How cells (might) sense microgravity

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ABSTRACT **This article is a summary of a lecture presented at an ESA/NASA Workshop on *Cell and Molecular Biology Research in Space* that convened in Leuven, Belgium, in June 1998. Recent studies are reviewed which suggest that cells may sense mechanical stresses, including those due to gravity, through changes in the balance of forces that are transmitted across transmembrane adhesion receptors that link the cytoskeleton to the extracellular matrix and to other cells (e.g., integrins, cadherins, selectins). The mechanism by which these mechanical signals are transduced and converted into a biochemical response appears to be based, in part, on the finding that living cells use a tension-dependent form of architecture, known as tensegrity, to organize and stabilize their cytoskeleton. Because of tensegrity, the cellular response to stress differs depending on the level of pre-stress (pre-existing tension) in the cytoskeleton and it involves all three cytoskeletal filament systems as well as nuclear scaffolds. Recent studies confirm that alterations in the cellular force balance can influence intracellular biochemistry within focal adhesion complexes that form at the site of integrin binding as well as gene expression in the nucleus. These results suggest that gravity sensation may not result from direct activation of any single gravioreceptor molecule. Instead, gravitational forces may be experienced by individual cells in the living organism as a result of stress-dependent changes in cell, tissue, or organ structure that, in turn, alter extracellular matrix mechanics, cell shape, cytoskeletal organization, or internal pre-stress in the cell-tissue matrix.—Ingber, D. How cells (might) sense microgravity. *FASEB J.* 13 (Suppl.), S3–S15 (1999)**

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THIS PAPER IS BASED on an invited lecture I presented at the Belgium National Academy of Sciences in the opening symposium of the European Space Agency (ESA)[2]/National Aeronautics and Space Administration (NASA) Workshop on *Cell and Molecular Biology Research in Space*, which convened in Leuven, Belgium in June 1998. The organizers chose the title of my lecture: *How cells (MIGHT) sense microgravity.* The word, "might," was probably added as an afterthought by a wise meeting organizer. This was a prudent choice because although it is clear that physical forces, such as those due to gravity, are fundamental regulators of tissue development, little is known about how living cells sense these signals and convert them into a biochemical response. This transduction process, which is at the core of gravity sensation, is known as mechanotransduction; and this is what I will focus on today.

Past work on mechanotransduction has revealed that certain cells have evolved specialized crystal structures that respond directly to the force of gravity. These dense crystals are called statoliths, literally "standing stones," or otoliths, as in the case of the sensory cells of the inner ear. When we move our heads, these dense crystals slide over the receptor cells like tiny lead weights, and it is the resulting localized distortion of the cell surface and interconnected cytoskeleton (CSK) that is somehow sensed by the cell. The statolith represents an elegant mechanism for mechanotransduction, however, it does not explain how all of the cells in the body sense gravity.

One of the most common changes observed in astronauts who undergo long-term spaceflight is bone resorption. In fact, it has been known for over a century that bone matrix is deposited in distinct patterns that precisely map out engineering lines of tension and compression for any structure of that size and shape under similar loading conditions (1). If the loading pattern is altered or an astronaut is placed in microgravity, the bone immediately remodels. We now know that living cells within bone (osteoblasts, osteoclasts) are responsible for this remodeling. This means that individual cells must be able to sense changes in physical forces in their local environment that are caused by gravity and that they respond in the most efficient manner possible: by putting new matrix where it is needed and removing

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[2] Abbreviations: ESA, European Space Agency; NASA, National Aeronautics and Space Administration; CSK, cytoskeleton; ECM, extracellular matrix; CE, capillary endothelial; FAs, focal adhesions.

it from where it is not. However, no one has ever found statoliths in bone cells or in cells within other tissues that are equally sensitive to perturbation by mechanical stresses (e.g., muscle, skin, blood vessels, lung, cartilage, nerve, etc.). In fact, sensitivity to mechanical forces now seems to be a general property of all living tissues and all cultured cells (2–6).

To understand how gravitational forces alter cell function, we must place this form of developmental control in context of what we have learned in recent years about other forms of cell regulation. Understanding mechanotransduction in the context of cell and tissue morphogenesis has been the major focus of my laboratory for the past 20 years. When I first entered the morphogenesis field, all of the attention was on the identification and isolation of regulatory factors. This led to the discovery of the importance of soluble growth factors and insoluble extracellular matrix (ECM) molecules, in addition to mechanical forces, as critical regulators of tissue growth and development. However, in recent years, the focus has shifted from discovery of mitogens and morphogens to analysis of the mechanism by which extracellular stimuli elicit an intracellular response, or what is known as "signal transduction."

Over the past few years I have been to many conferences and workshops that focus on signaling. Unfortunately, each lecturer seems to have his or her favorite signaling pathway: a few arrows leading to MAP kinase, a particular tyrosine kinase, or a G protein is often offered as an answer to how a complex function, such as cellular growth, is controlled. It seems unlikely, however, that we will ever fully understand cell regulation if we focus on any single signaling mechanism in isolation. The reality in living tissues is that cells normally sense multiple simultaneous inputs. For example, at the same time a cell in a healing wound binds a growth factor, it may be forming new ECM adhesions at its base, and through these adhesions it may feel the pull of the surrounding tissue, due in large part to the force of gravity. However, each individual cell produces only one concerted response: it either grows or moves or differentiates or it dies locally. Thus, the key is not which pathways are turned on, but how all these signals are integrated inside the cell. Furthermore, we have to understand how this integration occurs within the structural complexity of the living cell.

Let me provide you with the take-home message right now: we have found that living cells are literally hard-wired so that they can filter the same set of chemical inputs to produce different functional outputs and this mechanism is largely controlled mechanically, through physical distortion of transmembrane adhesion receptors on the cell surface that preferentially transmit stresses to the internal CSK. Cells in all tissues may sense changes in gravitational acceleration through associated alterations in the balance of forces distributed between their adhesions and the CSK, rather than through direct activation of any single "gravioreceptor" molecule. The basis for these claims will become clear from a review of recent work from our laboratory, as will be described below. A more thorough discussion of the mechanotransduction field can be found in recent reviews (2–6).

CONTROL OF CELL GROWTH AND FUNCTION BY CELL DISTORTION

A simple example of how mechanical forces can impact on cell behavior comes from studies in which we made cell distortion or cell shape an independent variable. It has been long known that cells deform from spherical to flat when they attach to an ECM substrate and that this distortion is mediated in part by the action of tractional forces (CSK tension) that cells exert on their own adhesions (7). Our concept for controlling cell shape was to create small adhesive islands that would prevent cell spreading but would promote local clustering of cell surface ECM receptors (integrins) and associated activation of signaling pathways inside the cell. Because of the presence of surrounding nonadhesive Teflon-like regions, the cells would be physically restricted in terms of how far they could extend and flatten. By including saturating amounts of soluble mitogens in the medium and a constant high concentration of immobilized ECM ligand, such as fibronectin, the size of the island could be varied independently while maintaining these other regulatory factors constant.

