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Dealing with mechanics: mechanisms of force transduction in cells

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Physical cues, such as forces applied to a cell membrane or the stiffness of materials to which cells adhere, are increasingly recognized as essential determinants of biological function, and mechanical stimuli can be as important as chemical stimuli in determining tissue fate or contributing to pathological states. The physical environment of the cell can act in concert with, or sometimes override, the signals given by proteins and other cellular ligands to change cell morphology, growth rates and transcriptional programs. Recent developments in technology and techniques have facilitated studies of how forces trigger cellular events on the molecular level. As the mechanisms of force transduction are identified, methods and concepts from the physical sciences might become as important as those of biochemistry in elucidating how cells function and how these functions might be altered or corrected in therapeutic and biotechnological contexts.

The influence of mechanical forces on tissues and individual cells has long been known, and the molecular mechanisms for MECHANOSENSING (See Glossary) are beginning to be unraveled. Among the most obvious mechanical responses are load-dependent growth of bone and the transduction of acoustic waves to cellular signals in the ear, but mechanical effects can influence several biological processes [1]. At the single cell level, force can initiate cell protrusion, alter motility and affect the metabolic reactions that regulate cell function, division and death. Observations on single cells include the dysfunction of lymphocytes in near-zero gravity [2], force-dependent acceleration of axonal elongation in neurons [3,4], force-dependent changes in the transcription of cytoskeletal proteins in osteoblasts and other types of cell [5], and altered transcription in endothelial cells where disturbed flow occurs [6]. In addition to responding to externally imposed forces, cells also exert internally generated forces on the materials to which they adhere, and some types of cell are exquisite detectors of material stiffness, changing their structure, motility and growth as they interrogate the mechanical properties of their surroundings [7,8]. This article focuses on findings that suggest possible mechanisms by which cells detect forces, and discusses how recent advances in measuring and modeling the viscoelastic properties of cells and subcellular structures can help identify the force sensors.

Soft tissues are subject to a wide range of force

The types of STRESS (i.e. force per unit area; see Box 1) to which different tissues respond are many, and it is therefore likely that different structures are responsible for different forms of mechanical sensing. For example, cartilage typically experiences stresses of 20 MPa, and the chondrocytes within it alter their expression of gycosaminoglycans and other constituents as they deform in

Glossary

Elastic: A response to force in which the material deforms instantaneously by an amount proportional to the stress, maintains this deformation independent of duration, and recovers to its original unstrained shape when the stress is removed.
Elastic modulus: The ratio of stress to strain, representing a kind of 'spring constant' for a material. Because strain has no units, the SI unit for elastic moduli is the Pascal.
Mechanosensing: The ability of a cell or tissue to detect the imposition of a force. Examples of such forces include gravitational pressures, shear stresses caused by fluid flow, acoustic waves and contractile forces exerted from one cell to another.
Microrheology: Measurement of viscoelasticity on micron-sized pieces of material. Most microrheological methods rely on the use of a small probe particle to determine the local elastic properties of a medium. The motion of the probe particle reflects the nature of the material in which it is embedded. If the particle is surrounded by a fluid, then its motion will be that of a diffusing particle whose diffusion coefficient depends on the viscosity of the fluid: that is, the motion of the particle will be diffusive in nature. If the particle is enclosed in an elastic network, by contrast, it will still move but its motion will be restricted by the surrounding network: that is, the amount of motion will directly reflect the elasticity of the network. Thus, by measuring the motion of the particle due either to its thermal fluctuations or to its response to a driving force, the elastic modulus of the local environment of the particle can be probed.
Stress: Force per unit area. The direction at which a force is applied to a surface defines whether it is a shear stress (parallel to the surface), compressive stress or elongational stress (perpendicular to the surface). The SI unit for stress is the Pascal (Pa) = N/m^2.
Strain: A quantitative measure of the amount by which a material is deformed. This unitless quantity is calculated from changes in the dimensions of a piece of material before and after the application of a force and it depends on the size and shape of the original material.
Viscoelastic: A combination of elastic and viscous responses. Because most soft materials have both elastic and viscous responses, their deformation changes over time but not at a rate that is proportional to the stress, and their strain is partially recoverable when the stress is removed.
Viscous: A response to force in which the material deforms without limit at a rate proportional to the stress, increases its deformation in proportion to the duration that the stress is applied, and remains in its fully strained state when the stress is removed.

