Cellular control lies in the balance of forces

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Mechanical tension generated within the cytoskeleton of living cells is emerging as a critical regulator of biological function in diverse situations ranging from the control of chromosome movement to the morphogenesis of the vertebrate brain. In this article, we review recent advances that have been made in terms of understanding how cells generate, transmit and sense mechanical tension, as well as how they use these forces to control their shape and behavior. An integrated view of cell regulation that incorporates mechanics and structure as well as chemistry is beginning to emerge.

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Abbreviations
ECM extracellular matrix
FAC focal adhesion complex

Introduction

Recent studies have revealed that mechanical tension generated through molecular interactions within the cytoskeleton is critical for control of cell form and function. Cellular behaviors that are modulated by cell-generated forces or associated changes in cell shape include growth, differentiation, apoptosis, motility, signal transduction, gene expression, chromosome movement and extracellular matrix (ECM) remodeling, to name a few. Furthermore, when these same forces are transmitted across the cell surface they can influence tissue development. Even the formation of the most complex of organs, the vertebrate brain, seems to be guided by mechanical forces [1]. At the heart of this process lies the response of individual cells to mechanical tension [2,3] and, ultimately, the response of the molecules that make up the cells.

Understanding how mechanical forces influence cell behavior requires a new paradigm. In classic stimulus–response coupling, a soluble signal-bearing molecule ligates a receptor and initiates an intracellular response. In contrast, the major effect of cytoskeletal tension is to establish and maintain a cellular force balance. Tensional forces generated within contractile microfilaments pull inward on the surface membrane and the cell's internal components. These inward-directed forces are resisted by external adhesions to the ECM and to other cells, by internal molecular struts within the cytoskeleton itself and by the surface membrane when stiffened by osmotic pressure. Thus, at any point in time the cell exists in a state of isometric tension.

To understand how mechanical forces regulate tissue development, we must therefore address the question of how cells sense and respond to changes in this cellular force balance. This involves the questions of how mechanical stresses are transmitted through cells and brought into balance, how these forces influence molecular structure and biochemical activities and how the cell orchestrates these changes simultaneously at many locations, both inside and outside itself. Major advances have been made in all of these areas over the past year and these are the focus of this article.

Bringing life into balance

All living cells generate tension within contractile microfilaments in their cytoskeleton using an actomyosin filament sliding mechanism and they exert this tension on their surface membrane. But it is now clear that physical forces are not transmitted equally at all points across the cell surface. Rather, mechanical signals are transmitted over preferred molecular pathways and, specifically, across transmembrane receptors that mediate cell–ECM [4] and cell–cell adhesion [5,6]. Among these molecules, transmembrane ECM receptors called integrins have so far occupied center stage.

When mechanical stresses are applied directly to cell-surface receptors by magnetically twisting surface-bound microbeads, the integrin linkages are much stiffer than those formed by other transmembrane molecules (for example, metabolic receptors) and thus greater force is required to deform the cell [4]. Both integrin clustering and ligand binding-site occupancy are required for this mechanical coupling [7••,8]. Formation of the focal adhesion complex (FAC) is also central to this response. The FAC is a macromolecular scaffold that mechanically couples the cytoplasmic portion of integrins to the internal actin cytoskeleton [4]. It contains actin-associated molecules such as vinculin, talin and α-actinin, as well as many signaling molecules that mediate stimulus–response coupling, including tyrosine and serine protein kinases, inositol lipid kinases, ion channels and even a subset of growth factor receptors [9,10]. Vinculin may have a central role in transmembrane mechanical coupling because the mechanical stiffness of the structural linkage through the FAC is greatly reduced in F9 embryonic carcinoma cells that lack vinculin, whereas replacement of vinculin through transfection restores normal transmembrane mechanical coupling [11••].