We accomplished this through the use of a soft lithography-based micropatterning technology (8, 9) that was developed by George Whitesides' laboratory (Department of Chemistry, Harvard University) as an alternative method for creating microchips for the computer industry (10). When we plated capillary endothelial (CE) cells on circular islands 20 μm in diameter that were separated from neighboring islands by wider (40 μm) nonadhesive (polyethylene glycol-coated) regions, we obtained spherical cells that remained limited to the size, shape, and position of the engineered island (**Fig. 1**, ***A*** and ***B***). When we plated the same cells on square islands, we produced square cells with 90° corners (Fig. 1, *C* and *D*).

To explore the effects of cell distortion on cell function, Christopher Chen, a student in my laboratory, cultured cells on individual square adhesive islands of varying size. Using this approach, he was able to demonstrate that CE cells can be geometrically switched between gene programs for growth

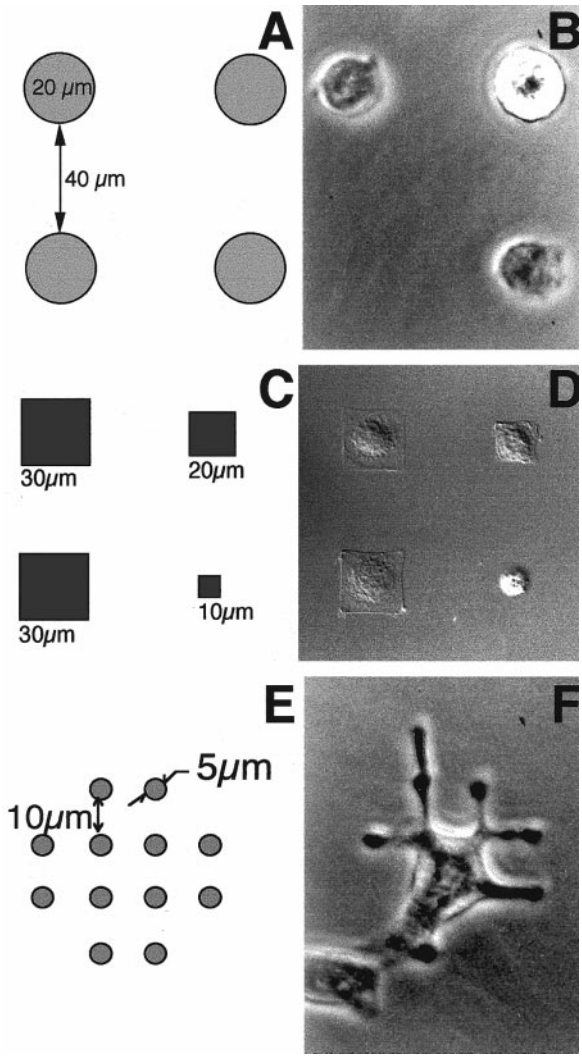

Figure 1. Control of cell shape using micropatterned substrates. *A*) Pattern with 20-μm-wide circular islands; *B*) CE cells grown for 24 h on 20-μm circular islands; *C*) pattern with different sized square islands of indicated size; *D*) cells grown on corresponding square islands; *E*) pattern with multiple, small (5 μm) circular adhesive islands that are more closely spaced (10 μm); *F*) single cell spread over many similar sized circular islands shown in *E* (see ref. 9 for more details).

and apoptosis (programmed cell death) (9). Cell growth increased in an exponential fashion as the adhesive area available for cell spreading increased from approximately 1000 to 3000 μm^2. Conversely, as cell spreading was restricted to smaller sizes and growth was turned off, apoptosis was switched on. Cell distortion, rather than a particular form (e.g., round vs. square), was the critical element here because cells that spread to a similar degree (equal projected cell area) exhibited similar behavior. Recently, Laura Dike and Chris Chen extended these studies by showing that linear substrates that hold CE cells in a moderate degree of extension, which induces neither growth nor apoptosis, switch on differentiation (capillary tube formation) in CE cells (Dike, L., Chen, C., and Ingber, D., unpublished observations). Similar results were obtained earlier by plating CE cells on dishes coated with varying ECM densities that differed in their ability to resist cell tractional forces (11). Thus, mechanical interactions between cells and their ECM appear to play a critical role in cell regulation by switching cells between different gene programs (**Fig. 2**).

ADHESION COMPLEXES AS SITES FOR SIGNAL INTEGRATION

How can this work? How can cell adhesion and spreading shift cells between different gene programs in the presence of a saturating amount of soluble growth factors? Part of the explanation appears to be based on how cells restructure their CSK when they adhere to ECM. Cell attachment to ECM is mediated by binding of transmembrane integrin receptors that cluster in spot weld-like anchoring sites, known as focal adhesions (FAs), where they physically interconnect with the actin CSK (12). This connection is mediated by binding interactions between the cytoplasmic face of the integrin receptors and actin-associated molecules in the cytoplasm, such as vinculin and talin.

We (13, 14) and others (15, 16) discovered that many of the signaling molecules that are turned on by integrins and mitogenic receptors are not floating around in lipid bilayer; rather, they are immobilized on the CSK and specifically concentrated within the FA at the site of integrin binding. This includes tyrosine kinases, such as c-src and FAK kinase, as well as the Na^+/H^+ antiporter, inositol lipid kinases, and others. In fact, we even found that a subset of high-affinity growth factor receptors (FGF receptors) are concentrated within the FA (13). Thus, part of

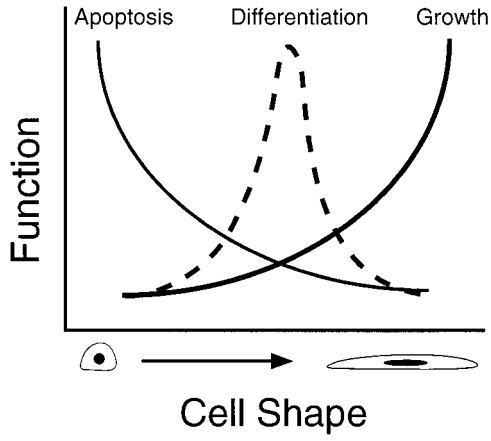

Figure 2. Schematic diagram showing mechanical switching between different gene programs in CE cells. CE cells grow when spread, die when fully retracted, and differentiate into capillary tubes if maintained at a moderate degree of extension while also forming cell-cell contacts (e.g., on thin micropatterned lines).

the mechanism of signal integration appears to be based on the spatial organization of signaling molecules within the cell and their physical association with CSK elements within the FA. Similar integration likely occurs in cell-cell adhesions because signaling molecules also concentrate in the CSK adhesion complexes that connect to transmembrane adhesion receptors (e.g., cadherins) in these regions (12).