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Box 1. Rheological measurements

Rheology relates to the measurement of the mechanical properties of a material. These properties can be measured by applying strain to one side of the material, thereby deforming it, and then quantifying the resultant stress on the other side of the material, as shown in Figure I. If the material is elastic, the resultant stress is proportional to the strain, similar to the way in which the force on a spring depends on the amount that the spring is stretched. If the material is a viscous fluid, by contrast, the resultant stress is proportional to the rate of strain or to how fast the material is deformed. Most materials have contributions that are both solid-like and fluid-like.

To measure the response of a material, an oscillatory strain is applied at a frequency, ω. The solid-like response results in a stress that exactly follows the motion of the applied strain, and it provides a measure of the elastic or storage modulus, $G'(\omega)$. The fluid-like response results in a stress that is proportional to the speed of the applied motion, and it provides a measure of the viscous or loss modulus, $G''(\omega)$. Together, these two moduli define the elastic response of the material.

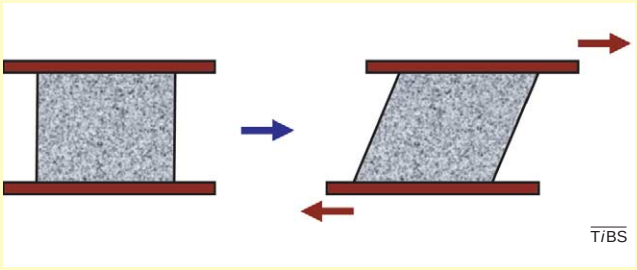

Figure I. To make a rheological measurement, a material (gray shaded area) is confined between two surfaces (brown bars) that can be moved relative to each other. A typical configuration for a shear measurement is shown, in which two parallel surfaces attached to the top and bottom of the sample are moved relative to each other in the parallel plane (right). Measurements of the extent of deformation and the force required to make the movements during an oscillatory deformation determine the viscoelastic properties of the sample.

response to such large forces [9]. Bone and the osteocytes within respond to similarly large stresses [10]. At the other extreme, endothelial cells respond to shear stresses of <1 Pa [11,12], and neutrophils activate in response to even smaller shear stresses [13].

The geometry and time course of mechanical perturbations are also crucial factors. For example, endothelial cells lining blood vessels respond to changes in stress or to turbulence, rather than to a specific magnitude of stress [6,14]. Cells that line blood vessels and epithelial cells in the lung experience dilation forces over large areas, and in both of these settings both the magnitude and the temporal characteristics of the force determine cell response [15]. How cells respond to mechanical stress depends not only on specific molecular sensors but also on their internal mechanical properties, because these material parameters determine how the cell deforms when subjected to force.

Cellular structures that can transmit force

A cellular mechanoresponse requires at least two distinct components: an element or structure that is directly altered by the applied force, and a second element that transmits the information from this 'mechanical sensor' to the ultimate target, which might be, for example, a transcription initiation site in the nucleus or cytoplasmic proteins that remodel the cytoskeleton. The second element might also be mechanical or it might involve the same set of intracellular reactions that mediate cell activation by biochemical signals.

One model of mechanosensing proposes that cells are 'hard wired'; that is, the cytoplasmic space is interconnected with a network of ELASTIC fibers [16] that enable internal structures such as chromatin to respond directly and immediately to forces applied to the cell membrane [17]. Such connections might explain the observation that the engagement of transcription factors on supercoiled DNA can be altered by applying force to the DNA *in vitro* [18]. Mechanosensing does not, however, require that the signals must be different from those initiated by chemical stimuli. Several recent studies show that more traditional second messages, such as increases in cytoplasmic Ca^{2+} concentration [19,20] or changes in protein phosphorylation [21–24], occur after mechanical stresses, suggesting that mechanical stimuli can signal their cellular effects by intracellular messages that are used by soluble ligands.

Although the chemical signals required for mechanosensing are beginning to be identified, the initial sensor of mechanical force is not yet known. Cellular structures that are thought to sense force directly include both proteins and lipids. Increasing characterization of the intracellular signal initiated by force will help to differentiate among the possible sensors, and the magnitude of force required for different mechanical responses will also tell apart the various models. Just as a transmembrane protein with micromolar affinity is unlikely to be the receptor responsible for signaling from a hormone that is active at nanomolar concentrations, it is also unlikely that a very stiff element can respond to small shear stresses or that very compliant proteins can trigger responses only at high stresses.