FAC formation is itself controlled mechanically. When a cell attaches to an ECM substrate, it attempts to

retract that substrate until forces come into balance, much like a bow and a bowstring. By physically restraining microbeads bound to integrins using an optical trap, proportional strengthening of the cytoskeletal linkages has been demonstrated [7••]. Tension across integrins also can be increased by stretching flexible culture substrates [12], by pulling the cell away from its fixed ECM adhesions through application of fluid shear stress [13], or by enhancing mechanical coupling between integrins and the cytoskeleton in vinculin-lacking cells by replacing vinculin protein [11••]. All of these mechanical perturbations enhance recruitment of FAC proteins (for example, $\alpha 5\beta 1$ integrin, talin, vinculin) to the site of ECM binding.

The mechanical force balance across integrins also can be controlled chemically by activating the small molecular weight G protein, Rho. Rho promotes actomyosin filament sliding and tension generation by activating Rho-kinase [14] which, in turn, phosphorylates myosin light chain, thereby activating its ATPase activity. Rho-kinase also further augments the contractile response by phosphorylating myosin phosphatase, thus inhibiting myosin light chain dephosphorylation [15•]. The associated rise in isometric tension that results from increased contraction against the cell's fixed ECM adhesions promotes integrin clustering and FAC formation [16••,17•]. Increased isometric tension also promotes cytoskeletal realignment, resulting in the formation of long bundles of contractile microfilaments (stress fibers) along the lines of applied stress stretching between different FACs [16••,18]. This response can be similarly altered by modulating the mechanical stiffness of the integrin–cytoskeleton linkages that resist the cytoskeletal tension, for example, by varying vinculin expression levels in the cell [11••].

Tension generated in the actin cytoskeleton is not borne only by cell surface adhesions, it is also resisted from within by internal molecular struts within the cytoskeleton itself. In time-lapse microscopic studies of cells transfected with microtubule-associated proteins labelled with green fluorescent protein, individual microtubules can be seen to buckle under compression as they extend and push against surrounding cytoskeletal elements [19••]. Microtubule buckling ceases when cells are treated with actomyosin inhibitors [20], confirming that contractile microfilaments generate the force that compresses these microtubule struts. Intermediate filaments that transfer tension from microfilaments to microtubules and ECM adhesions also may help to bear some of these loads [4,21••]. Disassembly of intermediate filaments using specific mimetic peptides from the helix initiation domain of these proteins results in destabilization of microtubule and microfilament networks as well as dramatic changes in cell shape [22]. The surface membrane along with its closely associated submembranous cytoskeleton also may resist tensional forces generated within the actin cytoskeleton when the cell is osmotically stressed [23].

The finding that compressed microtubules balance the tension generated by contractile microfilaments helps to explain why disruption of microtubules using pharmacological inhibitors (such as colchicine and nocodazole) results in enhanced contraction of ECM [24] as well as increased formation of stress fibers and FACs [25•]. In simple terms, loss of the microtubule struts results in transfer of the same mechanical loads onto the cell's ECM adhesions, as well as a rise in tension within the actin cytoskeleton. Microtubule depolymerization may also alter tension generation chemically by activating myosin light chain phosphorylation [24]. Nonetheless, at least in cardiac myocytes, microtubule disassembly results in immediate changes in cellular mechanics independently of any measurable change in microfilament contraction (sarcomere shortening) as quantitated directly using laser diffraction analysis [26].

Importantly, the same tensional forces that are balanced by ECM and cytoskeletal struts are also transmitted to the nucleus. When an ECM-coated micropipette was bound to cell-surface integrin receptors and pulled away from the membrane in cultured endothelial cells, cytoskeletal filaments reoriented, nuclei distorted, and nucleoli redistributed along the axis of the applied tension field within one second after force application [21••]. Interestingly, the actin lattice was found to be able to mediate force transfer to the nucleus independently of microtubules or intermediate filaments, although only at low levels of deformation. Only intermediate filaments could transfer mechanical forces to the nucleus at high levels of deformation. Direct micromanipulation of the cytoskeleton (harpooning of the cytoplasm using ultrafine micropipettes) combined with engineering analysis confirmed that, because of their ability to transfer tension, intermediate filaments and microfilaments act as molecular 'guy wires' that mechanically stiffen the nucleus and anchor it in place [21••].