There are two important points here. The first is that proximity between different transducing molecules provides a way to integrate integrin and growth factor signaling pathways at the cell surface. For example, Helen McNamee, Martin Schwartz, and I found that integrins can regulate inositol signaling by controlling synthesis and local availability of the phosphatidylinositol-*bis*-phosphate substrate within the FA, whereas growth factors (e.g., platelet-derived growth factors) control downstream signaling by regulating the breakdown of this inositol lipid through activation of phospholipase C (14, 17).

The second point is the finding that these signaling molecules essentially function when in a "solid state," that is, when immobilized on insoluble CSK scaffolds. This finding is recurring again and again in cell biology: many of the enzymes and substrates that mediate DNA synthesis, RNA processing, protein synthesis, and glycolysis also appear to function when immobilized on insoluble scaffolds in the cytoplasm and nucleus (18). This finding provides a possible mechanism to explain the incredible efficiency of biochemical reactions that are observed in living cells and cannot be mimicked in a test tube. However, it also has important implications for how stress-induced changes in cell shape and CSK structure could alter biochemical reactions and change gene expression, given that the FA represents a preferred site for mechanical signal transfer across the cell surface (19–21), as I will describe below.

FUNCTIONAL CONTROL BY CELL SHAPE AND CYTOSKETAL TENSION

The integrin signaling elicited within FAs has been shown to be critical for control of cell growth and function (12, 22). Thus, one could argue that cell shape and mechanical distortion of the CSK are not important; instead, it might be the increase in total area of cell-ECM contacts and associated integrin binding that dictates whether cells will grow or die on large versus small adhesive islands. To explore this further, we effectively broke up a single small adhesive island (which would not support spreading or growth) into many smaller FA-sized islands (3–5 μm in diameter) that were spread out and separated by nonadhesive barrier regions (Fig. 1*E*) (9). When cells were plated on these substrates, their processes stretched from island to island and the cells exhibited an overall extended form similar to cells on large islands (Fig. 1*F*). However, the total area of cell-ECM contact was identical to that exhibited by non-growing cells on the smaller islands. The question was: which is the most critical element for cell growth: the ECM contact area available for integrin binding or cell shape?

The answer is that cell shape is the most critical determinant of cell function, at least in the presence of optimal growth factors and high ECM binding. DNA synthesis was high in the cells that spread over multiple small islands, whereas apoptosis was completely shut off (9). Thus, cell shape *per se* appears to govern how individual cells will respond to chemical signals (soluble mitogens and insoluble ECM molecules) in their local microenvironment, as first proposed by Folkman and Moscona (23). This mechanism for establishing local growth differentials may play a critical role in morphogenesis in all developing systems (24, 25).

What is the mechanism by which cell shape exerts its effects on cell function? Clearly, the full answer is unknown. However, recent studies have revealed that cell shape exerts its effects on cell cycle progression many hours after early growth signals are turned on. When synchronized CE cells are presented with an optimal ECM coating concentration, saturating growth factors, and a substrate that promotes cell spreading, the cells synchronously enter S phase approximately 16–20 h later. Yet, even with optimal growth factor and integrin stimulation and associated early signaling pathways turned on, growth can be inhibited if the cells are rounded up any time before 12 h (e.g., using trypsin or cytochalasin D) (26, 27). This finding is consistent with work from other laboratories which show that adhesion and CSK integrity are required for cells to pass through the G1/S transition (28, 29).

Recent work by Sui Huang in my group has confirmed that even with early signaling cascades (e.g., MAP kinase) fully activated, CE cells will not enter S phase unless cell spreading is also promoted (27). Furthermore, cell shape exerts these effects on growth by harnessing the cell's molecular machinery that is normally responsible for control of the late G1/S restriction point, including the key cell cycle regulators: cyclin D1 and the cdk inhibitor, $p27^{kip1}$. The importance of cell shape appears to be that it represents a visual manifestation of an underlying balance of mechanical forces that in turn convey critical regulatory information to the cell. For instance, we were able to show that pharmacological inhibitors of actomyosin-based tension generation can produce the same cell cycle arrest as cell rounding, without altering global cell shape (27). Thus, changes in mechanical stresses, including those im-

posed by gravity, could impact on cellular growth and biochemistry by altering the net balance of forces within the cell and its CSK.

CELLULAR TENSEGRITY AND ESTABLISHMENT OF THE CELLULAR FORCE BALANCE

These are interesting experimental results, but the question remains: how could altering stresses applied to ECM or the cell surface change CSK shape and alter cell function? This is a difficult mechanism to understand if one accepts the conventional view of the cell being composed of a viscous fluid cytosol surrounded by an elastic surface membrane. Instead, we have found that living cells are hard-wired by a continuous series of molecular struts (microtubules, cross-linked microfilament bundles), cables (contractile microfilaments), and ropes (intermediate filaments) in the form of a discrete CSK network that mechanically connects specific transmembrane adhesion receptors on the cell surface to discrete contacts on the nucleus in the center of the cell (19, 20, 30). Furthermore, we have found that cells use a particular form of architecture, known as tensegrity, to organize and mechanically stabilize this CSK network (31).

Tensegrity comes out of the Buckminster Fuller school of geodesic architecture. It emerged, in part, from Fuller trying to understand why the geodesic dome is so efficient in terms of its ability to carry a given load with a minimal amount of building materials. He realized that it is not what it is made of: it could be constructed from wood struts or aluminum poles. Rather, it is how these elements distribute and balance mechanical stresses in three dimensions that determine the dome's stability. Most man-made buildings are compression-dependent in that the weight of one brick, due to gravity, compresses down on the brick below and it is through this continuous compression that stability results. However, if one were to drive a truck into the side of a brick wall, the wall would shatter into pieces. In contrast, Fuller realized that the dome gains its omnidirectional stability from continuous tension that is resisted locally by a subset of its structural elements (31, 32).