Protein-mediated mechanosensing

The structure that is most commonly implicated in mechanosensing is the cytoskeleton or, more precisely, the link between the extracellular matrix and the cytoskeleton formed by transmembrane proteins. In this complex, three mechanically sensitive proteins are linked in series, and any one of them might respond to force by unfolding part of their structure to reveal a cryptic binding site that initiates a signal. Figure 1 shows schematically how this mechanotransduction might occur. In the resting state, the cell is attached to an extracellular matrix protein (e.g. fibronectin) by a bond to a transmembrane protein (e.g. an integrin), which in turn is linked at its intracellular domain to the cytoskeleton (e.g. by linkage to F-actin mediated by α-actinin or talin). When force is applied to the extracellular matrix protein, one of three protein transitions might occur to activate the cell.

First, a domain of the extracellular protein might unfold and expose a cryptic site that acts as an activating ligand for an adjacent receptor. Fibronectin, as well as other extracellular proteins with homologous domains, unfolds specific modules under forces of a few tens of pico-Newtons applied by an atomic force microscope to reveal cryptic sites that promote the assembly of fibronectin

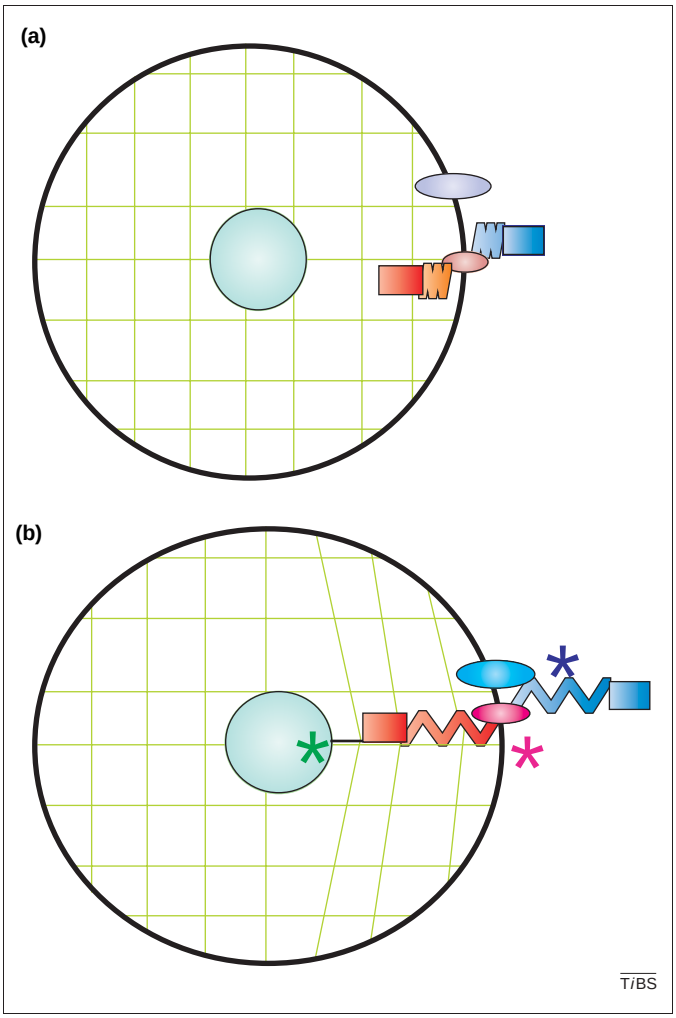

Figure 1. Mechanotransduction at the membrane–cytoskeletal interface. **(a)** In this cell, the cytoskeleton is in an initial configuration that surrounds the nucleus and is attached to the cell membrane by a linker protein (e.g. talin, vinculin or α actinin; shown in red), which is attached to a transmembrane protein (e.g. integrin or cadherin; pink oval), which in turn is bound at its extracellular domain to an extracellular matrix protein (e.g. fibronectin, collagen or laminin; blue). **(b)** When a force is applied to the extracellular protein at the cell membrane, the cell becomes deformed and several hypothetical changes in protein configuration take place (asterisks). Possible changes include stretching of the extracellular matrix protein to activate a new receptor (blue oval), activation of the transmembrane receptor (magenta oval) that is linked to the cytoskeleton, stretching of the intracellular protein (red) that links the cytoskeleton to the transmembrane protein, and transmission of the force directly to the nucleus (green asterisk).

fibrils [25,26], which are essential for the proper activation of integrins [27].

