, we need, at this point, to understand the way that mechanical cues from the microenvironment are interpreted by cells in the more complex microenvironments found in tissues, organs and organisms. On the basis of the examples of the interplay between cell mechanics and

intercellular communication that we have discussed, our knowledge of single-cell responses, while informative, are clearly not enough for a more complete understanding of pivotal processes such as morphogenesis and pathogenesis. Therefore, the field of mechanically induced cell-cell communication holds the potential to provide cell and tissue researchers with important insights into physiological processes at the macro-, micro- and nano-scales.

Cells, in all their complexity, have adapted ways to sense and respond to a multitude of mechanical cues in the microenvironment. In this review, we have discussed many of the known details about mechanobiological processes in mammalian cells and have attempted to present several examples from the literature illustrating the deep involvement of mechanical forces in fundamental cell-cell communication pathways. This emerging new knowledge has given rise to intense study at the cell, multi-cellular, tissue and organismal levels, leading to the emerging view that mechanical cues are critical determinants of cell fate. Moreover, mechanical signals probably also act as an independent means of signal transduction, both inside and between cells, under physiological conditions.

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References

- Archambault JM, Elfervig-Wall MK, Tsuzaki M, Herzog W, Banes AJ (2002) Rabbit tendon cells produce MMP-3 in response to fluid flow without significant calcium transients. *J Biomech* 35:303–309
- Baker BM, Chen CS (2012) Deconstructing the third dimension; how 3D culture microenvironments alter cellular cues. *J Cell Sci* 125:3015–3024
- Barczyk M, Carracedo S, Gullberg D (2010) Integrins. *Cell Tissue Res* 339:269–280
- Beningo KA, Wang YL (2002) Flexible substrata for the detection of cellular traction forces. *Trends Cell Biol* 12:79–84
- Berk BC, Fujiwara K, Lehoux S (2007) ECM remodeling in hypertensive heart disease. *J Clin Invest* 117:568–575
- Bershadsky A, Kozlov M, Geiger B (2006) Adhesion-mediated mechanosensitivity: a time to experiment, and a time to theorize. *Curr Opin Cell Biol* 18:472–481
- Besschetnova TY, Kolpakova-Hart E, Guan Y, Zhou J, Olsen BR, Shah JV (2010) Identification of signaling pathways regulating primary cilium length and flow-mediated adaptation. *Curr Biol* 20:182–187
- Bhattacharya R, Gonzalez AM, Debiase PJ, Trejo HE, Goldman RD, Flitney FW, Jones JC (2009) Recruitment of vimentin to the cell

- surface by beta3 integrin and plectin mediates adhesion strength. *J Cell Sci* 122:1390–1400
- Boucher PA, Joos B, Morris CE (2012) Coupled left-shift of Nav channels: modeling the Na(+)-loading and dysfunctional excitability of damaged axons. *J Comput Neurosci* 33:301–319
- Brown AE, Discher DE (2009) Conformational changes and signaling in cell and matrix physics. *Curr Biol* 19:R781–R789
- Buschmann I, Pries A, Styp-Rekowska B, Hillmeister P, Loufrani L, Henrion D, Shi Y, Duelsner A, Hoefler I, Gatzke N, Wang H, Lehmann K, Ulm L, Ritter Z, Hauff P, Hlushchuk R, Djonov V, van Veen T, le Noble F (2010) Pulsatile shear and Gja5 modulate arterial identity and remodeling events during flow-driven arterio-genesis. *Development* 137:2187–2196
- Butcher DT, Alliston T, Weaver VM (2009) A tense situation: forcing tumour progression. *Nat Rev Cancer* 9:108–122
- Buxboim A, Ivanovska IL, Discher DE (2010) Matrix elasticity, cytoskeletal forces and physics of the nucleus: how deeply do cells “feel” outside and in? *J Cell Sci* 123:297–308
- Carmignac V, Durbeek J (2012) Cell-matrix interactions in muscle disease. *J Pathol* 226:200–218