It is difficult to visualize continuous tension in the fully triangulated geodesic dome; however, it is clearly observed in the tensegrity sculptures of Kenneth Snelson who was one of Fuller's students. Snelson's tensegrity masts are composed of multiple steel girders that can rise more than 60 feet in the air, yet none of the girders physically touch. Instead, they are held up against gravity and suspended in mid-air through interconnection with a continuous series of high tension cables that follow geodesic

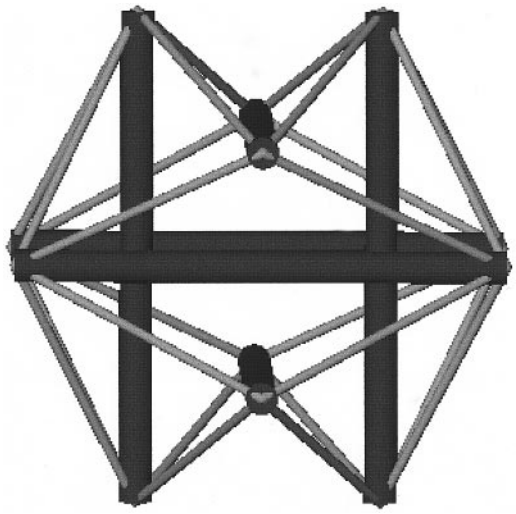

Figure 3. A computer-generated diagram of a 6-strut and 24-cable tensegrity model in its equilibrium form. Note that the struts to do not physically contact one another; the structure is stabilized by the presence of a continuous series of tension cables that provide tensional continuity and pre-stress the entire structure (kindly provided by Molecular Geodesics, Inc.).

(minimum path) lines. Spherical models can be constructed using similar building rules (**Fig. 3**). These sculptures may look strange, however, this is how our bodies are constructed. If you were to visit an anatomy laboratory, you would see a human skeleton composed of bones that must be wired together and hung from a stand in order to take on an upright human form. In reality, our bodies are composed of 206 compression-resistant bones that are pulled up against the force of gravity and stabilized through interconnection with a continuos series of tensile muscles, tendons, and ligaments. Furthermore, the mechanical stiffness of our bodies is determined by the level of tone or pre-stress in our muscles and not by osmotic forces. This is true for all types of animals, independent of size or species. In an identical way, the stability of a stick-and-string tensegrity structure (Fig. 3) depends directly on the pre-stress (preexisting tension) in its filament system.

Many years ago, when I was a student at Yale, I constructed a simple tensegrity model made out of sticks and elastic string (**Fig. 4**) and was able to show that this model can mimic many of the behaviors of complex living cells (24, 31, 33). Because of the internal tone in the elastic strings of this model, it takes on a spherical shape when unanchored, just as living cells do in suspension. However, when the model is anchored to a rigid resisting substrate, it spontaneously spreads out and takes on a flattened form, like an adherent cell. Furthermore, when the same model is similarly attached to a flexible substrate, the model pulls that substrate into compression wrinkles and takes on a more rounded form. Nearly identical behavior has been observed in living

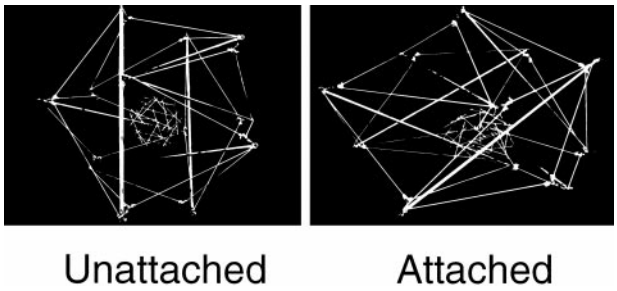

Figure 4. A nucleated tensegrity cell model shown in its unattached and attached forms. The larger cell model was constructed from aluminum poles and elastic cord in the arrangement shown in Figure 3. The nucleus is a geodesic sphere composed of applicator sticks and white elastic strings; the nucleus is connected to the larger cell model by black elastic threads that cannot be seen because of the black background. *Left*) the cell and nucleus in the unattached model both exhibit spherical forms. *Right*) coordinated spreading of the cell and nucleus, along with basal reorientation of the nucleus, are observed when the model is anchored to a rigid substrate.

cells cultured on flexible silicone rubber substrates (7). We also showed that a model built with a nucleus that was connected to the surface by additional elastic strings nicely mimicked the coordinated changes in cell and nuclear shape, as well as the polarization of the nucleus to the base, that are observed in cells when they spread on a rigid substrate (24, 33–35) (Fig. 4). More recently, we and others have confirmed that the mechanical stiffness (ability to resist shape distortion) of the cell depends directly on the level of pre-stress within the CSK (36–39) as well as on the maintenance of tensional continuity (20, 30), as predicted by the tensegrity model.

It turns out that geodesic architecture is also utilized at the molecular level in cells. Many people assume that the CSK transforms from gel to sol when cells detach from a substrate and round, and that the cell effectively behaves like a balloon filled with molasses. In reality, cells can transform between fully spherical and fully flattened forms over a period of minutes to hours without altering the total amount of F-actin or microtubule polymer in the cell (40, 41). In fact, high-resolution freeze etch electron microscopy has shown that the cytoplasm of round cells is filled by a continuous filamentous actin network that is initially isotropic (non-oriented) but progressively realigns into linear bundles (stress fibers) along its base when the cell adheres to a rigid adhesive substrate (42). Meanwhile, in the apical region of the cells, this same filamentous network can rearrange into polygonal actin nets that sometimes can extend to form highly triangulated structures, including molecular geodesic domes composed of actomyosin filaments (**Fig. 5**) (42–44). This finding is a clear example of how cells use tensegrity at the molecular level. It is interesting to note that these structural interconversions between an isotropic network and either a linear bundle or geodesic dome can be predicted using a simple kinematic model that can transform from an unstable network into fully triangulated tensegrity forms without disruption of network continuity (33). In fact, using this simple kinematic model, we created structures that precisely mimicked forms observed in the actin CSK of living cells, including strut for strut and vertex for vertex correspondence at the molecular level, as defined by electron microscopy (33).

More recent computer simulation studies carried out in collaboration with Robert Ezzell, Keith Oslakovic, and Ryota Matsuura (Molecular Geodesics, Inc.; Cambridge, MA) reveal how the pull of actomyosin filaments can induce buckling or bending within interconnected CSK struts that bear some of the compressive loads (**Fig. 6**). In fact, local compressive buckling of microtubules has been visualized directly within the CSK of living cells through the use of microtubules labeled with green fluorescent protein (45). Microtubule curvature (buckling) also has been shown to decrease when cells are treated with inhibitors of actomyosin-based tension generation (46). In addition, mechanical measurements in individual cultured cardiac myocytes confirm that increasing the total mass of polymerized microtubules compromises the contractility of these cells by physically resisting compressive (inward-directed) forces in the CSK lattice, even though Z-band movement

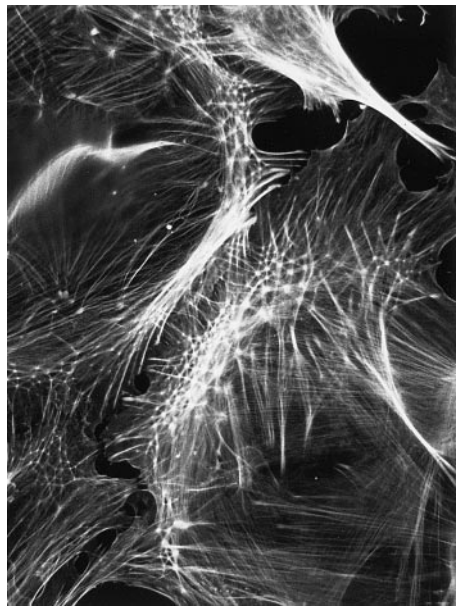

Figure 5. The filamentous actin cytoskeleton of a cultured fibroblast visualized by rhodaminated-phalloidin staining. A partial arc-like region of a molecular geodesic dome is observed within the cytoskeleton of the center cell; note that the filopodia appear as short linear spikes that initiate only at the vertices of this triangulated lattice. (This image was kindly provided by Alan Hall and Kate Nobe, University College London).

