Matrix elasticity, cytoskeletal forces and physics of the nucleus: how deeply do cells 'feel' outside and in?

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Summary

Cellular organization within a multicellular organism requires that a cell assess its relative location, taking in multiple cues from its microenvironment. Given that the extracellular matrix (ECM) consists of the most abundant proteins in animals and contributes both structure and elasticity to tissues, ECM probably provides key physical cues to cells. In vivo, in the vicinity of many tissue cell types, fibrous characteristics of the ECM are less discernible than the measurably distinct elasticity that characterizes different tissue microenvironments. As a cell engages matrix and actively probes, it senses the local elastic resistance of the ECM and nearby cells via their deformation, and – similar to the proverbial princess who feels a pea placed many mattresses below – the cell seems to possess feedback and recognition mechanisms that establish how far it can feel. Recent experimental findings and computational modeling of cell and matrix mechanics lend insight into the subcellular range of sensitivity. Continuity of deformation from the matrix into the cell and further into the cytoskeleton-caged and -linked nucleus also supports the existence of mechanisms that direct processes such as gene expression in the differentiation of stem cells. Ultimately, cells feel the difference between stiff or soft and thick or thin surroundings, regardless of whether or not they are of royal descent.

Key words: Cell mechanics, Matrix, Nucleus

Introduction

Mesenchyme in embryos consists of loosely packed cells that adhere within a gelatinous extracellular matrix (ECM); these cells can develop into bone, cartilage, muscle and other mesenchymal tissues. Triggers of the very initial steps of differentiation from this mass are mostly unknown, but definitive mechanical differences between mesenchymal tissues eventually do emerge. In the case of bone, osteoblasts adhere to a compliant collagenous ECM known as osteoid (Sodek and McKee, 2000), which is microns thick, on top of calcified and rigidified collagenous bone (for a glossary of biophysical terms, see Box 1) (Fig. 1A). Within cartilage, chondrocytes are surrounded by a compliant pericellular matrix that separates these cells from a stiff and fibrous collagen matrix (Poole et al., 1987). In muscle, resident stem cells known as satellite cells adhere to a thin basement membrane of compliant ECM that surrounds each striated muscle fiber (Fig. 1B). These cellular microenvironments differ not only in their composition and in the crosslinking between fibrous and non-fibrous proteins, but also in terms of their elasticity, E, which quantifies the resistance to deformation of ECM and nearby cells. As reviewed here, cells can push and pull within these microenvironments and feel mechanical differences – at least over a finite range of elasticities and distances, which we elaborate on below.

In the last dozen years, it has become increasingly clear that matrix or tissue elasticity has an influential role in regulating numerous cell functions. Cell contraction (Discher et al., 2005), migration (Hadjipanayi et al., 2009b; Lo et al., 2000), proliferation (Hadjipanayi et al., 2009a; Winer et al., 2009), organization (Krieg et al., 2008) and even cell death (Wang et al., 2000) are modulated by cell-generated, actin-myosin forces that depend – in a feedback fashion – on matrix elasticity. The mechanical feedback is analogous to lifting a barbell with your arm, in that your muscle feels the

weight and exerts sufficient force to lift the weight at a desired rate; E is equivalent to that weight. Cytoskeletal assembly and gene expression during the differentiation of muscle cells (Engler et al., 2004b; Yip et al., 2009), osteoblasts (Kong et al., 2005) and mesenchymal stem cells (MSCs) (Engler et al., 2006) are likewise directed by tissue levels of E through myosin-dependent

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Box 1. Glossary

To understand the definitions below, grab a latex glove from the nearest lab bench.

Compliance: one glove is more compliant than two gloves held and stretched in parallel.

Compressibility: fill the glove with water and tie it off like a water balloon. You can still stretch the glove, but its volume does not change because water lacks compressibility.

Deformation: stretch the latex glove between your two hands.

Elasticity: the latex glove completely recovers after you stretch and release.

Force: the weight that you would apply to the glove to stretch it by the same amount that you achieved by hand.

Rigid: your benchtop does not stretch significantly compared to your latex glove.

Stiffness: two gloves held together in parallel and stretched are twice as stiff as one glove.

Strain: the normalized length change of the glove. For fun, draw a grid on the glove with a permanent marker pen and see how the grid distorts when you stretch the glove.

Stress: the force per cross-section of the latex glove that you stretched. If your friend cut your stretched gloves like cutting a ribbon, the stress would be relieved (and equal zero).

Tension: synonymous with stress.

Traction: put the glove on and rub the benchtop with your palm.
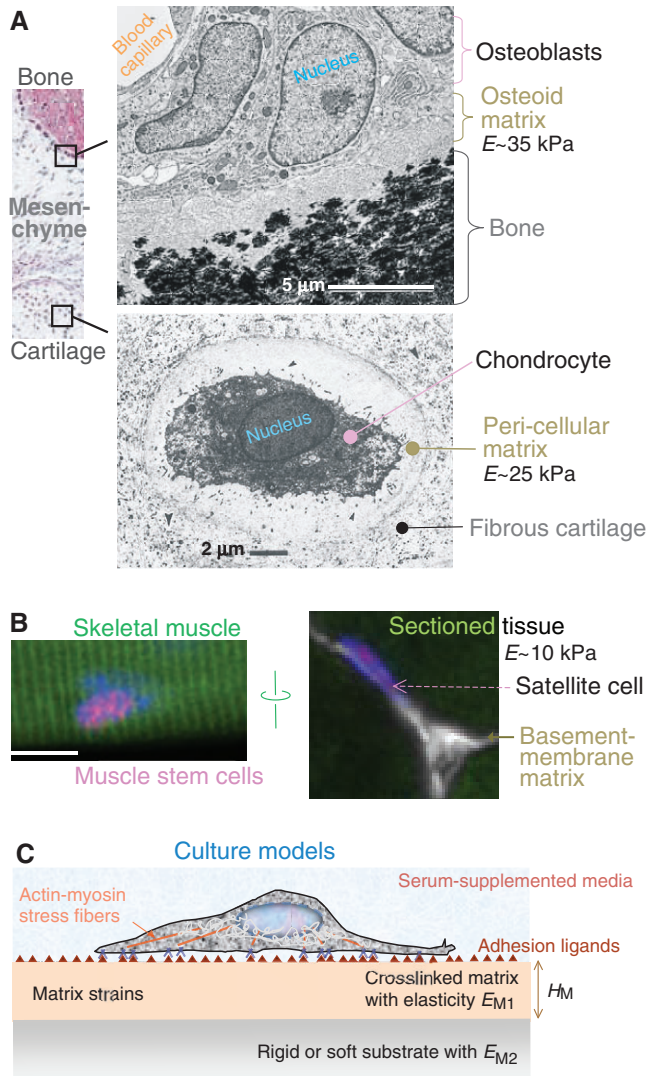
Fig. 1. Mesenchymal cells, matrix elasticity and culture models. (**A**) Left: development of mesenchyme into both cartilage and bone (images courtesy of Marc D. McKee, McGill University, Quebec, Canada). Right: transmission electron micrographs of nascent bone with a layer of cells and matrix on top of bone (upper) or mature cartilage (lower) with a single cell surrounded by matrix. E denotes the elasticity of matrix (see main text). (**B**) Left: longitudinal view of muscle stem cells (pink marker) on striated skeletal muscle. Right: transverse section showing stem cells adhering to matrix (Cerletti et al., 2008). (**C**) Culture models use gels to mimic the thinness and elasticity of natural matrices.

predicated on numerous physical features that ultimately dictate how far we see. Physical optics of the eye are of course crucial to how far we see, but so are many aspects of our everyday surroundings, including the brightness of lighting, the clarity of the air and even the color of the objects present. It is these environmental characteristics that have driven the evolution of eye structures, photoreceptors, optical transduction pathways and even chromatin organization (Solovei et al., 2009). The latter might seem surprising, but in fact retinal rod cells of animals that can see in the dark have recently been shown to possess an unusual chromatin organization that is well-suited to night vision. Similar principles for evolved function probably apply to tissue mechanics down to the level of the cell and its matricellular (i.e. matrix-plus-cell) microenvironment.

