

# Cell mechanics and mechanotransduction: pathways, probes, and physiology

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**Huang, Hayden, Roger D. Kamm, and Richard T. Lee.** Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 287: C1–C11, 2004; 10.1152/ajpcell.00559.2003.—Cells face not only a complex biochemical environment but also a diverse biomechanical environment. How cells respond to variations in mechanical forces is critical in homeostasis and many diseases. The mechanisms by which mechanical forces lead to eventual biochemical and molecular responses remain undefined, and unraveling this mystery will undoubtedly provide new insight into strengthening bone, growing cartilage, improving cardiac contractility, and constructing tissues for artificial organs. In this article we review the physical bases underlying the mechanotransduction process, techniques used to apply controlled mechanical stresses on living cells and tissues to probe mechanotransduction, and some of the important lessons that we are learning from mechanical stimulation of cells with precisely controlled forces.

cytoskeleton; micromanipulation; cell signaling

ALL LIVING ORGANISMS face mechanical forces, from the fluid forces around a bacterium to the high forces in a human knee during stair climbing. The process of converting physical forces into biochemical signals and integrating these signals into the cellular responses is referred to as mechanotransduction. Although this review cannot cover all that has been discovered about mechanotransduction, we discuss the molecule- and cell-level structures that may participate in mechanotransduction, provide an overview of prominent techniques currently used for exerting mechanical stresses on cells, and conclude with an overview of the tissue-level response to mechanical signaling.

## FORCE TRANSDUCTION PATHWAYS AND SIGNALING

*Force transmission pathways at the cellular and subcellular scales.* Understanding the molecular basis for mechanotransduction requires knowledge of the magnitude and distribution of forces throughout the cell at the molecular scale. At present, we have sufficient information to measure molecular scale forces in only a very few cases. We can, however, analyze the mechanisms by which force is transmitted throughout the cell and use that as a basis for speculation about the molecular mechanisms of mechanotransduction. A variety of different methods have been used to mechanically stimulate a cell, and the cellular response is multifaceted and diverse. Similarly, there are likely to be a variety of sensing mechanisms and locations within the cell where forces can be transduced from a mechanical to a biochemical signal. Despite this apparent complexity, it is probable that cells stimulated in different ways are activated by similar mechanisms at the molecular level. To identify these commonalities, it is useful to consider how externally applied forces are transmitted into and through-

out the cell, as well as the magnitudes and distribution of force corresponding to these different methods of stimulation.

Both continuum and microstructural approaches have been used to determine force distributions. In the case of a continuum model, the details of the microstructure are ignored, and the forces transmitted via the individual microstructural elements are described in terms of stresses and corresponding strains, each assumed to be averaged over a distance many times greater than the characteristic dimension of the microstructure. One advantage of this approach is that stress distributions can then be determined by more established methods of analysis that have been used for more traditional materials. Among the several disadvantages are that no account is taken of the true biological character of the cell and that many simplifying assumptions are made either to make the problem more tractable or for lack of sufficient knowledge about the material. A very few of these studies have focused on stress distributions in the context of mechanotransduction by either magnetic twisting cytometry (43) or linear force magnetocytometry (47). With both of these approaches, and in an analytic model of linear force application by Bausch et al. (5), the deformations and stresses were predicted to be highly localized, decaying in a distance of  $\sim 10 \mu\text{m}$  from the site of force application by a tethered microbead. Experiments, however, have shown that the strain field is far from homogeneous, is widely distributed throughout the cell, and tends to be focused at regions around focal adhesions, so there are clearly additional structural features that need to be incorporated into the models. One aspect, the discrete nature of the force-transmitting filaments, is included in the theoretical analyses of tensegrity structures (17). Other factors associated, for example, with the localized attachment of the cell via focal adhesions also need to be considered in all these models.

Continuum models have frequently been used to obtain an estimate of cell stiffness, generally characterized by a Young's modulus or shear modulus, from experiments in which the cell is deformed by external force application. For studies of

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mechanotransduction, such models are also useful in determining the distribution of stresses to regions of the cell remote from the site of force application and, from that, the force levels acting within individual subelements. Davies et al. (21) first proposed that mechanotransduction could be viewed as occurring at either the site of force application or at more remote locations in the cell due to the transmission of force to the nucleus, cell-matrix adhesions, or cell-cell junctions. Although the general trend is for forces to become more diffuse and lower in magnitude with increasing distance from the site of force application, this is not necessarily always the case. As an example, in force application by fluid shear stress, forces at isolated focal adhesions on the basal cell surface can experience stresses considerably higher than the average shear stress acting on the luminal surface. Evidence for this comes from several recent studies in which the deformations within the cell have been mapped (37, 38, 43). Using displacement of the intermediate filament network as a measure of strain resulting from external fluid shear stress, Helmke and colleagues (37, 38, 43) identified regions of highly localized strain. A similar effect was observed when mitochondrial displacement was measured in response to forces applied by magnetic twisting cytometry (43), where the elevated strains were apparently found in the vicinity of focal adhesions. In contrast, membrane displacements tracked by means of microbeads attached to the cell membrane decay with distance from the point of linear force application by magnetic beads (5, 44). However, the resolution of displacements with these membrane-tethered beads was not sufficient to detect localized regions of high strain. Also, despite the general tendency for displacements to decrease with increasing distance, quite a wide range of variability was seen, with some marker beads very close to the magnetic bead undergoing little or no deformation and some farther away exhibiting unusually high displacements.

The pattern of cytoskeletal strain associated with hemodynamic shear stress is much more complex than one would expect. Using confocal imaging of fluorescently labeled intermediate filaments, Helmke et al. (37) were able to obtain strain maps throughout one plane within the cell, showing that, contrary to what would be observed in an elastic continuum, displacements occurred in all directions and appeared to be only weakly correlated with the direction of stress application. All these results suggest that the transmission of force is complex and that the nonhomogeneous nature of the intracellular milieu needs to be considered in the interpretation of strain measurements.