- Charras GT, Horton MA (2002) Single cell mechanotransduction and its modulation analyzed by atomic force microscope indentation. *Biophys J* 82:2970–2981
- Choudhury N, Bouchot O, Rouleau L, Tremblay D, Cartier R, Butany J, Mongrain R, Leask RL (2009) Local mechanical and structural properties of healthy and diseased human ascending aorta tissue. *Cardiovasc Pathol* 18:83–91
- Ciapetti G, Granchi D, Baldini N (2012) The combined use of mesenchymal stromal cells and scaffolds for bone repair. *Curr Pharm Des* 18:1796–1820
- Ciobanaru C, Faivre B, Le Clairche C (2012) Actin dynamics associated with focal adhesions. *Int J Cell Biol* 2012:941292
- Cowin SC (1998) On mechanosensation in bone under microgravity. *Bone* 22:119S–125S
- Cox TR, Erler JT (2011) Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech* 4:165–178
- Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, Burke B, Stahl PD, Hodzic D (2006) Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol* 172:41–53
- Dahl KN, Ribeiro AJ, Lammerding J (2008) Nuclear shape, mechanics, and mechanotransduction. *Circ Res* 102:1307–1318
- Dechat T, Pfliegerhaer K, Sengupta K, Shimi T, Shumaker DK, Solimando L, Goldman RD (2008) Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev* 22:832–853
- Dufrene YF (2002) Atomic force microscopy, a powerful tool in microbiology. *J Bacteriol* 184:5205–5213
- Dufrene YF (2008) Towards nanomicrobiology using atomic force microscopy. *Nat Rev Microbiol* 6:674–680
- Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S (2011) Role of YAP/TAZ in mechanotransduction. *Nature* 474:179–183
- Dupres V, Alsteens D, Andre G, Dufrene YF (2010) Microbial nanoscopy: a closer look at microbial cell surfaces. *Trends Microbiol* 18:397–405
- Engler AJ, Griffin MA, Sen S, Bonnemann CG, Sweeney HL, Discher DE (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J Cell Biol* 166:877–887
- Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–689
- Eyckmans J, Boudou T, Yu X, Chen CS (2011) A hitchhiker's guide to mechanobiology. *Dev Cell* 21:35–47
- Farge E (2011) Mechanotransduction in development. *Curr Top Dev Biol* 95:243–265
- Farsad K, De Camilli P (2003) Mechanisms of membrane deformation. *Curr Opin Cell Biol* 15:372–381
- Fletcher DA, Mullins RD (2010) Cell mechanics and the cytoskeleton. *Nature* 463:485–492
- Fliegau M, Benzing T, Omran H (2007) When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* 8:880–893
- Formigli L, Meacci E, Sassoli C, Chellini F, Giannini R, Quercioli F, Tiribilli B, Squecco R, Bruni P, Francini F, Zecchi-Orlandini S (2005) Sphingosine 1-phosphate induces cytoskeletal reorganization in C2C12 myoblasts: physiological relevance for stress fibres in the modulation of ion current through stretch-activated channels. *J Cell Sci* 118:1161–1171
- Franke WW (2009) Discovering the molecular components of intercellular junctions—a historical view. *Cold Spring Harb Perspect Biol* 1:a003061
- Fudge DS, Gardner KH, Forsyth VT, Riekel C, Gosline JM (2003) The mechanical properties of hydrated intermediate filaments: insights from hagfish slime threads. *Biophys J* 85:2015–2027
- Geiger B, Spatz JP, Bershadsky AD (2009) Environmental sensing through focal adhesions. *Nat Rev Mol Cell Biol* 10:21–33
- Ghajar CM, Bissell MJ (2008) Extracellular matrix control of mammary gland morphogenesis and tumorigenesis: insights from imaging. *Histochem Cell Biol* 130:1105–1118
- Gilula NB, Satir P (1972) The ciliary necklace. A ciliary membrane specialization. *J Cell Biol* 53:494–509
- Glogauer M, Ferrier J, McCulloch CA (1995) Magnetic fields applied to collagen-coated ferric oxide beads induce stretch-activated Ca²⁺ flux in fibroblasts. *Am J Physiol* 269:C1093–C1104