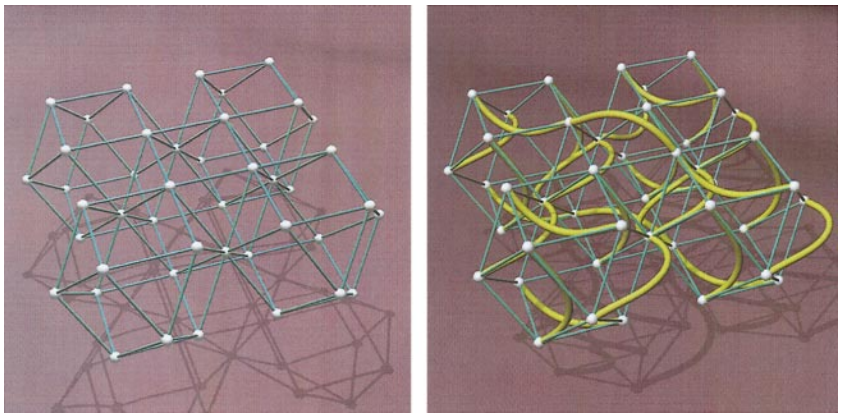

Figure 6. Tensegrity-based integration in the cytoskeleton. *Left*) a computer-generated image of a model cytoskeletal network composed of multiple polyhedral modules, with each strut representing a single actomyosin filament. This network can convert between polygonal, linear bundle, and geodesic dome forms (31, 33), however, it is unstable in the form shown here. *Right*) when stiffer struts that can bear higher compressive loads are placed across the diagonals of the square faces of the polygonal network, each individual module takes on the form of the tensegrity structure shown in Figure 3 and the entire lattice self-stabilizes. Note that the model suggests that the contraction of the actomyosin filaments would cause buckling in the interconnected microtubules (right). Thin lines, actomyosin filaments; thick yellow struts, microtubules.

(actomyosin-based tension generation) proceeds normally under these conditions (47).

Taken together, these data provide direct support for the conjecture that microtubules bear some of the compressive loads in a cellular tensegrity system (24, 48). However, microtubules are not the only compression-bearing elements in the CSK. For example, highly stiffened, cross-linked bundles of actin that form the cores of filopodia (49) also bear compression caused by the centripetal pull of the surrounding cell cortex and surface membrane in migrating cells (33). Based on computer simulations, it is possible that rapidly polymerizing (elongating) actin filaments in the leading edge may help to mechanically stabilize local regions within the lamellipodium by pushing out against the surrounding microfilament lattice and thereby pre-stressing the network without active contraction.

CELLULAR TENSEGRITY AND MECHANOTRANSDUCTION

Tensegrity does more than predict pattern formation. It also helps to explain how cells sense and respond to external mechanical signals. For example, if one were to pull on a single wood beam in the frame of a house, local bending and breakage would result. In contrast, when a single element in a pre-stressed tensegrity structure is stressed, all of the interconnected elements undergo global structural rearrangements, even at a great distance (**Fig. 7**). This results in a linear stiffening response: the mechanical stiffness of these structures increase in direct proportion as the level of applied stress is raised (**Fig. 8*A***). To our amazement, in 1993 we found that living cells exhibit this precise behavior (19).

Ning Wang in my group developed a method to measure CSK stiffness in living cells by allowing cells to bind to small (5 μm diameter) ferromagnetic spheres coated with ECM ligands, applying controlled twisting forces (shear stresses) to the bound surface receptors through the beads, and simultaneously quantitating the degree of bead rotation (angular strain; **Fig. 9**) (19, 36–39, 47, 50–52). First, he observed that twisting on bound metabolic receptors (acetylated low-density lipoprotein receptor) (19) or histocompatibility antigens (52) was met by very little resistance, even though they spanned the plasma membrane. In contrast, twisting bound integrin receptors (which form FAs that connect to the actin CSK) resulted in a proportional increase in cellular stiffness (Fig. 8*B*). In other words, living cells behaved as if they were tensegrity structures. The importance of this observation may be even more

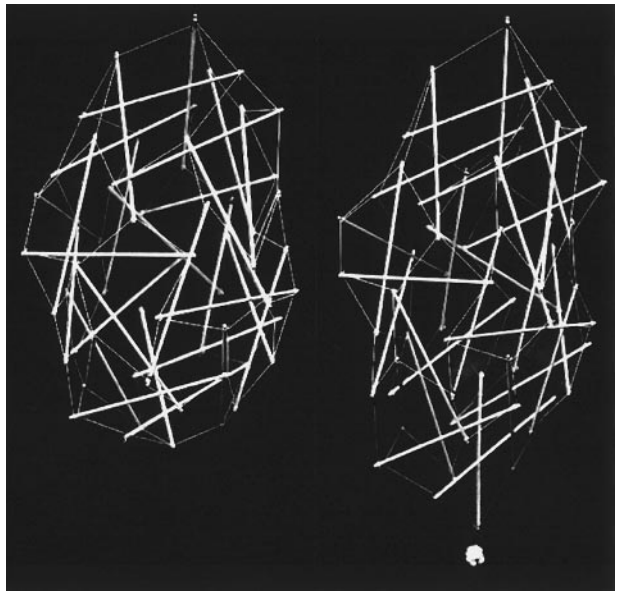

Figure 7. Tensegrity provides action at a distance. Application of a local stress to the bottom element of a stick and elastic string tensegrity structure (left) results in long-distance stress transfer and global structural rearrangements throughout the entire model (right). This response is accompanied by linear stiffening behavior (Fig. 8*A*).