One common pathway for force transmission is via the focal adhesions. Integrins, which form bonds with various extracellular proteins such as fibronectin or vitronectin, constitute a primary pathway for intracellular force transmission and therefore have been viewed as likely candidates for the initiating mechanosensing event. On the intracellular side, many proteins tend to localize to focal adhesions (Fig. 1), binding directly to either the  $\alpha$ - or  $\beta$ -subunits of the integrin heterodimer. These include, for example, paxillin, focal adhesion kinase (FAK), and caveolin. Paxillin and FAK take on greater significance because each has multiple binding partners. Other proteins have been identified that link integrins to the actin cytoskeleton, having binding domains for both integrin and actin. Among these are tensin, talin,  $\alpha$ -actinin, and filamin (54).

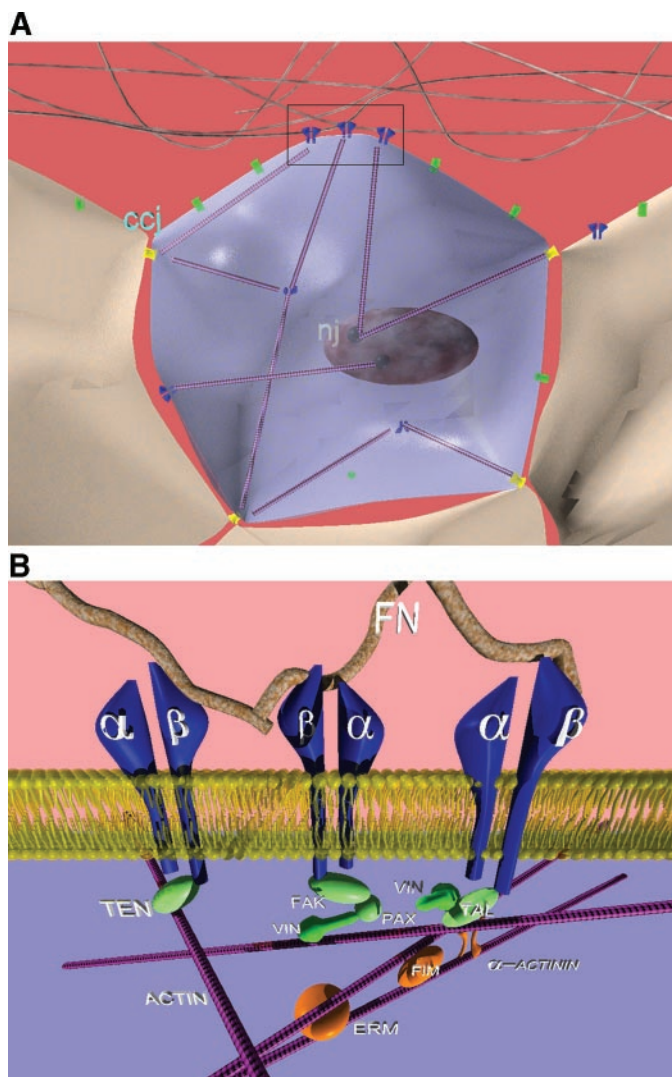


Fig. 1. A: cell-cell and cell-matrix interactions are transmitted to the internal cellular structures such as the cytoskeletal components. Transmembrane proteins include mechanosensitive ion channels (green) and signaling molecules such as integrins (blue), which form focal adhesions (in black box) against the extracellular matrix (brown fibers at top). Some pathways of mechanotransduced signals lead to gene activation at the nucleus (large brown ellipsoid) via nuclear junctions (nj; gray spheres). Pathways can also involve cell-cell junctions (ccj; yellow). Area inside the black box surrounding the top three integrins represents the area of detail in B, but note that the cytoskeletal fibers shown here are not meant to be identical to those shown in B. The proportion of the components illustrated is not meant to reflect actual numbers of the components, and the image is not drawn to scale. B: protein interactions link the external mechanical environment to the cellular cytoskeleton. Clusters of integrins (blue) form focal adhesions, which play a vital role in mechanotransduction. FN, fibronectin;  $\alpha$  and  $\beta$ :  $\alpha$ - and  $\beta$ -integrin dimer subunits; TEN, tensin; FAK, focal adhesion kinase; VIN, vinculin; PAX, paxillin; FIM, filamin; TAL, talin; ERM, ezrin-radixin-moesin proteins. Image is not drawn to scale.

Many of these proteins have more than one actin binding site and can therefore also serve to cross-link actin filaments, thereby strengthening the structures that distribute force away from the focal adhesions [e.g.,  $\alpha$ -actinin, ezrin-radixin-moesin (ERM) proteins, and filamin]. The structure of a focal adhesion incorporates a wide diversity of proteins, so it has been difficult to identify the precise pathway for force transmission. It is

likely, however, that many of these proteins listed above and shown in Fig. 1 are subjected to forces that could give rise to conformational change.

*Levels of force needed to elicit a response.* Forces can be exerted on a cell by a variety of experimental techniques, and each type of force application, if of sufficient magnitude, is capable of eliciting a biological response from the cell. To the extent that the same mechanosensors are being activated, it should be possible to show equivalence among these different methods in terms of the magnitude of local forces experienced at the site of transduction. In the case of fluid shear, the critical level of stress for a variety of biological responses has been observed to be  $\sim 1$  Pa. Integrated over the entire apical surface of a vascular endothelial cell with a surface area of  $1,000 \mu\text{m}^2$ , this produces a total force of 1 nN. Other experiments with forces applied via tethered beads also exhibit a threshold value of  $\sim 1$  nN. If the total applied force is balanced solely by the forces in focal adhesions that occupy only 1% of the basal surface area, then the stress on the focal adhesions amplifies 100-fold to 100 Pa.