These studies suggest that molecular connections between integrins, cytoskeletal filaments and nuclear scaffolds provide a discrete path for mechanical signal transfer throughout living cells, as well as a mechanism to bring tensional forces into balance. Because of a preexisting balance of forces distributed througout the entire cell, forces applied at one pole of the cell can produce action at a distance — at the opposite pole as well as in the depth of the nucleus. This may explain how application of directional fluid shear stress to the apical surface of endothelial cells results in coordinated directional remodeling of FACS at the cell base [27•].

In summary, recent studies reveal that the stability of cytoskeletal structure and cell shape results from the cell's ability to bring internal tensional forces into balance, thereby creating a prestress that stabilizes molecular architecture in the cell. This type of building system,

which incorporates local compression members that balance globally transmitted tensional forces and thereby creates a prestress that stabilizes the whole structure is known as 'tensegrity' [18,28••]. The key features of tensegrity — continuous tension [21••,29•], local compression [19••] and the dependence of shape stability (mechanical stiffness) on prestress [30•,31] — have all been demonstrated experimentally in living cells. The findings that a local stress can produce proportional mechanical stiffening [4,7••], global structural rearrangements [21••] and biochemical alterations [27•] at a distance in living cells also are all predicted by tensegrity [4,18,28••].

Tensegrity may even be used to stabilize the mitotic spindle and to regulate chromosome position [18,32••]. Laser ablation of microtubules in the spindle results in movement of the spindle pole toward the equator on the irradiated side [33•] and immediate buckling of the remaining microtubule struts [32••,33•]. Apparently, the microtubules push against the surrounding molecular lattice to create the prestress necessary to keep the spindle from collapsing. The surrounding tensile net may be composed of nuclear matrix [34], myosin filaments [35], or even actin filaments [36] in certain cells. The finding that all of the chromosomes in the genome are mechanically coupled by DNA [29•] raises the possibility that this chromosomal network may also resist the pushing forces of the spindle microtubules and hence play into this tensegrity force balance. Importantly, a mathematical model of cell mechanics based on tensegrity has been developed recently and can predict the mechanical behavior of living cells starting from first mathematical principles [37,38]. This engineering approach may provide a handle that will allow us to define the cellular and nuclear force balance more quantitatively in the future.

Shifting the balance alters cell behavior

Because of the existence of a cellular force balance focused on integrins, changes in ECM mechanics or in mechanical stresses applied to the cell surface can produce immediate alterations in cytoskeletal structure inside the cell leading to changes in intracellular biochemistry and gene expression. For example, pulling on microbeads bound to integrins with magnetic forces results in release of intracellular calcium, whereas pulling on other transmembrane receptors has no effect [39••]. Furthermore, the effects of fluid shear stress on endothelial cells [40], mechanical strain on vascular smooth muscle cells [41], substrate distortion on human bone cells [42], and stretching on the neuromuscular junction [43•] all can be inhibited using integrin antagonists. Even mechanotransduction in *Caenorhabditis elegans* seems to involve transmembrane molecules that physically link cytoskeletal elements to the ECM, in addition to functioning as stress-sensitive ion channels [44].

Interestingly, the biochemical changes that are produced inside the cell seem to be sensitive to the direction of the applied force as well as to its magnitude, as seen, for example, when apically applied fluid shear stresses result in directional remodeling of FACs at the cell base (that is, where the cell's fixed adhesions would experience the greatest local distortion) [13,27•]. Furthermore, the release of calcium and neurotransmitter in response to transfer of mechanical tension across integrins in the neuromuscular junction occurs within 1–2 milliseconds after stress application [43•]. The rapidity of this response suggests a direct mechanical effect that likely occurs close to the site on the cell surface where the force was applied. Thus, the FAC may represent a point of convergence for transduction of all three extracellular signals: ECM, soluble factors and mechanical forces [28••].

