Cellular mechanotransduction: putting all the pieces together again

Donald E. Ingber[1]

Vascular Biology Program, Departments of Pathology and Surgery, Harvard Medical School and Children's Hospital, Boston, Massachusetts, USA

ABSTRACT **Analysis of cellular mechanotransduction, the mechanism by which cells convert mechanical signals into biochemical responses, has focused on identification of critical mechanosensitive molecules and cellular components. Stretch-activated ion channels, caveolae, integrins, cadherins, growth factor receptors, myosin motors, cytoskeletal filaments, nuclei, extracellular matrix, and numerous other structures and signaling molecules have all been shown to contribute to the mechanotransduction response. However, little is known about how these different molecules function within the structural context of living cells, tissues, and organs to produce the orchestrated cellular behaviors required for mechanosensation, embryogenesis, and physiological control. Recent work from a wide range of fields reveals that organ, tissue, and cell anatomy are as important for mechanotransduction as individual mechanosensitive proteins and that our bodies use structural hierarchies (systems within systems) composed of interconnected networks that span from the macroscale to the nanoscale in order to focus stresses on specific mechanotransducer molecules. The presence of isometric tension (prestress) at all levels of these multiscale networks ensures that various molecular scale mechanochemical transduction mechanisms proceed simultaneously and produce a concerted response. Future research in this area will therefore require analysis, understanding, and modeling of tensionally integrated (tensegrity) systems of mechanochemical control.—Ingber, D. E. Cellular mechanotransduction: putting all the pieces together again *FASEB J.* 20, 811–827 (2006)**

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GIVEN THE DOMINANT focus on genomics and its success over the past three decades, it could be asked why we should care about the effects of mechanical forces on cells. Yet it is becoming increasingly clear that epigenetic factors, particularly mechanical and structural cues that influence cell behavior, have a central role in embryogenesis and tissue physiology, as well as in a wide variety of diseases. At the same time, great technological advances in areas such as nanotechnology, micromanipulation, biological imaging, and computer modeling have enabled us to analyze mechanotransduction: how forces affect the biochemical activities of individual molecules, both in isolation and within living cells. For these reasons, mechanoregulation is once again becoming a central focus in fields ranging from molecular biophysics and cell biology to human physiology and clinical medicine.

Advances in the most fundamental research areas have been impressive—numerous molecules and subcellular structures have been shown to mediate force sensation and mechanochemical conversion at the nanometer scale (**Fig. 1**). But it remains unclear how the whole cell processes this molecular scale information and orchestrates a physiologically relevant response in the context of the multiscale architecture of our whole bodies. Thus, the time is now ripe, as in the old Humpty-Dumpty nursery rhyme, to put all the pieces together again and to understand how cells react to mechanical stimuli in their normal tissue context. To do this, we need to consider work from researchers in a wide range of fields—biophysics, molecular cell biology, physiology, anatomy, developmental biology, engineering, computer science—that are often unaware of each other's findings, even though they might be highly pertinent. The goal of this article is to place all these data within a structural context that has physiological relevance and hence to help integrate and focus future research in this field.

MECHANOCHEMICAL CONVERSION AT THE NANOMETER SCALE

The field of mechanobiology has been driven by a search for specialized mechanotransducer molecules that change their chemical activity state when they are mechanically distorted, and thereby convert mechanical energy into biochemical energy. However, the function of virtually every molecule could potentially be altered by mechanical stress because all bioactive molecules move between extended and contracted forms,

[1]Correspondence: Vascular Biology Program, Karp Family Research Laboratories 11.127, Children's Hospital, 300 Longwood Ave., Boston MA 02115, USA. E-mail: donald.ingber@childrens.harvard.edu

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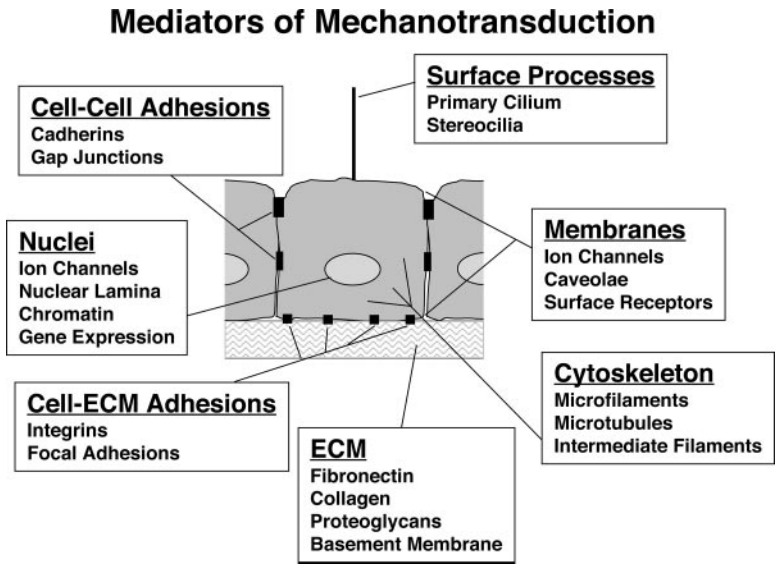

Figure 1. Mediators of cellular mechanotransduction. Many molecules, cellular components, and extracellular structures have been shown to contribute to mechanochemical transduction. These transduction elements include ECM, cell-ECM, and cell-cell adhesions, membrane components, specialized surface processes, cytoskeletal filaments, and nuclear structures. The challenge for the future is to understand how cells orchestrate all these transduction mechanisms in the context of living tissue anatomy to produce a concerted response to mechanical signals.

rearrange internal domains, or alter their motion on the nanoscale in the process of carrying out their biochemical activities (1–3). Mechanical distension of cell membranes, for example, using patch-clamp techniques, modulates the cation-transporting activity of stress-sensitive ion channels by producing conformational changes that alter their opening or closing rates through distortion of the associated lipid bilayer (3, 4) or through force-induced displacements of intramolecular "gating domains"(**Fig. 2*A*, *B***) (5, 6). The movement of molecular motors, such as myosin, and the activity of enzymes (e.g., RNA polymerase) can be inhibited by applying an opposing stalling force using optical tweezers (7, 8). This is because the conformational changes that these molecules undergo comprise the rate-limiting step for their translocation or catalytic activities (Fig. 2*C*). Single molecule force spectroscopy studies show that individual peptide domains within proteins found in the cytoskeleton (9), cell-cell adhesions (10), and the extracellular matrix (ECM) (11) undergo stepwise unfolding when they are mechanically extended (Fig. 2*D*). There is also specificity to this response in that mechanical forces and temperature induce protein unfolding by distinct mechanisms (12), and the degree of unfolding depends on where the load is applied to the protein (13).

Because of their effects on molecular conformation, physical forces can modulate the kinetics of protein-protein or protein-ligand binding in living cells. The force generated by myosin motors and exerted on actin filaments feeds back to prolong the lifetime of the bound crossbridge, whereas its release is accelerated