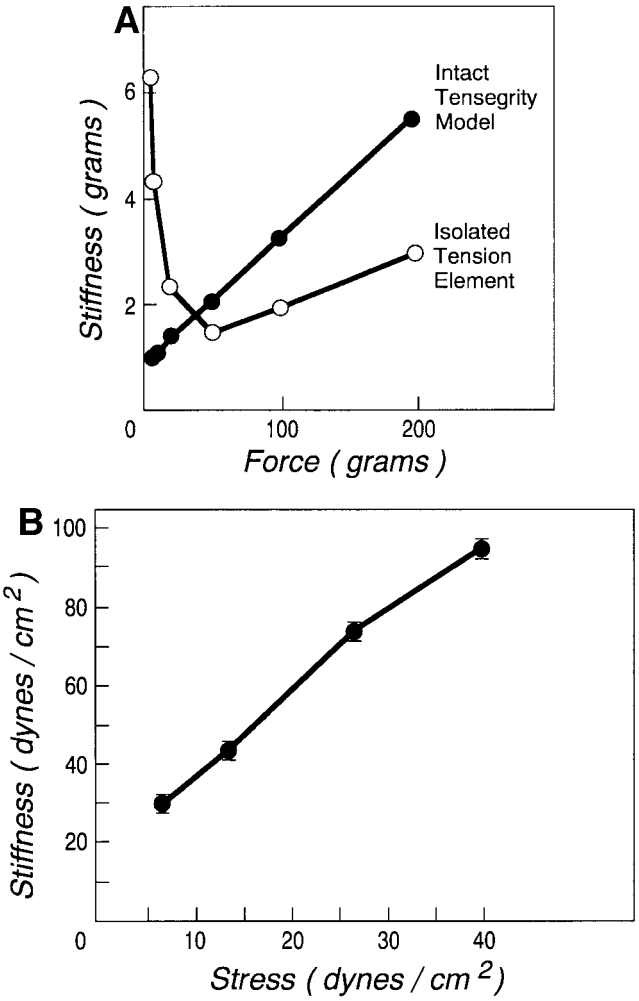

Figure 8. Linear stiffening behavior in a tensegrity model (*A*) and living CE cells (*B*). The stiffness of both structures increases in direct proportion as the level of the applied stress is raised (methods by which these data were obtained can be found in ref. 19). Note that an isolated tension element from the tensegrity model does not exhibit this behavior (*A*).

significant than seen at first glance. For example, bioengineers have known for many years that most normal tissues also exhibit linear stiffening behavior, however, this response could never be explained starting from first mathematical principles. Working in collaboration with Dimitrije Stamenovic, we now can explain this fundamental property of living cells and tissues mathematically, using tensegrity as a mechanistic basis (53, 54).

The major point here is that if cells behave as tensegrity structures, then changes in CSK and nuclear structure may be driven by altering the balance of forces transmitted across the cell surface. However, the model predicts and our data confirm that mechanical stresses are not transmitted equally across all points on the surface membrane. Rather, mechanical signals appear to be transferred into the cells specifically at sites where the cell mechanically connects its internal scaffolds to its external supporting structures, that is, within specialized adhesion complexes, including FAs and cell-cell junctions. Experimental studies have confirmed that integrins and other adhesion molecules (e.g., cadherins, selectins) provide preferred paths for mechanical signal transfer across the cell surface (19, 36–39, 47, 50–52), as suggested by the tensegrity model (2, 24). The finding that the FA mediates mechanical signal transfer is especially interesting given that the FA represents a major site for signal processing inside the cell. By focusing diverse stimuli on a common site, mechanical signals may be simultaneously integrated with signals from ECM and growth factors to produce a concerted response (**Fig. 10**). However, the overall response to stress may still be modulated by altering the pre-stress in the CSK-ECM lattice.

HARD-WIRING IN THE CYTOSKELETON

Even with all of these results, many people still have a problem with the tensegrity model. They prefer to view the cell as a viscoelastic fluid surrounded by an elastic membrane. If cells are hard-wired as suggested by the tensegrity model, then pulling rapidly on integrin receptors on the cell surface would be expected to induce structural changes in the nucleus in the center of the cell. In contrast, if the cytoplasm was filled with a viscous fluid, these stresses would rapidly dissipate in the cytosol. Indeed, Andrew Maniotis in my lab demonstrated hard-wiring in living cells by pulling on cell surface integrins with ECM-coated micropipettes and inducing nucleolar realignment within the center of the nucleus, many micrometers away from the site of stress application (20). He and Krzysztof Bojanowski also demonstrated mechanical continuity within the nucleus, including physical connections between all of the chromosomes in the human genome (55). It is interesting to note that DNA appeared to be responsible for this mechanical connectedness.

Finally, other groups have shown that the mitotic spindle is itself a tensegrity structure (56). By rapidly disrupting one microtubule within the mitotic spindle using laser ablation, they were able to demonstrate immediate buckling in the remaining spindle microtubules. These results suggest that the microtubules act as compression struts that push out and pre-stress the surrounding chromatin network and associated nuclear matrix, thereby creating a tensegrity force balance that stabilizes the spindle. This is a beautiful example of the use of hierarchical organization in cellular tensegrity (31, 33). Both the spindle and the whole cell independently exhibit properties of tensegrity. Yet, at the same time, the spindle remains tensionally coupled to integrins on the

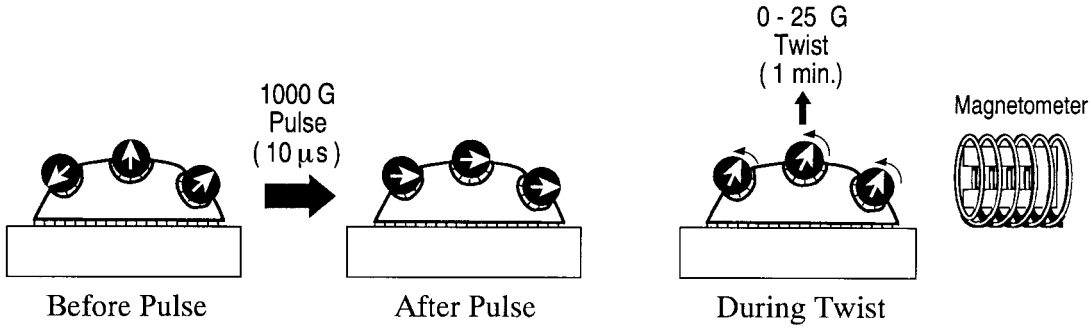

Figure 9. A schematic diagram demonstrating how cytoskeletal mechanics can be measured non-invasively in living cells through the use of ligand-coated magnetic beads in combination with a magnetic twisting device. Ferromagnetic microbeads (5-μm diameter) were allowed to bind to cell surfaces for 10 min and unbound beads were removed before magnetic manipulation was initiated. Brief application of a strong external magnetic field (1000 Gauss for 10 μs) resulted in magnetization and alignment of the magnetic moments of all surface-bound beads. Defined mechanical stresses (0–68 dynes/cm^2) were then applied without remagnetizing the beads using a weaker "twisting" magnetic field (0–25 Gauss) applied perpendicular to the original field. The average bead rotation (angular strain) induced by the twisting field was quantitated using a magnetometer. In the absence of force transmission across the cell surface, the spherical beads would twist in place by 90° into complete alignment with the twisting field. In contrast, transmission of force to the CSK would result in increased resistance to deformation. Thus, we can measure changes in cytoskeletal mechanics in living cells by measuring changes in the rate and degree of bead rotation (See refs. 19 and 50 for more details).

surface of the mitotic cell (20). Hence, both the smaller and larger system also function as one single, integrated tensegrity network.

