

Tensegrity II. How structural networks influence cellular information processing networks

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Summary

The major challenge in biology today is biocomplexity: the need to explain how cell and tissue behaviors emerge from collective interactions within complex molecular networks. Part I of this two-part article, described a mechanical model of cell structure based on tensegrity architecture that explains how the mechanical behavior of the cell emerges from physical interactions among the different molecular filament systems that form the cytoskeleton. Recent work shows that the cytoskeleton also orients much of the cell's metabolic and signal transduction machinery and that mechanical distortion of cells and the cytoskeleton through cell surface integrin receptors can profoundly affect cell behavior. In particular, gradual variations in this single physical control parameter (cell shape distortion) can

switch cells between distinct gene programs (e.g. growth, differentiation and apoptosis), and this process can be viewed as a biological phase transition. Part II of this article covers how combined use of tensegrity and solid-state mechanochemistry by cells may mediate mechanotransduction and facilitate integration of chemical and physical signals that are responsible for control of cell behavior. In addition, it examines how cell structural networks affect gene and protein signaling networks to produce characteristic phenotypes and cell fate transitions during tissue development.

Key words: Mechanobiology, Mechanotransduction, Biocomplexity, Bioinformatics, Integrins

Introduction

Biology and medicine are currently undergoing a paradigm shift. Up until now, we have focused on identifying the molecular components that comprise life, with the hope that rigorous characterization of all the parts will lead to understanding of the whole. As a result of sequencing the genomes of multiple organisms, including the human, it is now clear that there is more to the equation: the whole is truly greater than the sum of its parts. Thus, biology is shifting away from reductionism and towards the development of methods and approaches necessary to deal with 'biocomplexity'. The challenge is to understand how complex cell and tissue behaviors emerge from collective interactions among multiple molecular components at the genomic and proteomic levels and to describe molecular processes as integrated, hierarchical systems rather than isolated parts.

Another driving force behind this paradigm shift is the resurgence of interest in mechanical forces, rather than chemical cues, as biological regulators. Clinicians have come to recognize the importance of mechanical forces for the development and function of the heart and lung, the growth of skin and muscle, the maintenance of cartilage and bone, and the etiology of many debilitating diseases, including hypertension, osteoporosis, asthma and heart failure. Exploration of basic physiological mechanisms, such as sound sensation, motion recognition and gravity detection, has also demanded explanation in mechanical terms. At the same time, the introduction of new techniques for manipulating and probing individual molecules and cells has revealed the

importance of the physical nature of the biochemical world. Enzymes such as RNA polymerase generate as much force as molecular motors (Mehta et al., 1999); cells exert tractional forces on microparticles greater than those that can be applied by optical tweezers (Schmidt et al., 1993); and behaviors required for developmental control, including growth, differentiation, polarity, motility, contractility and programmed cell death, are all influenced by physical distortion of cells through their extracellular matrix (ECM) adhesions (Folkman and Moscona, 1978; Ben-Ze'ev et al., 1980; Ingber et al., 1986; Li et al., 1987; Ben-Ze'ev et al., 1988; Ingber and Folkman, 1989; Opas, 1989; Ingber, 1990; Mochitate et al., 1991; Singhvi et al., 1994; Chen et al., 1997; Lee et al., 1997; Dike et al., 1999; Parker et al., 2002). These insights teach us that, if we truly want to explain biological regulation and to confront the complexity problem, we must consider how molecular signaling pathways function in the physical context of living cells and tissues.

But how does a physical force applied to the ECM or cell distortion change chemical activities inside the cell and control tissue development? The answer lies in molecular biophysics; however, it also requires that we take an architectural perspective and consider both multi-molecular and hierarchical interactions (Ingber and Jamieson, 1985; Ingber, 1991; Ingber, 1997; Ingber, 1999; Chen and Ingber, 1999). First, it is critical to point out that many of the enzymes and substrates that mediate protein synthesis, glycolysis and signal transduction appear to be immobilized on insoluble molecular networks within the cytoskeleton that provides shape to the cell (Ingber,

1993; Wang et al., 1997; Janmey, 1998). Regulatory molecules involved in DNA synthesis and RNA processing similarly associate with analogous scaffolds in the nucleus (Pienta et al., 1991). This type of 'solid-state' biochemistry differs from our conventional view of cell regulation because it is not diffusion limited and insoluble biochemical activities may be regulated independently from those that act freely in the cytosol. Moreover, an analogous system is observed at the tissue level, where growth factors and tissue-remodeling enzymes are similarly immobilized on insoluble ECM scaffolds (Folkman et al., 1988) or on the external surface of transmembrane ECM receptors (Boger et al., 2001).

