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TENSEGRITY: THE ARCHITECTURAL BASIS OF CELLULAR MECHANOTRANSDUCTION

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ABSTRACT

Physical forces of gravity, hemodynamic stresses, and movement play a critical role in tissue development. Yet, little is known about how cells convert these mechanical signals into a chemical response. This review attempts to place the potential molecular mediators of mechanotransduction (e.g. stretch-sensitive ion channels, signaling molecules, cytoskeleton, integrins) within the context of the structural complexity of living cells. The model presented relies on recent experimental findings, which suggests that cells use tensegrity architecture for their organization. Tensegrity predicts that cells are hard-wired to respond immediately to mechanical stresses transmitted over cell surface receptors that physically couple the cytoskeleton to extracellular matrix (e.g. integrins) or to other cells (cadherins, selectins, CAMs). Many signal transducing molecules that are activated by cell binding to growth factors and extracellular matrix associate with cytoskeletal scaffolds within focal adhesion complexes. Mechanical signals, therefore, may be integrated with other environmental signals and transduced into a biochemical response through force-dependent changes in scaffold geometry or molecular mechanics. Tensegrity also provides a mechanism to focus mechanical energy on molecular transducers and to orchestrate and tune the cellular response.

INTRODUCTION

The question of how organisms sense mechanical signals and transduce them into biological responses has always intrigued biologists. At the beginning of this century, many scientists speculated that mechanical stresses played a

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key role in the determination of tissue growth and form. More recent studies confirm that physical forces, including gravity, tension, compression, pressure, and shear, influence growth and remodeling in all living tissues and show that these effects are exerted at the cell level. Cell growth, differentiation, secretion, movement, signal transduction, and gene expression all can be altered by applying mechanical stresses directly to cultured cells. Yet, we still do not fully understand how individual cells perceive physical forces nor how they choreograph the molecular performance that results in mechanotransduction: the conversion of a physical signal into a biological or chemical response.

Although studies that analyze the effects of mechanical forces on cells commonly assume that other stimuli are constant, living cells likely receive many simultaneous inputs. For example, at the same time an endothelial cell is exposed to a change in fluid shear stress, it also may bind to a different growth factor or adhere to another extracellular matrix (ECM) molecule. Growth factors and ECM independently activate the same intracellular signaling pathways that mechanical stresses have been reported to influence through binding to their own cell surface receptors. Furthermore, many of the molecules that transduce these signals (e.g. ion channels, protein kinases, lipid kinases) are physically immobilized on the insoluble cytoskeleton (CSK), the major structural framework of the cell, which is itself highly sensitive to mechanical deformation. Nevertheless, living cells are able to simultaneously sense all these signals and yet produce only one concerted response: They grow, or differentiate, or die locally (32, 33). Thus to fully understand the mechanism of mechanotransduction and its relevance for regulation of tissue development and remodeling, we must explain how all these signals are integrated inside the cell.

The point of this chapter is not to provide an extensive review of recent experimental advances in the field of cellular mechanotransduction nor to enumerate the different signaling mechanisms that become activated when living cells are mechanically stressed. Excellent reviews of this type can be found in the recent literature (8, 13, 16, 27) and in the accompanying chapters in this volume. Instead, I hope to present a theoretical framework that places all the potential molecular mediators of mechanotransduction (e.g. ECM, stretch-sensitive ion channels, signaling molecules, CSK filaments) within the context of the structural complexity that exists in living cells and tissues.

In this chapter, I review work suggesting that the structural organization and interconnectedness of the CSK provides a physical basis for translating mechanical forces into a biochemical response, as well as a mechanism for integrating these signals with those generated by growth factors and ECM. This concept emerged from studies with cell models built from sticks and string using tensegrity architecture (28, 31, 34, 74). Tensegrity models predict that

living cells and nuclei may be hard-wired to respond immediately to mechanical stresses transmitted over cell surface receptors that physically couple the CSK to ECM or to other cells. Tensegrity also offers a mechanism to explain how the CSK remodels in response to stress and, hence, how signaling molecules that are immobilized on this insoluble scaffold might change their distribution and function when force is applied to the cell surface. Results of studies with living cells that provide direct support for the tensegrity hypothesis are reviewed. Finally, I describe potential molecular mechanisms that cells may utilize to convert mechanical energy into chemical energy at the molecular level, as well as how tensegrity may be used to tune this transduction response.

THE TENSEGRITY MODEL AND MECHANOTRANSDUCTION

The precise molecular mechanism of mechanotransduction remains unclear; however, there are a few clues. For example, cell surface ion channels have been identified that become activated or inactivated when the cell membrane is mechanically tensed in many cells. However, G protein activation, release of chemical second messengers (e.g. arachidonic acid, cyclic AMP, inositol trisphosphate, calcium), protein phosphorylation, secretion of growth factors, CSK alterations, remodeling of cell-ECM adhesions, and changes in gene expression also occur within seconds to minutes following mechanical perturbation (3, 9, 24, 43, 46, 59, 61–63, 73). In fact, activation of mechanosensitive ion channels is often not required for many of these effects (46, 62). Furthermore, the same mechanical stimulus may produce a different response depending on the presence of soluble hormones or the type of ECM substrate on which the cell adheres (63, 79). Thus it has been extremely difficult to dissect out cause and effect when analyzing the molecular basis of mechanotransduction.

In *C. elegans*, a genetic approach was used to map the mechanical signaling cascade that begins with a physical touch and ends with a change in movement. This work led to identification of multiple genes that encode the molecules required for mechanotransduction. Some of these genes encode new ion channels that may be stress sensitive; however, others encode ECM molecules and CSK proteins (12, 26). These elegant studies hit home the fact that molecules present in all three cellular domains—extracellular, membrane, and cytoplasmic—play critical roles in the mechanotransduction response. Biochemical analysis of the gravity-sensing mechanism in plants reveals a similar paradigm (11, 78). Thus we must place our current knowledge about molecular transducers within the structural framework that exists in living cells and tissues.

Initial Assumptions

When my laboratory first approached this problem, we started with a few basic assumptions. The first was that to understand how individual cells sense and respond to forces, we must first map the molecular path by which mechanical signals are transferred across the cell surface. As in any architectural system, mechanical loads should be transferred across points where the structure is physically anchored to its underlying support foundation. Living cells are anchored to insoluble ECM scaffolds (e.g. basement membranes, interstitial matrix) that join together cells and mechanically stabilize all living tissues. They also interconnect with neighboring cells. We therefore assumed that mechanical stresses must be transferred to adherent cells through their adhesive contacts with surrounding ECM or through their junctions with neighboring cells (27, 34).

