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The mechanical cell

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Many decisions we make are based on our ability to probe the mechanical properties of materials and to measure forces applied to us. We choose ripe fruits in part by squeezing them, make inferences based on the firmness of a person's handshake, and are often attracted or repelled by whether something is soft or sticky, a response of great interest to product designers and cosmetic manufacturers. These sensory abilities depend on our capacity to function as rheometers: we apply forces of controlled magnitude and duration and detect the resulting deformation or rate of flow. That is, we are simultaneously aware of stress (force/area) and strain (deformation) or strain rate when we judge how an object feels or how hard we are pushed or pulled. Cells appear to be equally sensitive to information about force, stiffness and adhesivity. The range of force and stiffness to which different cell types respond and the nature of their responses as they encounter materials with stiffness different from that of the tissue in which they normally reside are as individual as their responses to chemical stimuli.

The ability of cells to respond to external forces or to detect the mechanics of their substrates as they apply internally generated forces depends on the mechanical properties of the cells themselves. The same methods and instrumentation used to measure the mechanical properties of synthetic materials — the province of rheology — have been applied to tissues and isolated cells. In the latter case, modification of traditional methods and invention of new methods have been needed to cope with the small size and fragility of an individual cell. In this primer we shall attempt to summarize some of the current findings in cell mechanics and how they are thought to affect how cells function or malfunction *in vivo*.

Why physics matters for cells

The idea that the physical properties of cells are important for their biological function is nothing new. Many early cell biologists emphasized that defining the physical features of cells is essential to understanding how they function [1]. Cell functions that are defined by the mechanical work done by the cell such as motility and cytokinesis have especially motivated studies of the cell's mechanical properties and mechanisms of force production. But beyond those processes that, like muscle contraction and cell locomotion, clearly do mechanical work and require an elastic cytoplasm or a gelation-solation transition to perform that work, the physical state of the cell has sometimes appeared to be merely a side-effect of the structures and reactions required for the more important genetic and biochemical processes that guide cell function. Recently, however, interest in the physics of cells has been stimulated by evidence from a wide range of studies that external force applied to a cell, and the resistance that extracellular matrices exert on cell-derived forces, also generate signals that are as potent as those of chemical stimuli to direct cell growth, survival, differentiation, and function [2]. Changes in those physical features or in the cell's response to them are beginning to be taken seriously as contributing factors and not just consequences of pathologies such scarring, fibrotic disease and cancer [3].

A few examples of the importance of external forces are the ability to promote axonal elongation by applying pN to nN scale forces to the tips of the neuronal growth cone, the effects of fluid flow on the morphology and signaling of vascular endothelial cells, and the abrupt loss of bone or muscle mass when forces due to gravity or exercise are reduced. To understand how these forces are transmitted throughout the molecular structures of the cell, and how they might be transduced into biochemical reactions, requires detailed quantitative characterization of the mechanical properties of the cells at the points where these forces are applied. Just as the three-dimensional atomic structure of a hormone receptor is needed to fully understand how that chemical stimulus activates a cellular function, so too is it necessary to define how cells and macromolecules within them deform when forces are applied.

Not only do cells respond to forces applied from the outside, but they are also often highly responsive to the passive resistance of their substrates to the active forces that they themselves generate. This field of investigation, stimulated largely by a study that showed how differently fibroblasts looked and moved on gels with the same chemical characteristic but different stiffness [4], has revealed many instances where the mechanical properties of the environment modify or even override strong chemical signals. For example, sarcomeres will not form in cultured myocytes unless they grow on materials with the approximate stiffness of a muscle, and mesenchymal stem cells cannot be made to differentiate efficiently into osteocytes when they grow on soft materials even when provided with the chemical factors appropriate to this cell type [5].

How cells respond to forces, which signaling pathways are used, which genes are upregulated, and so on, is now beginning to be unraveled in some cases, such as endothelial cells in disturbed flow environments, but which molecules first respond to the force is often still unknown. Even less is known about how cells probe their mechanical environment — that is, how they function as rheometers.

Force sensing is different from stiffness sensing

Fundamentally, a cellular force sensor can be a *passive* component that merely responds to changes in forces. Perhaps the best-known example of a physiological force sensor is the hair cell in the ear that translates sound waves into neural impulses initiated by changes in membrane ion channel activity. A stiffness sensor, or durosensor, however, must *actively* apply a stress and measure strain or *vice versa* and then compute the ratio of these two variables. To perform this stress-strain assay, the durosensor must use molecular motors to apply a force to its substrate, measure the resulting movement, and compute the stress/strain ratio to read out elasticity.