Cell attachments to the ECM or to other cells contribute not only to the cohesion of tissues and organs but also to the capacity of a cell to recognize and be regulated by microenvironments. For example, dynamic protein complexes known as focal adhesions (FAs) physically link the actin-myosin cytoskeleton to various ECM proteins – collagens, fibronectin and laminin (Zaidel-Bar et al., 2007) – and provide a means to transmit forces during cell migration and stationary contraction (Beningo et al., 2001; Giannone et al., 2004). Externally applied forces are known to regulate FA size, shape (Riveline et al., 2001) and composition (Galbraith et al., 2002; Zaidel-Bar et al., 2004). FAs are thus mechanosensitive, converting forces into biochemical signals (Alenghat and Ingber, 2002; Geiger et al., 2001; Leong et al., 1995; von Wichert et al., 2008). Physical properties of matricellular microenvironments – particularly stiffness, E and geometry – can therefore be probed by cells that apply actin-myosin stresses to ECM via adhesion contacts (Discher et al., 2005). The magnitude of cell-induced matrix deformations is determined by an interplay between the traction forces that are applied by the cells and the overall compliance of structures in the microenvironment, which is determined by elasticity, shape and the means by which the structures are physically coupled to any boundaries. Basement membranes, such as those found in muscle (Fig. 1B), are ubiquitous, being found below endothelial cells in blood vessels and below epithelial cells in other tissues. The finite thickness of basement membranes prompts the question of whether cells on either side of a thin matrix can communicate mechanically with each other.

Cell-induced deformation of ECM propagates a finite distance into the matrix and is invariably accompanied by cell deformation (Engler et al., 2008). The latter probably contributes to the feedback mechanisms that regulate cell contractility and help to maintain a basal level of cell pre-stress (tension). The basal tension implies that ECMs are constantly under stress, which allows cells to continuously probe the mechanics of their microenvironment – analogous to having your eyes open at all times. To illustrate the feedback mechanics another way, envision yourself in a swimming pool: if someone else jumps in, you can feel the waves they create, but if you yourself swim close to a wall, the waves you make will be reflected and will allow you to sense the presence of the wall. Mechanical obstacles and defects in microenvironments include interfaces, ECM fibrolysis or rigidification (Gunther et al., 1999; Sugimoto et al., 2006); the sensing of such obstacles by cells might trigger repair or disease. Distortions of normal patterns of stress and strain propagation across the matrix as a result of cell contraction or motile processes might contribute to pathological processes such as the epithelial-to-mesenchymal transition (Paszek et al., 2005), in which normal monolayers of epithelial cells become more three-dimensional mesenchyme and contribute to tumor progression.

mechanisms. MSCs initiate osteogenesis on firm, collagen-coated gels that mimic the elasticity of osteoid (E~35 kPa), and MSCs initiate myogenesis on softer, collagen-coated gels that mimic the elasticity of muscle (E~10 kPa), whereas collagen-coated glass (which is rigid compared with any soft tissue) has no clear inductive effect.

The conversion of mechanical cues into biochemical signals (Bershadsky et al., 2006; Discher et al., 2005; Vogel and Sheetz, 2006) presumably establishes the cellular sensitivity to E and also determines the distance that cells can sense into their surrounding environment. To use vision as an analogy, conversion of light into biochemical processes and distinctive neurological signals is

Determining the distance that cells can 'feel' is important for understanding cellular processes in development, wound healing, tumor-cell invasion (metastasis) and tissue regeneration. In this Commentary, we focus on physical principles that determine cellular responses in the context of the ECM, rather than on detailed molecular mechanisms that underlie these responses. We discuss the elasticity of tissue and the stress and strain across finite, structured elastic media – as illustrated in a prototypical cell-culture model in which matrix biochemistry is separated from matrix physics (Fig. 1C). We discuss how deeply cells feel into the matrix, and how the organization of cell populations is directed by static and dynamic mechanical cues. Furthermore, we briefly describe theoretical models that predict how cells respond to these signals on a purely mechanical basis. Finally, we survey possible models for outside-in mechanical coupling of mechanical cues to processes in the cell nucleus, and suggest how gene regulation might be influenced by nucleus deformation.

Elasticity of physiological microenvironments

A recent, award-winning documentary film entitled 'The English Surgeon' (made by Geoffrey Smith in 2007) takes the viewer inside an operating theater during surgery to remove a brain tumor. With the patient awake and the top of his skull removed, the surgeon provides a vivid physical description of what he is touching. He says: "Normal brain has the consistency of very smooth cream cheese. … And the tumor makes it more rubbery … stickier and thicker." A key issue for the cell biologist is whether the softness of such tissue – which contrasts with the rigidity of glass coverslips and tissue-culture plastic (polystyrene) – influences cell structure and function.

Tissues are elastic in that they typically return to their original form after external forces deform the tissue for minutes or longer. The overall contours of the ECM and the way it physically couples to adjacent tissues can help to direct deformations of matrix and tissue so that cells can feel their way through tissue. Mechanical distortions are indeed directional and fast propagating, even at the cellular level (Na et al., 2008). By comparison, cytokine and growth factors deliver more diffuse signals that tend to be highly specific and transient. Such fundamental differences between mechanical and chemical signals might explain why mechanical molecules such as myosin-II play such important roles in embryonic development (Krieg et al., 2008; Pouille et al., 2009).