Our second assumption was that we could not answer the question of how cells sense and respond to mechanical forces if we viewed the cell as a viscous protoplasm surrounded by a membrane. Instead, we had to take into account that all cells contain an internal filamentous framework or CSK that stabilizes cell shape. Furthermore, the CSK is not a passive structure: All living cells generate active tension within their internal CSK via an actomyosin filament sliding mechanism similar to that used in muscle. This is not obvious in cells cultured on rigid plastic dishes; however, it is easily visualized by placing the same cells on malleable substrata such as silicone rubber, which they mechanically contract and pull up into folds (22). Isometric tension also can be measured in nonmuscle cells (e.g. endothelial cells, fibroblasts) cultured on flexible collagen gels (42), and release of these gels results in spontaneous contraction (24). These results indicate that all living cells have an internal prestress (pre-equilibrated stress) analogous to the stress within a tensed bow or catapult that is harnessed to send a projectile flying through space. This CSK prestress also corresponds to the basal tone that can be measured in resting muscle cells before being chemically stimulated to contract.

This conceptual approach led to a few observations that have important implications for cellular mechanotransduction. The first was that CSK tension is the major force acting on living cells, and thus all external mechanical loads are imposed on a pre-existing force balance. Therefore, the cellular response to stress is more like that of a violin string to tuning than a conventional stimulus-response coupling in which the signal (e.g. growth factor) is absent before it is added externally. This suggested that we must change the way we think about mechanosensation.

The second point was that forces may not be transmitted continuously across the entire cell surface, as assumed by conventional engineering models of cells that rely on continuum mechanics theory (17). This is because the membrane

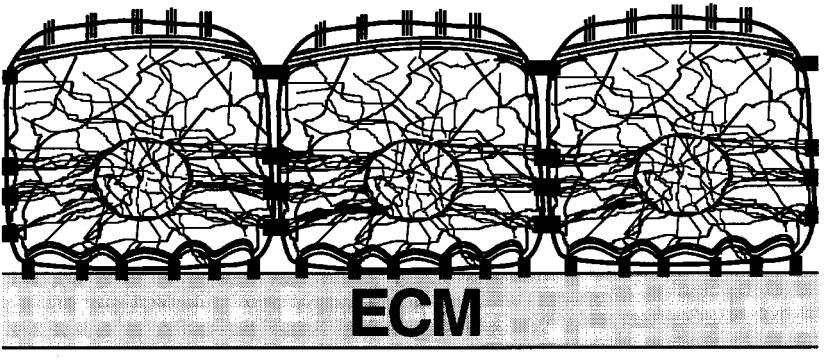

Figure 1 The intracellular cytoskeleton interconnects with the underlying extracellular matrix and neighboring cells through focal adhesion complexes at the cell base and specialized junctional complexes at the lateral cell borders, respectively. Because of the presence of this molecular continuum, distant molecules in the ECM, cytoplasm, and nucleus may be mechanically coupled.

is not evenly glued to the ECM or to neighboring cells. Rather, a cell anchors to underlying ECM and surrounding cells by physically coupling its tensed CSK filaments to specific receptors that cluster within localized adhesion sites (Figure 1). These molecular spot welds are called focal adhesion complexes (FACs) when they mediate cell-ECM adhesion at the cell base (Figure 2) and junctional complexes (e.g. adherens junctions, desmosomes) when they mediate cell-cell adhesion along the lateral cell borders (18).

FACs contain clusters of transmembrane ECM receptors called integrins (1). Integrins are heterodimeric proteins comprised of different α and β subunits. There are more than 20 different types of each chain (e.g. $\alpha 2$, $\alpha 5$, αV, $\beta 1$, $\beta 2$, $\beta 3$, etc); the specific combination of the different subunits defines the molecular binding specificity (e.g. integrin $\alpha 5\beta 1$ binds fibronectin, whereas $\alpha 2\beta 1$ binds collagen). In addition, the FACs contain multiple actin-binding proteins (e.g. talin, vinculin, α-actinin, paxillin) that also interact with the cytoplasmic tail of the integrin and thereby form a molecular bridge that stretches continuously from ECM to the internal CSK (18). Intermediate filaments also may insert on these adhesion complexes in certain cells (5).

Specialized cell-cell adhesion molecules (e.g. cadherins, selectins, CAMs) use some of the same actin-associated proteins (e.g. vinculin, α-actinin) to physically couple to the actin CSK, but not others (e.g. not talin) (18, 80). Specialized CSK linker proteins, known as catenins, also interconnect cadherins to the actin CSK within adherens junctions at the lateral cell borders (19). Desmosomes represent sites in which the intermediate filament systems of

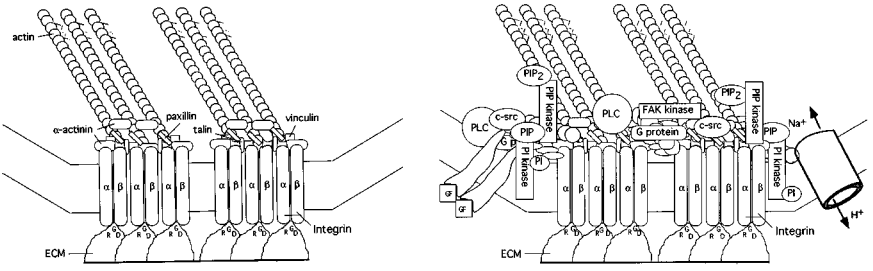

Figure 2 The cytoskeletal framework of the focal adhesion complex (*left*) comprised of clustered integrins and actin-associated molecules (e.g. vinculin, talin, paxillin, α-actinin) physically interconnects the extracellular matrix to intracellular actin microfilaments (ends of stress fibers). These structural interconnections represent a preferred molecular pathway for transmembrane mechanical signal transfer. Many signal transducing molecules that mediate the cell's response to growth factors and ECM binding function when immobilized on the same molecular framework (*right*). Thus the focal adhesion complex may represent a major site for integration of chemical and mechanical signals.

neighboring cells are mechanically coupled; however, the molecular basis of transmembrane coupling is not as well understood.