Transfer of forces across the FAC also may lead to creation of new functional microcompartments inside the cell. For example, binding of ECM microbeads leads to recruitment of the protein translation machinery, including mRNA and ribosomes, to the periphery of the FAC [45••]. This recruitment can be inhibited by interfering with cytoskeletal tension generation using myosin light chain kinase inhibitors and promoted by applying mechanical stress to the surface-bound microbeads using magnetic forces. The existence of this translational microcompartment at the site of integrin binding may mediate the rapid increase in protein synthesis that occurs in response to mechanical stress application [46].

Importantly, altering the cellular force balance also can influence biochemistry on the outside of the cell and, specifically, ECM remodeling that is critical for pattern formation during tissue development. For example, fibronectin fibril assembly can be stimulated by lysophosphatidic acid which increases cytoskeletal tension generation through activation of Rho or by depolymerizing microtubules using nocodazole which both activates Rho and transfers forces from microtubules onto cell–ECM adhesions [47•]. The activity of enzymes that are involved in ECM degradation, such as metalloproteinases, may be similarly altered by changing cytoskeletal structure and tension transmission [48,49].

Changing the mechanical strength of the cell's adhesions can alter its ability to transmit the tractional forces necessary to drive cell migration [7••,50•]. For instance, cell surface molecules such as the urokinase plasminogen activator receptor and plasminogen activator inhibitor-1 seem to modify the rate of cell migration by altering the adhesiveness of αVβ3 integrin [51,52]. Interestingly, αVβ3 integrins physically connect the complex of plasminogen activator inhibitor-1, urokinase and the urokinase receptor to the cytoskeleton [53], therefore it is possible that force transfer across this integrin may modulate the activity of this ECM remodeling complex and *vice versa*.

The finding that integrin–cytoskeleton linkages strengthen as the ability of the ECM to resist cell tension is increased

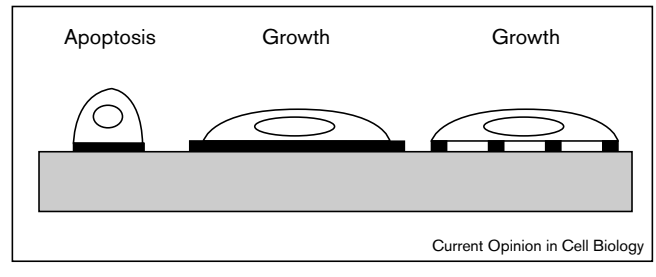
[4,7••] helps to explain why the rate of cell movement varies depending on the adhesivity of the ECM [50•]. For a cell to migrate, there also must be an asymmetry in traction between the front and the rear; forward adhesions must stabilize whereas rear adhesions must be released. By plating cells on a silicon chip micromachined to contain mechanosensors only microns in size, tractional forces were measured directly in the front and rear areas of moving cells and this point was confirmed directly [54•]. Thus, both the direction and the level of force transferred across integrins must be considered when attempting to understand how complex cellular behaviors are controlled.

One of the most important effects produced by altering the force balance across cell–ECM adhesions is to change cell shape. If the stiffness of the ECM substrate is greater than the stiffness of the cell's cytoskeleton, then the cell will deform (flatten and spread) as it pulls against its ECM adhesions. In this process, the entire cell and nucleus change in a coordinated manner because they are hard-wired together by tensed cytoskeletal filaments [21••]. Although the small molecular weight G proteins Rho and Rac clearly have an important role in regulating cell shape, even their effects may be largely mechanical in nature. For example, the effects of Rho on cell shape and cytoskeletal organization appear to be mediated by cytoskeletal tension generation in cultured fibroblasts and epithelial cells [16••,17•]. Similarly, neurite extension can be induced in Rac-deficient PC12 cells, which lack this activity, simply by pulling on the surface of the neurite with micropipettes [55]. In fact, tension may normally drive neurite outgrowth *in vitro* [2] as well as *in vivo* [3].