Most, but not all cell types tested thus far can respond to a wide but limited range of substrate elasticities, and their durosensors must be able to actively control this stress-strain assay, presumably by changing their initial stress or strain input. Stiffness sensing is highly variable among different cell types, and the amount of stress they apply to their substrate also varies widely. Most tissue cell types spread better on harder substrates but some, such as neurons, extend much better on soft matrices that resemble the elasticity of brain and are inhibited from spreading on rigid surfaces. The quantitative and qualitative differences in durosensing and durotaxis have many implications for wound repair and pattern formation during development that are just beginning to be explored.

Minimal components for stiffness sensing

By analogy with rheometers, the cell's durosensor can be stress-controlled or strain-controlled. Although the mechanism is unknown, it seems plausible that after molecular motors generate tensions at the cell–substrate interface, the resulting strain leads to a structural change in a

protein or within the lipid bilayer, and this structural change activates some signaling pathway. This means that the durosensor requires an active stress or strain generator, a set of molecules or proteins to transmit stress or strain from the cell through its plasma membrane onto a ligand linked to the external substrate, and a sensory unit to measure the readout and convert that to a biochemical signal. Many experiments suggest that myosin is the active stress generator [6] and that the stress is transmitted through actin filaments onto flexible proteins such as talin or filamin which bind transmembrane proteins like integrins and then to an external ligand as illustrated in Figure 1.

Particular attention has focused recently on proteins that might act as clutches that mediate the tension between the inside and outside of the cell, especially in cases where their binding affinities to the cytoskeleton are increased or decreased by force. Some protein-protein contacts are characterized as catch bonds that are stabilized by decreased dissociation rates when force is applied, and others are slip bonds that dissociate faster under stress. How molecular clutches respond to mechanical stress might determine whether cells extend better on soft or hard substrates [7]. Proteins that are connected to integrins, focal adhesions or actin, or that are embedded nearby in the lipid bilayer are all possible durosensors, especially those that can unfold to expose cryptic activation sites, open or close ion channels, or recruit/expel proteins as a result of increased or decreased membrane curvatures. Another possibility is that the tension generated by the putative clutch protein, mediated by either slip or catch bonds, might affect protein binding affinities on, for example, actin filaments and thus constitute a readout for elasticity. Because the structures most likely to deform are those in the softest parts of the intracellular–extracellular link, the stiffness of the cell relative to its surroundings is critical to the cell's durosensing capability.

How stiff are cells?

Many different methods have been used to apply forces to cells and measure the resulting displacement, but providing an unambiguous value for cell stiffness is elusive. The stiffness of a cell or a tissue is not a simple scalar quantity like temperature or growth factor concentration, but rather a function that depends on time scale, the degree of deformation, the direction of imposed force, and spatial distribution of the deformation. Cells and extracellular matrices are neither simple solids nor liquids, nor are they homogeneous materials. Indeed, a defining characteristic of most cellular and tissue stiffnesses is that they are *nonlinear*; their stiffness typically increases with increasing deformation [8]. Moreover, the presence of molecular motors and other active processes within cells leads to the conversion of chemical to mechanical energy, further complicating the mechanical behavior [9]. This complexity is compounded by the fact that nearly all studies of cell mechanics *in vitro* apply to cells grown on rigid surfaces.

A remarkable feature of at least some tissue cells is that they tune their stiffness to match that of the substrate to which they adhere, at least over a limited range of stiffness, as illustrated in Figure 2. The mechanisms by which they alter stiffness include increased cytoskeletal assembly, activation of crosslinkers and generation of internal tension that exploits the strain-stiffening non-linear elasticity of the cytoskeleton. Perhaps the most that can be clearly inferred from rheological studies of cells is an upper limit to their elastic modulus that is probably defined by the maximal concentration of crosslinked cytoskeletal networks they can produce. Measured on a time scale of approximately one second and a strain of a few percent, this value seems to be near 20 kPa for a typical animal cell, but for other regions within the cell and under different conditions, this value can be much less.

Are cells liquids or solids?