Based on the above assumptions, we expected that both cell-generated stresses and external mechanical forces should converge on these localized adhesion sites. Thus we suggested that transmembrane receptors that physically couple internal CSK networks to external support scaffolds provided specific molecular pathways for mechanical signal transfer across the cell surface (27, 34). In other words, ECM receptors and cell-cell adhesion molecules could act as mechanoreceptors.

Cellular Tensegrity

There was one final assumption: Cells are physically built to respond immediately to mechanical stress. The corollary is that understanding how cells stabilize their structure and shape may help to explain how cells sense and respond to mechanical signals. At the time we initiated our studies on cell structure, it was assumed that cell shape was controlled by either membrane surface tension, osmotic forces, CSK viscosity, or molecular polymerization. In contrast, we based our model of cell organization on the possibility that cells stabilize their extended forms by incorporating compression-resistant elements, either internal molecular struts or localized regions of the underlying ECM, to resist the otherwise global pull of the contractile CSK (34).

There is a known building system that self-stabilizes through use of isolated compression struts, which place the surrounding structural network under

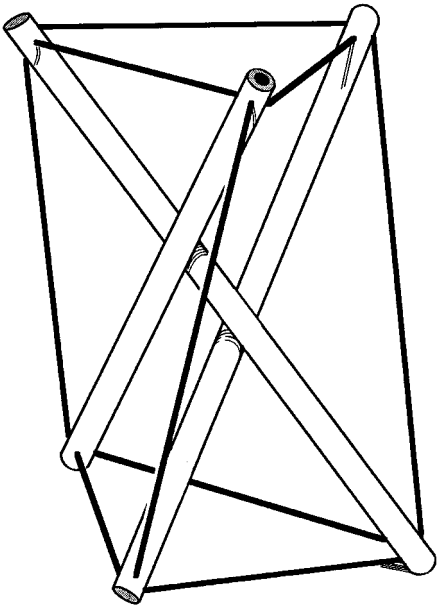

Figure 3 Tensegrity structures contain a series of isolated compression-resistant elements (*white struts*) that resist the pull of surrounding tensile elements (*black cables*) and impose a prestress that stabilizes the entire network. These structures may contain different size, shape, and number of building elements, and they may be organized hierarchically. Thus they can exhibit a wide range of forms that differ from this simple conceptual depiction.

tension and thereby create an internal prestress (Figure 3). This form of architecture is known as tensegrity because of its dependence on tensional integrity (30). In fact, this is the way our bodies are constructed: Our bones are held up against the force of gravity and mechanically stabilized through their tensile linkages with surrounding muscles and tendons. Even insects, which have an exoskeleton, and plants, which impose a global prestress through generation of local turgor pressures within individual cells, use a similar mechanism of structural stabilization.

Analysis of tensegrity cell models constructed by interconnecting multiple wood dowels with a continuous series of elastic strings revealed some interesting behavior (28, 31, 34). These cell models appeared round when unattached because of their internal tension; however, they spread out and flattened when they were attached to a rigid foundation. When the rigid foundation was then made flexible, the same model spontaneously contracted the substrate and returned to a round configuration, again because of the presence of internal

tension. In other words, this simple stick and string tensegrity model mimicked the behavior of living cells cultured on malleable substrates.

These studies suggested that cell spreading may result from a transfer of CSK stresses onto the surrounding ECM and a concomitant shift in internal force distributions within the CSK, rather than from a net increase in the amount or length of CSK filaments. Mooney, working in my laboratory, recently approached this question directly by simultaneously measuring cell spreading kinetics and changes in microtubule and actin microfilament mass in liver epithelial cells (hepatocytes) plated on different ECM substrata (54). These studies demonstrated that spreading and flattening of the entire cell body is not driven directly by net polymerization of either microfilaments or microtubules. Instead, ECM proteins promote cell spreading by resisting cell tension and thereby promoting structural rearrangements within the CSK. These studies also provided evidence to suggest that microtubules do act as internal support struts (i.e. local compression elements) that resist the pull of the surrounding contractile actin CSK in these cells, as previously demonstrated in many other cell types (reviewed in 28). Stiffened (cross-linked) bundles of actin filaments also bear local compression when they push out against the surrounding tensed CSK and interconnected cortical membrane to form thin finger-like projections called filopodia in migrating cells (66). Thus living cells appear to use tensegrity to stabilize their form.

HIERARCHICAL FEATURES Tissues are organized as a structural hierarchy; they are composed of groupings of individual cells that, in turn, contain specific arrangements of smaller organelles exhibiting their own mechanical stability. For example, the nucleus contains its own internal structural framework or nuclear matrix (56) and, in fact, nuclei can be physically transplanted from one cell to another without loss of function. Yet, when a cell spreads on a culture dish, its nucleus also extends in parallel (35).

Importantly, tensegrity can provide a mechanical basis for this hierarchical behavior. This can be visualized by constructing a model in which tensile threads are stretched from the surface of a large tensegrity structure to a smaller tensegrity sphere placed at its center. When this round model attaches and spreads on a rigid substrate, the cell and nucleus extend in a coordinated manner (Figure 4). The nucleus also polarizes basally because stress concentrates at the cell base. In vitro experiments confirm that living cells and nuclei spread and polarize in a similar manner when they adhere to ECM (36).

The tensegrity structures used here are conceptual models that represent a mechanism of form stabilization that should be independent of scale and thus may apply equally well at the organ, tissue, cell, organelle, or molecular levels (30). Furthermore, all the interconnected molecular elements in these hierarchical assemblies feel the same pull (albeit to varying degrees) and respond in