In Part I of this two-part Commentary, published in the last issue of JCS (Inger et al., 2003), I described how a mechanical model of cell structure based on tensegrity architecture (Fuller, 1961) explains how the mechanical behavior of cells emerges from collective interactions among multiple molecular filaments within the cytoskeleton. A mathematical formulation of this theory has led to a priori predictions of static and dynamic mechanical behaviors that closely mimic those exhibited by living cells. The model also revealed that the critical determinants behind the emergence of these complex behaviors are architecture (three-dimensional arrangement of the elements) and the level of prestress (isometric tension) in the cytoskeleton. Both of these parameters are, in turn, controlled by adhesive (mechanical) interactions between cells and ECM. In addition, the tensegrity model provides a physical basis to explain the behavior of molecules in context of the structural hierarchies in which they normally function (i.e. multicellular organisms composed of organs, tissues and cells). Here, in Part II of the article, I discuss how combined use of tensegrity and solid-state mechanochemistry by cells might mediate mechanotransduction and integrate the various physical and chemical signals that are responsible for control of cell behavior. I also explore how these structural networks influence cellular information-processing networks to produce characteristic phenotypes and cell fates during tissue morphogenesis.

Adhesion receptors as mechanoreceptors

It has been known for more than a century that mechanical forces influence pattern formation in various tissues, organs and organisms (Wolff, 1892; Koch, 1917; Thompson, 1952). Although it is clear that individual cells mediate the response to stress, the molecular basis of mechanotransduction – the mechanism by which mechanical forces are transduced into a biochemical response – has remained an enigma. Past models of mechanoregulation assumed that mechanical forces influence cell behavior as a result of generalized distortion of the cell membrane. However, many types of signaling molecule, including various stretch-sensitive ion channels, protein kinase C, focal adhesion kinase (FAK), extracellular signal-regulated protein kinase (ERK), Rho, heterotrimeric G proteins and adenylyl cyclase, can be involved in the chemical signaling response that is elicited by a mechanical stimulus (Ingber, 1991; Ingber, 1997; Davies, 1995; Janmey, 1998; Chicurel et al., 1998a; Alenghat and Ingber, 2002). Moreover, most of these are intracellular, and even the ion channels that are exposed on the cell membrane do not appear to sense physical forces under physiological conditions directly.

Instead, the stress-sensitive channels seem to sense mechanical signals from inside the cell, through their molecular linkages to the cytoskeleton (reviewed in Alenghat and Ingber, 2002).

The tensegrity model of cell structure described in Part I of the article explains how the cytoskeleton responds to mechanical stress (Ingber, 2003). In the model, cells generate their own internal tension or prestress in the actin cytoskeleton, which is balanced by internal microtubule struts and external ECM adhesions. In other words, adherent cells exist in a state of isometric tension, and thus any external mechanical load is imposed on a pre-existing cellular force balance. Thus, the cellular response to stress may differ depending on the level of tension in the cell, much like tuning a guitar string alters the tone it creates when strummed. The tensegrity model is a mechanical paradigm, and hence it does not per se explain chemical behavior in living cells. However, it does provide a mechanism to distribute and focus mechanical forces on distinct molecular components throughout the cell.

Because the tensegrity model indicates that the molecular filament networks that form the cytoskeleton and link to transmembrane adhesion receptors are the primary load-bearing elements in the cell, it provides a potential mechanism to link mechanical stresses applied at the tissue and organ level to changes in molecular chemistry inside the cell. Specifically, pursuit of the tensegrity theory led to the concept that mechanical signals that are transferred across cell surface ECM receptors, such as integrins (Wang et al., 1993), can be transduced into a chemical response through distortion-dependent changes in cytoskeletal structure either locally at the site of receptor binding or distally at other locations inside the cell (Ingber and Jamieson, 1985; Ingber, 1991; Ingber, 1997).