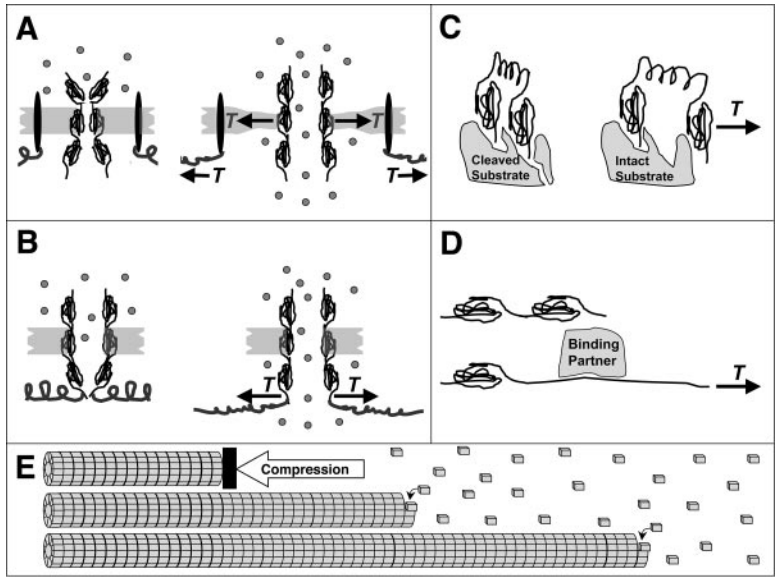

Figure 2. Molecular mechanisms of mechanochemical transduction. *A)* Diagrammatic representation of a stretch-sensitive ion channel that alters its conformation and changes its opening and closing rates when the membrane bilayer distorts, thereby exerting tensional forces (*T*; arrow indicates direction) on the channel molecule. Membrane distortion may be produced by surface shear forces or by cytoskeletal distortion of transmembrane molecules that tightly associate with the lipid bilayer (black ovals), such as integrins or other integral membrane proteins. *B)* Another stretch-sensitive ion channel that experiences tensional forces that are transmitted directly from the internal cytoskeleton. These forces stimulate ion flux by tugging on the cytoplasmic portion of the channel that acts as a "gating spring" and opens the pore when tensed. *C)* Tension-dependent distortion of an enzyme (as shown here) or molecular motor can alter its catalytic activity and, for example, inhibit cleavage of a substrate by physically restricting molecular conformational changes. *D)* Tension application to individual proteins can produce unfolding of discrete peptide domains within the molecules. This may influence their elastic properties, as well as expose previously masked binding sites that can alter biochemical activities. E*)* The chemical potential of proteins that form biopolymers may alter when the filaments are compressed or tensed. Release of end-on compression of a cytoskeletal microtubule, for example (as shown here), results in tubulin monomer addition and polymer elongation.

when the force is dissipated (14). The binding kinetics of adhesion molecules, such as cell surface receptors that mediate leukocyte adhesion and rolling on endothelium, vary depending on the concentration, rate, and history of force application. For instance, the same L-selectin–carbohydrate bonds that dissociate over a time scale of milliseconds and mediate leukocyte–endothelial cell adhesion under low shear stress can support cell adhesion and rolling at higher levels of shear because of force-induced stabilization of multiple bond formation and fast rebinding of broken bonds (15). P-selectin forms a molecular "catch-bond" when it binds to its glycoprotein ligand—the bond strengths are greater if first loaded by an abrupt rise in force (16). Conversely, in the case of integrin receptor-mediated cell adhesions to ECM, talin forms a molecular "slip-bond" by exerting a low concentration of cytoskeletal tension on closely packed integrins until several additional bonds form that stabilize the specialized anchoring complexes or "focal adhesions" that mechanically couple integrins to the actin cytoskeleton (17, 18).

Forces can influence chemical equilibria and molecular polymerization events as well. For example, application of tensional forces to cultured neurons or vascular smooth muscle cells through ECM adhesions results in force-dependent increases in microtubule polymerization (19, 20). This can be explained if cytoplasmic microtubules normally bear compressive forces that increase the chemical potential of tubulin monomers in the polymer relative to those in solution; decreased compression of a microtubule will lower the chemical potential of the polymer, as well as the concentration of soluble tubulin monomers that is necessary to maintain the equilibrium (21, 22). This will drive microtubule assembly until enough tubulin monomer is shifted from the soluble phase to the polymeric state to reestablish the equilibrium (Fig. 2*E*).

So there are numerous biophysical mechanisms by which mechanical energy might be translated into changes in biochemical activities at the molecular concentration. The more difficult challenge is to explain how forces that are transmitted through living organs and across multiple size scales are converted into biochemical alterations through specific transduction molecules, rather than altering the activities of all cellular components. We also need to understand how multiple molecular scale mechanisms are orchestrated simultaneously at the whole system level so that each cell can produce a concerted response to mechanical stress.

LESSONS FROM THE AUDITORY SYSTEM

Unfortunately, the mechanism by which mechanical forces applied at the macroscale influence specific molecular activities remains unknown in most somatic organ systems. However, analysis of specialized mechanosensory functions, such as hearing and balance, has revealed that mechanosensation depends as much on the architectural organization of the whole sensory organ and its specialized connective tissues as on the structure of individual sensory cells and mechanosensitive molecules. For example, hearing occurs through the transmission of sound vibrations that travel over long distances until they deflect the ear drum (tympanic membrane) and an interconnected series of millimeter-sized boney ossicles within the middle ear (**Fig. 3**). Both the ear drum and the bones are stabilized (stiffened) by the action of tiny muscles that "prestress" the entire network by placing it under isometric tension; this removes any slack in the system and makes it immediately responsive to mechanical stress. When hit by sounds, the coupled vibrations of the ear drum and bones rhythmically distort a smaller tensed membrane in the oval window of the inner ear that generates internal fluid waves and causes wave-like displacement

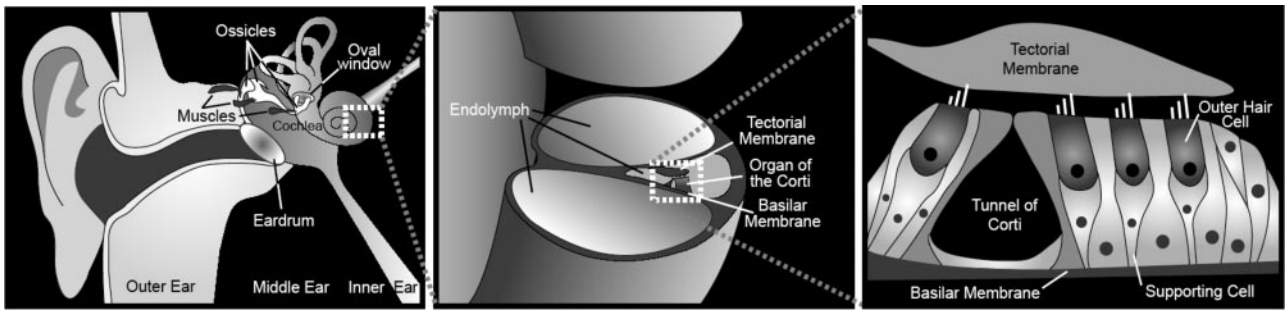

Figure 3. The hierarchical organization of the auditory system. Left: Sound vibrations that travel over long distances and pass through the ear canal induce rhythmic distortions of the tensed tympanic membrane (eardrum) of the outer ear. These movements propagate to the interconnected tensed muscles and boney ossicles of the middle ear that push and pull on the oval window opening to the cochlea of the inner ear. Middle: The movement of the oval window induces fluid pressure waves within the endolymph that flows through the spiral-shaped cochlea, which is shown in cross section at higher magnification in this view. These rhythmic changes in flow induce corresponding vibrations in the basilar membrane ECM that physically distort the adherent sensory epithelium within the organ of Corti of the inner ear. Right: Deflection of the basilar membrane and attached supporting cells results in bending of the inner hair cells and their apical stereocilia, which are fixed within the overlying, nonmotile tectorial membrane, as shown here at higher magnification. All of these structures must be prestressed (experience a resting tension) to be able to immediately respond to physical signals, and different hair cells sense different frequencies based on differences in the local mechanical compliance (stiffness) of the basilar membrane ECM (not shown).