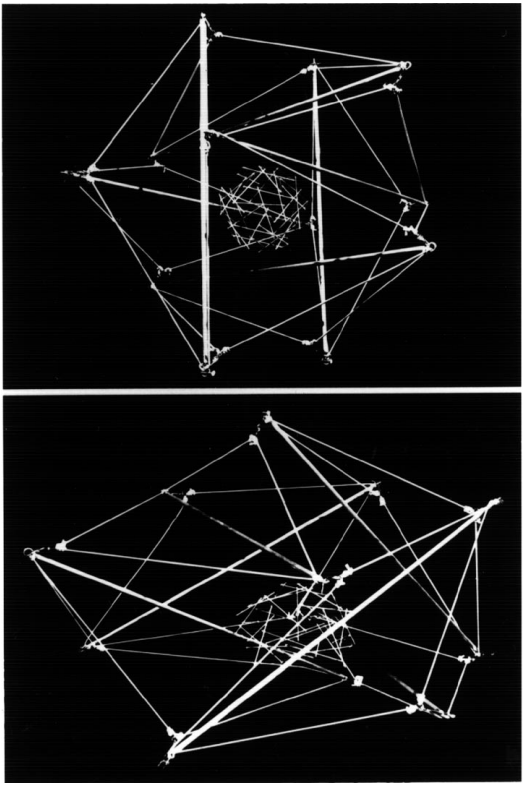

Figure 4 A two-tier hierarchical tensegrity model that mimics the behavior of nucleated cells. The larger model is constructed from aluminum struts and elastic cord. The smaller geodesic sphere at its center is composed of wooden sticks and thin white elastic thread. The two independently stable structures spread in a coordinated manner (bottom view vs top) and function as a single mechanically integrated unit because they are interconnected by thin black elastic threads that cannot be seen due to the black background.

an integrated manner, as visualized in the two-tier, nucleated tensegrity model (Figure 4). The flexibility and interconnectedness of this type of network could explain why large-scale movements (e.g. walking, running, sitting) can mechanically deform tissues, ECM, CSK, and nuclei without causing breaks or tears. More importantly in the present context, these modeling studies imply that living cells and nuclei might be hard-wired to respond directly to mechanical forces that are applied to specific cell surface receptors and, in particular, receptors that mediate cell adhesion. Thus we set out to test this hypothesis and to explore whether cells respond to external stresses through use of a tensegrity mechanism for force integration.

Molecular Pathways for Mechanical Signal Transfer

ADHESION RECEPTORS AS MECHANORECEPTORS To determine whether mechanical signals are transferred across the cell surface over discrete molecular pathways, we developed a device to apply controlled mechanical stresses directly to specific cell surface receptors without producing global changes in cell shape or altering fluid flow (74). Shear stress (torque) was applied to membrane-bound ferromagnetic microbeads (1–6 μm in diameter) that were coated with ligands or antibodies for different cell surface receptors by first magnetizing the beads in one direction and then applying a weaker twisting magnetic field that did not remagnetize the beads in the perpendicular orientation. The cellular deformation that resulted in response to stress application was determined by simultaneously quantitating bead rotation (angular strain) using an in-line magnetometer.

Using this magnetic twisting technique, we were able to demonstrate that applying shear stress to cell surface integrin receptors results in a stress-dependent increase in CSK stiffness (defined as the ratio of stress to strain) (Figure 5). As predicted by the tensegrity model, this response is mediated through higher order structural interactions between all the different CSK filament systems (i.e. microfilaments, microtubules, and intermediate filaments). In contrast, application of force to other transmembrane receptors that do not normally mediate adhesion (e.g. acetylated low-density lipoprotein receptor, HLA antigen) does not induce CSK stiffening (74, 80).

More recently, we confirmed that different integrins (β1, β3, αV, α5, α2) and cell-cell adhesion molecules (e.g. E-selectin, PECAM) can mediate force transfer across the cell surface and to the CSK, although the efficiency of coupling varies considerably from receptor to receptor (76, 80). In addition, we have been able to demonstrate that similar mechanical coupling between integrins and CSK is observed in many cell types, that this stiffening response does not require membrane continuity, and that dynamic changes in cell shape are driven by actomyosin-based tension generation and accompanied by coordinated changes in CSK mechanics (67, 75, 76). Other laboratories also have demonstrated that integrins physically couple to the CSK (65) and that they transfer CSK tension to the ECM (64). Importantly, both mechanosensation in animal cells and gravitropism in plants can be inhibited by interfering with integrin binding, using soluble synthetic peptides that are specific for these receptors (7, 74, 78, 79). Taken together, these results support our hypothesis that adhesion receptors such as integrins act as mechanoreceptors because they are among the first cell surface molecules to sense mechanical stress and thereby transmit these signals across the cell surface over a specific molecular pathway.

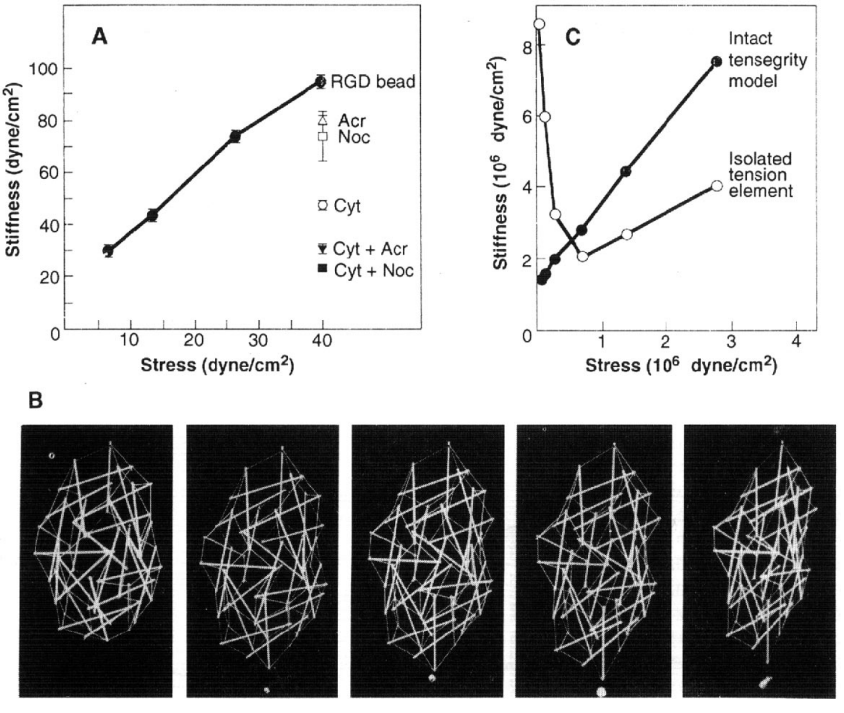

Figure 5 Analysis of the mechanical responsiveness of living cells and a tensegrity cell model. (*A*) The stiffness (ratio of stress to strain) of the CSK of living cells was measured using magnetic twisting cytometry. Nocodazole (Noc; 10 μg/ml), cytochalasin D (Cyt; 0.1 μg/ml), and acrylamide (Acr; 4 mM) each independently suppress the CSK stiffening response by interfering with microtubules, microfilaments, and intermediate filaments, respectively. (*B*) A tensegrity cell model under different mechanical loads. This model consists of a geodesic spherical array of wood dowels (0.3 $\times$ 15 cm) and thin elastic threads (0.06 $\times$ 6 cm). The model was suspended from above and loaded, from left to right, with 0, 20, 50, 100, or 200 g weights on a single strut at its lower end. (*C*) Stiffness of the stick and string tensegrity model is defined as the ratio of applied stress to strain (linear deformation of the entire structure). Similar measurements were carried out using an isolated tension element, i.e. a single thin elastic thread of similar size to that found in the model. (Reprinted with permission from Reference 74.)