- Green KJ, Getsios S, Troyanovsky S, Godsel LM (2010) Intercellular junction assembly, dynamics, and homeostasis. *Cold Spring Harb Perspect Biol* 2:a000125
- Grierson JP, Meldolesi J (1995) Shear stress-induced [Ca²⁺]_i transients and oscillations in mouse fibroblasts are mediated by endogenously released ATP. *J Biol Chem* 270:4451–4456
- Guolla L, Bertrand M, Haase K, Pelling AE (2012) Force transduction and strain dynamics in actin stress fibres in response to nanonewton forces. *J Cell Sci* 125:603–613
- Guvendiren M, Burdick JA (2012) Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat Commun* 3:792
- Hamill OP (2006) Twenty odd years of stretch-sensitive channels. *Pflügers Arch* 453:333–351
- Haswell ES, Phillips R, Rees DC (2011) Mechanosensitive channels: what can they do and how do they do it? *Structure* 19:1356–1369
- Hierck BP, Van der Heiden K, Alkemade FE, Van de Pas S, Van Thienen JV, Groenendijk BC, Bax WH, Van der Laarse A, Deruiter MC, Horrevoets AJ, Poelmann RE (2008) Primary cilia sensitize endothelial cells for fluid shear stress. *Dev Dyn* 237:725–735
- Hinz B (2010) The myofibroblast: paradigm for a mechanically active cell. *J Biomech* 43:146–155
- Hoey DA, Kelly DJ, Jacobs CR (2011) A role for the primary cilium in paracrine signaling between mechanically stimulated osteocytes and mesenchymal stem cells. *Biochem Biophys Res Commun* 412:182–187
- Hoey DA, Downs ME, Jacobs CR (2012) The mechanics of the primary cilium: an intricate structure with complex function. *J Biomech* 45:17–26
- Hoffman BD, Grashoff C, Schwartz MA (2011) Dynamic molecular processes mediate cellular mechanotransduction. *Nature* 475:316–323
- Hung CT, Allen FD, Pollack SR, Attia ET, Hannafin JA, Torzilli PA (1997) Intracellular calcium response of ACL and MCL ligament fibroblasts to fluid-induced shear stress. *Cell Signal* 9:587–594

- Hynes RO (2009) The extracellular matrix: not just pretty fibrils. *Science* 326:1216–1219
- Jaalouk DE, Lammerding J (2009) Mechanotransduction gone awry. *Nat Rev Mol Cell Biol* 10:63–73
- Janmey PA, Miller RT (2011) Mechanisms of mechanical signaling in development and disease. *J Cell Sci* 124:9–18
- Jensen CG, Poole CA, McGlashan SR, Marko M, Issa ZI, Vujcich KV, Bowser SS (2004) Ultrastructural, tomographic and confocal imaging of the chondrocyte primary cilium in situ. *Cell Biol Int* 28:101–110
- Johnson WA (2011) *Mechanobiology of cell-cell and cell-matrix interactions*. Springer, New York
- Kass L, Erler JT, Dembo M, Weaver VM (2007) Mammary epithelial cell: influence of extracellular matrix composition and organization during development and tumorigenesis. *Int J Biochem Cell Biol* 39:1987–1994
- Kaverina I, Krylyshkina O, Beningo K, Anderson K, Wang YL, Small JV (2002) Tensile stress stimulates microtubule outgrowth in living cells. *J Cell Sci* 115:2283–2291
- Kiehart DP, Galbraith CG, Edwards KA, Rickoll WL, Montague RA (2000) Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J Cell Biol* 149:471–490
- Kim S, Wong P, Coulombe PA (2006) A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* 441:362–365
- Kim DH, Wong PK, Park J, Levchenko A, Sun Y (2009) Microengineered platforms for cell mechanobiology. *Annu Rev Biomed Eng* 11:203–233
- Kirmse R, Otto H, Ludwig T (2011) Interdependency of cell adhesion, force generation and extracellular proteolysis in matrix remodeling. *J Cell Sci* 124:1857–1866
- Krepkiy D, Mihailescu M, Freitas JA, Schow EV, Worcester DL, Gawrisch K, Tobias DJ, White SH, Swartz KJ (2009) Structure and hydration of membranes embedded with voltage-sensing domains. *Nature* 462:473–479
- Kreplak L, Herrmann H, Aebi U (2008) Tensile properties of single desmin intermediate filaments. *Biophys J* 94:2790–2799
- Kunda P, Pelling AE, Liu T, Baum B (2008) Moesin controls cortical rigidity, cell rounding, and spindle morphogenesis during mitosis. *Curr Biol* 18:91–101