It is more difficult to compare these levels of force with those produced in experiments with imposed strain, because the associated forces of interaction between the cell and flexible substrate have not been measured. However, by taking a typical value for the Young's modulus of a cell to be  $\sim 1$  kPa, a stress of 100 Pa is sufficient to produce a 10% strain, a value in the range of those used in substrate strain experiments of mechanotransduction. In different experiments in which magnetic twisting cytometry was used and local strains by the relative displacements of fluorescently labeled mitochondria were observed, the local stresses were estimated to be in the range of several hundred pascals (43).

It is of interest to compare these stress levels with the stresses measured in unforced adherent cells. In these experiments, the stress exerted by individual focal adhesions is determined by measuring the deformation of the flexible substrate to which the cells are attached. From knowledge of the elastic properties of the substrate, the distribution of forces exerted at the focal adhesions can therefore be inferred. Balaban et al. (2) measured the adhesion stress in fibroblasts and found that for focal adhesions of various size, the contact stress proved to be fairly uniform, averaging  $5.5 \text{ nN}/\mu\text{m}^2$  ( $5.5 \times 10^3$  Pa). Assuming close packing of the integrins in the focal adhesion, this led to a conservative estimate that each integrin supports a force of a few piconewtons. If resting contact stresses of this level are typical, then it is difficult to see how changes in stress of even a few hundred pascals, as produced by fluid shear or bead force application, can induce a biological response. Because the stress perturbation would represent just a small fraction of the resting stress, the corresponding changes in molecular conformation would also be small and perhaps less likely to elicit a different biochemical signal, especially in the presence of thermal fluctuations.

The level of force needed to produce significant conformational change in the force-transmitting proteins can also be estimated. The force that causes the bond between two proteins to rupture establishes an upper bound. Several studies have measured the fibronectin/integrin bond strength, producing estimates in the range of 30–100 pN (50). Forces as low as 3–5 pN have also been shown to be sufficient to unfold certain subdomains in fibronectin (24). Although comparable studies

have not been conducted for intracellular proteins, fibronectin is a useful model system for force-facilitated binding because it contains cryptic binding sites that become exposed when the molecule is stretched, leading to fibril formation.

Finally, if external force is to be capable of producing a significant change in intracellular biochemical reaction rates, then the effect of force on protein conformation must exceed that associated with thermal fluctuations. Given that thermal energy,  $kT$ , is  $\approx 4$  pN·nm, and considering conformational changes with a characteristic length scale of 1–10 nm, the corresponding force levels would fall in the range 0.4–4 pN. This happens to coincide with the magnitude of force that can be produced by a single myosin molecule (28), consistent with the theory that active cellular contraction can induce cell signaling. Therefore, all this would suggest that the critical values of force exerted on a single molecule fall within the range of a few piconewtons to perhaps tens of piconewtons.

*Molecular transducers: the molecules and mechanisms implicated in mechanotransduction.* It is now clear that cells transduce mechanical force by using a variety of mechanisms. Of these, mechanosensitive ion channels have perhaps been studied most extensively. Some, such as MscL (mechanosensitive channel of large conductance, with a large pore diameter and low ion selectivity), are apparently regulated by changes in membrane tension, as can be demonstrated by patch-clamp experiments. Membrane tension, controlled by the suction pressure in a micropipette attached to a small region of the cell membrane, elicits a change in conductivity when forces on the channel are of sufficient magnitude (36). In the case of MscL, this occurs at a level of membrane tension ( $\sim 10^{-2}$  Pa·m) just below that which would cause the membrane to rupture ( $\sim 6 \times 10^{-2}$  Pa·m), thereby providing a useful "pressure relief valve" in the event of extreme osmotic swelling, for example. Molecular dynamic simulation of MscL based on its crystal structure (11, 36) has shown that changes in membrane tension are capable of producing changes in pore dimension on the order of 0.5 nm (34), although in vitro experiments suggest that the open pore diameter is closer to 3–4 nm (63). In other cases, such as the calcium ion channels in the stereocilia of hair cells in the inner ear, the mechanism of excitation is somewhat less obvious, and the channel is likely to be activated, in this instance, by tension in the tip links that span the distance between two stereocilia (16) and transmit force to the channel either directly via extracellular connections or via the internal cytoskeleton [see Hamill (36) for a recent review]. In the case of vascular endothelial cells, it has also been shown that mechanosensitive ion channels can mediate fluctuations in intracellular ion concentration (56), although the mechanism of control or the pathway of force transmission to the channel has not been elucidated. Speculation has suggested that these channels might be activated by forces transmitted either directly via extracellular contacts, via tension in the cell membrane, or indirectly via forces transmitted into the cell through integrin receptors to the cytoskeleton and then to the channel proteins. However, as suggested in a recent review, "the mechanisms involved in the control of open/closed ion channel conformations by shear remain obscure" (51).

Although it has been postulated that conformational changes in intracellular proteins might also be a method of mechanosensing used by cells, direct evidence for this is scarce. At least one example exists, however, of a biochemical reaction that

can be mediated by force-induced conformational change. As discussed above, cryptic binding sites on fibronectin can be exposed by force-induced lengthening of the molecule, leading to the formation of fibrils. This process has been extensively studied by experiment and molecular dynamic simulation (18, 29), and the results of these studies show that forces of 3–5 pN are sufficient to unfold individual fibronectin modules and that a force of 5 pN is sufficient to stretch the molecule to five times its initial length (29, 87). These levels of force are comparable to those estimated above for the conformational changes that could lead to intracellular mechanotransduction.

Though somewhat less studied, several intracellular proteins (e.g., Src family kinases, vinculin, mDia, ROCK, or WASP) have also been identified as possible “molecular switches,” undergoing conformational change that exposes protein-binding sites in response to an external force (30). Essentially any protein in the force transmission pathway from extracellular contacts through to the cytoskeleton is a possible candidate for a mechanosensor, and unfolding of both integrins (14) and integrin-associated proteins (98) has been implicated. Focal adhesion proteins are prime candidates, especially in view of recent experimental evidence showing that stretching of detergent-treated cells (to remove the cell membrane) on a compliant substrate can lead to enhanced binding of FAK and paxillin to focal adhesions (73). Because the cell membranes are absent in these experiments, transmembrane ion channels clearly are not involved. Multiple pathways are likely to exist, however, because it has been shown that talin1 is essential for force-dependent focal adhesion remodeling but not for activation of the Src family kinases (31).