In fact, results from many studies with various cell types and model systems show that mechanical stress application to integrins can alter cytoskeletal structure and activate signal transduction and gene expression in a stress-dependent manner (Wang et al., 1993; Schmidt et al., 1993; Wilson et al., 1995; Yano et al., 1996; Chen and Grinnell, 1997; Glogauer et al., 1997; D'Angelo et al., 1997; Salter et al., 1997; Chicurel et al., 1998b; Lynch et al., 1998; Pavalko et al., 1998; Low and Taylor, 1998; Chen et al., 1999; Schwartz et al., 1999; Meyer et al., 2000; Lee et al., 2000; Wozniak et al., 2000; Chen et al., 2001; Jalali et al., 2001; Wang et al., 2001b; Maroto and Hamill, 2001; Goldschmidt et al., 2001; Riveline et al., 2001; Liu et al., 2002; Urbich et al., 2002). These studies also show that mechanochemical transduction proceeds, at least in part, on the cytoskeletal backbone of the focal adhesion complex that forms at the site of integrin binding (Glogauer et al., 1997; Chicurel et al., 1998b; Chen et al., 1999; Meyer et al., 2000; Wozniak et al., 2000; Riveline et al., 2001). Analysis of mechanical signaling within the motor nerve terminal similarly reveals that stress-dependent activation of calcium signaling is mediated by integrins and the effect is so rapid (<10 mseconds) that forces must be transferred directly from the integrins to adjacent calcium channels in the synaptic adhesion complex (Chen and Grinnell, 1997). Mechanical forces applied to integrins, but not to control transmembrane proteins such as metabolic acetylated-low density lipoprotein (Ac-LDL) receptors, growth factor receptors or HLA antigens, also activate intracellular cAMP signaling and recruit components of the protein synthesis machinery (e.g. ribosomes and polyA-mRNAs) directly at the site of integrin binding (Chicurel et al.,

1998b; Meyer et al., 2000; Riveline et al., 2001). In addition, forces applied to integrins produce a stress-dependent increase in focal adhesion assembly that is mediated through the small GTPase, Rho (Riveline et al., 2001; Balaban et al., 2001; Galbraith et al., 2002), and the sustained response of endothelium to fluid shear stress appears to require continuous formation of new integrin-binding complexes (Jalali et al., 2001). Importantly, stress-activated signals transmitted by integrins are distinct from signals elicited by integrin receptor clustering alone (Chicurel et al., 1998b; Meyer et al., 2000), although both signaling mechanisms appear to require focal adhesion formation and, thus, associated cytoskeletal rearrangements (Plopper et al., 1995; Miyamoto et al., 1995).

Cytoskeletal restructuring in response to external stress through integrins also normally requires maintenance of cytoskeletal tension generation (i.e. cellular prestress) (Chicurel et al., 1998b; Wang et al., 2001a; Balaban et al., 2001). Forces transmitted across transmembrane integrin receptors thus appear to be converted into chemical and electrical signals inside the cell as a result of their transmission across discrete cytoskeletal linkages and associated changes in the cytoskeletal force balance (Fig. 1).

Although there has been less emphasis on the role of other transmembrane adhesion receptors in mechanotransduction, it is clear that they also preferentially mediate transmembrane force transfer to the internal cytoskeleton relative to non-