MECHANICAL COUPLING TO THE CYTOSKELETON Transmembrane force transfer across integrins correlates with recruitment of the FAC proteins, vinculin, α-actinin, and talin and, thus, physical linkage of integrins to the actin CSK (74). In collaboration with Ezzel (14), we recently compared the mechanical properties of mutant cells that lack vinculin with wild-type cells and two different vinculin-transfected clones. Interestingly, cells that lacked vinculin retained the ability to form filopodia and contained normal levels of total polymerized and cross-linked actin, yet they could not form lamellipodia, assemble stress fibers, or efficiently spread when plated on ECM. The loss of vinculin also resulted in inhibition of FAC formation and in a decrease in the mechanical stiffness of the integrin-CSK linkage, as measured using cell magnetometry. Furthermore, when vinculin was replaced by transfection, the efficiency of transmembrane mechanical coupling, stress fiber formation, and cell spreading were all restored to near wild-type levels.

Vinculin, therefore, may represent one of the downstream molecules mediating the mechanical signaling cascade that begins with a tug on an integrin and results in stress-dependent changes in CSK structure and associated changes in cell shape. However, integrins may use multiple mechanisms for mechanical coupling. For example, integrin $\alpha 2$ appears to bind directly to actin filaments (40). This integrin also exhibits an enhanced ability to mediate collagen gel contraction when compared with other integrin subtypes (64). Future studies will be necessary to fully map the molecular pathways that mediate force transfer within the FAC, as well as in cell-cell adhesion complexes.

A LOCAL SIGNAL PRODUCES A GLOBAL RESPONSE Magnetic cytometry studies show that living cells respond to increasing levels of applied stress by getting stiffer. The most interesting result, however, is that the shape of the stiffening response is linear: the mechanical stiffness of the CSK increases in direct proportion as the level of applied stress is raised (Figure 5). Cultured endothelium exhibits a similar response when exposed to fluid shear stress (71). This behavior is also a fundamental property of many living tissues, including muscle, mesentery, cartilage, skin, and bone, yet there is no known mechanical or mathematical explanation for this behavior (17).

When we applied mechanical stresses to a tensegrity model, we found that it also exhibited linear stiffening (Figure 5) (74). This response was due to a novel structural property of tensegrity systems: all the interconnected structural elements globally reorient in response to a local stress (Figure 5). Cells and tissues may similarly exhibit high mechanical strength because stresses applied locally are distributed over thousands of interconnected molecular support elements. More recent modeling studies have revealed that tensegrity also can explain how different mechanical stress distributions can generate specific molecular

patterns (e.g. stress fibers, triangulated nets, geodesic domes) within the actin CSK, independently of any change in filament polymerization (28, 31).

Working with Stamenovic (69), we have developed a mathematical basis for the linear stiffening response exhibited by living cell and tissues based on tensegrity, starting from first principles. This approach confirms that two major parameters determine the mechanical stability of tensegrity structures: prestress and architecture. Prestress determines the initial stiffness of the structure and assures that the system will respond immediately when externally stressed. It also determines the characteristic frequency of vibration (harmonic oscillation) that the structure will exhibit. In contrast, architecture refers to the number of building elements, as well as how they distribute forces in space. This geometric feature determines how the interconnected structural elements rearrange and thus how the entire structural assembly stiffens in response to stress.

Both the original stick and string tensegrity cell models and this mathematical analysis incorporate elastic tensile elements to model dynamic changes in filament length. Actin filaments and microtubules are non-extensible; however, intermediate filaments are highly entangled polymers that progressively extend and straighten as the cell spreads. Recent studies also show that a highly elastic protein filament called titin exists in the CSK of many cells (39). In any case, even tensegrity structures that contain non-extensible filaments exhibit a linear stiffening response, although over a narrower range of extension. Furthermore, a tensegrity network composed of non-extensible sticks and strings can exhibit both linear stiffening and high flexibility if the stiff compression elements are allowed to buckle like microtubules do in living cells (M Coughlin & D Stamenovic, personal communication).

LIVING CELLS AND NUCLEI ARE HARD-WIRED If cell surface integrin receptors, CSK filaments, and nuclear scaffolds are hard-wired together, as suggested by the tensegrity model, then mechanical stresses could be transferred well into the depth of the cell over specific molecular pathways. Maniotis, Chen & Bojanowski, working in my laboratory, recently found that living cells and nuclei are indeed hard-wired such that a mechanical tug on cell surface integrin receptors can immediately change CSK organization and alter the arrangement of molecular assemblies in the depth of the nucleus, in time periods much faster than those necessary for polymerization (49). This ability to produce an action at a distance was found to be specific for integrins, independent of cortical membrane distortion, and mediated by discrete linkages between the CSK and nucleus. Also, filamentous linkages could be demonstrated between different nucleoli in interphase cells and different chromosomes in mitosis (48, 49).

Analysis of the molecular basis of this transcellular mechanical coupling reveals that actin microfilaments alone are sufficient to mediate force transfer

to the nucleus at low strains; however, intermediate filaments are required to maintain mechanical coupling at high deformation. These CSK cables also function as molecular guy wires to anchor the central nucleus in place and to control its mechanical stiffness. In contrast, microtubules act to stabilize the cytoplasm and nucleus against lateral compression.

In summary, these studies confirm that although the CSK is surrounded by membranes and penetrated by viscous cytosol, it is this discrete filamentous network that provides cytoplasm's principal mechanical strength, as well as the main path for mechanical signal transfer from the cell surface to the nucleus. Furthermore, the efficiency of force transfer depends directly on the mechanical properties of the CSK and nucleus which, in turn, are determined through cooperative interactions between microfilaments, intermediate filaments, and microtubules, just as was predicted by the tensegrity model. This prestressed system of molecular connections may therefore provide a discrete path for mechanical signal transfer through cells, as well as a mechanism for producing integrated changes in cell and nuclear structure in response to stress.