Second, the force might be transmitted by the extracellular protein to the transmembrane protein, which might undergo an activating conformational change analogous to that proposed for mechanosensitive channels triggered by changes in pressure in the bilayer. Pulling on integrins bound to collagen-laminated beads, or pulling directly on the cell membrane with a micropipette [28] or laser trap [29], shows that signals can be generated without necessarily activating a second receptor [19], suggesting that structures in the receptor can be directly activated by force.

Third, the extracellular and transmembrane proteins might remain unchanged but transmit the force to a protein bound to the receptor, which would be then activated in the same manner proposed for the above two cases. At the intracellular interface, several crucial cytoskeletal linkers including α-actinin [30] and vinculin [31,32] contain cryptic active sites that are occluded by intra-domain bonds and exposed by ligands such as phosphoinositides or Cdc42 [33], and it is possible that applied force might also reorient the polypeptide and expose these active sites. Similarly, the intracellular focal adhesion protein talin has emerged as an essential mediator of the force response of integrins [34].

Alternatively to these three types of protein transition, the force might be transmitted farther through a more decentralized region of the cytoskeleton to a distant part of the cell. Evidence in support of this hypothesis is provided by the remarkable observation that the proteins that are capable of binding to detergent-extracted cytoskeleton before stretching differ from those that can bind after the cytoskeletons are stretched on a flexible substrate. Some proteins bind better to stretched cytoskeletons, whereas other proteins lose their affinity after the cytoskeleton is deformed [35]. Other recent studies have shown a large-scale reordering of the cytoskeleton and the movement of internal organelles after the application of force, which has led to the proposal that the first chemical signal can be generated away from the application point of the force [36–38].

Lipid-bilayer-mediated mechanosensing

The lipid bilayer of the cell membrane has at least two, probably related, ways in which to mediate mechanosensing. The amphipathic nature of the phospholipids produces two lateral forces in opposite directions in the membrane. An outward-directed force, which tends to increase membrane area, is due to configurational entropy gained as the hydrophobic chains randomize their conformations; this gain in entropy arises because the hydrophobic chains in a lipid bilayer in aqueous solution are much straighter than they would be in the configurations most likely to be adopted in pure acyl chain melts or hydrophobic solvents. Opposing this expansion is an inward-directed force that is due to the free-energy cost associated with exposing hydrophobic acyl chains to water at the membrane interface. Transmembrane proteins inserted in a bilayer are at mechanical equilibrium with these forces, which depend strongly on the nature of the lipid chains and the curvature of the membrane [39–41].

When a membrane is deformed by a force, two things can happen (Figure 2). First, the change in lateral forces in the lipid bilayer can couple to a change in the conformation of a transmembrane protein, leading to its activation. Recent molecular dynamics simulations of mechanosensitive ion channels in bacteria have shown that appropriate activating transitions can occur in protein complexes when the membrane is subjected to realistic changes in lateral pressure [42,43]. In this model, the activity is in the protein and the lipids do not necessarily rearrange.

Second, because the membrane contains many different lipids, forces at the cell membrane, either from outside or from links to a contractile cytoskeleton, might cause high local curvature that could reorganize the membrane chemically, because lipids that are more stable in curved

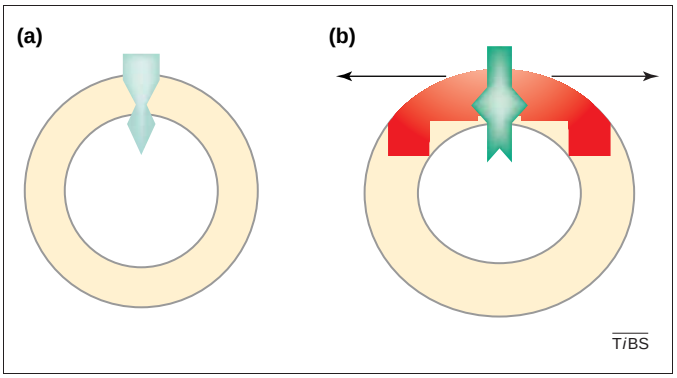

Figure 2. Mechanotransduction in the membrane bilayer. **(a)** In this cell membrane, a transmembrane protein (e.g. an ion channel in the closed position; shown in green) is in a resting state in the lipid bilayer (yellow). **(b)** Changes in the lateral pressures of the lipid bilayer resulting from membrane deformation (arrow) cause a redistribution of lipids in the bilayer (red patch), which in turn promotes conformational changes in the transmembrane protein that might be associated with changes in structure (e.g. channel opening).