Many physiological microenvironments that surround cells are soft, even within hard tissues. Osteoid on bone and pericellular matrix in cartilage provide clear examples of hierarchically structured microenvironments in which the various elements have
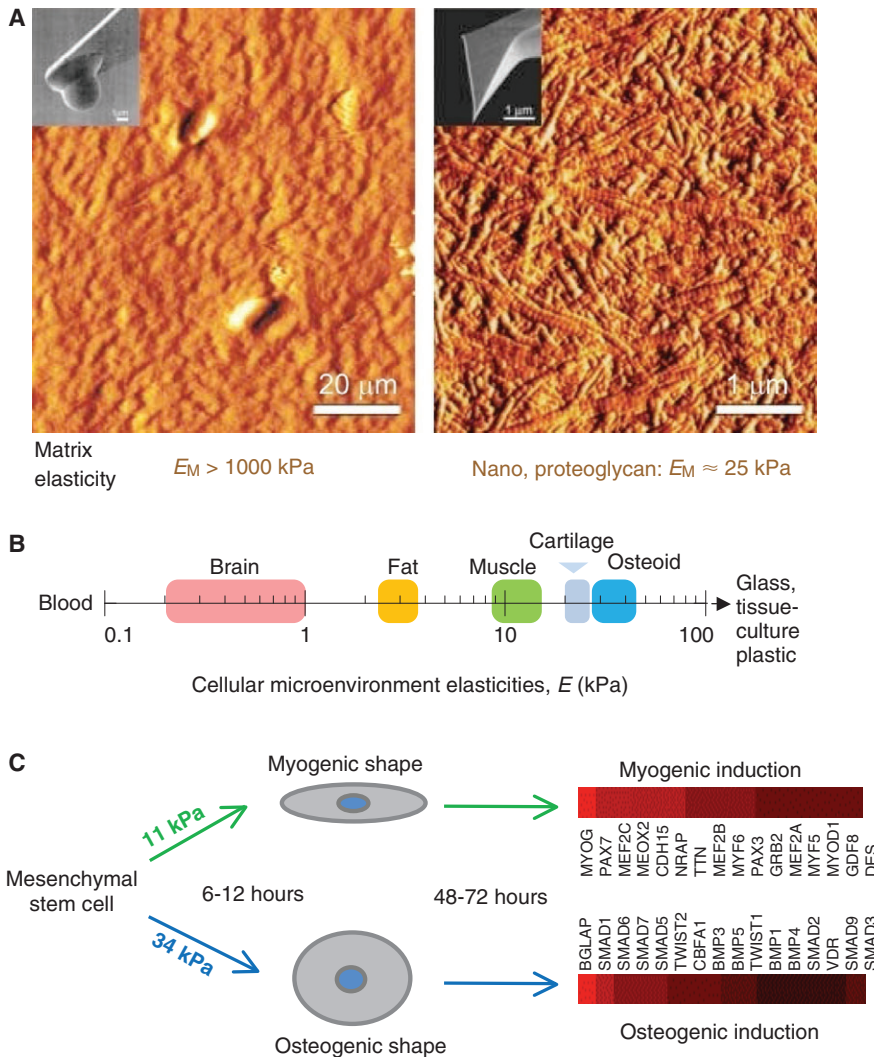
Fig. 2. Elasticity of normal tissues. (A) Articular cartilage imaged by rastering with an AFM, either at the micron scale using a 5-μm-diameter spherical tip (left) or at the nanometer scale with a sharp pyramidal tip (right) (Stolz et al., 2004). Cartilage matrix elasticity E_M depends on length scale: micron-scale and macroscopic measurements probe the stiff collagen-fiber network, whereas the nm-sharp tip probes the softer proteoglycans. (B) Tissue cells are located in a wide range of elastic microenvironments that vary from soft brain and fat through the intermediate stiffness of muscle to relatively rigid cartilage and osteoid. (C) Mesenchymal stem cells attach to elastic substrates and take on phenotypic morphologies within hours of plating. Days later, the cells begin to express lineage markers that are determined in part by the elasticity of the substrate on which they are grown (Engler et al., 2006).

a range of mechanical properties at multiple length scales (Fig. 1A). Stress-bearing bone and cartilage must have sufficient strength and durability to sustain the large forces that are applied at a 'macroscopic' scale of tissue function. However, the mechanics of the microenvironments around the osteoblasts and chondrocytes might be very different from the macroscopic properties of the tissue. Although few tools are suited to probing the mechanics of cellular microenvironments, the atomic force microscope (AFM) is emerging as a powerful tool suited to such studies. An AFM consists of a cantilever with an attached tip (Fig. 2A, insets) that is pressed into a tissue or other material, and the bending of the cantilever, which relates to the material's stiffness, is then precisely measured by monitoring a laser beam that reflects off of the back of the cantilever. Rastering of the AFM tip also produces images; these can either be coarse if using a 2.5-μm sphere or well-resolved if using a ~10-nm sharpened tip. The two tips generate images of, for example, the articular cartilage surface that appear different from each other – both in imaging and in elasticity (Fig. 2A) (Stolz et al., 2009; Stolz et al., 2004). Fibrous collagen II cannot be resolved at the coarser scale (imaged with the 2.5-μm sphere), whereas the sharpened tip reveals the fine structure. The sphere is sufficiently coarse that, when the AFM tip is pressed hard into the cartilage, the stress is distributed among multiple collagen fibers, and the measured value for E is similar to that commonly measured at a macroscopic scale (E_{macro}~1 MPa) (Alexopoulos et al., 2005). By contrast, the nano-sharp tip can probe the proteoglycan matrix between collagen fibers and a much smaller measurement for E is obtained (E_{nano}~25 kPa). To use an analogy of the structure of an office building, the large-diameter collagen fibers are like the rigid steel beams that provide a framework and essential (tissue) support, whereas the walls and carpeting that occupants of the building come into contact with are much softer materials – like proteoglycans and perhaps small-diameter collagen fibers around cells. The cited findings for cartilage are indeed in agreement with some of the most recent micropipette-aspiration-based measurements of the pericellular matrix that can be co-isolated with chondrocytes from cartilage (Guilak et al., 2005). Equally important, disease states such as osteoarthritis lead to either increases or decreases in E_{nano} but little to no change in E_{macro} (Stolz et al., 2009; Stolz et al., 2004).

On the basis of these and other studies, physiological microenvironments around cells should be classified according to their elasticity. Although more micro-scale measurements by methods in addition to AFM measurement certainly need to be made, results to date suggest that an elasticity scale for solid tissue ranges from less than 1 kPa for soft brain, to over 1 kPa for fat and striated muscle, to dozens of kPa for stiffer cartilage and pre-calcified bone (Fig. 2B) [respectively: brain (Flanagan et al., 2002; Georges et al., 2006; Kondo et al., 2005), fat (Patel et al., 2005), muscle (Engler et al., 2004b; Ferrari et al., 1998), cartilage (Stolz et al., 2004), bone (Andrades et al., 2001; Engler et al., 2006; Holmbeck et al., 1999; Morinobu et al., 2003)]. Furthermore, considerable data have suggested that cells sense matrix stiffness (reviewed in Discher et al., 2005), and that some very basic responses – including lineage specification of MSCs towards neurons, myoblasts and osteoblasts – is based in part on elasticity, as is evident from cell morphology and the expression of lineage markers (Fig. 2C) (Engler et al., 2006). Work with differentiated cells has further shown that neuron branching is promoted on very soft matrices that mimic brain tissue (Flanagan et al., 2002), whereas cardiomyocyte maturation (Jacot et al., 2008) and beating (Engler et al., 2008) have been shown to occur optimally on stiff but non-rigid matrices that mimic striated-muscle stiffness. For these latter two cell types, the respective rigidification of glial scars (Georges et al., 2006) and infarct scars (Engler et al., 2008) has also been shown to inhibit normal phenotypes. Therefore, accurate measurements of tissue elasticity in both normal and diseased states seem increasingly important for understanding the effects of microenvironments on cells, and perhaps vice versa.

Cell-induced matrix deformations

Contractile cells use myosin-II to deform their microenvironment by transmitting actomyosin stress via adhesive contacts. Indeed, any soft matrix will deform when stress is applied, and an elastic matrix will also regain its original shape when external forces are removed. Importantly, even when a physiological matrix contains visible fibers [as in articular cartilage with 200-nm diameter collagen-II fibers embedded in proteoglycan (Fig. 2A)], the micro-scale mechanics that are relevant to cells seem well described by a single, direction-free value for the elasticity, E. For cartilage, such thick fibers are largely absent from the proteoglycan pericellular matrix that surrounds chondrocytes (Fig. 1A), which again highlights the need to carefully assess microenvironment structure and not assume that ECM relevant to cells is always fibrillar. When a physiological matrix is isotropic (as in osteoid and pericellular matrix), the relationship between stress (force per area) and strain (relative extension) in small deformation (up to about 10% strain) can be completely described using two elastic moduli. The elasticity E (which is also formally called the Young's modulus) has been emphasized thus far, and soft-tissue matrices are controllably mimicked in their elasticity by various crosslinked hydrogel systems such as polyacrylamide gels coated with collagen. However, one must also measure either the compressibility (which measures resistance to volume changes) or the Poisson ratio (which measures lateral contraction during extension). Folded proteins have compressibilities that are orders of magnitude greater than E, as does water, which makes up ~70% of biological mass. So, it is commonly assumed that E is a more important parameter than compressibility in cell mechanics.