It is important to note that the term, hard-wiring, is used here in the context of cell and nuclear structure to indicate mechanical connectedness at any point in time when a force is applied to the cell surface. The cell and CSK are also highly dynamic; individual molecules and connectors may come and go. However, tensegrity defines how and where these forces are transmitted and thus it helps to establish and maintain characteristic molecular patterns. The hierarchical nature of cellular tensegrity systems also may permit entire subunits to be removed and replaced (e.g., integrins in FAs, CSK filaments in the cytoplasm, cells in whole tissues) without compromising structure or function at a larger scale.

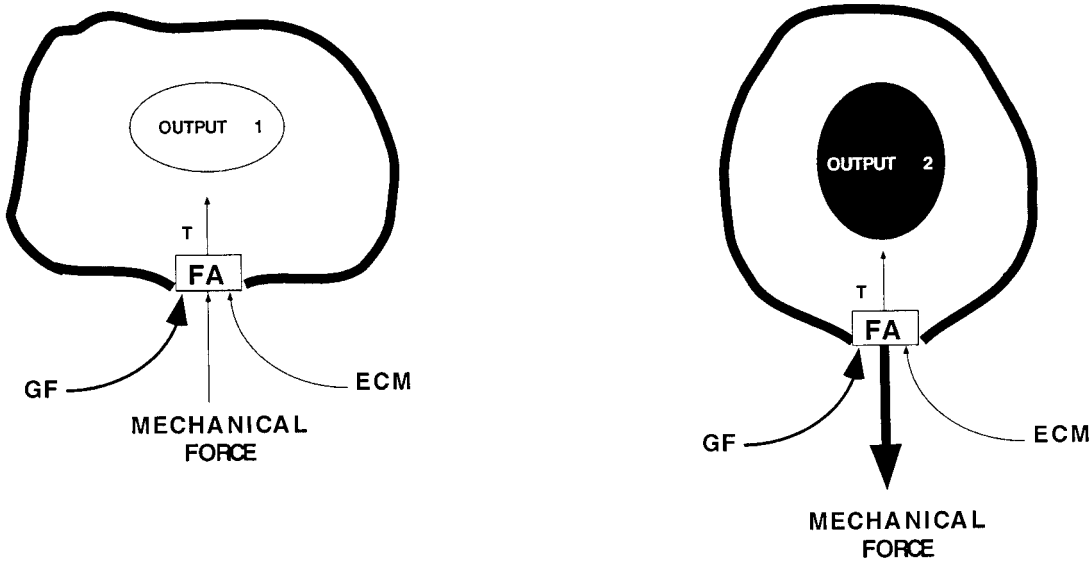

Figure 10. A schematic diagram depicting signal integration in cellular adhesion sites. Signals from growth factors, ECM, and mechanical stresses all converge on common sites where cells physically anchor to extracellular matrix (focal adhesion) and to other cells (not shown). Because living cells always generate active tension in their cytoskeleton, an exogenous mechanical stress is imposed on a preexisting force balance. By altering the balance of forces transmitted across the adhesion site, the signaling machinery that is oriented on the cytoskeletal backbone of the adhesion complex can be altered, thereby producing different functional outputs, even though growth factor and ECM signals remain constant. GF, growth factor signals; ECM, signals from extracellular matrix; FA, focal adhesion complex; T, cytoskeletal tension.

CELLULAR CONTROL LIES IN THE BALANCE OF FORCES

In summary, the importance of these results and of the tensegrity model is that they suggest that cellular control lies in the balance of forces that are transmitted across cell surface adhesion receptors, through the CSK, and into the nucleus (63). In this sense, we can think of integrins and other transmembrane adhesion receptors as mechanoreceptors because they are among the first cell surface molecules to sense externally applied mechanical stresses and because they transmit these signals across the surface over a specific molecular pathway. Integrins within FAs, and both cadherins and selectins within their own respective adhesion complexes (12, 52, 57), may be viewed as part of a larger mechanochemical transduction machinery or signaling organelle because they provide a way to interconvert mechanical signals into a chemical response at the site of force application to the cell surface (2, 3, 12). For example, our studies with CE cells revealed that while occupancy and clustering of integrin receptors is alone sufficient to activate intracellular signaling cascades and gene expression (13–16, 57, 58), it is not sufficient for CE cell survival. These cells require integrin signaling and a slight degree of mechanical distortion (change in forces balanced across the FA) to survive. If the spreading is not extensive, these cells enter what is essentially a default differentiation cascade. To further shift cells from differentiation to growth, the balance of forces transmitted across integrins, through the FA, and to the CSK once again must be altered. In other words, the same inputs produce different functional outputs depending on changes in the level of forces transmitted across the FA (Fig. 10).

Transmission of force across the cell surface and throughout a tensionally integrated lattice would simultaneously result in CSK rearrangements at multiple locations inside the cell and nucleus as well as subsequent changes in molecular mechanics. In fact, chemically or genetically deleting the FA protein, vinculin, disrupts mechanical force transfer through the FA, interferes with these CSK rearrangements, and prevents complex cell behaviors, such as movement, even though local integrin binding and cell adhesion are unchanged (21, 59). All of these normal behaviors return when normal vinculin levels are restored through transfection.

If biochemistry does function in a solid state, then changing molecular architecture and mechanics within the FA, CSK, or interconnected nuclear scaffolds could have major effects of cellular biochemistry due to local alterations in thermodynamic or kinetic parameters (3). For example, if one were to rapidly pull on a molecular filament or scaffold without breaking the structure, then some or all of the molecular constituents that constitute that structure must undergo some degree of local distortion. Changing the shape of a molecule alters its kinetic and thermodynamic behavior and hence, changes biochemistry (3, 60).

Is this true? Can mechanics control biochemistry in living cells? The answer is clearly yes. A simple example is the observation that the actin bundles that form the cores of the filopodia that lead cell migration are not formed randomly. Rather, they preferentially polymerize specifically from the vertices of actin geodesic domes or triangulated nets that form in more central regions of the cell (43, 44). In other words, while increasing levels of free actin monomer or cleaving actin filaments may chemically promote new actin polymerization, it is the tension-dependent restructuring of the CSK that dictates where these new bundles form and how they integrate with the rest of the cell. This is a clear example of how cell mechanics and structure can locally impinge on a biochemical reaction as well as on cell polarity.