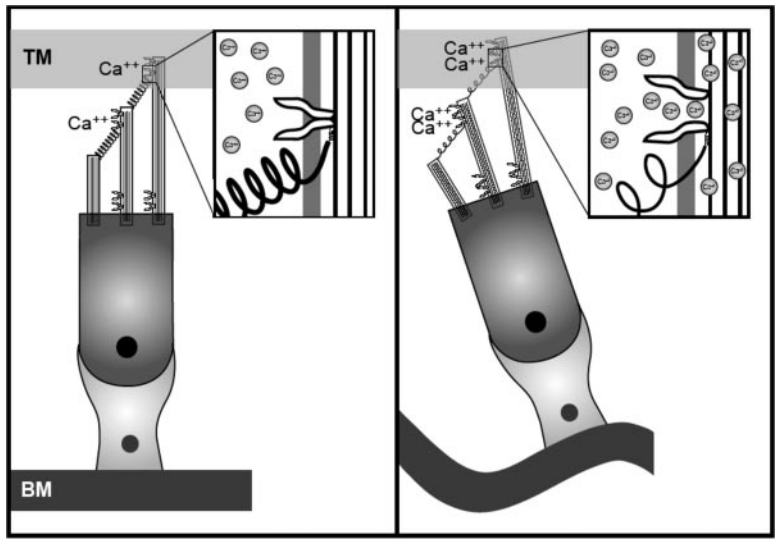

Figure 4. Mechanochemical conversion at the nanometer scale in the auditory system. A schematic of a hair cell of the inner ear from the right view of Fig. 3 shown at higher magnification in the absence (left) or presence (right) of a sound stimulus, which causes the basilar membrane (BM) to undulate. When the stereocilia bend because their tips are fixed in the overlying immobile tectorial membrane (TM), the forces are focused through cadherins that form tensed tip-links that span the surface membrane and connect to myosins and actin filaments within the neighboring cilia (indicated by a large spring that extends with force in right vs. left insets). Increased tension on the membrane or on the actin filaments that may link to the cytoplasmic regions of stretch-sensitive ion channels results in opening of these channels and Ca^{2+} ion influx near the tip-link insertion sites and at the basal pivot point of each stereocilium. This results in membrane depolarization that activates auditory nerve fibers (not shown) and conveys signals to the brain. In this manner, channeling of forces across multiple size scales facilitates the cellular mechanotransduction events that mediate sound detection.

of a specialized ECM—the basilar membrane—at the micrometer scale within the organ of Corti (Fig. 3 and **Fig. 4**). This planar ECM is also pretensed through the action of contractile supporting cells that anchor to it along their base. The sensory hair cells attach to the upper surfaces of the supporting cells; thus, their stiffened cell bodies and apical stereocilia move at the same frequency as the basilar membrane. However, the hair cells' apical stereocilia are embedded in another ECM (the tectorial membrane), which is immobile, and so mechanical shear stress is produced at the upper cell surface when the basilar membrane and attached cells deform; this causes the stereocilia to bend (Fig. 4).

The stereocilia are microvillar projections that contain rigid actin filament bundles that extend apically from the internal cytoskeleton that provides shape stability to the cells of the cochlea (23). Each stereocilium is joined to ~20 to 30 neighboring stereocilia and physically integrated into a single functioning unit (the stereocilia bundle) through interconnection at an even tinier size scale by a series of fine molecular filaments known as "tip links." These tip links are composed of the cell-cell adhesion protein cadherin 23 that forms a complex with unconventional myosin motors that pull against rigid actin filament bundles within the adjacent stereocilia (Fig. 4) (24, 25), and thereby prestress (and stiffen) the entire stereocilia array. Loss of myosin function or cadherin 23 leads to disruption of the stereocilia bundle architecture (24, 26) and abnormal gating of ion channels in response to stress (27).

When sound vibrations induce stereocilia deflection, they specifically focus mechanical energy through these tensed tip-links, and induce opening of mechanically gated ion channels, such as the transient receptor potential (TRP) ion channel, TRPA1 (28), located near the point of insertion of the tip-link on the surface membrane (Fig. 4). These or other stretch-sensitive channels are also activated at the base of each stereocilium where it inserts into the apical cell surface (29), and where stresses concentrate when the stereocilium's long lever arm is deflected. It remains unclear whether the tip link transfers force directly to the channels or indirectly through its connections to the internal cytoskeleton (i.e., through myosin and actin), which may in turn link to the helical cytoplasmic tail of the TRP channel formed by multiple tandem ankyrin domains (6). Regardless of the path of force transmission, these mechanically induced ion fluxes trigger the cell depolarizations that activate auditory nerve fibers and convey sound signals to the brain.

This multiscale mechanism must incorporate additional architectural features in order to explain how hair cells sense sounds of different frequencies, and distinguish between loud noises and whispers. The hair cells modulate the frequency of stereocilia vibration and their sensitivity to mechanical stimuli by varying the concentration of isometric tension in their cytoskeleton, much like tuning a guitar string. The vibration of the stereocilia is actually tuned to a particular frequency of oscillation that is positioned precisely at a critical point such that any additional influx of mechanical energy due to increase in sound volume induces a loss of state stability and immediately increases the amplitude of oscillation (30). It is through this nonlinear process that individual hair cells can sense signals well below the concentration of background thermal noise, and actively amplify faint sounds. By keeping the resting tension on the channels constant, hair cells can also adapt over a slower time scale to prolonged deflections of the stereocilia to maintain optimum sensitivity (31), whereas disruption of the tensed tip links and dissipation of the prestress in the molecular network results in impaired sound sensation (24).

The basilar membrane also vibrates at the same frequency of the stimulus precisely where it is maximally sensitive to that particular frequency so that each

position along its length responds to a different frequency of sound vibration. This behavior is based on local variations in the physical properties (mechanical compliance) of this ECM, and on active voltage-dependent shortening and lengthening of another set of outer hair cells that contract and relax at the same frequency as the sound stimulus (32). By pushing or pulling on the same basilar membrane and altering the basal tension in this ECM at a larger size scale, these cells can amplify or dampen vibrations, alter the concentration of mechanical force that is experienced by the inner hair cells, and thereby modulate their sensitivity to sound. It is likely because of the critical importance of these different molecules, tension-generating mechanisms, and multiscale structures that deafness can result from mutations in such a wide variety of ostensibly unrelated genes, including those that encode ion channels, cytoskeletal proteins, cell adhesion molecules, and ECM components.

MECHANOTRANSDUCTION HIERARCHIES: FROM MACROSCALE TO NANOSCALE

Thus, analysis of the auditory mechanism teaches us that nature has developed an ingenious strategy for mechanotransduction that involves use of structural hierarchies (systems within systems) that span several size scales and are composed of tensed networks of muscles, bones, ECMs, cells, and cytoskeletal filaments that focus stresses on specific mechanotransducer molecules (Figs. 3, 4). Moreover, the level of isometric tension at all levels within this network tunes the whole system and governs how it will respond to external mechanical loads. This raises the question of whether somatic organs use analogous mechanisms to sense and respond to mechanical force.

In fact, virtually all organs and tissues are organized as prestressed structural hierarchies that exhibit immediate mechanical responsiveness and increase their stiffness in direct proportion to the applied mechanical stress (33). In the musculoskeletal system, for example, prestress arises from a balance between contractile forces that are generated within the cytoskeletons of muscle cells and the ability of rigid bone matrix to resist these forces at the macroscale. On a smaller size scale, these forces are distributed between adjacent muscle bundles, blood vessels, and nerve tracts through myofascial and ECM connections, and removal of tensile and shear effects on neighboring tissues alters tissue adaptation to mechanical stimuli (34). At a cellular size scale, the shape stability of individual muscle bundles and blood vessels requires that an analogous force balance be established between tractional forces exerted by the parenchymal cells and resisting forces that are exerted by stiffened ECMs (such as cross-linked collagen bundles, tensed basement membranes), surrounding connective tissue cells, and other microenvironmental forces (e.g., effects of gravity, movement, hemodynamic stresses). Muscle and tendon tissues adapt to stresses applied at the level of the whole musculoskeletal system and protect themselves against injury rearranging on many size scales, including by rearranging the molecular components that comprise the tensed ECMs and interconnected cytoskeletal elements within adherent cells (**Fig. 5**) (35, 36).