To estimate cell-induced strains and stresses within gel matrices, various methods referred to as traction force microscopy have been widely used. Input measurements for calculations of strain and stress include either the displacements of embedded marker beads (Dembo and Wang, 1999; Lee et al., 1994; Merkel et al., 2007), the distortion of micro-patterned elastomers (Cesa et al., 2007; Riveline et al., 2001) or changes in photoelastic patterns (Curtis et al., 2007; Zimberlin et al., 2008). Whereas such substrates are continuous gel matrices and somewhat similar to a homogeneous ECM, discrete structured substrates composed of bendable elastic pillars are also useful for estimating cell traction forces (du Roure et al., 2005; Tan et al., 2003). Similarly, when granules or other particles undergo reversible displacements within cells, as seen in beating cardiomyocytes (Engler et al., 2008), the elastic strains and stresses within cells can also be estimated and compared with matrix strains. If the strains within the cells exceed the strains in matrix – as is the case with cardiomyocytes beating on rigid glass – then all of the cell-generated strains are sustained by the cell and the cell tends to forcibly remodel its own cytoskeleton. Indeed, in a study of cardiomyocytes, the striations were lost and the cells de-differentiated only on substrates that were much stiffer than physiological substrates (Engler et al., 2008).

A formal theoretical basis for using cell-induced matrix deformations to calculate cell tractions relies on superposing the

solution for a point force, f, acting at the surface of an isotropic substrate that is either of semi-infinite (Boussinesq, 1885) or finite (Maloney et al., 2008; Merkel et al., 2007) thickness. More complex geometries such as arbitrarily thin gels that are either flat or perhaps curved (per Fig. 1A,B) cannot be readily solved analytically but can be computed using finite element models (see below). Limitations in such analyses have restricted the quantitative insights from otherwise elegant studies of Harris and co-workers, who pioneered studies of cell-driven wrinkling of thin silicone films on top of a fluid (Harris et al., 1980). Similar limitations pertain to gels of collagen fibers (e.g. Grinnell, 1982), although much has been learned from such gel systems. Theoretical and computational approaches are important not only for calculations of the cell traction forces that cause matrix and cell deformations, but also for determining the length scales that cells probe by actively propagating stresses and strains into their adjacent matrix. Ultimately, because cells lack eyes to see and ears to hear, a cell's ability to feel into its surroundings not only provides important cellular cues, but also defines the cell's tactile microenvironment.

How deeply do cells 'feel'?
Experimental approaches
Cells feel their physical environment by applying traction stresses to matrix and then sensing mechanical response(s) at or near the cell-matrix interface. The propagation of cell-derived deformations into an elastic and homogeneous media occurs over a relatively long range; displacements decay inversely to the distance between the force source and the cell, although cells might not be sensitive to much of the displacement field. To revisit the analogy to vision, photons can be present for the eye to collect, but sometimes there are not enough photons to clearly visualize an object at a great distance. Similarly, for thin substrates affixed to a second underlying rigid substrate (e.g. glass in Fig. 1C), the long-range propagation of displacements will generally be affected; however, a thin matrix is subjectively thin only if the cell senses the rigid substrate (Maloney et al., 2008; Merkel et al., 2007). For example, cardiac fibroblasts cultured on synthetic gels of equal E that are either a few microns thin (Fig. 3A) or of cellular dimensions in thickness (~100-μm thick, Fig. 3B) on top of glass apply similar stresses to the gels, but the interfacial deformations (i.e. strains) are smaller on the thinner substrate.

The spread area of a cell is a simple morphological metric of cell state and can be used to identify the critical thickness at which cells start to feel a rigid substrate that is hidden beneath a compliant matrix. In general, cells spread more and generate more stress

(pre-stress or tension) on matrices with higher E (Fig. 3C) (Engler et al., 2004a; Solon et al., 2007). A thin gel matrix on rigid glass is expected to be effectively stiffer than a thick layer of the same matrix. The first study that aimed to assess how deeply cells can feel showed that smooth-muscle cells do not change their spread area whether they are plated on collagen-coated polyacrylamide gels of 5- or 70-μm thickness (Engler et al., 2004c). However, subsequent work with MSCs showed that cell spreading on matrices softer than E~5 kPa occurs to a greater extent on gels of sub-micron thickness than on thicker substrates (Engler et al., 2006). More recently, fibroblast cell lines were shown to behave similarly (Maloney et al., 2008). We have observed that such differences in cell morphology extend into the cell to the nucleus, and that increasingly distinctive nuclear changes occur on soft substrates thinner than ~5 μm (A.B. and D.E.D., unpublished). The experimental results to date thus show that mechanosensitivity – at least in terms of depth perception by cells on collagen-functionalized gels that mimic tissue elasticity – is limited to subcellular length scales.
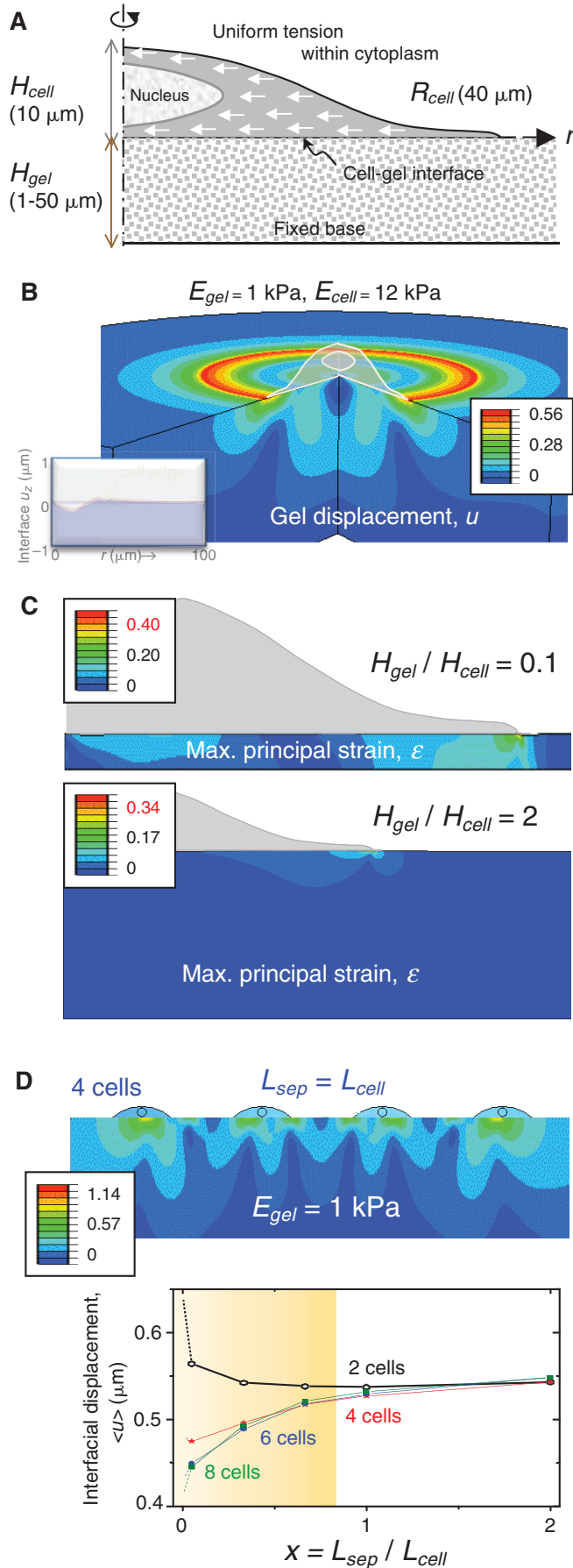
Further information about the possible range of mechanical effects that occur within thin synthetic gel substrates has been provided in a study that used gel indentation with the cantilever of an AFM. Gels of varying thickness, H, were made at the same time with the same polyacrylamide gel solutions to maintain a constant E of tissue-like (~1 kPa) elasticities, and the gels were all indented by ~1-2 μm at forces that bend the cantilever in the nano-Newton (nN) range (Engler et al., 2004c). An apparent elasticity, E_{app}, was obtained in this AFM experiment by fitting the force f versus indentation depth d with a generalized Hertz model:

$$f = \alpha\, E_{app}\, d^m .$$

In this equation, α and m are dictated by the geometry of the tip of the AFM cantilever, which is either a sphere or a pyramid shape (e.g. Fig. 2A, insets). The equation has the general form of a spring law, with the force required to indent increasing with displacement and with substrate stiffness. For gels of thickness H<10-20 μm (and d=0-1 μm), the estimated E_{app} was found to exceed the real E, and this difference between real and apparent – which only reflects the fact that the strains in the thin matrices are restrained by the nearby rigid glass – was more pronounced for 1-kPa gels than for 8-kPa gels. Importantly, all of the length scales and forces determined by measurements of polyacrylamide gels are relevant to cell-matrix mechanics. Therefore, boundary conditions, elasticity and perhaps also embedded fibers can modulate the apparent elasticity of compliant matrices that cells are likely to sense.
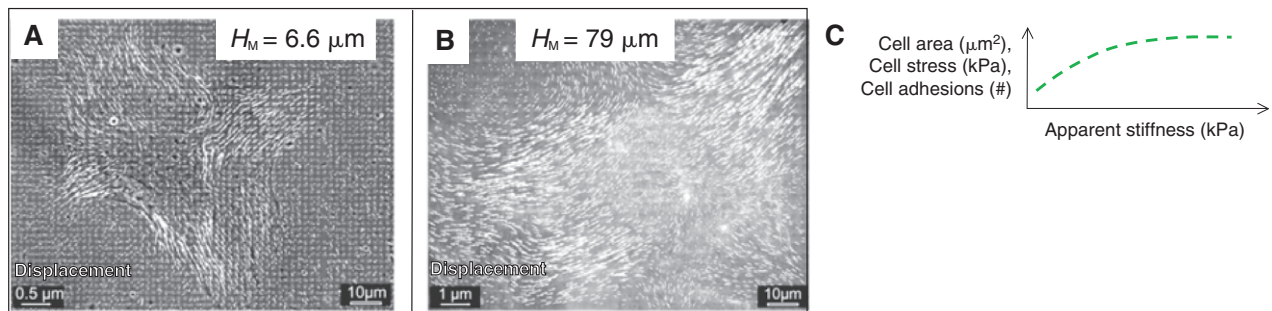
Fig. 3. Cell-induced substrate deformations show that decreasing substrate thickness effectively stiffens the substrate. Cardiac fibroblasts contract and deform thin (**A**, 6.6 μm) or thick (**B**, 79 μm) bottom-fixed substrates (Merkel et al., 2007). The white lines indicate surface displacements of magnitude indicated by the leftmost scale bars; the rightmost scale bars indicate image size. (**C**) Cell spreading and cell stress (e.g. stress-fiber assembly) increase with substrate stiffness, which increases with substrate elasticity E and decreases with substrate thickness H_M.

A

H_{cell} (10 μm)

H_{gel} (1-50 μm)

Uniform tension within cytoplasm

Nucleus

R_{cell} (40 μm)

r

Cell-gel interface

Fixed base

B

$E_{gel} = 1$ kPa, $E_{cell} = 12$ kPa

Gel displacement, u

0.56
0.28
0

Interface u_z (μm)

cell edge

r (μm) →

C

0.40
0.20
0

$H_{gel} / H_{cell} = 0.1$

Max. principal strain, ε

0.34
0.17
0

$H_{gel} / H_{cell} = 2$

Max. principal strain, ε

D

4 cells

$L_{sep} = L_{cell}$

1.14
0.57
0

$E_{gel} = 1$ kPa

Interfacial displacement, $\langle u \rangle$ (μm)

0.6

0.5

0.4

2 cells
4 cells
6 cells
8 cells

0   1   2

$x = L_{sep} / L_{cell}$

Computational approaches

To begin to understand the implications of these experimental findings for cells, a mean-field type of finite element method (FEM) model of contractile cells attached to elastic gels of varying thickness has recently been introduced (Sen et al., 2009). Unlike traction-force-microscopy computations, the input data for this FEM model consists of parameters such as the elasticity of the matrix, E_M, the elasticity of the cell, E_{cell}, tension in the cell and size of the cell. The goal of the model is to predict matrix displacements, strains and stresses, particularly as a function of matrix thickness. An axisymmetric cell with a 40-μm radius is modeled as uniformly contractile, with a pre-stress (tension) in the cytoplasm that exerts stress on the underlying compliant gel (Fig. 4A). The nucleus lacks any contractility and is treated as a purely elastic body, which is relevant to further discussion of the physics of the nucleus (see below). When the mechanical equilibrium state (i.e. elastostatics) is computed by the FEM, it is found that the cell-matrix interface deforms both tangentially and vertically, and that displacements decay around or below the cell well within 40 μm of the cell boundaries (Fig. 4B). This is an indication that cells are unlikely to feel on length scales that are much larger than themselves. Cells can thus be considered blind, deaf and highly restricted in their sense of touch.

In agreement with intuition, on thin gels, computed strains are blunted by the underlying rigid substrate (Sen et al., 2009) (Fig. 4C). Mean interfacial displacements δ as a function of gel thickness H ($=H_{gel}$) fit remarkably well to a simple saturable function:

$$\delta = a + bH / (H_{crit} + H) ,$$

which yields a half-saturation length scale for thickness, H_{crit}. H_{crit} can be thought of as the gel thickness below which cells start to feel the underlying rigid surface. H_{crit} was found to be ~1 μm, and the effect on H_{crit} of varying E_M was non-linear, with the strongest dependence on $E_M < 8$ kPa. The collective results from the FEM modeling are similar to results from limited studies in which the spread area of MSCs was measured for a set of nominally ~1-μm thin gels versus thick gels (Engler et al., 2008). Importantly, the stiffness ranges for mechanosensitivity match matrix compliance at which various cell types – smooth-muscle cells (Engler et al., 2004c), fibroblasts (Rajagopalan et al., 2004) and malignant phenotypes (Paszek et al., 2005) – show many distinctive responses in cytoskeletal organization, signaling, etc.