Like all complex materials, cells and tissues are neither pure solids nor pure liquids. Whether they appear solid (usually defined by their ability to recover shape) or liquid (defined by their irreversible shape change) depends on how long and how much they are deformed. For example, on a timescale of seconds, most cells and tissues recover shape and are therefore solids, but when subjected to prolonged stresses, they remodel, by both active and passive processes and therefore appear liquid. Even on a subcellular level, the ability of amoeboid cells to undergo solid–liquid transitions as judged by the presence or absence of Brownian motion in liquid or glassy parts of the cytoplasm was recognized centuries ago to be an essential feature of locomotion. The precise functional form of how elastic moduli scale with time has attracted much attention from cell biologists and soft matter physicists.

An interesting feature of plots of elastic modulus *versus* time (or equivalently shear storage modulus *versus* frequency from oscillatory measurements) is that, on a logarithmic scale, plots of modulus *versus* time are linear with very shallow slopes. This so-called power law behavior is seen from the scale of intracellular particles to whole cells and tissues, and is also reported in studies of reconstituted cytoskeletal networks. The molecular explanation for this power law behavior and its significance for the cell have stimulated much interest and debate [10]. However, the biological significance of a cell's time-dependent elasticity seems clear. To optimize the design of a helmet to protect the brain from rapid trauma-induced deformation, the relevant quantity is the brain's elastic modulus on a sub-second time scale, but when trying to understand how a neuron or glial cell probes its environment, brain rheology on a much longer time scale is relevant.

Future directions

Mechanobiology and in particular elasticity sensing are increasingly seen to be important epigenetic factors that can influence cell behavior and play a central role in gene induction, protein synthesis, cell growth and differentiation. Recent advances in making soft biocompatible materials and in instrumentation capable of measuring cell-scaled deformations and forces have produced many examples of how physical factors alter cell biology. The molecular mechanisms of mechanosensing, or at least some proteins that appear to be important for this function, are beginning to be identified. Many basic features for stiffness and force sensing remain unknown, such as the time that it takes or the amount of deformation needed before a cell judges a material too soft, too hard, or just right. It seems very likely that many surprising results will emerge from studies of cellular physics that will help define how to optimize biomimetic materials and how cells can be controlled when their normal functions fail.

Box 1. Brief glossary for cell mechanics

Force

The quantity that causes an object with mass to change its velocity. It is a vector that has both magnitude and direction. Newton defined force formally to be directly proportional to mass and acceleration. Its SI unit is the newton, N. On a single cell scale a useful unit is the pN, and a single motor protein produces a maximal force of a few pN.

Stress

The force exerted on an object normalized by the area over which the force is acting. For example, when we slam a hammer on a piece of solid oak, we will at most make a shallow indentation, but by maximizing stress using a nail with a very small area, we can easily pierce the oak. The SI unit of stress is the pascal, Pa, or N/m^2. 1 Pa = 1 $pN/\mu m^2$. Stresses

vary widely in biology, from < 1 Pa for shear stress due to blood flow to > 10^6 Pa on knee cartilage every time an average adult person stands up.

Strain

A dimensionless number which is the formal definition of deformation; it reports the geometric change in shape of a material under stress. Very approximately, it is the distance a material is stretched or compressed relative to its resting length. Cells typically undergo strains of 10–100 % during lung expansion, muscle contraction, and so on.

Elastic modulus

This is a measure of stiffness. Formally it is the ratio of stress to strain, and therefore also a vector, and indeed most biological materials are anisotropic and therefore stiffer in one direction that another. An important complication is that most biological materials are viscoelastic rather than simply elastic, and therefore the elastic modulus is not a simple spring constant, but also depends on the amount of time, and the degree to which the ample is deformed. The unit is also Pa, and most soft tissues have elastic moduli between 10 and 50,000 Pa, measured on a time scale of one second and a strain of one percent . Elastic modulus is often measured by imposing constant strain and measuring the stress, but can also be measured by applying a controlled stress and measuring the resulting strain.

Viscosity

For liquids, this is the ratio of stress to the rate of strain (or flow rate). The SI unit is Pa.s = 10 Poise. The viscosity of water for example, is approximately 1 mPa.s.

Viscoelastic

The combination of viscosity and elasticity in a material. Viscoelastic materials exhibit significant time-dependent flow like liquids, but also have some ability to recover their initial shape after a deforming stress is removed (the hallmark of elasticity).

Compliance

The capacity to change shape in response to stress. Roughly, compliance is the inverse of elastic modulus, and its unit is correspondingly 1/Pa. Compliance is usually measured by applying a constant stress and measuring the resulting strain, which can slowly increase with time as the sample creeps.