Given that much intracellular signaling results from reactions between membrane-bound proteins, enhanced diffusion in the plane of the membrane has been proposed as another potential mechanism of mechanotransduction (45). Experiments in which fluorescence recovery after photobleaching (9) and a molecular probe of membrane viscosity, 9-(dicyanovinyl)-julodine (35), were used demonstrated a rapid (<10 s) shear- and direction-sensitive change in the lipid lateral diffusion coefficient,  $D$ , with shear stresses in the range of 1 Pa. Interestingly, the response to shear depends on whether shear stress is increased in a stepwise or ramp fashion; in the case of a stepwise increase, shear induces an increase in  $D$  upstream of the nucleus (on the portion of the membrane that the flow first encounters) and a reduction downstream, whereas the ramp in shear decreases  $D$  everywhere. These changes are also linked to ERK and JNK activation, but only in the case of a stepwise increase in shear (10). It is not yet clear, however, how the stresses from fluid shear cause changes in membrane fluidity.

The well-established finding that force can lead to changes in gene expression, combined with the observation that forces applied via membrane-based receptors can cause nuclear deformation (57), has led to speculation that force might directly influence transcription through force-mediated conformational changes in chromatin. Forces, in this instance, would be transmitted via the cytoskeletal network to the nuclear envelope and from there, via the lamin network, to chromatin. Other studies have shown that external forces transmitted to the cytoskeleton can cause rupture of microtubules, which in turn can initiate a biochemical signaling response (61).

## TECHNIQUES FOR MECHANICALLY PROBING CELLS

Exploration of the models of mechanotransduction discussed above relies on the use of different methods to apply mechanical forces to living cells. One of the central achievements in mechanotransduction has been the development of carefully designed devices to impose mechanical forces. Although these devices are often conceptually simple, they rely on rigorous bioengineering principles to ensure that precise forces are imposed with the desired distribution. There are two general approaches to studying the response of cells to mechanical forces. First is the use of a cohort of cells; in this case, cells are subject to some deformation or physical stress and then assayed as a group. These methods are amenable to many molecular biology techniques that provide results from hundreds of thousands or millions of cells in aggregate. More recent approaches are used to study individual cells with precisely imposed forces.

To evaluate molecular signaling and the physical response of groups of cells, techniques were developed to mimic stresses inherent in native physiological environments. Shear stress, stretch, and pressure changes are typical stimuli applied to cohorts of cells. These methods are generally well-established because they have been in use for over a decade, and they have many variants, combinations, and published results.

*Membrane stretch.* Applying a fixed strain to a group of cells is usually achieved by culturing the cells on an elastic substrate (often silicone elastomer) and then applying a known strain to the substrate. The coating of the substrate can be altered depending on the adhesion characteristics of the cells, but fibronectin and collagens are typically used. Strain rates commonly vary from 0.1 to 10 Hz, and the strain percentage ranges from <1% to >30%.

One dimensional (1-D) stretch is based on pulling a membrane in one direction. Some 1-D strain devices allow the membrane to distort freely in the direction perpendicular to the strain, whereas others constrain the two free edges so that there is no compression as a result of the membrane stretch. One-dimensional strain may be used to study reorientation based on asymmetric strain direction (59). Two-dimensional (2-D) strain devices are an extension of the 1-D strain devices, except the membrane is strained in two directions at once, allowing a more uniform, “biaxial” strain field.

The two major types of biaxial strain devices are the piston strain device and the pressure strain device. In the former case, a piston moves in the vertical direction relative to the membrane. The device allows the piston to slide relative to the fixed membrane so that the piston induces membrane strain. In pressure strain devices, the membrane is sealed, and introducing and removing gas from beneath the membrane results in transmembrane pressure changes and subsequent deformation. These devices may be designed so that the strain transmitted to the cell is nearly identical in all directions and is therefore independent of cell orientation. The strain will vary close to the edge of the membrane where the membrane is mounted, but generally few cells grow at the periphery of the membranes.

Biaxial strain devices are particularly well suited to many types of biochemical and molecular techniques that provide an average readout from millions of cells. For example, stretching smooth muscle cells and studying MAP kinase activation revealed JNK/SAPK and ERK activation and the dependency

of activation on the type of matrix used to coat the membranes (68). Gene induction, such as induction of the biomechanically responsive gene *egr-1*, is readily shown by methods that similarly impose strain (93). Cell stretchers are particularly useful for harvesting large amounts of RNA necessary for genomic studies (27). It is important to recognize that cell stretching necessarily imposes a fluid shear stress to the cell monolayer due to flows induced in the media above the cells; this fluid shear stress can be complex and difficult to predict (7).

**Shear stress.** Most commonly used on endothelial cells, the application of shear stress to a monolayer is accomplished by moving fluid through a flow chamber. There are two main designs, a pressure-driven flow chamber that typically results in a fully developed parabolic laminar flow profile and a cone-and-plate flow chamber in which the cone is rotated relative to a fixed plate that results in a linear flow profile and uniform shear stress. There are many variants on each main design; for example, the pressure-driven flow chamber can have a rectangular cross section or a circular cross section. Flow can be either steady or unsteady to generate a time-varying shear stress. Investigators have also designed geometric changes within these flow chambers to create a recirculation region in which to simulate disturbed flow regions, such as near arterial bifurcations, for example. Care must be taken to keep flow rates sufficiently low to maintain laminar flow, unless turbulence is being studied as a factor, to maintain a constant level of uniform shear stress. Typical shear stress levels for studying endothelial cell effects range from 1 to 20 dyn/cm<sup>2</sup> (0.1–2 Pa). Shear-induced signaling cascades and changes in endothelial cell morphology have been reviewed extensively (20, 21, 55). Shear stress can induce changes in endothelial cell proliferation (92), membrane fluidity (9), and cell morphology (62, 96).