Fig. 4. Computing cell-induced matrix deformations, depth sensing and matrix-mediated cell-cell interactions. (A) Cells on gels are modeled as axisymmetric and homogeneously pre-stressed (i.e. with uniform tension) coupled continuously to compliant gels (Sen et al., 2009). The cell height and radius are H_{cell} and R_{cell}, respectively, and matrix thickness is H_{gel}. **(B)** Matrix displacements in finite-element computations are maximal at the cell edge and include vertical displacements of the cell-matrix interface (inset). The color scales are for the indicated variable. Matrix and cell elasticity are E_{gel} and E_{cell}, respectively; the vertical displacement of the gel is u_z. **(C)** The strain field propagates across thin gels (top) to the rigid base at bottom, whereas strain localizes to the cell edge for thick gels (bottom). **(D)** Cell-cell interactions are facilitated by elastic deformations of the matrix. Matrix deformations are suppressed in the middle of a string of cells (four cells in this case) due to tractions applied by adjacent cells of width L_{cell}, separated by distance L_{sep}. With increasing cell number, mean displacement decreases owing to opposite tractions at the middle of the string, and this trend saturates to a value that is independent of string length. The color scales are for the indicated variable, which is the mean interfacial displacement.

Intensive computation is not the only means by which to theoretically assess how far cells feel. A type of theory known as the finite-layer theory has been used to predict the critical gel thickness through which cells might feel an underlying rigid substrate. Matrix deformations induced by individual micron-scale contacts suggest a critical thickness of 1.5-2.0 μm (Maloney et al., 2008), whereas estimates that are based instead on tractions applied by cells suggest longer length scales in the order of tens of microns (Merkel et al., 2007). The differences might reflect differences in substrate materials, including both E and the compressibility, but there is qualitative agreement that cells feel on subcellular length scales in these types of gel systems. Results for fibrous matrix systems might be very different, although it should be noted once again that not all ECM is fibrous, even for cells in collagen-rich tissues (e.g. Fig. 1A). What is clear is that thin gels are useful model systems in which length scales of active cell mechanosensitivity can be understood.

Matrix-mediated cell-cell interactions

The field of cell mechanics has for many years been focused on the responses of cells to externally imposed forces rather than cell-generated forces. Model systems that involve stretching a silicone sheet with cells attached, for example, are relevant to tissues such as lung and blood vessels, both of which undergo periodic dilation. Systems in which media is flowed over cells are also relevant to settings such as endothelial cells lining blood vessels, and bone cells that have long projections in narrow channels in which flow couples to bone compression. However, it is also the case that tissue cells in vivo attach to an ECM that the cells pull on and propagate forces into; these cell-generated forces can physically affect neighboring cells. Such cell-generated forces generally add to external forces, and cell-matrix-cell interactions are no doubt complex.

Matrix-mediated cell-cell interactions have been examined theoretically in terms of force-dipole interactions that can lead to strings of cells (Bischofs et al., 2004; Bischofs and Schwarz, 2003; Schwarz and Safran, 2002). Experiments have shown that myoblasts are highly contractile, spindle-shaped force dipoles (Sen et al., 2009). In vitro, these ~100-μm-long cells seem to feel each other laterally at a distance, attract and then reorganize into aligned cells that eventually fuse into multi-nucleated myotubes (Engler et al., 2004b). Co-alignment of myoblasts occurs when cell centers are within ~100 μm on a matrix with E=8 kPa; this distance extends to ~200 μm when the elasticity of the matrix is E=1 kPa. In addition to inducing the cooperative organization of contractile cells, matrix-mediated elastic interactions can also induce coordinated cell migration. The ability of an isolated cell to sense and migrate in response to a gradient in elasticity is referred to as 'durotaxis'. However, fibroblasts growing at high density seem to interact more with one another than with the matrix (Lo et al., 2000). Migration is likewise hindered when endothelial cells are near each other, although this only occurs with compliant gels that mimic physiological stiffness (Reinhart-King et al., 2008). These results thus suggest that cells can feel each other through a shared matrix.

Perhaps the best worked-out example to date of cell coupling through a shared matrix is that of outer hair cells (OHCs) in the cochlea. OHCs can sense a wide range of sound amplitudes with a selective frequency tuning. Groups of stereociliary bundles extend upwards from the OHCs and physically couple to the tectorial membrane above them; in the mouse, the tectorial membrane matrix has a gradient in E (Richter et al., 2007; Shoelson et al., 2004) that ranges from ~20-200 kPa (Gueta et al., 2006). Elastic coupling of hair bundles gives rise to a cooperative response that suppresses uncorrelated noise and increases amplification gain, improving frequency selectivity as compared with individual hair bundles (Dierkes et al., 2008). OHCs thus feel their environment in the context of transducing an external mechanical stimulus (sound waves) into electrophysiological excitation of neurons (hearing).

FEM modeling with the same methods that have been used to understand thin versus thick substrates (described above) has also been used to compute the effects of cell separation and cell number on matrix strains (Sen et al., 2009). When an increasing number of cells are placed closer to one another than about one-cell radius on a 1-kPa gel, substrate displacements increasingly cancel one another out: cells located on opposite sides of a given cell pull on the matrix against each other (Fig. 4D). Cells found in the middle of a field of cells experience less matrix displacement compared with that experienced by isolated cells, which implies that the middle cells are not only interacting with each other but thereby are more decoupled from the matrix. At the outer cell edges, deformations are also enhanced, which provides a basis for understanding edge effects – for example, the enhanced proliferation of cells at the edges of patterned islands (Nelson et al., 2005) – although such effects have only been reported for cells adhering to fibronectin that is loosely attached to rigid substrates. The dependence of such effects on the physical properties of the matrix have yet to be verified experimentally, but the results to date collectively suggest that the broad influence of matrix physical properties constitutes a newly discovered principle in cell organization and tissue formation.

Cell morphology and cytoskeletal forces are directed by extracellular mechanical cues

It has been shown by several groups that many cell types spread, change shape, orient themselves and organize their adhesions and cytoskeleton according to ECM stiffness, strain and stress. With increasing E, from ~100 Pa to ~100 kPa, cells spread more (Fig. 3C) and exhibit more developed adhesive contacts (Engler et al., 2004a; Pelham and Wang, 1997). As E increases, stress fibers not only increase in abundance, which indicates more force generation by cells, but the stress fibers also tend to align and orient the cell, leading to cell polarization (Engler et al., 2004a; Pelham and Wang, 1997). These responses are evident as long as matrix ligand (such as collagen) exceeds a limiting density; these responses are clear within a few hours of plating cells on the matrix. In addition, localized versions of these responses can be seen within ~5-10 minutes after forces are focally applied to a cell using a bead or a glass rod to contact the cell (Choquet et al., 1997; Riveline et al., 2001; Wang et al., 1993).

Cyclic strains that are externally applied to a silicone sheet with cells on top reveal a rate dependence to cell responses (Jungbauer et al., 2008). Slow stretching of the film induces cell orientation that is parallel to the direction of applied stretch (Collinsworth et al., 2000; Eastwood et al., 1998) but, when the stretch rate exceeds ~1 Hz, cells reorient nearly perpendicular to the stress direction (Kurpinski et al., 2006; Smith et al., 1997; Takemasa et al., 1997). Accordingly, Safran and co-workers have proposed for cells a homeostatic stress in terms of a 'force dipole' at the cell-matrix interface, which cells are programmed to reach over time (De et al., 2007). For stretching that is slower than the required relaxation timescale, cells align parallel to the applied stress. For rapid stretching that is faster than relaxation timescales, cells cannot adapt and will tend to orient perpendicular to the direction of the applied stretch. This approach to driving the orientation of cells is as fundamental to understanding polarized cells

as is the net charge dipole on an α-helix or any other complicated macromolecule.