- Kuo JC, Han X, Hsiao CT, Yates JR 3rd, Waterman CM (2011) Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. *Nat Cell Biol* 13:383–393
- Kurth F, Eyer K, Franco-Obregon A, Dittrich PS (2012) A new mechanobiological era: microfluidic pathways to apply and sense forces at the cellular level. *Curr Opin Chem Biol* 16:400–408
- Levayer R, Lecuit T (2012) Biomechanical regulation of contractility: spatial control and dynamics. *Trends Cell Biol* 22:61–81
- Long SB, Campbell EB, Mackinnon R (2005) Crystal structure of a mammalian voltage-dependent Shaker family K⁺ channel. *Science* 309:897–903
- Mammoto A, Mammoto T, Ingber DE (2012) Mechanosensitive mechanisms in transcriptional regulation. *J Cell Sci* 125:3061–3073
- Matthews BD, Thodeti CK, Tytell JD, Mammoto A, Overby DR, Ingber DE (2010) Ultra-rapid activation of TRPV4 ion channels by mechanical forces applied to cell surface beta1 integrins. *Integr Biol (Camb)* 2:435–442
- Mazumder A, Shivashankar GV (2010) Emergence of a prestressed eukaryotic nucleus during cellular differentiation and development. *J R Soc Interface* 7 (Suppl 3):S321–S330
- McCullen SD, Haslauer CM, Loba EG (2010) Musculoskeletal mechanobiology: interpretation by external force and engineered substratum. *J Biomech* 43:119–127
- Mejillano MR, Kojima S, Applewhite DA, Gertler FB, Svitkina TM, Borisy GG (2004) Lamellipodial versus filopodial mode of the actin nano-machinery: pivotal role of the filament barbed end. *Cell* 118:363–373
- Meyer CJ, Alenghat FJ, Rim P, Fong JH, Fabry B, Ingber DE (2000) Mechanical control of cyclic AMP signalling and gene transcription through integrins. *Nat Cell Biol* 2:666–668
- Mitrossilis D, Fouchard J, Pereira D, Postic F, Richert A, Saint-Jean M, Asnacios A (2010) Real-time single-cell response to stiffness. *Proc Natl Acad Sci USA* 107:16518–16523
- Mizutani T, Haga H, Kawabata K (2004) Cellular stiffness response to external deformation: tensional homeostasis in a single fibroblast. *Cell Motil Cytoskeleton* 59:242–248
- Monnerie H, Tang-Schomer MD, Iwata A, Smith DH, Kim HA, Le Roux PD (2010) Dendritic alterations after dynamic axonal stretch injury in vitro. *Exp Neurol* 224:415–423
- Muller DJ, Dufrene YF (2011) Force nanoscopy of living cells. *Curr Biol* 21:R212–R216
- Myers KA, Applegate KT, Danuser G, Fischer RS, Waterman CM (2011) Distinct ECM mechanosensing pathways regulate microtubule dynamics to control endothelial cell branching morphogenesis. *J Cell Biol* 192:321–334
- Na S, Collin O, Chowdhury F, Tay B, Ouyang M, Wang Y, Wang N (2008) Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proc Natl Acad Sci USA* 105:6626–6631
- Ng CP, Hinz B, Swartz MA (2005) Interstitial fluid flow induces myofibroblast differentiation and collagen alignment in vitro. *J Cell Sci* 118:4731–4739
- Nilius B, Owsianik G, Voets T, Peters JA (2007) Transient receptor potential cation channels in disease. *Physiol Rev* 87:165–217
- Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol* 9:60–71
- Pajerowski JD, Dahl KN, Zhong FL, Sammak PJ, Discher DE (2007) Physical plasticity of the nucleus in stem cell differentiation. *Proc Natl Acad Sci USA* 104:15619–15624
- Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, Weaver VM (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–254
- Pedersen JA, Boschetti F, Swartz MA (2007) Effects of extracellular fiber architecture on cell membrane shear stress in a 3D fibrous matrix. *J Biomech* 40:1484–1492
- Pelling AE, Horton MA (2008) An historical perspective on cell mechanics. *Pflugers Arch* 456:3–12
- Pelling AE, Veraitch FS, Chu CP, Mason C, Horton MA (2009) Mechanical dynamics of single cells during early apoptosis. *Cell Motil Cytoskeleton* 66:409–422
- Petrie RJ, Gavara N, Chadwick RS, Yamada KM (2012) Nonpolarized signaling reveals two distinct modes of 3D cell migration. *J Cell Biol* 197:439–455