membranes diffuse into, whereas those seeking flat membranes diffuse out of, local sites of either negative or positive curvature [44–46]. Inositol phospholipids, which are the main determinants of the actin cytoskeleton and especially unstable in flat membranes, are among the membrane constituents that might form local clusters as a result of forces [33].

A major issue in this field is differentiating the effects that are directly dependent on lipids from those that are exerted on proteins and alter lipid distribution as a consequence. A related issue is whether the magnitude of force required to alter channel properties *in vitro* is encountered *in vivo*, especially in eukaryotic systems. The force exerted, for example, by osmotic pressure on a bacterial membrane is likely to be greater than local forces exerted on eukaryotic cells, and the presence of lipid-mediated sensors of force in eukaryotes is still a contested hypothesis. However, some membrane proteins, such as members of the TRP channel superfamily, seem to be good candidates for mechanosensing in eukaryotic cells [47], although a clear distinction between direct physical regulation or regulation by other proteins or inositol phospholipids [48] remains to be clarified.

In addition to limited changes in membrane structure caused by small forces, cells in multicellular organisms also experience forces large enough to damage the membrane, and various mechanisms that promote membrane resealing or biochemical reactions against temporary loss of the permeability barrier represent another important form of mechanosensing [49].

Mechanosensing without protein or lipid conformational changes

Response to deformation, especially in a multicellular system, does not necessarily require a change in protein or lipid structure at the molecular level, but rather might result from redistribution of the space between signaling centers or enzymes and their substrates [50]. A recent study in which compressive stresses deformed a monolayer of epithelial cells found that signaling by autocrine factors was altered because the intercellular space between cell membrane containing epidermal growth factor (EGF) receptors decreased to enable greater receptor occupancy by EGF [51].

Internal versus external stress

Not only does mechanosensing involve a response to external forces, but cells also use internally generated stresses to probe the mechanical properties of their environment and show a wide range of responses to extracellular stiffness. Early studies showing that cells can wrinkle the elastic membranes on which they are adhering [52] have been recently expanded by using protein-laminated hydrogels of variable stiffness [7], microfabricated polymer surfaces [53], cantilevers [54] and pedestals [55] to quantify the stress that cells exert on the surface to which they adhere.

A striking finding is that cells pull harder on stiffer surfaces, resulting in large differences in cell shape, motility, growth rate and intracellular signaling dependent on the substrate stiffness. The mechanism by which cells can measure the stiffness of their surrounding is not known, but it is likely to require both an intact cytoskeleton and appropriate complexes at the cell membrane that might be identical to those used for sensing externally generated forces.

Measuring intracellular mechanics

To clarify force transmission in cells and therefore to evaluate which elements are most likely to deform [56], it is essential to measure the mechanical properties of the cell interior. Cells, like almost all 'soft' materials, are characterized by a combination of mechanical properties with features of both solids and liquids. To disentangle this complex behavior and to distinguish between the liquid and solid behavior, mechanical properties are often measured as a function of frequency. A frequency-dependent force is applied, and the resultant motion is measured.

The fluid component of the mechanical properties arises from the background fluid in the cell. This fluid is generally thought to be VISCOUS, because small particles are able to diffuse freely through the cell [57]. A fluid is characterized by its viscosity: the greater the viscosity, the lower the diffusion coefficient of the small particles and the slower they move through the cell. The fluid component is distinct because its motion depends on how fast the force is applied, rather than directly on the magnitude of the force. But a cell does not normally flow without limit, and thus overall a cell must be a solid.