Mechanotransduction through integrins

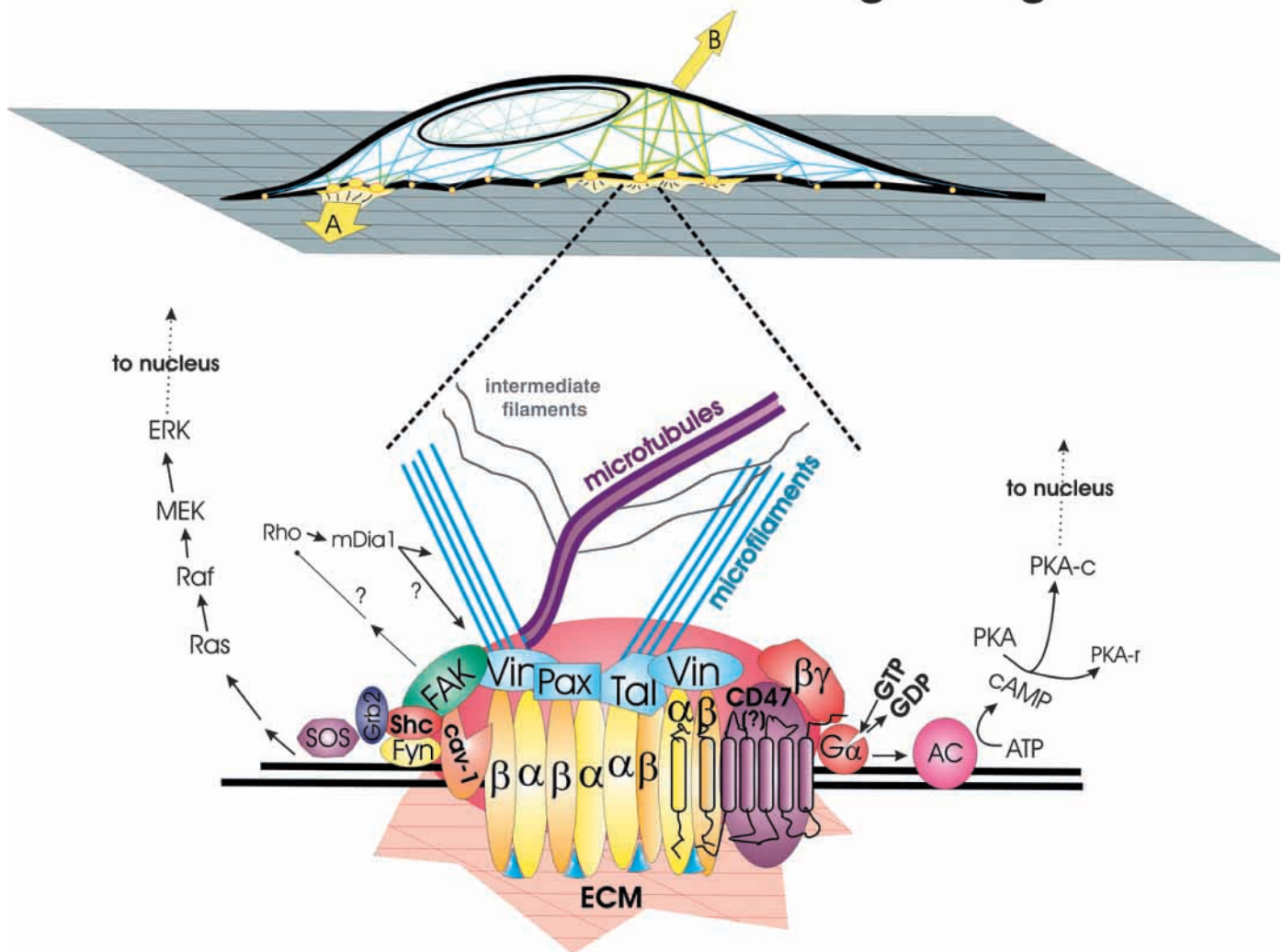


Fig. 1. A schematic diagram of how forces applied via the ECM (A) or directly to the cell surface (B) travel to integrin-anchored focal adhesions through matrix attachments or cytoskeletal filaments, respectively. Internally generated tension and forces transmitted via cell-cell contact similarly reach focal adhesions through the cytoskeleton. Forces concentrated within the focal adhesion (magnified at bottom of the figure) can stimulate clustering of dimeric ($\alpha\beta$) integrin receptors and induce recruitment of focal adhesion proteins [e.g. Vinculin (Vin), Paxillin (Pax), Talin (Tal)] that connect directly to microfilaments and indirectly to microtubules and intermediate filaments (certain integrins can also connect directly to intermediate filaments, for example, within hemidesmosomes). Forces applied to this specialized cytoskeletal adhesion complex activate integrin-associated signaling cascades, which among others, include such protein as focal adhesion kinase (FAK), extracellular signal-regulated protein kinase (ERK), Shc, Rho, mDia1, caveolin-1 (cav-1), CD47, heterotrimeric G-proteins, adenylate cyclase (AC) and protein kinase A (PKA).

adhesion receptors. For example, E-selectin mechanically couples to the internal cytoskeleton by forming a specialized adhesion complex that shares some components with focal adhesions (Yoshida et al., 1996). Cadherins that link to the actin cytoskeleton also mediate transmembrane mechanical coupling in epithelial cells (Potard et al., 1997). Thus, similar mechanochemical conversion mechanisms may proceed in junctional adhesion complexes and focal adhesions; however, this remains to be demonstrated directly.