- Praetorius HA, Spring KR (2003) Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* 191:69–76
- Puliafito A, Hufnagel L, Neveu P, Streichan S, Sigal A, Fygenson DK, Shraiman BI (2012) Collective and single cell behavior in epithelial contact inhibition. *Proc Natl Acad Sci USA* 109:739–744
- Rapraeger AC, Krufka A, Olwin BB (1991) Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* 252:1705–1708
- Rho JY, Kuhn-Spearing L, Zioupos P (1998) Mechanical properties and the hierarchical structure of bone. *Med Eng Phys* 20:92–102
- Rodriguez JP, Gonzalez M, Rios S, Cambiazio V (2004) Cytoskeletal organization of human mesenchymal stem cells (MSC) changes during their osteogenic differentiation. *J Cell Biochem* 93:721–731
- Rohatgi R, Snell WJ (2010) The ciliary membrane. *Curr Opin Cell Biol* 22:541–546
- Rouleau L, Tremblay D, Cartier R, Mongrain R, Leask RL (2012) Regional variations in canine descending aortic tissue mechanical

- properties change with formalin fixation. *Cardiovasc Pathol* 21:390–397
- Ruegg C, Veigel C, Molloy JE, Schmitz S, Sparrow JC, Fink RH (2002) Molecular motors: force and movement generated by single myosin II molecules. *News Physiol Sci* 17:213–218
- Sachs F, Morris CE (1998) Mechanosensitive ion channels in non-specialized cells. *Rev Physiol Biochem Pharmacol* 132:1–77
- Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 17:153–162
- Schwarz US, Gardel ML (2012) United we stand: integrating the actin cytoskeleton and cell-matrix adhesions in cellular mechanotransduction. *J Cell Sci* 125:3051–3060
- Seko Y, Seko Y, Takahashi N, Shibuya M, Yazaki Y (1999a) Pulsatile stretch stimulates vascular endothelial growth factor (VEGF) secretion by cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 254:462–465
- Seko Y, Takahashi N, Tobe K, Kadowaki T, Yazaki Y (1999b) Pulsatile stretch activates mitogen-activated protein kinase (MAPK) family members and focal adhesion kinase (p125[FAK]) in cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 259:8–14
- Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, Bazellieres E, Butler JP, Fredberg JJ, Trepast X (2012) Mechanical waves during tissue expansion. *Nat Phys* 8:628–634
- Shi ZD, Tarbell JM (2011) Fluid flow mechanotransduction in vascular smooth muscle cells and fibroblasts. *Ann Biomed Eng* 39:1608–1619
- Shivashankar GV (2011) Mechanosignaling to the cell nucleus and gene regulation. *Annu Rev Biophys* 40:361–378
- Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci USA* 102:3318–3323
- Simske JS, Hardin J (2001) Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. *Bioessays* 23:12–23
- Solon J, Kaya-Copur A, Colombelli J, Brunner D (2009) Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell* 137:1331–1342
- Stewart MP, Helenius J, Toyoda Y, Ramanathan SP, Muller DJ, Hyman AA (2011) Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* 469:226–230
- Storch U, Mederos y Schnitzler M, Gudermann T (2012) G protein-mediated stretch reception. *Am J Physiol Heart Circ Physiol* 302:H1241–H1249
- Sukharev S, Sachs F (2012) Molecular force transduction by ion channels: diversity and unifying principles. *J Cell Sci* 125:3075–3083
- Suki B, Bates JH (2008) Extracellular matrix mechanics in lung parenchymal diseases. *Respir Physiol Neurobiol* 163:33–43
- Swartz MA, Fleury ME (2007) Interstitial flow and its effects in soft tissues. *Annu Rev Biomed Eng* 9:229–256
- Swartz MA, Berk DA, Jain RK (1996) Transport in lymphatic capillaries. I. Macroscopic measurements using residence time distribution theory. *Am J Physiol* 270:H324–H329
- Ting LH, Jahn JR, Jung JJ, Shuman BR, Feghhi S, Han SJ, Rodriguez ML, Sniadecki NJ (2012) Flow mechanotransduction regulates traction forces, intercellular forces, and adherens junctions. *Am J Physiol Heart Circ Physiol* 302:H2220–H2229