Tension-dependent changes in cell shape are important because they can cause cells to switch between different genetic programs. Changing cells from flat to round by decreasing substrate adhesivity or the contact area of the ECM shuts off growth and turns on differentiation in many anchorage-dependent cells, including capillary endothelial cells [56••], mammary epithelial cells [57] and hepatocytes [58]. Changes in cell shape produce alterations in expression of differentiation-specific proteins [57] as well as critical cell cycle regulators such as cyclin D [59•]. These changes may be exerted at either the transcriptional [60,61] or post-transcriptional level [61,62].

By developing a technique to control cell extension independent of growth factors and local cell–ECM binding interactions, cell shape *per se* was shown to govern whether individual cells will grow or die [56••]. Capillary endothelial cells were cultured on micropatterned surfaces that contained ECM-coated adhesive islands only microns in size. The islands were surrounded by non-adhesive areas that prevented cell attachment. When cell spreading was progressively restricted by plating on smaller and smaller adhesive islands, DNA synthesis progressively decreased in parallel whereas cells on the smallest islands (10 μ in diameter) concomitantly switched on a death (apoptosis) program. By effectively breaking up the small adhesive islands into many smaller islands, each one a single FAC in size (3–5 μ in diameter), and separating these smaller islands by non-adhesive regions, the same cells could be made to spread out and flatten across multiple dot-like islands while maintaining the total area of cell–ECM contact constant (Figure 1). Using this approach, it was found that it is the degree to which the cell physically extends, and not its local ECM contacts, that dictates whether it will proliferate or die. Altering cell spreading and cytoskeletal distortion, in this case by plating cells on dishes coated with different densities of immobilized ECM molecules, also can modulate the contractility of individual cultured smooth muscle cells in response to soluble vasoconstrictors and vasodilators [31].

Figure 1



Geometric control of cell life and death. Cell-shape-dependent switching between different gene programs was demonstrated by culturing cells on micropatterned substrates that contained ECM-coated adhesive islands of various sizes and shapes [56••]. Capillary endothelial cells underwent apoptosis when grown on small islands that prevented cell spreading, whereas they entered S phase and proliferated on similarly coated larger islands that permitted cell extension. By allowing cells to spread over multiple, smaller, FAC-size adhesive islands that provided the same amount of cell–ECM contact as a single small adhesive island, cell shape was shown to be the critical factor determining whether cells will grow or undergo apoptosis.

Importantly, growth free of anchorage and loss of cell-shape-dependent growth control can be induced by the mutation of a gene that is required for cell shape changes during *Drosophila* development (*lethal*(2)*giant larvae*) [63]. Furthermore, the transformed phenotype can be reversed in fibroblasts transformed with v-Ki-ras by restoring lost tropomyosin 2 through transfection, that is, by replacing a protein that normally mediates cytoskeletal tension generation [64]. Neoplastic transformation also can be reversed using β1 integrin blocking antibodies [65•], which may similarly influence the cellular force balance and alter cytoskeletal structure.

Even biochemical activites in the nucleus are sensitive to changes in force balances. For example, the decrease in tension (prestress) that occurs when a chromosome detaches from the mitotic spindle causes chromosome movements to cease throughout the entire spindle, a