Molecular mechanics in mechanism: from forced unfolding to heat-shock proteins

The various mechanosensitive responses outlined above occur far too quickly to be explained by changes in gene expression and instead originate from a combination of signaling cascades – such as phosphorylation events in FA signaling (e.g. Bershadsky et al., 2006) – and coupled changes in protein-protein interactions that are intrinsic to cytoskeleton remodeling. Deciphering the detailed molecular kinetics of matrix mechanotransduction – that is, exactly how and when mechanical cues are translated into biochemical signals and, in turn, a biological response – remains a major challenge. The concept of forced unfolding and extension of protein domains is one of the most intriguing hypotheses that is increasingly supported by recent results. Work with the purified FA components talin and vinculin has shown, for example, that forced extension of talin leads to unfolding and exposure of cryptic sequences for binding to vinculin (del Rio et al., 2009). Such a process should activate talin and vinculin for F-actin assembly and thereby regulate FA growth and cytoskeleton remodeling. Documentation in living cells of the step-by-step force-driven kinetics of FA assembly is desperately needed. In the case of talin, the molecule must bind into an adhesion at two points so that force can displace the two points and then unfold a domain that recruits vinculin to promote FA growth:

talin binds an FA at two points
→ forced unfolding of talin domain(s)
→ vinculin binds talin domain
→ FA growth .

In future, evidence of mutant talin proteins that fold or unfold with different kinetics under force would provide some of the clearest structure-function data in support of such a model.

Phosphorylation-modulated activation of kinases such as FA kinase (FAK) is also well known to involve conformational changes (e.g. Liu et al., 2002). An indirect role for force in such activation is implicated by the finding that a change in FAK phosphorylation occurs in parallel to a cell-tension-dependent switch of binding states of $\alpha5\beta1$ integrin to its ligand, fibronectin (Friedland et al., 2009). In addition, the phosphorylation of some proteins, such as p130Cas, has now been shown to depend on protein extension (Sawada et al., 2006). Data at the cellular level for stretching of p130Cas has thus far been obtained using a conformation-specific antibody, but such antibodies are unfortunately too rare to provide broad insight into the many possible force-dependent changes in the folding and mechanical remodeling of other proteins in cells.

To broadly reveal structural changes within proteins in living cells, a proteomic-scale method known as 'cysteine shotgun' mass spectrometry (Johnson et al., 2007) has been developed. This methodology exploits two main principles: first, Cys residues in cytosolic proteins are relatively hydrophobic and therefore are often buried within protein folds; second, membrane-permeable dyes for use in viable cells covalently react with Cys residues and can be imaged in cells and then mapped to primary sequences of proteins by mass spectrometry. In some of the earliest experiments that used this method, MSCs were labeled with a Cys-reactive dye and myosin-II was inhibited or not with blebbistatin. Several proteins showed significant differences in labeling with differential myosin-II activity, and specific Cys sites were tentatively identified in non-muscle myosin-IIA, vimentin and filamin (Johnson et al., 2007). Labeling kinetics of cell lysates under native and urea-denaturing conditions has confirmed numerous sites in these same proteins as 'cryptic' because they are slowly labeled under native conditions. Many more cryptic sites in several other proteins, including talin, have been identified in lysates (D.E.D., unpublished), although these sites require further study to understand their kinetics in intact cells.

To understand molecular mechanisms in mechanotransduction, we ultimately need to know what happens first in the signaling cascade – in other words, we must understand the kinetics of transitions within multi-component structures. However, this is complicated within a cell, owing to the mechanical networking that transfers forces from the ECM to FAs to many interconnected cytoskeletal proteins. In addition, on the basis of almost two decades of single-molecule studies of force-driven transitions, it is known that the rate k of any unfolding or dissociation in almost any given molecular transition (over a distance x) increases exponentially with force, f:

$$k=k_0 \exp(f x / k_B T) .$$

In the equation above, the zero force rate k_0 and the transition work [force (f) times distance (x)] scaled by the random thermal energy $k_B T$ are characteristic of specific molecules and perhaps specific domains. This equation has been shown to fit forced unfolding of domains in the cytoskeletal protein spectrin within cells (Johnson et al., 2007), thus demonstrating that any kinetic studies in mechanobiology indeed require careful attention to the forces.

As highlighted by the rate equation for k, force and temperature (or work and heat) are physically intertwined in cell biology. The recent studies discussed above implicate force-driven protein structural changes in cells that are mechanically stressed, but we already have a hint of biological significance in studies of heat-shock proteins. Heat-shock proteins function as chaperones that are activated by temperature increases of just a few degrees above 37°C (e.g. fevers of ~40°C), as well as by other environmental stresses. This sensitivity suggests that many human proteins are poised to unfold, even at body temperature. The small heat-shock protein Hsp27 is especially interesting in that it not only binds to and stabilizes many cytoskeletal proteins, including F-actin (An et al., 2004), but it can also translocate into the nucleus and modulate gene transcription (Friedman et al., 2009). Members of the Hsp70 family exhibit similar mechanosensitive responses, including nuclear translocation (Jagodzinski et al., 2006).

Physics of the nucleus: a linked lamina around a fractal fluid of chromatin

Much is known about translocation through nuclear pores, but comparatively little is understood about the collective physics of the nucleus. Given that cells can feel the matrix that surrounds them, do cytoskeletal stresses and strains on the ECM also propagate into the cell nucleus to affect gene expression? The nucleus is caged by the cytoskeleton near the center of most cells (Fig. 5A), and recent studies show that nuclear movement during polarized cell migration results from actin-myosin contraction (Gomes et al., 2005). Electron-microscopy images further suggest that the nucleus is a highly deformable body (e.g. Fig. 1A), whereas micromechanical experiments provide physical measures of nuclear plasticity. As shown in Fig. 5B, aspiration of a single nucleus into a micropipette has demonstrated that the nucleus flows and chromatin reorganizes when stresses are similar in magnitude to those that cells apply to the ECM (~1-10 kPa)
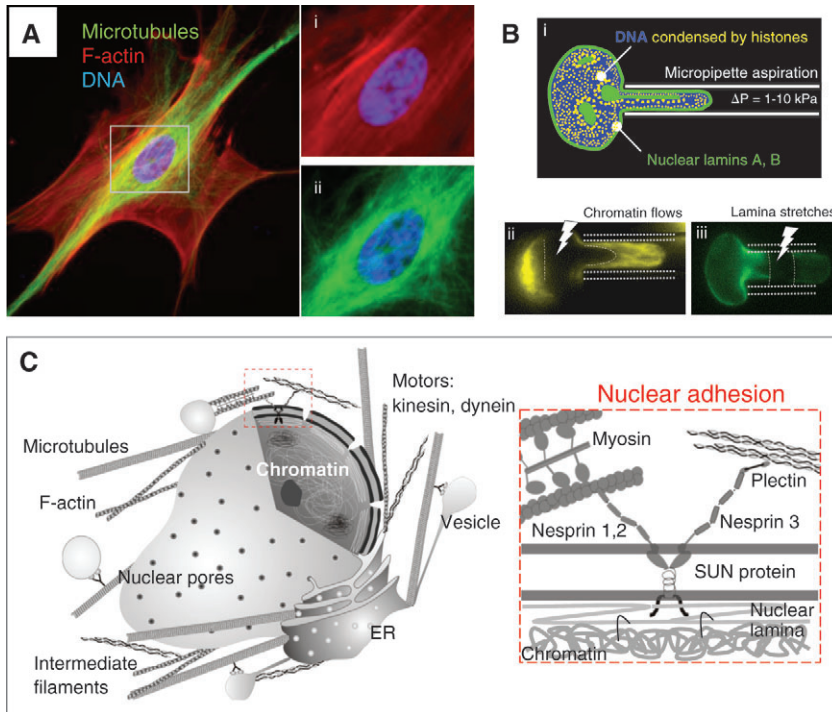
Fig. 5. The nucleus: caging by the cytoskeleton, deformability, and molecular components of nuclear adhesions. (A) Typical cytoskeletal architecture around the nucleus of a mesenchymal stem cell on a 34-kPa collagen-coated polyacrylamide gel substrate. The actin cytoskeleton is shown in red (phalloidin staining; i), microtubules are shown in green (anti-α-tubulin; ii) and nuclear DNA is stained blue with Hoechst. (B) Micropipette aspiration mimics the large deformations observed in vivo (e.g. Fig. 1A). Experimental aspiration pressures (i) are typical of the stresses that cells generate (Pajerowski et al., 2007). Such pressures deform the nucleus, as evidenced by chromatin flow (ii) and lamina stretching (iii). Photobleaching is indicated by the lightning bolt and reveals the respective flow and displacement profiles. (C) Schematic of nuclear organization and interaction with selected major cell components. The nuclear envelope consists of the inner and outer nuclear membrane, the latter of which is continuous with the endoplasmic reticulum (ER). Trafficking of ER on the microtubule network is depicted. The lamina interacts with chromatin and inner membrane proteins such as SUN proteins, which also bind nesprins that span the outer membrane and cross into the perinuclear space. The nesprins link to the various cytoskeletal components and provide a means to transmit cell stress to the chromatin via a nuclear adhesion.