Other organs use different mechanisms to generate a stabilizing prestress. For instance, in the lung the residual filling pressure that remains after expiration is responsible for tensing and stiffening the ECMs (basement membranes, collagen fibers, elastin bundles) that surround each alveolus, and for resisting surface tension forces acting on the epithelium; this force balance stabilizes the alveoli in an open form (37). Lung expiration and inspiration influence this force balance

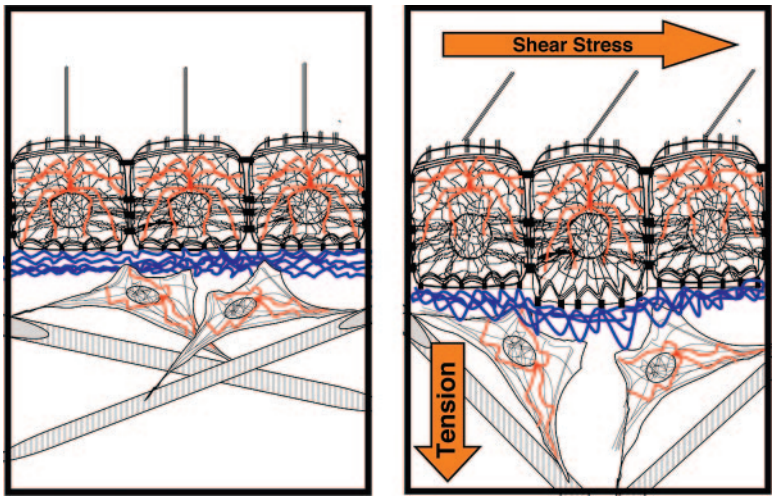

Figure 5. Cellular mechanoresponsiveness and physical connectivity between ECM, cells, cytoskeletal networks, and nuclei. Mechanical loads exerted at the macroscale trickle down to produces changes in connective tissue and ECM that result in production of stresses at the microscale that produce global structural rearrangements of the individual molecular components that comprise the ECM, as well as the interconnected cytoskeletons and nuclei of adherent cells. These schematic diagrams show that tension-dependent changes in the orientation of collagen bundles produce cell, cytoskeletal, and nuclear rearrangements within adherent fibroblasts, as well as distortion of interconnected basement membrane scaffolds, which in turn produce similar cytoskeletal and nuclear rearrangements within associated endothelial cells. At the same time, fluid shear stresses applied at the apical surface of certain endothelial and epithelial cells can also influence cytoskeletal structure and cellular biochemistry by deflecting the primary cilium found on the surface of many cells, and by exerting drag on the plasma membrane and apical cell-cell junctions. Because these shear stresses are also channeled through the cytoskeleton to basal cell-ECM adhesions, they can alter the structure and function of underlying connective tissue as well. The entire cellular response to stress may therefore vary depending on the structural integrity and organization of the whole cytoskeleton-cell-ECM lattice, as well as the concentration of isometric tension in the network prior to load application.

and produce complex micromechanical responses in the lung parenchyma, including lengthening and shortening (and tension and compression) of alveolar walls depending on the direction of the applied stress (38–40). This is accompanied by extension and linearization of some collagen fibers on inspiration, as well as buckling of the same fibers on expiration (39). Breathing also causes the lateral intercellular spaces between epithelial cells to reversibly shrink and expand without compromising the structural integrity of the tissue. This form of reversible mechanical deformation might activate intracellular signaling within surrounding alveolar cells by altering the local concentration of soluble ligands for epidermal growth factor receptors (41).

On the other hand, cartilage is prestressed owing to a combination of tensile forces in the collagen network and the physical swelling of proteoglycan molecules at the molecular scale, which push outward against the nonextensible collagen fibers due to their energy of hydration (42, 43). Changes in ion concentrations in the surrounding interstitium can influence whole tissue mechanics by altering this swelling pressure in cartilage (44, 45). When cartilage is compressed (e.g., due to the force of gravity), coordinated responses are again produced at multiple size scales, including reorientation of proteoglycans throughout the ECM network (46) as well as changes in cell shape, cytoskeletal organization, and nuclear from within chondrocytes (47). In addition, because this mechanical network is composed of multiple discrete structural elements (e.g., ECM components, cells) with much free space, compressive forces may induce fluid flow in the lattice, which can shear cell surfaces and alter electrochemical potentials (44, 45). Similar effects are observed when bone is compressed as a result of cyclical muscle contractions (acting against gravity) even though it has a higher material density because of the presence of Haversian canals (48). All these physical changes can alter cellular signal transduction and thereby induce connective tissue remodeling. The key point is that because they are prestressed (and hence, there is no slack in these structures), the ECMs that hold cells together within any tissue react immediately as an integrated mechanical system when the whole organ is stressed (Fig. 5).

Because cells use specific transmembrane receptors, such as integrins, to mechanically couple their cytoskeletal network to the ECM (and so form one extended physical lattice; Fig. 5) (1, 49), the cell senses distortion of the ECM, or an associated increase in its rigidity, as a tug on these adhesion receptors (50, 51). Integrins connect to the cytoskeleton through focal adhesions that contain multiple actin-associated proteins such as talin, vinculin, paxillin, and zyxin (17). The cytoskeleton in turn responds mechanically to forces transferred over the ECM and channeled through integrins by rearranging its interlinked actin microfilaments, microtubules and intermediate filaments, as well as associated organelles (e.g., mitochondria) and nuclei, on even smaller scales (Fig. 5), thereby strengthening the whole cell against the potential deleterious effects of mechanical distortion (35, 50, 52–56).

The use of transmembrane adhesion receptors and linked cytoskeletal filament networks for force transmission provides a way for cells to channel and focus stresses applied at the cell surface so that they concentrate on focal adhesions, as well as at distant sites in the cytoplasm (e.g., mitochondria) and nucleus, and on the plasma membrane at the opposite pole of the cell (52, 53, 57–60). By contrast, mechanical forces only produce local effects at the surface membrane when they applied to other transmembrane molecules that fail to form strong cytoskeletal connections (e.g., growth factor receptors, histocompatibility antigens) (52, 53). This "action at a distance" might explain how mitochondria that associate with microtubules can sense mechanical strain in endothelial cells, release reactive oxygen species and activate signaling molecules (e.g., NF-κB, vascular cell adhesion molecule-1) that contribute to inflammation and atherosclerosis (61). Long distance force transfer over integrins and through the cytoplasm requires an intact cytoskeleton and varies depending on the concentration of cytoskeletal prestress as well as the direction of force application (58, 62, 63). Conversely, increases in cytoskeletal tension inside the cell that are transmitted outward across integrins can feed back to promote structural changes in the surrounding ECM, such as unfolding of peptide domains within fibronectin molecules that promote fibril assembly (64); this adds strength to the tissue at a higher systems level.

The cytoskeleton of each cell is also prestressed because tensional forces that are generated within contractile microfilaments and transmitted throughout the cell are balanced by internal microtubules that resist being compressed, as well as by extracellular adhesions to ECM (**Fig. 6**) and to other cells (49, 53, 54, 65–68). This allows cells to shift compressive forces back and forth between microtubules and ECM adhesions, such that microtubules bear most of the prestress in rounded cells with few anchoring points whereas the ECM bears most of the load in spread cells on highly adhesive substrates (Fig. 6) (68). The existence of this complementary force balance in the cytoskeleton explains how external forces that are applied at the cell surface can alter the chemical potential of tubulin and thereby control microtubule polymerization in cells (Fig. 2*E*) as is required for nerve outgrowth or directional cell migration, as well as why cytoskeletal tension and ECM adhesions contribute to this response (19, 20, 22, 69). Similar mechanical interactions between microfilaments, microtubules, and cell substrate adhesions govern the shape and stiffness of the cells and their linked ECMs (Fig. 6) (49, 50, 54). On a smaller size scale, the shape stability of specialized membrane processes (e.g., filopodia, stereocilia, primary cilia, flagella) are similarly stabilized through the establishment of a force balance between stiffened cytoskeletal struts (cross-linked bundles of microfilaments or microtubules) that resist compressive forces created by the