**Other techniques for mechanically stimulating groups of cells.** Cells can be subjected to elevated hydrostatic pressure by several means; the simplest is to use compressed air or a column of fluid above the cells being stimulated. Alternatively, cells grown on a porous but stiff substrate can be exposed to a transmembrane pressure, an elevated pressure on the apical surface relative to that in the region beneath the cells (67). To simulate certain physiological conditions, cells can be embedded in gels or tissues and subjected to compression with the use of pistons linked to motors or actuators (78). Alternatively, excised vessels can be internally pressurized with media, producing simultaneous vessel wall strain and hydrostatic pressure (52). Pressure studies are used, for example, to examine the effects of hypertension in arteries and stresses on osteoblasts. Ultrasound vibrations have been implicated in the up-regulation of some immediate-early response genes known to be mechanosensitive (60).

**Single cells.** Assays on single cells or small numbers of cells have the advantage that the mean response can be determined while preserving the variability of data from individual cells. Some of the different methods for stressing single cells are examined below. Unlike methods that impose stresses on large numbers of cells, the stresses applied on cells from single-cell techniques can be focused on a given region of the cell. Most of the forces range from 1 pN to 10 nN; for comparison, a spherical cell with a radius of 10  $\mu\text{m}$  weighs  $\sim 40$  pN. Precise forces can be applied to cell surfaces by attaching particles to

the cells with the use of an adhesive ligand or antibody to a specific receptor and then generating a force on the particle. Most of these particles are in the range of 0.1 to 10  $\mu\text{m}$  in diameter. One advantage of using particle-based stressing is the ability to coat the bead with a specific ligand or antibody to apply a stress via the bead on a specific group of receptors. Other advantages include the ability to apply either a fixed displacement (using optical trapping) or fixed force (using magnetic trapping) to the cell and the flexibility to apply either very local stresses by using few beads or nearly global stresses by using a large number of beads. As with the methods described above, the force application can be either steady or time varying. However, there are also disadvantages to the use of particles, including internalization due to phagocytosis, which typically occurs after many hours, and the rather non-uniform distribution of beads across many cells. In addition, the effectiveness of the coatings can be influenced by variations in the level of antibody or ligand coating coupled with degradation over time, incorrect orientation of the coated molecule, the presence of nonspecific binding, and variabilities in the strength of ligand attachment to the bead. It is also difficult to determine the contact area between the bead and cell membrane, and this can lead to uncertainties in the level of stress acting locally. Despite these shortcomings, the use of particles to apply highly controlled forces in a time-dependent manner to cells has enjoyed a surge of popularity.

**Optical traps.** Optical traps (also called optical tweezers or laser traps) consist of a laser directed at a micrometer-scale object, such as beads or organelles, and used to control their position. For typical object sizes (0.5–10  $\mu\text{m}$  in diameter) used, the force is generated by the refraction of the laser within the bead coupled with the difference in photon density from the center to the edge of the beam (Fig. 2). Very small objects do not trap well because the trapping force decreases with decreasing object volume.

Optical traps can generate tens to hundreds of piconewtons of force per bead. Their main limitation is that with one beam path, only one bead at a time can be controlled. Typically, optical traps are used to control particle position, but the force acting on a bead can also be determined on the basis of the distance between the center of the particle and the laser focal point. This requires high spatial resolution of particle position, however, and feedback control if force is to be specified. Despite these limitations, optical traps have been used to study membrane and cell elasticity and local responses in a diverse array of cell types, including neurons and red blood cells (15, 19, 75). Optical traps are also suitable for studying movement or force generation by structures at the molecular scale, such as kinesin motors (6, 49).

**Magnetic force application.** Magnets can be used to apply either a linear force or twisting torque to a particle. In the case of linear force application, the device is constructed with one or more electromagnetic poles consisting of a para- or ferromagnetic core wrapped with wires. When current is passed through the wires, the pole becomes magnetized, and small para- or ferromagnetic beads are attracted to the pole. The force can be amplified by designing the pole to converge to a sharp tip (Fig. 3). To generate uniform forces across an area, multiple tips can be arranged with different current amplitudes and directions to generate a nearly uniform magnetic force field

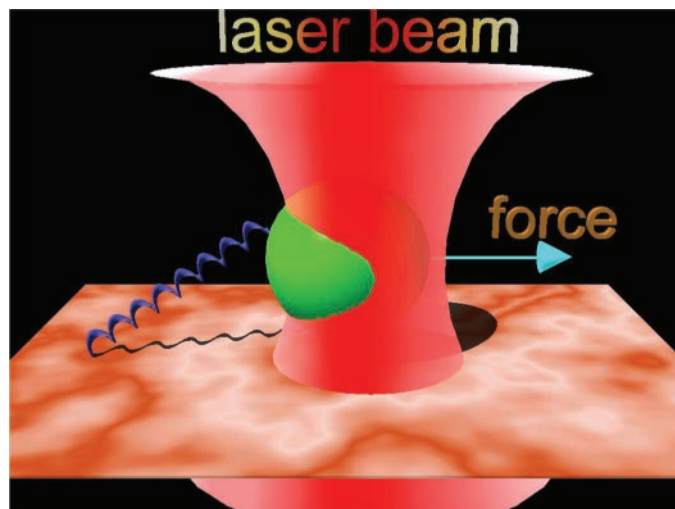


Fig. 2. Optical trapping can be used to apply strains at low forces ( $\sim 10$  pN) to adhesive molecules attached to the trapped bead. Direct force application of beads attached to cells is also possible. For large beads, the force is generated by refraction of the laser coupled with the lower intensity of the beam near the edges. Image is not drawn to scale.

in any direction. Alternatively, a blunt pole can be used, which will generate a much smaller force over a larger area.