The primary solid component of the cell is the cytoskeleton, which is a gel formed by entangled networks of biopolymers (Figure 3). The cytoskeleton is characterized by its mesh size, that is, the average distance between points where the polymers cross and are chemically linked together. In the case of F-actin, crosslinks are introduced by specific proteins. Some proteins, such as α-actinin, might have a role in mechanosensing, whereas others, for example filamin, are specifically upregulated in response to force, thereby strengthening the cell at points of mechanical stress [58,59]. Characteristics of solids can also result when objects are densely packed, leading to glassy behavior [60,61]. For example, if vesicles and other

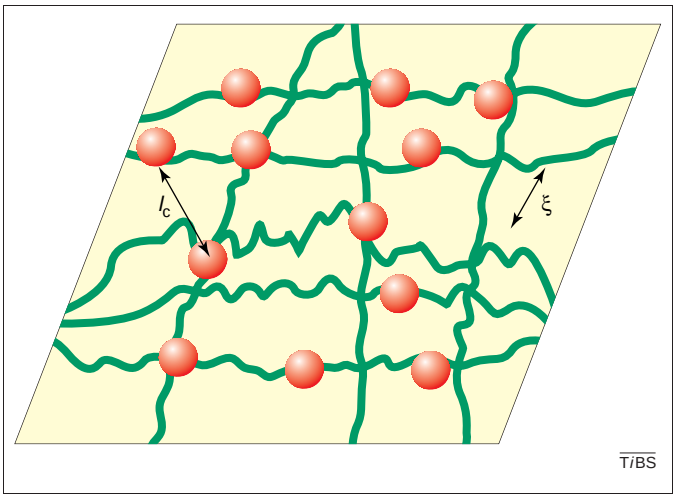

Figure 3. Model of the elasticity of actin networks. A crosslinked actin network is shown as a system containing slightly flexible actin filaments (green lines) that overlap each other but are also bound to other proteins such as filamin (red circles), some of which bind at filament intersections to make crosslinks. The network is characterized by two lengths: ξ, which quantifies the average distance between two filaments and is determined by the filament dimensions and their concentration; and l_c, the crosslink distance, which depends on the number and concentration of crosslinking proteins and the fraction of them that form an elastically effective link between two filaments. The elasticity of reconstituted actin networks is entropic in origin. It is much easier to bend an actin filament than to stretch it, and thermal fluctuations naturally cause the filaments to contain small bends. These bends shorten the length of the filaments. As a result, when a strain is applied to an actin network, the thermal fluctuations of the constituent filaments are stretched out. This stretching costs energy, leading to an elastic modulus. Because this modulus reflects the effects of thermal fluctuations, it is entropic in origin, much like the elasticity of more familiar polymers such as rubber bands. Once the fluctuations are stretched out completely, the intrinsic elasticity of the filaments themselves begins to contribute. As a result, the modulus can increase by several orders of magnitude as the network is strained.

organelles are densely packed within a confined region of the cell, their crowded packing causes them to behave collectively like a solid.

The prime difficulty in determining the mechanical properties of a cell is the inherent inhomogeneity. The cell is a very complex structure and different sections show vastly different mechanical properties. Two distinct approaches are typically followed to investigate cell rheology: first, bulk studies of purified cell components such as cytoskeletal proteins can provide insight into the behavior of the individual constituents of the cell; and second, highly localized measurements that probe the mechanical response of discrete regions of the cell have been recently developed to facilitate rheological measurements in the cell interior [62–66].

Viscoelasticity of purified cytoskeletal systems

Actin networks are an integral part of all cells and are among the most widely studied systems *in vitro*. The mechanical properties of reconstituted actin have been measured, both for entangled networks comprised only of actin and for crosslinked networks that incorporate crosslinking or bundling proteins [67–69]. Reconstituted actin forms a gel (Figure 3) where the mesh size, ξ, depends on the concentration of actin. The elasticity results from the effects of the thermal fluctuations of the actin filaments [70]. Because the actin filaments are so long and so thin, they inherently have thermal fluctuations that reduce their end-to-end length. When a STRAIN is applied, these fluctuations are straightened out. This response is similar to a folded piece of paper: when the paper is stretched, it is easier to pull out the folds than to stretch the paper itself. This reduction in the number of fluctuations costs energy and leads to an elastic response of the network. The origin of this elasticity, similar to that of an elastic band, is entropic: because the fluctuations represent different states that the filament can adapt, stretching the filament pulls out these fluctuations and reduces the number of these states.

The magnitude of the elastic response depends on the concentration of actin and on the concentration of crosslinks, because the number of crosslinks determines the effective length of the filaments. In addition, as the fluctuations are pulled out the intrinsic ELASTIC MODULUS of the filaments begins to dominate. As a result, the measured elasticity increases markedly as the strain increases. The details of the elastic response depend sensitively on the nature of the crosslinking protein and the role that it has in the formation of the network structure. The characteristic response of these networks, coupled with its dependence on actin concentration, crosslinker type and concentration, and the magnitude of applied strain, provides many means by which a cell can measure and can regulate its elastic properties.