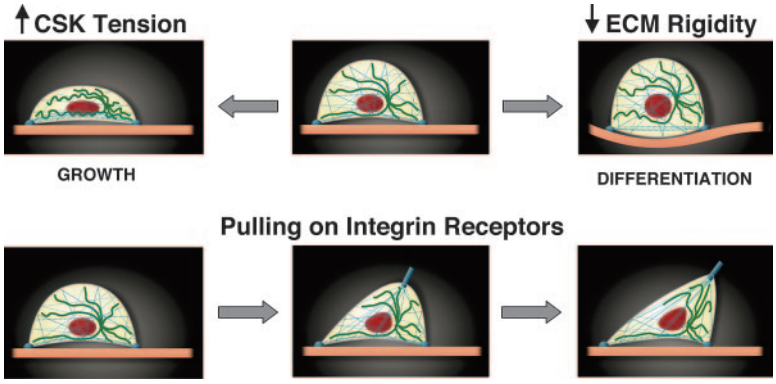

Figure 6. Integrated cell and cytoskeletal shape control through a complementary force balance between microfilaments, microtubules, and ECM. Top: Cell shape and the stability of the cytoskeleton depend on a mechanical force balance between microfilaments, microtubules and the ECM. In most anchorage-dependent cells, cell spreading on ECM is required for cell cycle progression and growth; abruptly increasing cytoskeletal tension in these spread cells results in cell flattening, increased bundling of actin filaments within basal stress fibers, and enhanced buckling of some microtubules (left). Decreasing ECM rigidity to the point where it can no longer bear cell traction forces results in force transfer to internal microtubules, which increases their buckling and bending, as well as stress fiber disassembly and cell rounding; in most cells, cell retraction switches off growth and turns on differentiation or apoptosis (right). Bottom: Diagram of a cell adherent to a rigid ECM through two basal focal adhesions (semicircles) showing the response of cytoskeletal microtubules (red) and microfilaments (dashed blue lines) when tensional forces are applied to integrins that form a focal adhesion on its apical cell surface (cylinder). Pulling on integrin receptors results in coordinated structural rearrangements throughout the actin and microtubular cytoskeletons, as well as within the nucleus and basal focal adhesions. Pulling on other transmembrane receptors that do not mediate cell adhesion produces only a local membrane response.

surrounding tensed plasma membrane. Changes in molecular-level repulsive forces between neurofilaments may also contribute to the generation of prestress within individual cytoskeletal filament bundles (70).

The cortical cytoskeleton that supports the plasma membrane is yet another prestressed network structure that gains its mechanical stability by incorporating multiple rigid actin protofilaments that are held in place by a geodesic (triangulated) array of spectrin molecules that act like tensed cables suspended from the overlying lipid bilayer (71). Nuclear lamins and interconnected nuclear pore complexes form a smaller spherical geodesic lattice at the center of the cell that is pretensed by surrounding cytoskeletal filaments in spread cells (52, 54, 72). A mechanical force balance between compressed microtubules and a tensed network of chromosomes and nuclear scaffolds similarly stabilizes the mitotic spindle (57, 73).

The presence of these prestressed hierarchical networks, and their ability to channel mechanical forces over discrete molecular paths to sites deep inside the cytoplasm and nucleus, explain how cell distortion or mechanical stress application to ECM and bound cell surface integrins results in changes in nuclear shape and induces molecular organization within nucleoli on progressively smaller size scales (52). This coupling between integrins and the nucleus is largely mediated by intermediate filaments that extend from cell surface adhesion sites to the nucleus, and to a lesser degree by the actin cytoskeleton (52, 74), and the efficiency of this multiscale mechanical response is governed by cytoskeletal prestress (62).

MECHANOTRANSDUCTION THROUGH SOLID-PHASE MECHANOCHEMISTRY

If use of prestressed structural hierarchies helps to transfer force across many size scales and to focus stresses on specific load-bearing molecules in somatic cells, then we would expect to see mechanochemical conversion occur at sites where stresses are concentrated, such as in focal adhesions and cell-cell junctions that physically anchor cells to the ECM and other cells, as well as in linked cytoskeletal structures and nuclear scaffolds (Figs. 5, 6). In fact, much of cell signaling and metabolism is carried out using a form of solid-phase biochemistry in which many relevant enzymes and substrates physically associate with these insoluble scaffolds (75). For example, although integrins can trigger signaling transduction cascades and induce focal adhesion formation as a result of ECM ligand binding and associated changes in receptor conformation, application of mechanical forces to bound integrins can convey distinct signals to the cell (1, 17). Force application to bound integrins promotes focal adhesion assembly by activating the small GTPase Rho and stimulating its downstream targets mDia1 and Rho-associated kinase (ROCK), which promote actin filament polymerization and induce cytoskeletal contraction, respectively (76, 77). It is also mediated by integrin-associated, receptor-like tyrosine phosphatase-α (78) and changes of binding of the cytoskeletal linker protein, actin filament-associated protein (AFAP) that induce activation of Src kinases (79, 80). Certain structural molecules in focal adhesions also appear to be preferentially mechanosensitive. For example, zyxin increases its unbinding rate constant when cytoskeletal tension is dissipated (**Fig. 7**), whereas vinculin in the same focal adhesions is not affected (81). When released from focal adhesions, zyxin can translocate to the nucleus and alter transcription of genes, such as endothelin-1 (82). Stresses applied to integrins can also regulate gene expression post-transcriptionally by modulating the formation of protein synthetic complexes at focal adhesions (83).

This form of integrin mechanosignaling is bidirectional in that shifts in the cytoskeletal force balance

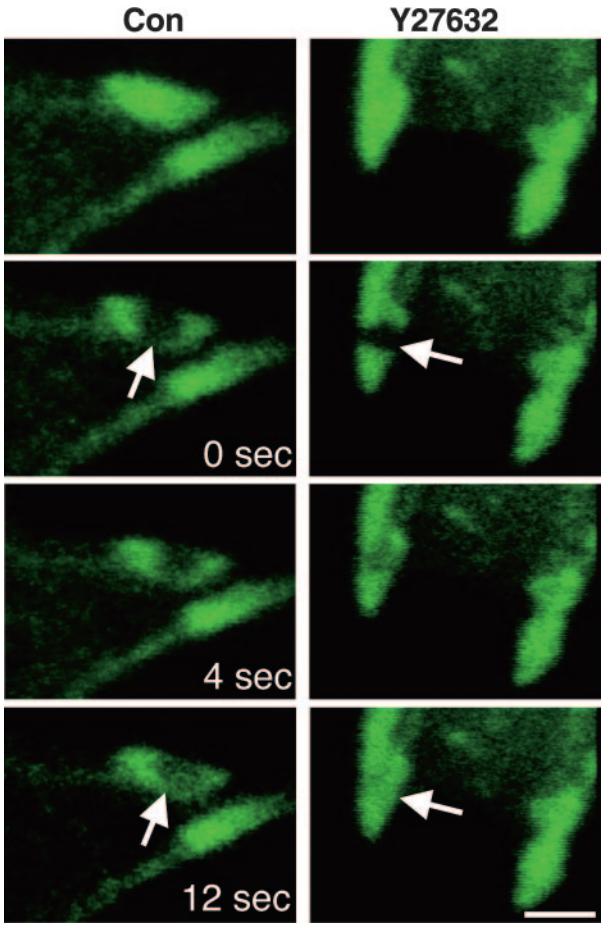

Figure 7. Mechanical control of focal adhesion remodeling through force-dependent changes of molecular binding kinetics. Fluorescence confocal microscopic images of focal adhesions from a fluorescence recovery after photobleaching study of endothelial cells expressing GFP zyxin cultured in the absence (control) or presence of a ROCK inhibitor (Y27632) that dissipates cytoskeletal tension. Note that decreasing cytoskeletal prestress accelerates zyxin recovery. Arrows indicate photobleached spots within individual focal adhesions that are analyzed over a period of 12 s after photobleaching; bar = 2 μm (81).

between microfilaments, microtubules, and ECM can feed back to alter Rho activation and focal adhesion formation (84–86). In addition, mechanical stress application to cells already bound to ECM can induce a new wave of integrin activation and binding and thereby trigger associated integrin-dependent chemical signaling events. The effects of apical fluid shear stress in endothelial cells on lamellipodia extension and cell realignment, as well as on gene expression mediated by Rho, its related small GTPase Rac, and extracellular signal-regulated kinase (ERK) (87–90) all require changes in dynamic binding interactions between integrins and their ECM ligands at the cell base (91).