Magnetic force application can be viewed as the mechanical complement to optical trapping; its main feature is that it generates a constant force. To apply a fixed, specific displacement to the particle, feedback is required. In addition, many beads are affected simultaneously, although with a single pole the forces on each bead are not necessarily the same, due to both nonuniformities in field strength and variations in particle composition and size. Current multipole designs allow for nearly constant field gradient over an area that spans tens to hundreds of cells. Bead selection is critical, however, because the magnetic contents of the particle influence the applied force. Ferromagnetic beads can generally exert much more force but retain some of their magnetization each time they are exposed to the field. Paramagnetic beads are less susceptible to magnetization but are limited to smaller forces. In general, paramagnetic beads can be used to generate hundreds of piconewtons per bead for an area magnetic trap and up to tens of nanonewtons for a single-pole trap, assuming one works within a distance of 10–100  $\mu\text{m}$  of the magnetic trap tip.

As an example, a magnetic tweezers was used to demonstrate that vinculin-deficient fibroblasts are less stiff than wild-type cells (1). Calcium signaling has been reported with beads tethered to integrin, but not transferrin, receptors on smooth muscle cells by using magnetic trapping (64). Phosphorylation of the MAP kinases have been demonstrated by using a low-force large-area magnetic trap (32). Models for the physical response of cells to local deformations from a magnetically forced bead have been proposed and fitted to experimental data (3–5).

**Magnetic twisting.** The second form of magnetic trapping is by the application of a torque. In this case, the beads are not pulled, but instead, a brief magnetic field is pulsed on the sample to magnetize the beads with a specific orientation. When a counterpulse is generated at a much higher magnitude and in a different direction, the beads then experience a

rotational force to realign with the new field. The resulting torque is described by the decay of the initial magnetization and the decrease in torque as a result of bead rotation.

Magnetic twisting has been used to demonstrate that integrins are more firmly attached to the cytoskeleton than acetylated LDL receptors (88). Twisting integrin receptors generates sufficient stress to activate mechanically sensitive genes (13). Models have been created to examine the amplitude and phase of bead rotation and displacement to estimate both solid and viscous characteristics (8, 25).

**Atomic force microscopy.** Atomic force microscopy (AFM), normally used for the purpose of imaging, can also provide structural information about the cell. The probe used in AFM consists of a fine pyramidal tip attached to a cantilever that flexes as the tip is pushed into the sample surface. By measuring the flexure in the cantilever with the reflection of a laser, it is possible to calculate the upward force acting at the tip (Fig. 4). In addition, the contours of the surface can be revealed by the vertical motion of the tip as it traverses horizontally back and forth over the sample. As a result, one can obtain both geometrical information about the surface being probed and the local stiffness of the surface. The most commonly used model for interpreting the depression-force relationship is referred to as the Hertzian model (40, 66) and assumes a semi-infinite, linearly elastic, homogeneous substance.

AFM typically produced estimates of cell stiffness in the higher ranges of measured values (hundreds of kilopascals, in contrast to most other techniques ranging from 0.1 to 10 kPa) but is perhaps the best method for demonstrating cell heterogeneity. AFM is particularly useful for mapping different locations of cells (such as actin fiber locations or the leading and trailing edges of cells) because the location of the indentation can be chosen (58, 69, 70, 74).

**Micropipette aspiration.** Pipette aspiration uses a subatmospheric pressure to partially aspirate a cell. The difference in pressure across the cell membrane is related to its deformation. This technique is most commonly used on white blood cells such as neutrophils, which do not adhere to most surfaces

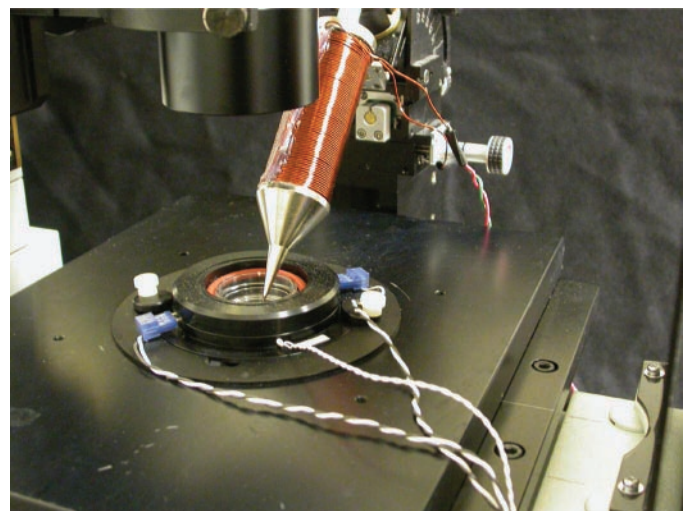


Fig. 3. A magnetic trap for high-force cell probing is typically composed of an electromagnet with a sharp tip on a micromanipulator. Imaging is performed on a microscope stage with a stage heater to maintain physiologically relevant temperatures.