Nonlinear elasticity

For an ideal elastic material, stress is proportional to strain, and the elastic modulus is the slope of the linear plot of stress vs. strain taken at any value of stress or strain. Many complex materials exhibit non-linear elasticity. That is, their elastic modulus changes with increasing strain. Such materials can be either strain-softening or strain-stiffening, as is the case for crosslinked cytoskeletal and extracellular filament networks.

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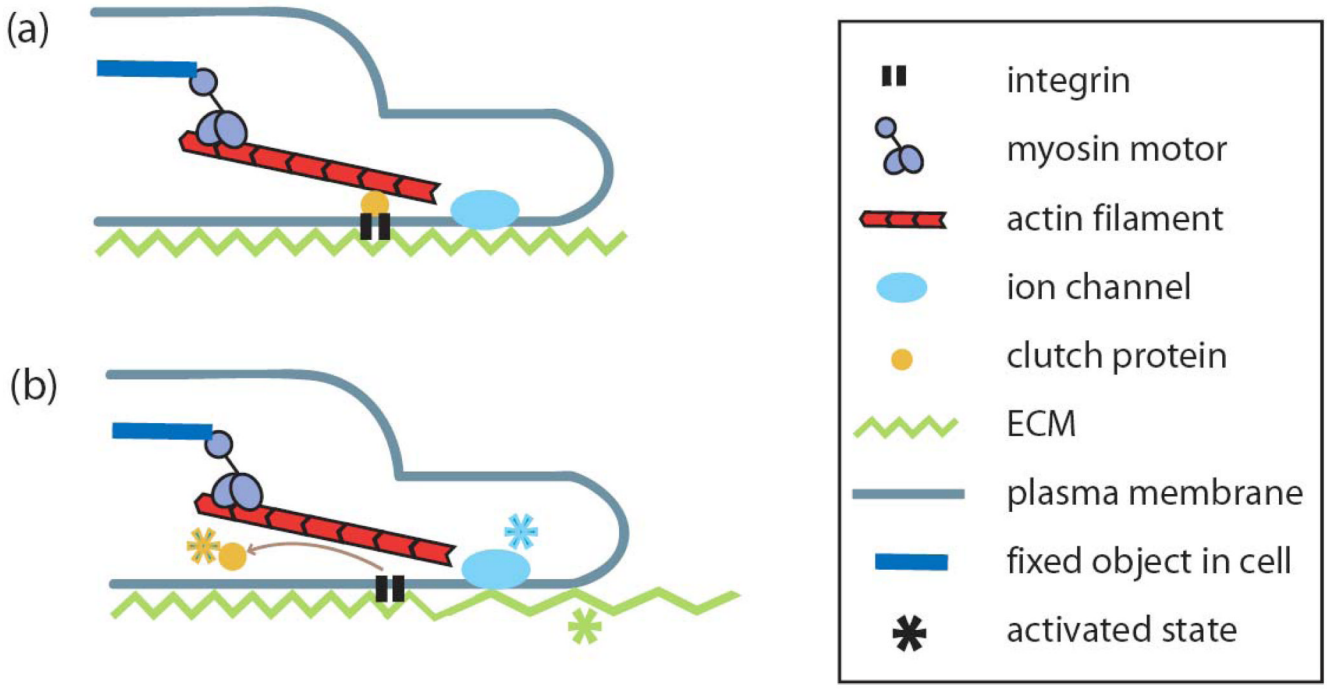

Figure 1. Cellular components proposed to be involved in stiffness sensing

(A) A cell in its resting state. Molecular motors such as non-muscle myosin walk on actin filaments to exert forces through focal adhesion proteins. Focal adhesion proteins are connected to transmembrane proteins such as integrins via slip or catch bonds. Thus traction forces are transmitted to the external substrate via integrin-ligand interactions. In principle, any protein along this 'force chain' of molecular motor to actin to focal adhesion protein to integrin to ligand to extracellular matrix can be stretched and therefore activated. Externally, ligands such as fibronectin or laminin might also be activated by force. On the plasma membrane, integrins or focal adhesion proteins can be stretch activated; the membrane itself may be deformed or sheared to induce protein clustering or recruitment. Internally, the tension on the actin filament itself might affect molecular motor affinity and hence transport of proteins; the nucleus might also be directly deformed to affect transcription. (B) A cell that applies a stress or a strain will get a response that is substrate rigidity dependent. The underlying theme is that the ability of cells to stretch proteins, generate tension and deform membranes or nuclei is strongly influenced by the elasticity of their substrate.