Forces that are channeled through transmembrane adhesion receptors and cytoskeletal filaments can activate stress-sensitive ion channels on the cell surface, and many mechanosensitive ion channels lose their normal regulated activities if the lipid bilayer separates from the underlying cortical cytoskeleton (92). Some integrins colocalize with putative stress-sensitive ion channels, including members of the ENaC family (93), and Ca^{2+} influx can be induced by force application to integrins, sometimes within milliseconds (56, 94, 95). Thus, forces appear to be directly transmitted from integrins to these channels either through cytoskeletal connections (Fig. 2*B*) or potentially through direct molecular associations. Members of the degenerin/epithelial-sodium channel (DEG/ENaC) family that mediate touch sensation in cells in a wide range of species, from *Caenorhabditis elegans* to mice, also function as transmembrane adhesion molecules that associate directly with cytoskeletal microtubules as well as with ECM components (3); hence, they likely experience forces transmitted directly from these structural elements. Direct binding of certain TRP channels to microtubules or actin filaments may be mediated by ankyrin repeats within their cytoplasmic domains (6).

By contrast, other mechanosensitive ion channels, including at least one member of the TRP family (TRPC1), display physiological responses in pure bilayers (3, 4). Activation of the ion-transporting activities of these channels may therefore result from stretching of the lipid bilayer within the surface membrane caused by external forces (e.g., drag due to fluid shear stress) (Fig. 2*A*). However, forces that are transmitted across integrins and associated cytoskeletal filaments also might influence the activity of these ion channels indirectly by distributing stresses to associated membrane lipids (either directly or through other integral membrane proteins), and thereby distorting the lipid bilayer (Fig. 2*A*).

In kidney epithelial cells, fluid shear stress produces Ca^{2+} influx by deflecting the primary cilium, which acts like a long, microscopic, vertical lever arm on the apical surface of these cells (96). This is a single elongated microvillar projection found on the surface of many epithelial and endothelial cells (Fig. 5) that is reminiscent of the stereocilium of inner hair cells (Fig. 4), except it contains a central bundle of microtubules instead of actin filaments. The mechanotransduction response in kidney cells is mediated by interactions between two members of the TRP family of mechanoregulated ion channels, polycystin-1 and polycystin-2, that colocalize with microtubules within the primary cilium (97).

Cells may also utilize other cell surface molecules to sense mechanical stress. For example, shear stress has been suggested to directly activate heterotrimeric G-proteins in whole cells by altering membrane fluidity (98) because their activities alter when they are placed in pure liposomes that are mechanically distorted (99). But this is complicated in living cells because direct stress application to integrins also activates large G-proteins and triggers signaling through cyclic AMP leading to activation of gene transcription, but this effect is not produced when the same force is applied to unligated integrins (using nonactivating antibodies) or other transmembrane receptors that do not form focal adhesions (55, 100). Thus, the mechanosensitivity of

large G-proteins in whole cells appears to require force transfer through integrins and discrete cytoskeletal linkages, although force transmission through the lipid bilayer could mediate this effect, as described above for certain ion channels (Fig. 2*A*).

Another important membrane-associated mechanotransduction mechanism involves lipid rafts. Endothelial cells respond to changes in fluid shear stress by activating various caveolae-associated signaling molecules, including tyrosine kinases, members of the Ras-Raf-mitogen-activated protein kinase pathway, and sphingomyelinase (101), which alters gene transcription by producing second messengers that stimulate Src kinases, ERK, and the Akt-NOS pathway (102). Disassembly of lipid rafts and caveolae through depletion of plasma membrane cholesterol inhibits mechanotransduction pathways in response to hydrostatic pressure and fluid shear stress in bone cells (103), suggesting that their activation by mechanical force involves direct bilayer distortion. However, lipid raft clustering cannot occur in the absence of reorganization of the actin cytoskeleton in T cells (104); the formation of lipid rafts that is required for the activity of Rac also depends on integrin-dependent signals from the ECM (105). Flow-induced redistribution of caveolin-1 on the surface membrane of endothelial cells is similarly associated with a redistribution of F-actin (106), which in turn is controlled by integrins (107). Most important, integrins and caveolin-1 directly associate in some cells through interactions with the Crk adaptor protein, and both the phosphorylation of caveolin-1 and the reorganization of actin that are induced by shear stress can be prevented by interfering with integrin binding (108). Thus, force-induced caveolae signaling might also involve channeling of forces through transmembrane adhesion receptors and the cytoskeleton that alter membrane deformation (Fig. 2*A*).

Integrin-containing focal adhesions are not the only cell membrane sites at which mechanochemical conversion takes place. Mechanical forces can be similarly transmitted across the cell surface and to the cytoskeleton through cadherins and other transmembrane molecules (e.g., selectins) that form intercellular junctional complexes, although the mechanical linkages formed by cell-cell adhesion molecules are generally not as strong as those mediated by integrins (35, 109, 110). Nevertheless, cadherins can mediate force-induced activation of Ca^{2+} influx through mechanosensitive ion channels and associated actin assembly (111), and application of fluid shear stress to osteoblasts causes the cadherin-associated junctional protein, β-catenin, to translocate into the nucleus, where it activates gene transcription (112). In endothelial cells, mechanical coupling through VE-cadherins feeds back to control integrin-dependent focal adhesion assembly, cell spreading, and growth, and this effect is mediated by Rho-dependent changes in cytoskeletal prestress (113). A mechanosensory cell-cell adhesion complex composed of VE-cadherin, PECAM-1, and vascular endothelial growth factor receptor-2 also mediates shear stress-dependent activation of integrins and downstream NF-κB in confluent endothelial cell monolayers (114). In bone cells, gap junctions mediate the release of the signaling molecule prostaglandin E2 in response to fluid flow (115), and membrane distortion induces opening of gap junction hemichannels in lens cells (116). Mutations of dystrophin, another transmembrane adhesion receptor involved in the mechanical anchorage of the cytoskeleton to the ECM in skeletal muscle cells, similarly leads to abnormal mechanotransduction, as measured by increased activation of stress-sensitive ion channels and ERK (70).

Focused stress transfer across membrane adhesion receptors and through the cytoskeleton may also activate signal transduction at distant sites because of the existence of discrete load-bearing networks and smaller scale structural hierarchies inside the cytoplasm. Apical shear stress produces nearly immediate remodeling of focal adhesions oriented in the direction of the flow at basal sites where transcellular forces concentrate (58, 63, 117). Forces transferred through the cytoskeleton to the nucleus can activate stress-sensitive ion channels on the nuclear membrane and thereby influence gene transcription (118); they could also potentially alter nucleolar function, chromatin folding, torsional strain within DNA, or access of key proteins (such as transcription factors or steroid hormones) to gene regulatory sites (1, 52). The importance of this mechanical connectivity between the surface, cytoskeleton, and nucleus could explain why disruption, mutations, or deletions of intermediate filament proteins or nuclear structural proteins (such as lamins) results not only in decreased mechanical stiffness of the whole cell and cell injury in response to mechanical stress, but also in reduced mechanical activation of gene transcription (52, 74, 119).

High levels of mechanical strain or very rapid physical perturbations can produce stress fracture of the membrane, which may alter cellular biochemistry by releasing cytoplasmic factors, such as fibroblast growth factor and angiotensin II, which can influence cell function in an autocrine or paracrine manner (120, 121). This is actually a physiological and reversible form of mechanotransduction that can occur in healthy people, for example, due to normal contractility of the heart (122) or peristaltic movements of the gut (120). The degree of membrane rupture produced by mechanical stress is governed by the dynamics of membrane resealing, which in turn is controlled by depolymerization of cortical F-actin (123). Hence, even this ostensibly passive response to mechanical stress is actually a regulated process that depends on the mechanical state of the prestressed cytoskeleton.

In summary, mechanochemical conversion occurs simultaneously at several sites inside cells, tissues, and organs (Fig. 1) because stresses are transferred over load-bearing networks including bones, muscles, fascia, ECMs, integrins, cell-cell junctions, cytoskeletal filaments and nuclear scaffolds that span many size scales (Figs. 3–6). In fact, individual cells within most somatic

tissues are too small to be distorted directly by subtle forces that are known to have potent effects on tissue form and function, such as gravity; that is, unless the cells contain dense organelles (e.g., otoliths, statoliths) that function like microscopic plumb-bobs (124). Cells therefore likely sense gravity and other generalized forces exerted on tissues and organs through their interconnections with ECM scaffoldings that experience stress and undergo deformation on a larger size scale; these structural changes trickle down to produce local cell and cytoskeletal distortion or changes of cellular prestress (Figs. 3–6).