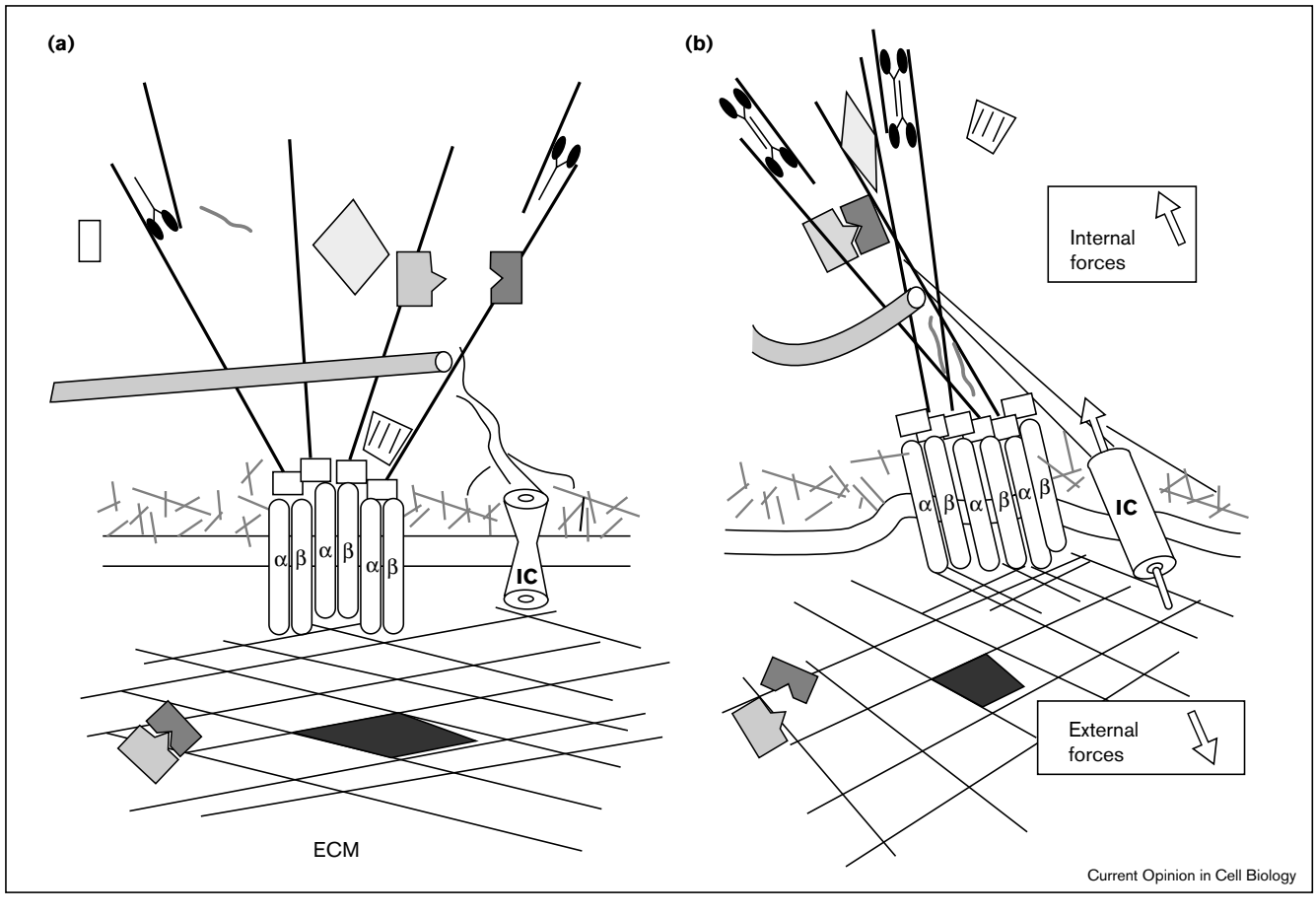
regulatory control which ensures fidelity of chromosome transfer [66,67••]. Release of tension results in phosphorylation of kinetochore proteins, a regulatory event that can be reversed by applying tension directly to chromosomes using a microneedle [66,67••]. A local change in tension on a single kinetochore may activate a phosphorylation cascade that, in turn, causes all the chromosomes to stop moving, even those at a distance. Alternatively, because the spindle uses a tensegrity mechanism to stabilize itself [32••], disconnection of one chromosome may decrease prestress throughout the entire spindle, thus causing a direct stress-induced change in phosphorylation in all of the remaining connected kinetochores.