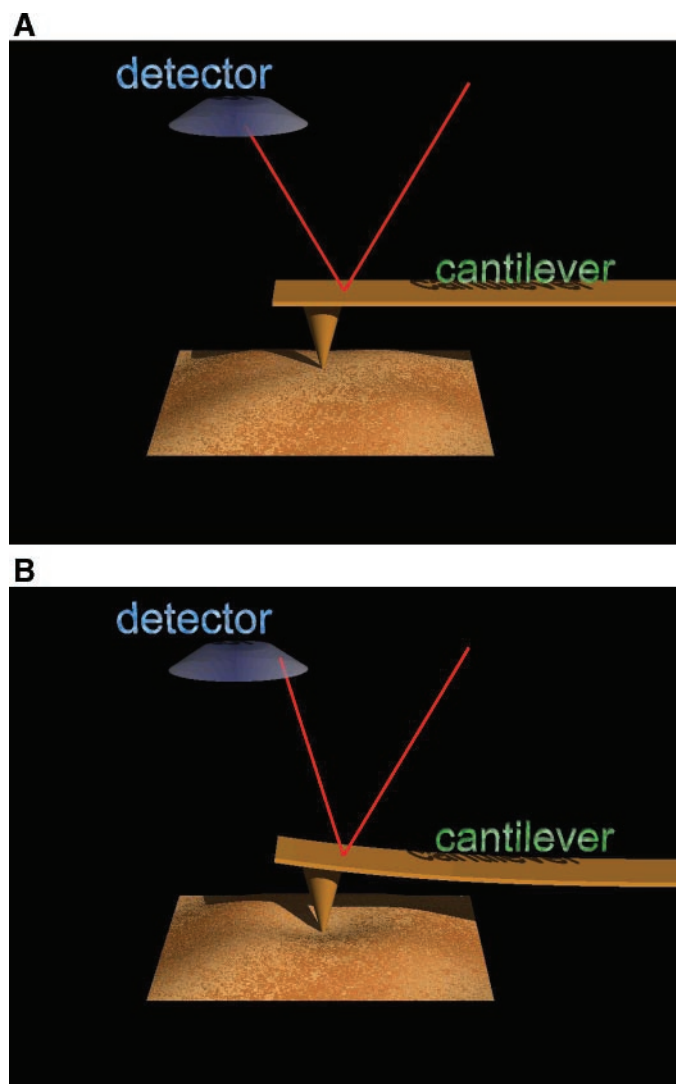


Fig. 4. Atomic force microscopy determines the deformation of a substrate by reflecting a laser off the cantilever. The detector is positioned so that when the cantilever is unstressed (A), the beam is in a neutral position. When the substrate is probed, the cantilever flexes (B) and the new beam reflection alters the position of the beam spot on the detector. By determining the bending of the cantilever using the beam spot information, it is possible to determine the local surface and underlying material properties with the use of appropriate models. Images are not drawn to scale.

without becoming activated and have extremely short working life spans compared with most other adherent cell lines (23, 85); however, pipette aspiration has also been used to study adherent cells (41, 57). Micropipette aspiration experiments have led to the development of models of neutrophils as a cortex exhibiting a constant surface tension filled with a viscous fluid. These properties can be altered with cytoskeleton-altering compounds such as cytochalasin-B (84). The cell cortical thickness has been estimated, using aspiration, to be on the order of  $0.1 \mu\text{m}$  (97). In addition, the presence of the nucleus affects neutrophil aspiration, especially under large deformations (46). The internal velocity field and changes in geometric parameters experienced by neutrophils during various deformation have been modeled using finite-element analysis, showing among other things a weakness in the classic

Brownian ratchet model to explain the magnitude of force generation (39).

*Other techniques.* Cytoskeletal mechanical properties can also be determined by squeezing the cell between two flat surfaces, although this approach has not been used extensively. In this method, a cell is placed between two parallel plates, which can then be moved closer together, pulled apart, or twisted, while the cell can be imaged to evaluate the distortion and subsequent mechanical response to the stimuli (80–82).

Microfluidics and micropatterning have given rise to studying the individual responses of cells to their mechanical environment by imposing physical constraints rather than applying external forces to the cell. These techniques demonstrate how cell geometry can influence the ability of the cell to migrate and interact with other cells. For example, cells undergo increased apoptosis when not permitted to spread; their viability when spread is maintained regardless of large variations in the actual contact area (12). Cell traction forces have been measured by using microfabricated pillars that bend according to the local traction generated by the cells (77) or by using fluorescent beads embedded in an extensible substrate (83). It is hoped that further studies with microfluidics will permit a more detailed and controlled study of individual cells and cell-cell interactions in small groups.

#### MOLECULAR RESPONSES OF CELLS AND TISSUES TO MECHANICAL STIMULI

With the diverse methods of mechanically stressing cells and the basic understanding of some of the molecular components thought to be physically involved in mechanotransduction, we now discuss how these results lead to an increased understanding of relevant physiological processes. The mechanisms by which most eukaryotic cells transform mechanical signals to biochemical signals remain elusive, and no single signal transduction pathway appears to mediate all mechanotransduction events, despite the prominence of common elements such as the cytoskeleton. This most likely reflects the interactions between signal pathways and context-specific signaling, rather than a true mechanical-biochemical coupling of each signal transduction pathway. Although we do not yet sufficiently understand mechanotransduction to predict cellular responses, it has been extremely instructive to study the global responses of specific cell types to mechanical stimuli. Presumably, cells within intact tissues react to mechanical stimuli with molecular responses that aim to protect the overall integrity of the tissue, at least in the short term. Although some cellular responses are altered by cell isolation procedures and growth factors used to maintain them (for example, fetal bovine serum itself stimulates transcription of some mechanically activated genes), many physiologically and pathophysiologically relevant molecular responses have been uncovered by mechanical stimulation of cultured cells.

Not surprisingly, most studies to date have focused on cells of tissues with primary structural functions such as bone, cartilage, and skin. These tissues are subjected to a wide range of mechanical loads; moreover, mechanical loading appears to be essential to maintaining normal function. Thus there may be a mechanical homeostasis, with cells responding to and interpreting growth factors and other biochemical signals within the context of mechanical forces to provide a structurally rational

structure (86). This theory has evolved in the bone field over the past century as an attempt to explain how daily loading changes bone structure, even though the magnitudes of deformation in bone are extremely small. Similarly, cartilage is subjected to widely varying stresses of up to  $2 \times 10^7$  Pa (200 atm) during activities like climbing stairs (33). Chondrocytes, the principal cells of cartilage that secrete the glycosaminoglycan-rich extracellular matrix that gives cartilage its mechanical properties, are highly responsive to dynamic stress (64). In fact, dynamic stress may be essential to tissue engineering of cartilage with mechanical properties suitable for implantation (65).