ORCHESTRATION OF THE CELLULAR RESPONSE

Given the central role of ECMs as force conduits in tissues, integrins have come to be viewed as critical mechanoreceptors, and focal adhesions as nanoscale mechanosensory organelles (1, 17, 50). Although a stress applied to integrins can produce a local response (e.g., focal adhesion remodeling and signaling) (17, 56, 76, 77, 100), the reality is that the whole cell is the mechanotransducer because it integrates these local signals with other environmental inputs before eliciting a specific behavioral response. This can be clearly seen in the way adherent cells generally respond to soluble mitogens by proliferating when they can physically spread on ECM, whereas intermediate-sized cells differentiate, and round or retracted cells undergo apoptosis when exposed to same soluble growth stimuli (125–127). The overall form of the cytoskeleton (128, 129) and the orientation of the cell relative to applied loads (such as uniaxial stretch) (87) also influence the direction in which cells will extend lamellipodia and move. This is the case even though these different shaped cells exhibit similar transmembrane signaling in response to growth factor receptor binding (130) or integrin ligation (131).

The global mechanical response of the cell varies depending on the concentration of prestress in its supporting structural network, just as the tension in the tympanic and basilar membranes of the ear influence the auditory response. Due to tensed cytoskeletal connections that link the cell's various mechanochemical transduction elements, variations of cellular prestress can influence how many different autonomously functioning components work together to produce a concerted response (e.g., analogous to a coupled harmonic oscillator). For example, stress-induced activation of cAMP signaling through integrins and large G-proteins occurs locally at sites of stress concentration within focal adhesions at the plasma membrane regardless of the concentration of cytoskeletal prestress (100). But changes in prestress govern how this cue is integrated with other signals elicited in the cell by microenvironmental stimuli to produce higher level functional responses, such as focal adhesion assembly, growth, contractility and directional motility (128, 132–135). The calcium signaling response triggered by force-induced deflection of the primary cilium on the apical surface of kidney epithelial cells also can be inhibited by disrupting cytoplasmic microfilaments, dislodging basal integrin adhesions, or dissipating cytoskeletal prestress (136).

Changes in cytoskeletal prestress also contribute to cell fate control. Culture dishes that promote cell spreading and growth (e.g., rigid dishes) support high levels of cytoskeletal tension, whereas flexible or poorly adhesive substrates that suppress growth and induce differentiation dissipate prestress (Fig. 6) (113, 132, 135, 137–139). In fact, cells seem to be tuned mechanically (compliance-matched) so that they preferentially differentiate on ECMs that have a mechanical stiffness similar to that of their natural tissues (140, 141). Cells also alter the concentration of forces they exert on the ECM to maintain tensional homeostasis that is necessary for the preservation and regeneration of normal functional tissue architecture (135, 142, 143), and disruption of this force balance may contribute to cancer formation (144, 145). Conversely, cells can be induced to remodel the form of adult tissues in their entirety (e.g., veins can be induced to change into arteries) by changing the basal stress concentration (146). Thus, cellular mechanotransduction may be controlled physiologically by altering the physical properties of the structural framework of the tissue (and cell), even though mechano-chemical transduction is mediated by specific nanoscale structures and molecular components.

MECHANICAL FORCES AS DEVELOPMENTAL REGULATORS

So although living cells might sense and respond to force locally through individual mechanosensitive molecules, they integrate physical and chemical signals at the whole cell level before they respond. For this reason local variations in cell shape owing to cell traction forces, regional variations in ECM mechanics, or differences in mechanical coupling between cell-cell junctions can influence embryogenesis and tissue pattern formation. For example, cell compression caused either by normal morphogenetic movements during mesoderm invagination or by external force application with a micropipette induces signal transduction events, including nuclear translocation of the transcription factor Armadillo and transcription of the Twist gene, which control formation of the dorso-ventral axis in the early gastrula-stage *Drosophila melanogaster* embryo (147). Physical interactions between cytoskeletal microtubules and microfilaments that alter cell shape also regulate cell fate and mediate position-specific control of epithelial architecture during wing development in these embryos (148). Cytoskeletal contractile forces that drive convergence and extension of multicellular populations similarly play a critical role in gastrulation and embryonic wound healing in *Xenopus laevis* (149, 150).

In later stages of tissue morphogenesis in mammals,

local increases in cell proliferation that drive the formation of epithelial buds and capillary branches during development of whole organs, such as lung, may result from regional increases in the mechanical compliance of the tensed basement membranes that alter tissue architecture and produce cell stretching or a rise in cytoskeletal tension within nearby adherent cells (151). Analysis of groups of cells cultured on microfabricated adhesive islands confirm that the physical form of a tissue can feed back to control cell growth patterns as a result of local variations of internal stresses that are distributed through the cytoskeleton and resisted by cell-cell and cell-ECM adhesions (152). Forces applied at the cell surface can also potentially affect tissue patterning by reorienting the cell division plane (57). In addition, cell shape distortion and mechanical stresses can regulate stem cell commitment (e.g., convert embryonic stem cells into vascular endothelium) and switch cells between different lineages (e.g., bone vs. fat) (153, 154). This process by which local changes in ECM mechanics or cytoskeletal prestress alter cell sensitivity to soluble factors and control cell fate switching in vivo is again reminiscent of the way in which regional variations in the stiffness of the basilar membrane, and associated local variations in cell tension, are used to modulate hair cell sensitivity to sound vibrations and tune individual cell auditory responses in the inner ear.

The importance of mechanical forces for organ development is made clear by the finding that mutation or deletion of the polycystin genes that encode mechanosensitive ion channels that mediate urine flow sensation in kidney epithelium leads to the development of polycystic kidney disease (97). Severe muscular dystrophies and cardiac myopathies similarly result from mutations of ECM molecules, integrins, adhesion molecules, cytoskeletal proteins or nuclear lamins that form the structural scaffolds that mediate force channeling in muscle (119, 155). Computational models similarly suggest that abnormal forces on the hip influence bone growth and ossification, as well as development of hip dysplasia in the fetus (156). Thus, the prestressed structural hierarchies that guide and orchestrate mechanotransduction throughout adult life also function to regulate their own formation and development in the embryo.

ARCHITECTURAL CONSIDERATIONS

Because cells, tissues, and organs are constructed as interconnected structural hierarchies composed of tensionally prestressed networks, forces applied at the macroscale that mechanically strain ECMs and deform cells and their internal cytoskeletons are able to filter down to smaller size scales (Figs. 3–6) to be focused on specific molecular components and to produce structural rearrangements within a subset of these molecules at the nanometer scale that change their biochemical activities (Fig. 2). Mechanochemical conversion may therefore occur in a concerted manner at numerous sites inside the cell because these forces are transmitted to multiple structures and molecules throughout the cytoplasm and nucleus, as well as on the surface membrane (Fig. 1). Individual protein molecules also appear to be prestressed structures that globally reorient their local stiffened domains (e.g., α-chains and β-strands) when mechanically stressed at the nanometer scale (Fig. 2) (157–159). This may in part explain how forces applied to tissues and cells can induce molecular conformational changes without disrupting cytostructural connectivity. Given the amplification scheme used by the tensed hair cells of the inner ear, it is possible that the tension exerted on specific molecules through their prestressed cytoskeletal linkages may similarly allow nonspecialized cells to sense signals in the cytoplasm in the presence of background thermal noise, and thus maintain optimal mechanosensitivity.

The levels of forces that cells encounter in vivo might not rise to the concentration necessary to produce conformation changes (e.g., molecular unfolding) in certain molecules necessary for changes in their biochemical activity (71). However, channeling of forces along discrete molecular filament networks (e.g., ECM, cytoskeleton) provides a way to concentrate stresses on specific molecules at particular locations while protecting most other cellular components from these same mechanical forces. In this manner, only a subset of mechanosensory molecules will experience force levels high enough to alter their biochemical activities. The process of cellular mechanotransduction in the context of living anatomy therefore might be more a manifestation of structural hierarchies and biological architecture, rather than being due to the action of any single "mechanotransduction molecule."