Mechanical and chemical signal integration at the whole cell level

One of the most important insights from the tensegrity model is that it suggests that mechanoregulation is not based on changes in the activity of any single mechanoreceptor or transduction molecule. Instead, mechanical signal processing and integration proceeds at the level of the whole cell (Ingber, 1999), because this is the level at which the cellular force balance is established (Ingber, 2003). For example, application of mechanical stress to integrins produces the same intracellular increase in cAMP within both round and spread endothelial cells (Meyer et al., 2000). Yet, the round cells integrate this signal with other inputs and switch on an apoptosis response, whereas the spread cells proliferate (Fig. 2) (Chen et al., 1997). Apoptosis rates also differ depending on the three-dimensional organization of tissue architecture, which again is associated with changes in cell shape and ECM mechanics (Weaver et al., 2002). Other cellular behaviors, including differentiation, motility, and contractility, can be similarly altered by changing cell shape or ECM rigidity (Li et al., 1987; Ben-Ze'ev et al., 1988; Opas, 1989; Singhvi et al., 1994; Mochitate et al., 1995; Lee et al., 1997; Dike et al., 1999; Parker et al., 2002). In general, in the case of most adherent cells, spread cells grow, retracted cells differentiate and fully round or detached cells undergo apoptosis, even though all may be stimulated with optimal levels of growth factors and ECM binding (Fig. 2). Thus, large-scale distortion of the cell produces signals that are distinct from those elicited by binding or stressing individual adhesion receptors. In this manner, the physical state of the whole cell facilitates higher-order signal integration that ultimately determines the physiological response.

Analysis of the mechanism by which cell distortion switches cells between different phenotypes has confirmed that cytoskeletal structure and prestress both contribute to this response. Cell cycle progression and motility (lamellipodia formation) can be inhibited by disruption of the actin cytoskeleton or inhibition of cytoskeletal tension generation (Iwig et al., 1995; Bohmer et al., 1996; Huang et al., 1998; Parker et al., 2002), whereas cytoskeletal disruption alone promotes apoptosis (Flusberg et al., 2001). Dissipation of cytoskeletal prestress also abrogates the effect of mechanical stress on gene expression in endothelial cells (Chen et al., 2001). In addition, ECM-dependent changes in the cellular force balance alter cytoskeletal tension generation (T. Polte and D.E.I., unpublished), cytoskeletal structure (Mochitate et al., 1991) and focal adhesion formation (Balaban et al., 2001), as well as cell growth, differentiation, motility and apoptosis in response to chemotherapeutic agents (Li et al., 1987; Ben Ze'ev et al., 1988; Opas, 1989; Mochitate et al., 1991; Weaver

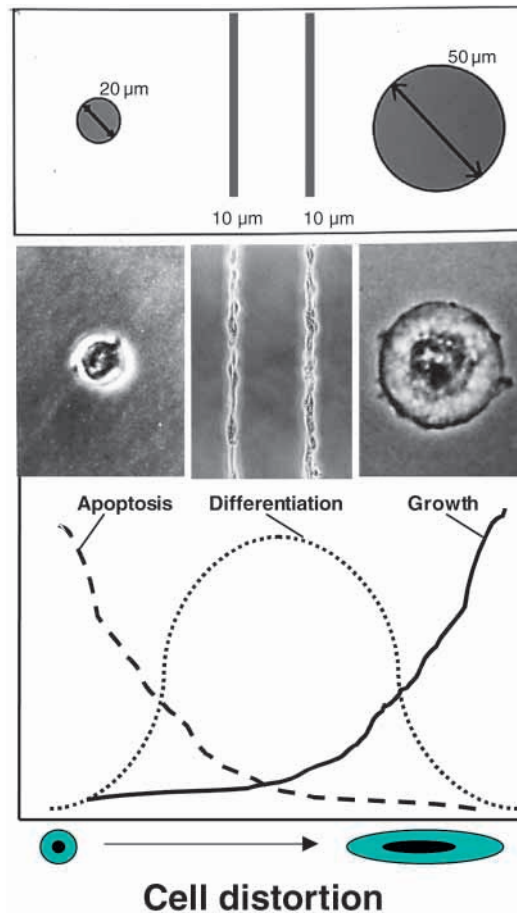


Fig. 2. Cell distortion-dependent switching between distinct cell fates. (Top) Phase contrast microscopic images of capillary endothelial cells whose shape, size and orientation were controlled using the corresponding micropatterned adhesive substrate designs (20 or 50 μm circles or 10 μm wide lines) shown above in gray that were coated with a constant fibronectin density. Although cells were cultured in the same growth-factor-containing medium, well spread cells on large circles proliferated, retracted cells on the small circles underwent apoptosis, and cells on linear patterns that exhibited an intermediate degree of spreading differentiated into hollow capillary tubes (for details, see Chen et al., 1997; Dike et al., 1999). (Bottom) A schematic summary of cell-distortion-dependent switching between growth, differentiation and apoptosis that has been demonstrated using micropatterned substrates of different size in various cell types, as described in the text.