(Pajerowski et al., 2007). Such results raise the possibility that matrix mechanics and cytoskeletal stresses might be directly transduced into nuclear function and gene expression, but further dissection of nuclear structure and transcriptional outputs is certainly warranted.

The nuclear lamina as a thin matrix

To stabilize fluid lipid bilayers in animal cells (which lack cell walls), a membrane skeleton generally forms. With plasma membranes, a network of spectrin-crosslinked actin is common, whereas with the nuclear envelope (Fig. 5C), the network present at the interface between chromatin and the inner nuclear membrane is composed of the intermediate proteins known as lamins (Dahl et al., 2008; Gruenbaum et al., 2005). Lamin-associated proteins, including lamin-binding receptor (LBR), help to anchor the lamins (Mattout-Drubezki and Gruenbaum, 2003). Expression of lamin B is constitutive and ubiquitous, whereas lamin A is expressed in many but not all terminally differentiated cells. A notable example of a cell in which lamin A is not expressed is the neutrophil, which has a multi-lobed or segmented nucleus that is required for these cells to crawl through tight spaces (Hoffmann et al., 2007). Micropipette aspiration of stem-cell nuclei, which also lack lamin A, together with RNA-interference experiments that knock down lamin A expression in epithelial-cell nuclei, have shown that the lamin meshwork stiffens the nucleus as the lamina stretches and the chromatin – which has a 'fractal globule' type of organization typical of long but unentangled polymers (Lieberman-Aiden et al., 2009) – is seen to distend and flow (Fig. 5B) (Pajerowski et al., 2007). These most recent measurements show that chromatin behaves as a fractal fluid as it flows irreversibly after about 10 seconds of applied stress. Beyond this timepoint, the contribution of lamin A to nuclear stiffness has been measured to be ~5 kPa ($\approx E_{lam}$). This stiffness approximates the elasticity of a typical soft-tissue microenvironment (Fig. 2B), although the thickness of the lamina

is also probably important in the same sense that ECM thickness H_M establishes whether cell-generated stresses can propagate through one medium and into another (Figs 1, 3, 4). These comparisons to the ECM are intriguing, as they suggest that cytoskeletal stress and strain can be transmitted inwards to the nucleus – provided there is physical linkage to the nuclear lamina.

The LINC complex constitutes a nuclear adhesion

The linker of nucleoskeleton and cytoskeleton (LINC) complex consists of nuclear-envelope spectrin-related proteins (nesprins) that bind at one end to the cytoskeleton and at the other end to nuclear-envelope proteins called SUN proteins, which attach to the lamina. Nesprins are large proteins (up to ~1 MDa in size) that are long and probably flexible based on homologies to spectrin. Various isoforms link to actin filaments, intermediate filaments and microtubules (Gruenbaum et al., 2005; Zhang et al., 2001), sometimes through molecules such as plectins (Foisner and Wiche, 1987). Nesprins possess a transmembrane domain that spans the outer nuclear membrane and binds to the SUN proteins within the 25- to 30-nm perinuclear space between the double bilayer of the nuclear envelope (Crisp et al., 2006). SUN proteins are integral membrane proteins that span the inner nuclear membrane and anchor to the lamins. Perturbations of LINC-complex proteins alter nuclear positioning (Grady et al., 2005). Analogous to FA complexes that span the plasma membrane and mediate both attachment and force generation by the cytoskeleton to the ECM, the LINC complex spans the nuclear membrane and mediates attachment and possibly force generation by the cytoskeleton into the nuclear matrix of lamins, chromatin and other components. It is therefore useful to consider these complexes as 'nuclear adhesions' (NAs).

Putting it all together: microenvironmental elasticity, cytoskeletal stress and gene organization

The relationship between chromatin dynamics and gene expression is an active area of study (Branco and Pombo, 2006; Kumaran

et al., 2008), and it is increasingly believed that genomes are not randomly organized within cell nuclei (Takizawa et al., 2008). Indeed, genes that are associated with differentiation processes have been observed to change position within the nucleus, with possible correlations between activity and position relative to the lamina (Kosak et al., 2002). This raises the possibility that matrix elasticity is physically wired via the cytoskeleton and NAs to the expression of at least some matrix-sensitive genes. Disease-causing mutations in various nuclear-lamina and LINC components include mutations that affect protein folding, as well as those that affect specific tissue lineages such as muscle (Shumaker et al., 2008). It is known that the nuclei of embryonic stem cells (ESCs) lack lamin A and are extremely soft (Pajerowski et al., 2007), and it is also known that ESCs proliferate but do not differentiate when non-muscle myosin-IIA is inactive (Conti et al., 2004). Given that myosins are a eukaryotic invention (Richards and Cavalier-Smith, 2005), myosin-mediated forces in multicellular organisms might have evolved key roles in accelerating rate-limiting steps in some of the most basic of multicellular processes, including lineage specification. The linkages that have been discovered between the actomyosin cytoskeleton, ECM and nucleus thus suggest many possibilities for physiological regulation.

Conclusion

In this Commentary, we have discussed several examples of how mechanical cues, particularly matrix cues, induce various cellular responses. Cytoskeleton assembly and linkage to FAs are key to cell shape, cell stress and how far cells feel. In addition, cytoskeletal caging of the nucleus and its tethering via NAs provide possible means of transmitting mechanical stresses to affect not only nuclear shape but also perhaps the inner nuclear matrix and gene expression (Webster et al., 2009). Mechanical coupling of the nucleus through the cytoskeleton to cell-surface integrins had been observed in earlier experiments that probed the cell surface with glass microneedles (Maniotis et al., 1997). Matrix elasticity and matrix thickness could couple to cell function in many physiological and regenerative contexts. For example, MSCs cultured on thick but not thin gel substrates (e.g. Fig. 1C) reveal matrix-elasticity-dependent effects on cell shape and cytoskeletal organization within hours, whereas changes in gene expression take days for integration of microenvironmental information (Engler et al., 2006). More recent results even document systematic changes in the nuclear shape of MSCs in response to matrix elasticity (A.B. and D.E.D., unpublished). Ultimately, although investigations of cell adhesion and ligand biochemistry remain essential, mechanotransduction at the whole-cell level seems possible only with carefully controlled cell-culture systems that mimic the measured elasticity of soft-tissue microenvironments.

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