Another example comes from the work of Steve Heidemann and Robert Buxbaum. They found that tensegrity-based transfer of mechanical loads between contractile actomyosin filaments, microtubule struts, and ECM tethers can explain how neurites extend their cell processes (48). Transferring a compressive load off the end of the microtubule and onto a newly formed ECM adhesion results in a change in the critical concentration of tubulin in the cell. If the levels of free tubulin remain constant, this would lead to the addition of tubulin monomers onto the end of the microtubule and hence, extension of the neurite process. Experimental studies confirm that neurites are pre-stressed structures and that neurite extension can be controlled by altering this type of cellular tensegrity force balance (61). A similar tensegrity mechanism appears to act in epithelial cells, however, alterations in this force balance manifest themselves through changes in tubulin protein half-life, rather than changes in total microtubule mass (62).

A third example comes from recent work by Marina Chicurel in my laboratory. Working in collaboration with Robert Singer (Albert Einstein Medical Center), we showed that altering the balance of mechanical forces transmitted across cell surface integrins can induce formation of a microcompartment specialized for protein synthesis in the region of the FA (63). We had previously shown that CE cells recruit many signaling molecules involved in growth control (e.g., Na^+/H^+ antiporter, FGF receptor) to the FA that forms at the site of integrin binding when they are allowed to bind to small ECM-coated microbeads (13). Because many of these molecules are also involved in control of

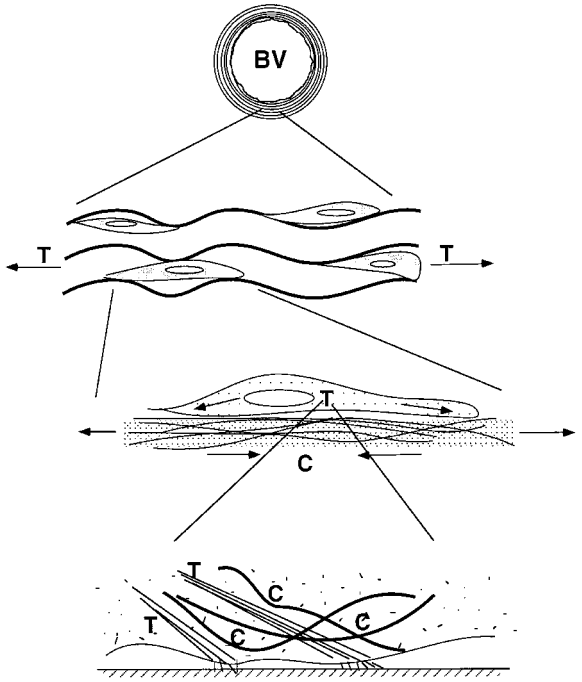

Figure 11. Hierarchical integration in tissues permits gravitation forces that act at the whole organ or tissue level to be sensed at the cellular and molecular levels. This integration is made possible through use of tensegrity architecture (3, 31). These diagrammatic views show the tensegrity structural hierarchy of a large blood vessel (BV) at progressively smaller size scales (from top to bottom). A pre-stressed balance of continuous tension (T) and local compression (C) elements stabilize the physical structure of this living material at all size scales. Contractile cells and local regions of tensionally-stiffened ECM (middle two views), and contractile microfilaments and microtubules (bottom) act as balanced tension and compression elements, respectively. Note that an element placed under tension (and stiffened) at one size scale (e.g., long collagen bundle) can act to resist local compression on a smaller size scale (e.g., between adjacent focal adhesions). A blood vessel is used only as a representative example; similar integration occurs in all tissues.

protein synthesis, we carried out high-resolution *in situ* hybridization with oligonucleotide probes for poly A^+ mRNA and ribosomes. We observed integrin-dependent recruitment of both probes to the bead-associated FA within minutes after bead binding. This recruitment could be inhibited by chemically interfering with actomyosin-based tension generation and enhanced in a stress-dependent manner by magnetically twisting the magnetic beads bound to surface integrin receptors but not to other transmembrane molecules (e.g., HLA antigen) (63). In other words, formation of this microcompartment was controlled specifically by transmitting stresses across the FA, thereby altering the CSK force balance. More recent studies show that cellular genes in the cAMP-dependent signaling pathway can be regulated in a similar stress- and integrin-dependent manner (C. Meyer and D. Ingber; unpublished results). Other studies similarly demonstrate that application of mechanical forces to integrins preferentially activates intracellular signaling mechanisms (reviewed in ref. 4). In the case of the neuromuscular synapse, transfer of stress across integrins results in an almost immediate (< 10 ms) release of calcium inside the cell; the effect is so rapid that it likely results from direct distortion of integrins and associated calcium channels at or very near the site of integrin binding (64).

IMPLICATIONS FOR GRAVITY SENSATION

What does this mean for how gravity influences cell and tissue development? First of all, local distortion in the CSK appears to be common to all mechanisms of cellular mechanotransduction. Certain specialized mechanosensory cells utilize highly dense organelles or microstructures (e.g., otoliths, stereocilia) to induce a localized stress differential and associated mechanical strain in the CSK in order to experience gravitational acceleration. Our experiments suggest that other nonspecialized cells may similarly feel the pull of gravity as a result of CSK distortion. However, this distortion may result indirectly through changes in the mechanics or flexibility of the cell's adhesions to ECM or to neighboring cells. These changes would be caused by large-scale forces acting at the whole tissue or organ level (e.g., bending of the femur, altered tone in muscles, posture-dependent effects on the vasculature and lung) that, in turn, alter the structure and form of their internal cellular and molecular components.

Taken together, these results suggest that we should discard the concept of a single gravity-specific receptor molecule and the idea that mechanical signals are transmitted equally at all points on the cell surface. Instead, we must try to understand gravity sensation in the context of the structural complexity of living cells and tissues. From this perspective, it is likely that in some cases the whole cell or even the whole tissue must be viewed as "the" gravity sensor. The importance of tensegrity is that it provides a way to focus mechanical energy on specific molecular components and to integrate part and whole, thereby tuning the entire cellular (and tissue) response (3, 31, 32). This tuning function may involve local changes in cellular rearrangements, cell shape, molecular architecture, chemical linkages, or modulation of internal pre-stress. Gravity acting on the whole organism is in fact a major contributor to pre-stress within individual tissues. When organisms are placed in microgravity, they experience an acute decrease in pre-stress on the macroscale which, due to the hierarchical organization of living tissues (31), should trickle down to produce corresponding changes in structure and

mechanics at the cellular and molecular level (**Fig. 11**). Clearly, other factors, including cosmic radiation, changes in fluid convection, vibration, and cabin conditions (e.g., constant temperature, artificial light, etc.) also could contribute to the effects of microgravity during spaceflight. However, future studies on gravity sensation should incorporate tensegrity-based considerations and strive to understand the minimal size of cellular aggregates necessary to sense gravity as well as the importance of tissue mechanics and CSK tone for the gravitropic response. FJ

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