At the same time as these events are going on in the spindle, the surrounding cytoskeleton is itself being remodeled by tensional forces. Using multimode light microcopy and a phosphorylation biosensor based on fluorescence energy transfer, the three-dimensional movements of myosin II and its phosphorylation were shown to map out the pattern of forces that drive cleavage furrow formation and contraction in dividing cells [35]. Tension also can still be transmitted from cell surface integrins to the mitotic spindle in dividing cells [21••]. In some unknown manner, these forces must interplay with those created in the mitotic spindle to coordinate karyokinesis and cytokinesis and to direct the pattern of cell division.

Orchestrating the cellular response

Taken together, these recent studies suggest that cell-generated forces provide key regulatory information to the cell. Cells stabilize their shape and structure by bringing these forces into balance using both external ECM adhesions and internal cytoskeletal struts and cables. Changes in cell tension may be produced chemically through alterations in signal transduction (for example,

Figure 2



Mechanical control of cellular biochemistry. **(a)** Integrins form a molecular bridge between ECM and the cytoskeleton that distributes mechanical stresses and helps bring these forces into balance. **(b)** Altering the cellular force balance by applying mechanical stresses, changing cytoskeletal tension or modulating ECM mechanics results in local distortion of molecular elements associated with the inside and outside of the FAC, as well as in global structural rearrangements throughout the cell. Associated changes in molecular structure and mechanics can influence cellular biochemistry by altering the relative distribution of interacting molecular components or modulating local thermodynamic and kinetic parameters [28••,68]. α, α integrin chain; β, β integrin chain; ECM, extracellular matrix; IC, stretch-sensitive membrane ion channel. Different shapes are schematic representations of various regulatory molecules that alter their association with cytoskeletal and ECM scaffolds, assembly dynamics or biochemical behavior in response to changes in the cellular force balance.

Rho activation) or mechanically by either applying external mechanical stresses to cells or altering the ability of ECM adhesions to resist cell-generated forces. Most importantly, shifting the force balance produces global changes in structure that result in coordinated alterations in biochemistry throughout the cytoplasm and nucleus.

Cells apparently use this mechanism, which integrates mechanics and chemistry, to produce different functional outputs given the same set of chemical inputs. They accomplish this by altering the cellular force balance and deforming the molecular scaffolds that form the continuous ECM–cytoskeleton–nuclear lattice. Altering the shape and orientation of these different molecular elements may change the relative positions of interacting regulatory components (for example, enzymes and substrates, kinases and phosphatases) that are immobilized on these scaffolds (Figure 2) and thereby, alter subsequent biochemical events. Furthermore, mechanically distorting any molecule will change its chemical potential and therefore alter its thermodynamics and kinetics [28••,68]. Stress-sensitive ion channels [69,70] and protein kinases or phosphatases in the kinetochore [66,67••] are examples of molecules that exhibit exquisite sensitivity to mechanical perturbation.

A tensegrity balance involving discrete networks of interconnected molecular filaments in the cytoskeleton, ECM and nucleus provides a means to distribute mechanical stresses simultaneously to key mechanotransducing molecules at many different locations and to tune the entire cellular response, both inside and outside the cell [28••]. This may work in the way sails are trimmed on a sailboat. By generating isometric tension, the cell effectively winches in on the interconnected molecules that feel this pull and stiffens these structures. This may explain why the behavior of stress-sensitive molecules, such as ion channels and G proteins, in liposomes [70] or in membrane patches that are torn free of normal cytoskeletal connections [69,71] is different from that in intact cells. In other words, the entire tensed cell may be both the sensing element and the controlling element that governs the ensuing response during mechanotransduction.

Acknowledgements

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