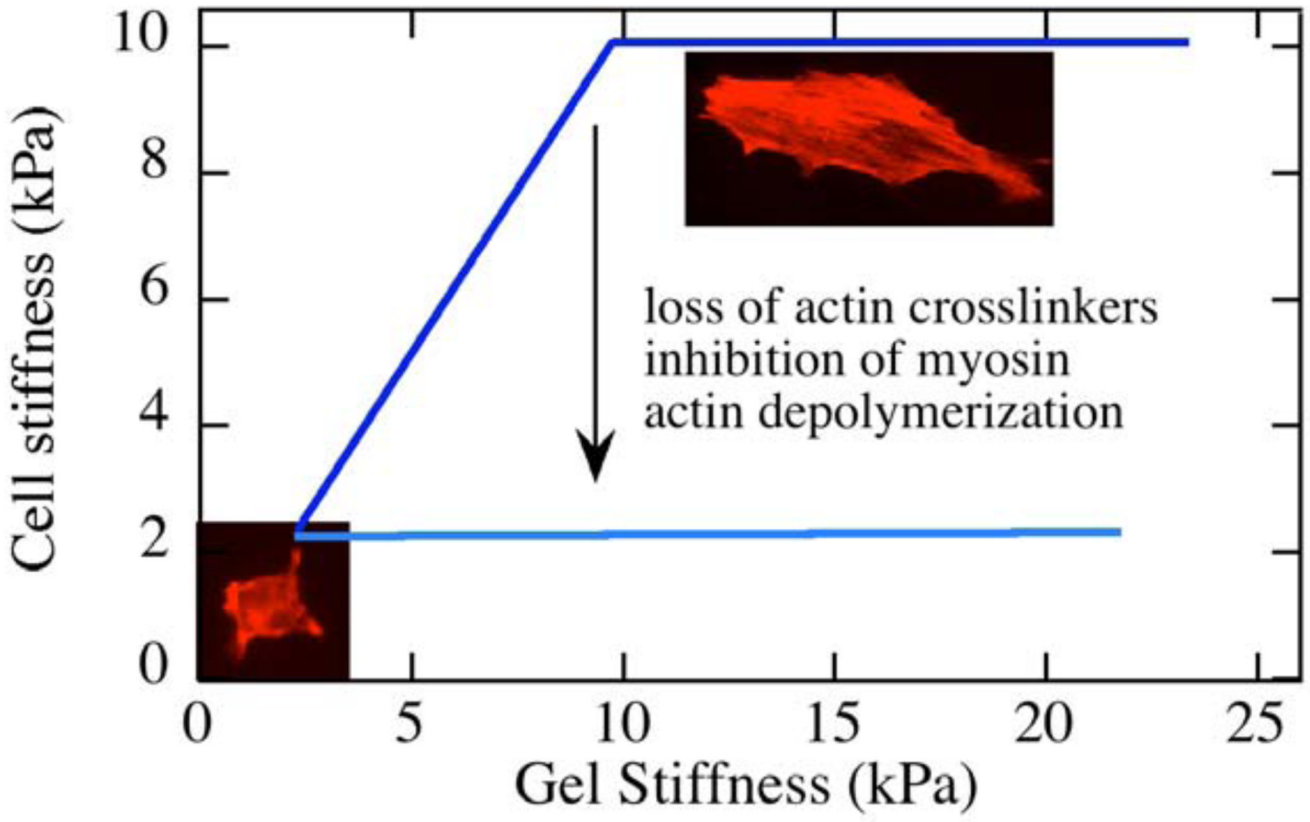

Figure 2. Change of a cell's shape and elastic modulus with the substrate stiffness

Many cell types change shape depending on the stiffness of their substrate, as illustrated by the two A7 melanoma cells stained for F-actin after incubation on collagen-coated polyacrylamide gels with stiffness of 500 or 15,000 Pa. Under some conditions, such as a fibroblasts or melanoma cell adhering to collagen-coated gels, the cells' apical stiffness measured with an atomic force microscope is approximately equal to that of the substrate as its stiffness is varied from 1 to 10 kPa at which the cell's stiffness reaches a maximum similar to that of the cell culture on glass or plastic, as shown by dark blue curve The stiffness matching response can be blunted or eliminated by inhibiting myosin, deleting actin crosslinkers like filamin A, or depolymerizing actin, as shown by light blue curve.