If the elasticity of an actin filament itself is increased, then the effect of thermal fluctuations will decrease. This can happen, for example, if the filaments are bundled together to form structures with larger diameters, because the elastic modulus of a rod increases as the fourth power of its diameter. If the filaments become stiffer, thermal fluctuations have a smaller role and the entropic contribution to the elasticity of the network is reduced. Similarly, if the actin or crosslinker concentration is increased, the entropic contribution to the modulus is reduced. Instead, the modulus is controlled by the mechanical deformation – either stretching or bending – of the filaments themselves [71]. This results in a large increase in the elasticity of the network. Other systems have also been measured *in vitro*, including microtubules and intermediate filaments [72], but the cortical stiffness of most cells is presumed to be dominated by actin.

Rheological measurements of intact cells

Measurements of the elastic properties of cells *in vivo* are much more difficult. Techniques are needed that can probe the elastic behavior on a very local basis with a resolution on the order of microns. A method that has been used for nearly 80 years is to monitor the motion of a small probe particle inserted in the cell [73]. If the particle is magnetic, it can be pulled or twisted by an external magnetic field and the resultant strain can be measured under a microscope as the stress is applied [74–76]. A stress can also be applied by using laser tweezers to force the bead to move. Alternatively, the Brownian motion of the bead can be measured [62,66]; in this setup, the thermal energy of the particle is responsible for the stress. In all experimental setups, the relationship of the stress to the strain can be determined. These methods probe the mechanical properties of the cell in the vicinity of the particle and have come

to be called 'MICRORHEOLOGY', reflecting the local nature of the probe.

Because the insertion or phagocytosis of a magnetic particle can disrupt the local environment of the cell, measurement of the motions of endogenous cytoplasmic structures has been used as an alternative method to probe intracellular rheology [66]. This measurement is much less disruptive to the cell structure, but it is dependent on the existence of local probe particles. More refined versions of this technique rely on the analysis of the correlated thermal motion of neighboring probe particles [77]; this measurement helps to reduce spurious effects due to local disruption of the probes and instead provides a more accurate measure of the properties of the cell between the probe particles.

Microrheological measures of the cell show that the cytoplasm is a viscous fluid, which is permeated by a gel network with a rather large mesh size; thus, particles of <50 nm in diameter can freely diffuse through the cell [78]. The viscosity of the cytoplasm is about 10–100 mPa s or roughly 10–100 times that of pure water; this increase presumably reflects the high concentration of proteins in the fluid [79]. The modulus of the actin network in the cell typically has a value of around 100–1000 Pa [71,80,81].

Such measurements are beginning to produce a stiffness profile of live cells that will enable us to map how forces are distributed in their complex structures. An interesting effect noted in the analysis of fluctuations of endogenous organelles is that their movements are inconsistent with pure diffusion driven completely by thermal motions; thus, the mechanical responses of live cells might be fundamentally different from the VISCOELASTIC response of materials in thermodynamic equilibrium [66].

Outstanding issues and future directions

The general idea that forces can elicit specific cellular responses is increasingly recognized, but the structures first responding to the force are not known. The situation is analogous to knowing that a soluble ligand provokes a specific cellular event but not knowing the receptor for that ligand. Decades of research were required to identify the molecular receptors for ligands as fundamentally important as endotoxin or thrombin, and the task of identifying specific receptors of forces is likely to be equally challenging. Possible candidates for force sensors include specific proteins at the membrane–cytoskeletal interface and the collective reorganization of membrane lipids coupling to changes in protein structure.

Another challenge is to provide quantitative data that support the schematic diagrams of force propagation throughout the cell. Many recent advances in microrheology and imaging are now quantifying cell mechanics and pinpointing the cellular structures that might determine mechanical properties, but a theoretical model that relates molecular structures to viscoelasticity – as exists for simpler flexible polymers – has not been fully developed for the larger, stiffer polymers of the cytoskeleton. The molecular origins of elasticity are often surprisingly subtle, and recruiting the methods and theoretical framework of polymer and colloidal physics will be crucial for solving the essentially biological problems involved in mechanosensation.

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