- Tojkander S, Gateva G, Lappalainen P (2012) Actin stress fibers—assembly, dynamics and biological roles. *J Cell Sci* 125:1855–1864
- Toyama Y, Peralta XG, Wells AR, Kiehart DP, Edwards GS (2008) Apoptotic force and tissue dynamics during *Drosophila* embryogenesis. *Science* 321:1683–1686
- Tremblay D, Cartier R, Mongrain R, Leask RL (2010) Regional dependency of the vascular smooth muscle cell contribution to the mechanical properties of the pig ascending aortic tissue. *J Biomech* 43:2448–2451
- Trepast X, Wasserman MR, Angelini TE, Millet E, Weitz DA, Butler JP, Fredberg JJ (2009) Physical forces during collective cell migration. *Nat Phys* 5:426–430
- Trichet L, Le Digabel J, Hawkins RJ, Vedula SR, Gupta M, Ribault C, Hersen P, Voituriez R, Ladoux B (2012) Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *Proc Natl Acad Sci USA* 109:6933–6938
- Vogel V, Sheetz M (2006) Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* 7:265–275
- Wang YL (2009) Traction forces and rigidity sensing of adherent cells. *Conf Proc IEEE Eng Med Biol Soc* 2009:3339–3340
- Wang J, Pelling AE (2012) An approach to visualize the deformation of the intermediate filament cytoskeleton in response to locally applied forces. *ISRN Cell Biol* 2012:9
- Wang J, Su M, Fan J, Seth A, McCulloch CA (2002) Transcriptional regulation of a contractile gene by mechanical forces applied through integrins in osteoblasts. *J Biol Chem* 277:22889–22895
- Wang JH, Thampatty BP, Lin JS, Im HJ (2007) Mechanoregulation of gene expression in fibroblasts. *Gene* 391:1–15
- Wang X, Veruki ML, Bukoreshtliev NV, Hartveit E, Gerdes HH (2010) Animal cells connected by nanotubes can be electrically coupled through interposed gap-junction channels. *Proc Natl Acad Sci USA* 107:17194–17199
- Weber GF, Bjerke MA, DeSimone DW (2012) A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev Cell* 22:104–115
- Whittard JD, Akiyama SK (2001) Positive regulation of cell-cell and cell-substrate adhesion by protein kinase A. *J Cell Sci* 114:3265–3272
- Wiig H, Swartz MA (2012) Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer. *Physiol Rev* 92:1005–1060
- Yasuda T, Akai Y, Kondo S, Becker BN, Homma T, Owada S, Ishida M, Harris RC (1996a) Alteration of cellular function in rat mesangial cells in response to mechanical stretch relaxation. *Contrib Nephrol* 118:222–228
- Yasuda T, Kondo S, Homma T, Harris RC (1996b) Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. *J Clin Invest* 98:1991–2000
- Young JL, Engler AJ (2011) Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. *Biomaterials* 32:1002–1009
- Zemel A, Rehfeldt F, Brown AE, Discher DE, Safran SA (2010) Optimal matrix rigidity for stress fiber polarization in stem cells. *Nat Phys* 6:468–473
- Zhang MZ, Mai W, Li C, Cho SY, Hao C, Moeckel G, Zhao R, Kim I, Wang J, Xiong H, Wang H, Sato Y, Wu Y, Nakanuma Y, Lilova M, Pei Y, Harris RC, Li S, Coffey RJ, Sun L, Wu D, Chen XZ, Breyer MD, Zhao ZJ, McKanna JA, Wu G (2004) PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc Natl Acad Sci USA* 101:2311–2316
- Zhuang J, Yamada KA, Saffitz JE, Kleber AG (2000) Pulsatile stretch remodels cell-to-cell communication in cultured myocytes. *Circ Res* 87:316–322
- Zieman SJ, Melenovsky V, Kass DA (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25:932–943
- Zink D, Fischer AH, Nickerson JA (2004) Nuclear structure in cancer cells. *Nat Rev Cancer* 4:677–687
- Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, Makita N, Iwanaga K, Zhu W, Kudoh S, Toko H, Tamura K, Kihara M, Nagai T, Fukamizu A, Umemura S, Iiri T, Fujita T, Komuro I (2004) Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* 6:499–506
- Zwarger M, Ho CY, Lammerding J (2011) Nuclear mechanics in disease. *Annu Rev Biomed Eng* 13:397–428