Mechanisms for Mechanochemical Transduction

Much of the cell's metabolic machinery functions in a solid state. The chemical reactions mediating protein synthesis, RNA transport, glycolysis, and DNA synthesis all appear to involve channeling of sequestered substrates and products from one immobilized enzyme to another along insoluble CSK filaments and nuclear matrix scaffolds (reviewed in 29). Signal transduction may be regulated in a similar manner. For example, we and others have shown that multiple signaling molecules that are activated by integrins and growth factors (e.g. phosphatidylinositol-3-kinase, Na^+/H^+ antiporter, phospholipase C, ${}_{pp}60^{c-src}$, ${}_{pp}125^{FAK}$) become physically associated with the CSK framework of the FAC within minutes after integrin clustering is induced (52, 57). A subset of growth factor receptors (e.g. FGF receptors) also can be found within the same insoluble complexes (57).

Thus the CSK framework of the FAC may represent a major site for signal integration between growth factor and ECM-based signaling pathways (Figure 2). Because these signaling molecules lie in the main path for mechanical force transfer, the FAC represents a potential site for translating mechanical stresses into biochemical responses and for integrating these responses with those activated by growth factor and ECM binding. This possibility is supported by the finding that mechanical stretch increases phosphorylation of the focal adhesion tyrosine kinase, ${}_{pp}125^{FAK}$ (21).

Some investigators have interpreted the finding that mechanical stresses can raise the level of inositol phosphates and release calcium from intracellular stores to indicate that the membrane-associated enzyme that produces this

product, phospholipase C, may be mechanosensitive. This is because growth factors commonly regulate this pathway by activating this enzyme. However, integrins can increase inositol phosphate production by another mechanism: They control the synthesis and, hence, availability of the inositol lipid substrate, phosphatidylinositol-bisphosphate (50). Interestingly, the phosphatidylinositol kinases that mediate this effect also are immobilized on the CSK within the FAC (51). This provides an excellent example of how mechanical stresses may be able to generate chemical signals through a variety of mechanisms and how difficult it is to interpret the molecular basis of mechanochemical transduction.

The findings presented above suggest that living cells and nuclei are literally built to sense and respond immediately to mechanical stresses applied to specific cell surface receptors such as integrins. Because of the presence of discrete CSK interconnections, mechanical stresses and vibrations may be preferentially transferred to distinct structures inside the cell and nucleus, including signaling molecules in the FAC, ion channels in the membrane, ribosomes, nuclear pores, chromosomes, and perhaps even individual genes. By distributing force over a relatively small number of support elements, this type of hard-wiring provides a mechanism to concentrate stress and focus mechanical energy on specific molecular elements that physically associate with the CSK. If cell and nuclear metabolism functions in a solid state, then stress-induced changes in scaffold geometry or mechanics could provide a mechanism to regulate and orchestrate the cellular response to force. This long-range force transfer could explain, for example, how cell stretching results in extension of the nucleus, physical expansion of nuclear pores, and associated increases in nucleocytoplasmic transport (15, 35).

GEOMETRIC REARRANGEMENTS Our studies with living cells demonstrate that pulling on integrins causes the internal CSK and nuclear scaffolds to immediately realign along the main axis of the applied tension field (48, 49). Resultant changes in the topology of these networks could alter cellular biochemistry directly. For example, mRNAs specifically localize to intersections between different actin filaments, rather than along their length (4). Vertices within highly triangulated microfilament networks (e.g. within actin geodesic domes) are also preferred sites for actin polymerization, as observed during formation of filopodia that lead cell movement (60). Thus stress-induced changes in CSK architecture (e.g. transformation of triangulated nets to linear bundles) could influence protein synthesis by destabilizing CSK-associated mRNAs or change the dynamics of actin polymerization and thereby alter cell spreading or movement.

Geometric remodeling of the CSK also may influence other biochemical or enzymatic reactions that channel along CSK or nuclear scaffolds (e.g. glycolysis, DNA synthesis) (Figure 6). For example, mechanical deformation of the

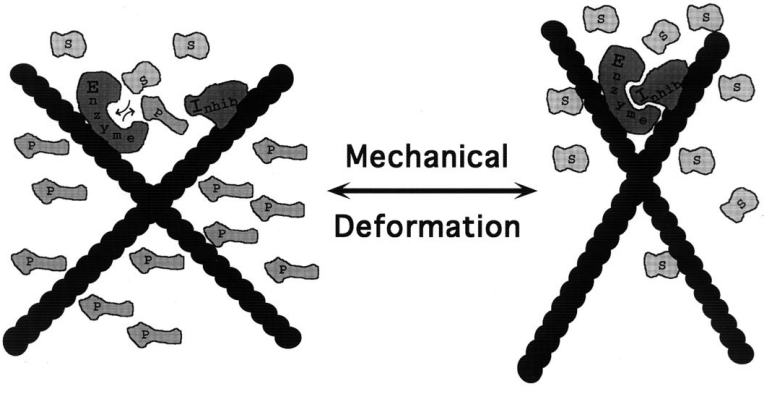

Figure 6 Mechanotransduction through geometric remodeling of CSK scaffolds. If biochemistry functions in a solid state, then changes in CSK network geometry may alter cell metabolism or signal transduction by changing the relative position of different regulatory molecules, hence altering their ability to chemically interact. Transduction also may be carried out through direct mechanical distortion of molecules that alters thermodynamic or kinetic parameters (not shown).

FAC may generate intracellular chemical signals by bringing immobilized kinases and substrates into direct juxtaposition, thereby facilitating downstream signaling. This type of direct mechanical coupling could explain how release of calcium and neurotransmitters from motor nerve terminals can be induced within 10 ms after integrins are stressed (7), as well as how $_{pp}125^{FAK}$ becomes phosphorylated in response to stretch (21).

CHANGES IN MOLECULAR MECHANICS Molecules that are incorporated within insoluble macromolecular scaffolds bearing mechanical loads transmitted from integrins will feel a pull exerted on the cell surface, whereas neighboring soluble molecules only nanometers away will not. The transfer of focused mechanical energy to these molecules will therefore alter their chemical potential, as well as their shape (e.g. protein folding) and motion through mechanical distortion. These are the very features of molecules that determine their chemical behavior. In fact, chemical mechanisms for altering enzyme activity or signal transduction, such as protein tyrosine phosphorylation, actually manifest themselves at the biophysical level by altering protein flexibility and conformation (72). Thus mechanical energy could be converted directly into chemical energy through stress-induced changes in molecular shape or mechanics.