One of the best-studied mechanotransduction paradigms is endothelial cells under the influence of fluid shear stress. Because abnormal shear stress patterns are spatially correlated with localization of atherosclerosis in humans, the responses of endothelial cells to changes in shear stress are highly relevant. This field has advanced rapidly because of the clear preservation of shear stress responsiveness of cultured endothelial cells on many levels, including changes in cell shape, adhesive properties toward leukocytes, and gene induction. Furthermore, the development of rigorous laboratory methods for imposing defined shear stresses to cultured monolayers has allowed the study of steady, oscillating, or turbulent shear stresses on cells; even cell-to-cell differences in gene expression have been identified (22). The imposition of rigorously defined shear stress on endothelial cells has revealed new pathways and new genes that may participate in atherosclerosis (89).

Even cells without obvious structural roles have interesting biomechanical responses to physical stimulus that may reflect *in vivo* functions. For example, the monocyte circulates in blood, but after attachment to the endothelium, monocytes migrate into tissues and become macrophages. Although the initial adhesion to the endothelium triggers defined signal transduction pathways, mechanical deformation of the monocyte after adhesion induces distinct molecular events including expression of the immediate-early response genes *c-fos* and *c-jun*, the transcription factor PU.1 (an *ets* family member that is essential in monocyte differentiation), and the macrocyte colony-stimulating factor receptor (95). Furthermore, mechanical deformation of monocytes promotes expression of the class A scavenger receptor, an important lipoprotein receptor in atherogenesis, and this induction could play a role in the exacerbation of atherosclerosis by hypertension (72). Thus even cells that do not play primary structural roles have biomechanical responses that can influence physiology and pathophysiology.

*Cardiomyocytes: a mechanotransduction genomics example.* As an example of the lessons we can learn from the study of mechanotransduction of specific cells, consider the cardiomyocyte. Mechanotransduction in the myocardium has been intensely investigated, in part because mechanical overload of the heart is highly clinically relevant (76). Most cases of heart failure are due to loss of normal myocardium (typically from a myocardial infarction), with resulting mechanical overload of the remaining viable myocardium. Mechanical overload causes growth of the cardiomyocyte that is presumably compensatory, allowing the enlarged myocyte to generate greater force, but eventually this can lead to mechanical dysfunction and failure of the tissue. Furthermore, mechanical overload of the heart in the laboratory is experimentally feasible, and the response is

typically reproducible. For example, the partial aortic occlusion or "banding" procedure to increase left ventricular systolic load is in widespread use throughout the world.

Studies of mechanically overloaded cardiac myocytes have revealed numerous useful pathophysiological pathways. Sadoshima et al. (71) found that cardiomyocytes could release angiotensin II, which acts as a paracrine growth factor. Other paracrine mediators such as endothelin and natriuretic factors are mechanically released. Mechanotransduction responses that are angiotensin II independent have been identified (94), and recent studies have pointed to the myocyte contractile elements and Z disks as mechanotransducers (48). However, it is worth noting that cytoskeletal networks are in a force balance with focal adhesions, the nucleus, and other cellular components such that isolating any component of the cell biomechanically and attributing mechanotransduction to that component is challenging. As might be anticipated from a cell whose mechanical properties depend on rapid changes in cytoplasmic calcium, cardiomyocyte mechanotransduction is tightly coupled to calcium-dependent signaling pathways such as CaM kinase II (79).

When cardiomyocytes are mechanically stimulated *in vitro*, cells enlarge and also activate genes such as brain natriuretic peptide and heparin-binding epidermal growth factor (53). These genes are also overexpressed *in vivo*. Because the response of cultured myocytes to mechanical overload shares many features with *in vivo* overload, exploration of new genes from mechanically overloaded cultured myocytes might be expected to yield discovery of novel pathways. With the use of microarray technology, an orphan interleukin-1 receptor family member called ST2 was found to be one of the most mechanically induced genes in cultured neonatal rat cardiomyocytes (90). ST2 is expressed at low levels as a membrane receptor on many cells; when cardiomyocytes are mechanically overloaded, they secrete a soluble, truncated form of this receptor through alternative promoter use of the ST2 gene. The secreted ST2 can be detected in serum from both mice and humans with mechanically overloaded hearts. Clinical and laboratory studies have confirmed that the soluble receptor is increased in the blood of patients with heart failure, and an increase in blood level over time reflects a worsening patient prognosis (91). The pathophysiology of ST2 in heart failure is undefined in part because the ligand for the receptor remains unidentified, but this genomic discovery approach shows how mechanically overloaded cultured cells can identify a new pathway and biomarker relevant to mechanically overloaded myocardium.

*Biological-mechanical environment.* It is increasingly apparent that biochemical and biomechanical signals communicate more closely than we have suspected. For example, blood flow plays a surprisingly important role in cardiac morphogenesis. When Hove et al. (42) studied cardiac fluid flow in zebrafish embryos, they found that occluding flow changed cardiac looping and valve formation (42). Thus altering fluid forces could cause congenital heart disease even in a genetically normal background, whereas traditionally congenital heart disease is considered to be guided by single or multiple genetic abnormalities in morphogenesis.

A fascinating experiment by Farge (26) further demonstrated the importance of mechanical forces in development. He studied the effects of mechanical forces on *Twist*, a gene in *Drosophila* that is normally expressed in restricted locations

such as the most ventral cells of the blastoderm embryo. With application of a 10% strain (essentially flattening the embryo), ectopic expression of *Twist* was induced over the entire dorsal-ventral axis, resulting in a deformed embryo. Thus mechanically active pathways interface with developmental pathways, suggesting that these transduction mechanisms are not simply adaptive mechanisms for structural compensation in fully developed tissues.

*Where are we going in mechanotransduction?* The scientific interface of biology and biomechanics is relatively young, although nature has been working on this relationship since the time of the earliest organisms. We are just beginning to develop the tools that allow the precise imposition and measurement of mechanical forces. Clearly, a major challenge is defining precisely how mechanical forces become biochemical signals; experimental findings to date suggest that cells use many pathways, although a specific pathway may be dominant in a given circumstance. However, even before we grasp how mechanotransduction occurs, cells are teaching us how they interweave mechanical signals into their fundamental molecular responses.

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