This use of structural hierarchies, tensile prestress, and geodesic (minimal path) structures for mechanical stability and integrated system-wide control is clearly key to mechanotransduction in whole living organisms; however, these features are also hallmarks of "tensegrity" (tensional integrity) architecture (**Fig. 8**) (54, 160). Tensegrity networks are composed of opposing tension and compression elements that balance each other and thereby create an internal prestress or resting tension that stabilizes the entire structure. Tensegrity can be seen at the macroscale in the way pulling forces generated in muscles and resisted by bones stabilize the shape of our whole bodies.

In hierarchical tensegrity structures containing multiple linked modules stabilized by similar rules, a local stress is borne by multiple prestressed elements that rearrange at multiple size scales, rather than deform and yield locally (Fig. 8). These rearrangements occur without compromising structural integrity, and they cause the entire network to physically strengthen when mechanically stressed (50, 54). Because tensegrities are composed of discrete networks of support elements (rather than a mechanical continuum like a hunk of rubber), they provide a way to transmit mechanical forces along specific paths and to focus or concentrate

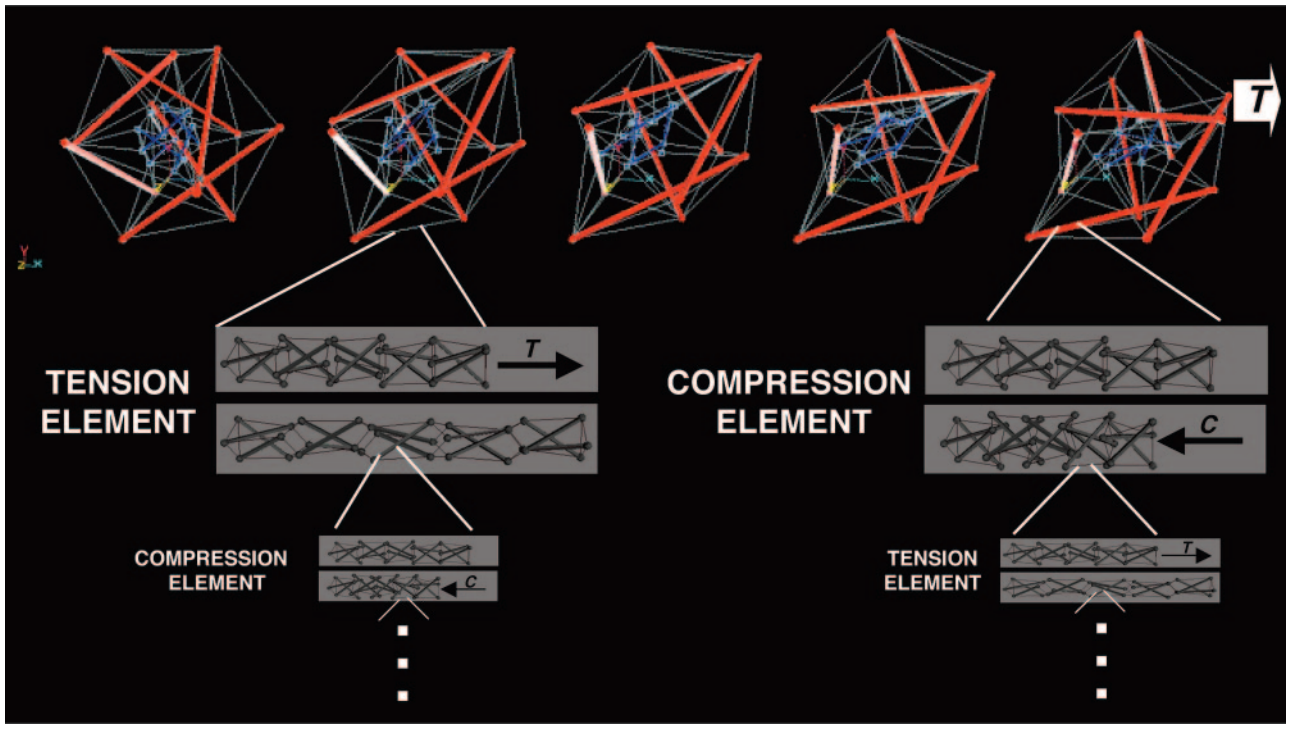

Figure 8. Computer models depicting multiscale structural rearrangements with a prestressed tensegrity hierarchy. Top: A two-tier hierarchical tensegrity composed of concentric larger (red) and smaller (blue) spherical (polyhedral) structures composed of struts and cables connected by tension elements. Note that the structure exhibits coordinated structural rearrangements of its internal elements as it extends to the right in response to tension (*T*) application (movies showing dynamic movements in tensegrities can be seen at: www.childrenshospital.org/research/Site2029/mainpageS2029P23sublevel24.html). Lower panels show how individual struts and cables of the structure may themselves be organized as compressed (*C*) and tensed (*T*) tensegrity mast structures at smaller and smaller size scales *ad infinitum*. A stress applied at the macroscale will result in global rearrangements at multiple size scales, rather than local bending or breakage, as long as tensional integrity and stabilizing prestress are maintained throughout the hierarchical network.

stresses on distant sites and at different size scales (Fig. 8) (1, 54). These are all features observed in whole organs as well as tissues, cells, membranes, cytoskeletal networks, subcellular organelles, nuclei, mitotic spindles, transport vesicles, viruses, and proteins (54, 72, 73, 158, 159, 161). Thus, use of this structural system for shape stability may explain how all these structures can immediately sense and respond to external mechanical signals. Recent advances in mathematical, engineering, and statistical mechanical models of static and nonequilibrium tensegrity structures (71, 162–167) and in the use of nanotechnology to create artificial tensegrities (168–170) may therefore provide new avenues of investigation into the fundamental biophysical mechanisms by which mechanical forces influence biological structures and produce biochemical responses at all size scales.

CONCLUSIONS AND PERSPECTIVES

Much has been learned about the molecular and cellular basis of mechanotransduction over the past decade. Integrins, cell-cell adhesion molecules, growth factor receptors, caveolae, mechanosensitive ion channels, cytoskeletal filaments, and numerous other signaling and structural molecules contribute to this response. However, it should be clear from this discussion that cellular mechanotransduction cannot be understood in isolation or defined entirely in terms of mechanosensitive molecules. Mechanical force sensing depends on the architectural context in which the cell lives. The cellular response will be governed by how mechanical forces are distributed throughout the organ and tissue of which it is a part, as well as by the level of preexisting tension in the ECM, cell, cytoskeleton, and membrane. Our ability to sense mechanical forces at the cellular level is therefore a direct manifestation of how our bodies are constructed. Thus, if we continue to seek out and study individual biological parts in isolation without considering contributions of multiscale architecture and invisible internal forces, we will never be able to fully understand how physical forces influence biological form and function. Future studies in the field of mechanical biology will need to focus on a whole new set of questions: How do molecular and biophysical interactions influence structures and biochemical activities locally, as well as at a distance? How do structural scaffolds in the membrane, cytoskeleton, and nucleus focus and channel forces in specific ways to provide particular functions? How do changes in the material properties or arrangement of multimolecular assemblies affect the biochemical activities of their

components, and *vice versa*? How can we measure or model changes in molecular shape and activities (e.g., binding kinetics) within the higher order context of multimolecular structural assemblies? How is internal prestress generated and controlled at each size scale, and what is its impact on these biochemical and mechanical responses?

The ultimate goal is to identify the specific structures and molecules that mediate this cascade of multiscale events during mechanotransduction in somatic organs, as we have begun to do in the auditory system. We will also need to understand the principles that govern the multiscale nature of these responses to be able to effectively model, predict and control synthetic nanostructured systems in the future. Success in this endeavor will require that molecular cell biologists form greater alliances with engineers, physicists, nanotechnologists, computer scientists, and even architects to develop new methodologies and predictive models. Through this collaboration, we may finally come to accept that life is not based on a structureless chemistry, and instead gain true insight into how multicellular organisms arise through interplay between mechanics and chemistry at all size scales. FJ

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