When a noncovalent adhesive bond between two proteins is either pulled or pushed, the chemical potential of that bond increases (i.e. the stable bond is at minimum energy) (reviewed in 45). Increasing the energy in the system may

change chemical activities by effectively changing the activation energy, thus altering the rate constant for a reaction. If the association and dissociation constants are equally affected, the thermodynamics (ratio between the constants) will not be altered, and the equilibrium will remain the same, but the kinetics will change. If the forward and reverse reactions differ in their sensitivity to this deformation, then the thermodynamics will be altered and the equilibrium will shift. Finally, the oscillatory motion of molecules, a key kinetic determinant of how they behave chemically, also can be altered by altering molecular shape or stiffness. Thus stress-induced changes in molecular mechanics can produce direct mechanochemical transduction in a number of ways.

Thermodynamic Regulation

An example of a thermodynamic transduction mechanism in living cells comes from analysis of the response of CSK microtubules to changes in mechanical stress. If the cytoplasm were entirely viscous, individual CSK filaments could not experience a tension or compression along their length. However, microtubules can feel compression because contractile microfilaments can insert at their ends or, in other words, because cells use tensegrity architecture.

A thermodynamic model was developed that incorporates tensegrity to explain how microtubule assembly is controlled within nerve cells (6). The formation of long nerve processes called neurites is mediated by elongation of CSK microtubules through polymerization. Microtubules are dynamic polymers of tubulin that rapidly polymerize and depolymerize in response to changes in the concentration of free tubulin monomer (41). Tensegrity predicts that each time a moving neurite forms a new adhesion, it transfers some compression from its internal microtubule struts to the external compression-resistant ECM. Decompressing a microtubule decreases its chemical potential, thereby lowering the critical concentration of tubulin required to maintain it in a polymerized form (25). Thus free tubulin monomers will be added to the ends of the microtubule until the tubulin monomer concentration decreases sufficiently to restore the equilibrium, or until the preexisting force balance is reestablished.

Analysis of living nerve cells has confirmed that microtubule polymerization is indeed sensitive to changes in tension in the surrounding actin CSK (38), as well as to alterations in cell-ECM adhesions (44). Microtubule assembly also can be induced by applying external tension directly to the surface membranes of these cells (10). Epithelial cells sense the same change in thermodyamic parameters in their microtubules when they attach and spread on ECM; however, they apparently have evolved a mechanism to produce a different molecular response (53). Rather than increasing microtubule polymerization, they react enzymatically by producing a concomitant increase in the rate of tubulin protein degradation when they transfer force from microtubules to the ECM. This results

in a net decrease in the concentration of free tubulin monomer until it matches the new lower critical concentration for tubulin. The net effect is that the total mass of polymerized microtubules remains constant when epithelial cells spread, whereas it increases during neurite extension.

The sensitivity of tubulin to tensegrity-based thermodynamic alterations may play an important role in signal transduction because many cellular functions (e.g. production of cAMP, actin filament organization) can vary depending on the state of microtubule polymerization (46, 47). Furthermore, dimeric tubulin can bind to specific G proteins, such as those that mediate adenylyl cyclase activation, and activate their GTPase activity via direct transfer of GTP (58). This is one explanation of how mechanical stresses and receptors that are not directly coupled to G proteins (e.g. integrins) may activate this signaling pathway.

Kinetic Regulation

One of the best examples of how mechanical stresses can alter reaction kinetics comes from analysis of cell adhesion and spreading (45). Because of the dynamics of cell adhesion, the equilibrium state may never be reached since the cell may not be able to form enough bonds fast enough to prevent detachment. This is particularly important in situations where cells that are trying to form new adhesions are exposed to mechanical stresses. For example, very fast on and off rates are required for leukocyte attachment and rolling on the endothelial cell surface at physiological shear stresses (2). Furthermore, all cells exert tractional forces on their adhesion receptors, and changes in receptor kinetics likely play a central role in most adhesive phenomena.

Analysis of how pulling or compressing the adhesive bond may alter chemical reaction rate constants has revealed that two types of dynamic behavior may result depending on the relative stiffness of the transition state and the bond (45). If the bond is stiffer than the transition state, bond dissociation will be accelerated by the application of stress and, hence, the bond will slip. If the bond is more flexible than its transition state, stressing the bond will actually decrease the dissociation rate and thus cause the bond to catch. Meanwhile, the forward rate constant (association rate) may or may not be altered. Analogous slide and catch bonds between different molecules may govern how higher order CSK scaffolds deform in response to stress. However, the main point is that mechanically stressing any molecule alters its behavior in a number of ways. The same mechanical stimulus may also produce an entirely different response depending on the structural properties of each molecular sensor.

Another kinetic feature that can be altered by mechanical stresses is molecular movement or vibration, one of the most important determinants of molecular function. To understand how mechanical stress alters molecular motion, think

of a molecule as a semiflexible spring that is fixed at its lower end and contains a dense ball bearing at its center. This spring exhibits a characteristic frequency of lateral vibration (harmonic frequency) that is a function of the inherent stiffness of the spring and the placement of its center of mass. If temperature and energy are constant, then shifting the center of mass closer to the lower fixed end of the spring or shortening its length (e.g. by altering protein folding) will cause the frequency of oscillation to increase and the amplitude (range of motion) to decrease, much like shortening the cable on a pendulum. Another way to alter the rate of vibration is to change the stiffness of the spring; decreasing its flexibility (e.g. by distending the molecule) will cause it to vibrate more quickly over a narrower range, much like a violin string does when it is stiffened (tensed). Furthermore, application of vibrational motion of the same frequency as the natural harmonic of the structure will result in an increase in the amplitude of vibration (range of motion) without changing the rate. Thus different molecules may be sensitive to different vibration frequencies. Of course, both frequency and amplitude also may be altered by adding energy to the system.

Therefore, stress-induced changes in molecular shape and mechanics can change a reaction rate, such as transport of an anion through a channel, by varying the frequency of channel opening or closing, or by changing the size of the pore opening at a given rate of vibration. Interestingly, many mechanosensitive ion channels respond to stress kinetically by altering their opening or closing rates (23). Moreover, normal mechanosensitive ion channel sensitivity and adaptation responses become deregulated when membrane-CSK linkages are disrupted (23, 70).

Tuning the Transduction Response

Tensegrity provides a mechanism to mechanically and harmonically couple interconnected structures at different size scales and in different locations throughout living cells and tissues (30, 34, 55). Thus cell and tissue tone may be tuned by altering the prestress in the system. This may be accomplished by altering the architecture of the system or the level of CSK tension (69). In either case, increasing the stiffness of the network will alter vibration frequencies and associated molecular mechanics of all the constituent support elements. This may, in part, explain how the part (molecule, cell) and whole (e.g. cell, tissue, organ, organism) can function as a single mechanically integrated system (30).

This tuning mechanism also may play an important role in mechanical signal amplification, as well as in the adaptation responses that are necessary to tune out certain signals. For example, recombinant mechanosensitive ion channels can be activated by direct mechanical deformation when placed in synthetic liposomes that lack any CSK interconnections (70). However, when compared with similar channels analyzed in in situ in living cells, these channels appear

to be hypersensitive and to lack normal adaptive responses (23, 70). If the mechanically stressed liposome containing the ion channel is viewed like a sail luffing in the wind, then the addition of CSK connections and associated transfer of tension may act to winch in the membrane and thus alter its range of motion and frequency of vibration as well as its stiffness. Any one of these changes may feed back to tune the mechanotransduction response, as seen in studies with intact cells (23, 70).

On a larger scale, alterations in CSK stiffness or in the number of load-bearing elements in the system will change how stress dissipates in the network before it reaches the molecular transducer. A cell that is very stiff may be able to sense lower levels of stress more quickly than a more flexible cell. Conversely, the more flexible cell may be able to sense larger strains. This adaptability may contribute to the different sensitivities exhibited by specialized mechanosensory cells; for example, the stiff hair cells of the inner ear sense small vibrations, whereas more flexible spindle cells of muscle recognize changes in length (stretch). A similar mechanism may explain why osteocytes, which contain highly extended (and hence stiffened) processes, preferentially respond to high frequency and low amplitude strains (13).

In addition, the way in which the CSK is organized (i.e. the architectural feature in the mathematical model) will itself alter the way in which the cell senses and responds to stress. For example, the shape of the mechanosensory cell may alter how it vibrates and thus determine its ability to sense particular vibration frequencies. The hair cell of the inner ear provides a beautiful example of this type of structural specialization. These cells contain numerous cylindrical projections on their apices called stereocilia, which contain cross-linked bundles of CSK filaments surrounded by a tightly apposed surface membrane. These rigid struts are, in turn, interconnected at their lateral borders by filamentous tip links. The presence of these lateral tensile connections pull together the individual stiffened stereocilia and create a local tensegrity force balance, thereby stabilizing the entire apical region of the cell. Their disruption results in both disorganization of cell architecture and loss of mechanosensitivity. Although it is commonly assumed that these tip links directly interconnect with their target mechanosensitive ion channels, recent studies suggest that these transducing molecules are located at a more distant site (20). This long-range effect may be explained by the use of tensegrity. Interestingly, conditions that cause mechanoreceptor cells in the fishing tentacles of sea anemones to tune their sensitivity to different vibration frequencies also produce changes in the length of their stereocilia and in the shape of their CSK (77).

Finally, because the ECM physically interconnects with the CSK, its mechanical properties also may contribute significantly to the mechanotransduction response. If the ECM is highly flexible, then a rapid deformation may

be sensed, whereas a sustained stress will dissipate before it reaches the cell. In fact, this very mechanism is used by Pacinian corpuscle mechanoreceptor cells of skin to filter out sustained signals (arising from continuous pressure or touch), a common form of receptor adaptation. If the ECM is less flexible, then stresses may be transmitted to and through the cell, only to be dissipated through movements in the CSK. For example, when fluid shear stress is applied to the apical membrane of endothelium, the cell and its CSK are immediately pulled against their fixed basal adhesions to ECM. This can be visualized by dynamic changes in FAC remodeling at the cell base, which occur within seconds to minutes after shear is exerted (9). The resistance imposed by the relatively inflexible ECM induces global rearrangements in the CSK through a tensegrity mechanism, as measured by a linear stiffening response (71). These changes in CSK mechanics, in turn, may serve to simultaneously modulate multiple signaling mechanisms. A similar response could be induced throughout the cell by a change in osmolarity that causes the cell membrane to pull outward or inward against its CSK tethers.

CONCLUSIONS

The purpose of this chapter is to review the architectural and molecular basis of cellular mechanotransduction, with particular emphasis on the process of signal integration. The experimental studies reviewed suggest that cell surface adhesion receptors (e.g. integrins, cell-cell adhesion molecules), interconnected CSK networks, and associated nuclear scaffolds function as a structurally unified system. This system gains its mechanical stability and long-range flexibility from tensional continuity, discrete load-bearing members, and the presence of internal prestress. These are the fundamental requirements of tensegrity architecture. Use of tensegrity could serve to concentrate stresses and focus mechanical energy on mechanochemical and mechanoelectrical transducing molecules that physically associate with the insoluble CSK. It also may provide a mechanism to orchestrate and tune the entire cellular response to stress.

These studies also suggest that the specialized anchoring complexes or FACs that mediate mechanical coupling between CSK, integrins, and ECM may represent a potentially important site for signal integration because molecules that transduce signals from ECM, growth factors, and mechanical stresses all appear to concentrate in this location. Similar signal integration also may occur within junctional complexes at the lateral cell borders (18). Mechanochemical transduction may, in turn, result from changes in the CSK geometry or mechanics that alter local thermodynamic or kinetic parameters. This type of direct mechanical coupling could serve to modulate slower diffusion-based chemical signaling pathways and coordinate functional changes throughout the depth of

the cytoplasm and nucleus. Thus, in simplest terms, the CSK may be viewed as mechanical filter: The same chemical or mechanical input will produce a different output (cellular response) depending on the geometry and mechanics of this structural framework. Use of tensegrity by cells may therefore help to explain how distortion of the cell or CSK caused by gravity, hemodynamic forces, pressure, stretch, or even cell tension (32, 37, 54, 68) can alter cellular biochemistry and switch cells between different genetic programs.

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