et al., 2002). These findings are consistent with the use of tensegrity by cells, and they show that, although local forces can produce local responses in cells (e.g. focal adhesion formation) (Chicurel et al., 1998b; Riveline et al., 2001; Balaban et al., 2001), global structural alterations at the level of the whole cytoskeleton govern the cell's physiological response to mechanical stress. A medically relevant example is the application of fluid shear stress to cultured endothelial cells. This activates multiple signal transduction pathways; however, signaling stops once the cells remodel their cytoskeletons and realign themselves with the flow, even though they experience the same shear stress on their surface membranes (Davies, 1995). Thus, it is the ability of the

endothelium to align its cytoskeleton along physiological stress field lines that prevents pathological responses, such as expression of inflammatory cytokines, under normal flow conditions.

Mechanochemistry at the molecular level

So how does a mechanical stress applied to the cell surface get transduced into a chemical response inside the cell? If cells use tensegrity, then, when a distending force is applied to cell surface adhesion receptors, the mechanical load will be transferred to linked cytoskeletal elements. These internal load-bearing filaments in the cytoskeleton will either distort or break. Experiments show that cytoskeleton-receptor linkages do not break when forces are applied to integrins, whereas breakage is observed when the same stress is applied to transmembrane metabolic (Ac-LDL) receptors (Wang et al., 2001a). If the cytoskeletal filaments and associated regulatory molecules distort without breaking when integrins are stressed, then some or all of the molecules that comprise these structures must similarly change shape.

When the shape of a molecule is altered, its biophysical properties change. For example, theoretical studies predict that extending or decompressing a microtubule will change the critical concentration of tubulin (a thermodynamic parameter that controls the balance between monomer and polymer) and thereby promote microtubule assembly (Hill and Kirschner, 1982). If cells use tensegrity, then ECM tethers and microtubule struts function in a complementary manner to resist cytoskeletal tension [Fig. 3 top; also see Fig. 2B in Part I (Ingber, 2003)]. Thus, when integrins are pulled, microtubules will be decompressed, and microtubule polymerization should be promoted (Fig. 3 bottom). In fact, this precise response has been demonstrated in various types of cultured cell, including nerve cells and smooth muscle cells (Joshi et al., 1985; Buxbaum and Heidemann, 1988; Dennerll et al., 1988; Dennerll et al., 1989; Putnam et al., 1998; Putnam et al., 2001; Kaverina et al., 2002). Although altering stresses across integrins does not alter microtubule polymerization in liver cells, the steady-state level of soluble tubulin monomer changes in a manner consistent with a similar stress-induced change in the critical concentration of tubulin (Mooney et al., 1994). Mechanical forces transmitted across adhesion receptors may therefore alter intracellular biochemistry by altering thermodynamic parameters locally (i.e. within load-bearing cytoskeletal scaffolds) in living cells (Ingber and Jamieson, 1985; Ingber, 1997). A similar form of mechanochemistry may occur on ECM scaffolds in the extracellular milieu, as shown, for example, by the requirement of

cell tension for fibronectin fibril assembly (Schwarzbauer and Sechler, 1999).

Altering molecular shape through application of stress to integrins and cytoskeletal filaments might also alter kinetic parameters. Imagine a spring fixed at its base that vibrates at a certain frequency: change the size, shape or center of gravity of this spring, and its vibration frequency will be altered, much like a metronome. Changing the shape of the molecule (e.g. through cytoskeletal distortion) will similarly alter its kinetic behavior and, hence, alter its chemical rate constant (Fig. 3 bottom). When stress-sensitive ion channels experience mechanical stress through their cytoskeletal linkages, they similarly alter the rate of their opening or closing. The use of tensegrity and prestress by proteins to stabilize their shapes at the molecular level (Ingber, 2003) may facilitate this response and provide a means to couple physical distortion owing to large-scale forces, such as gravity, at the macroscopic (tissue and organ) level to molecular shape changes on the nanometer

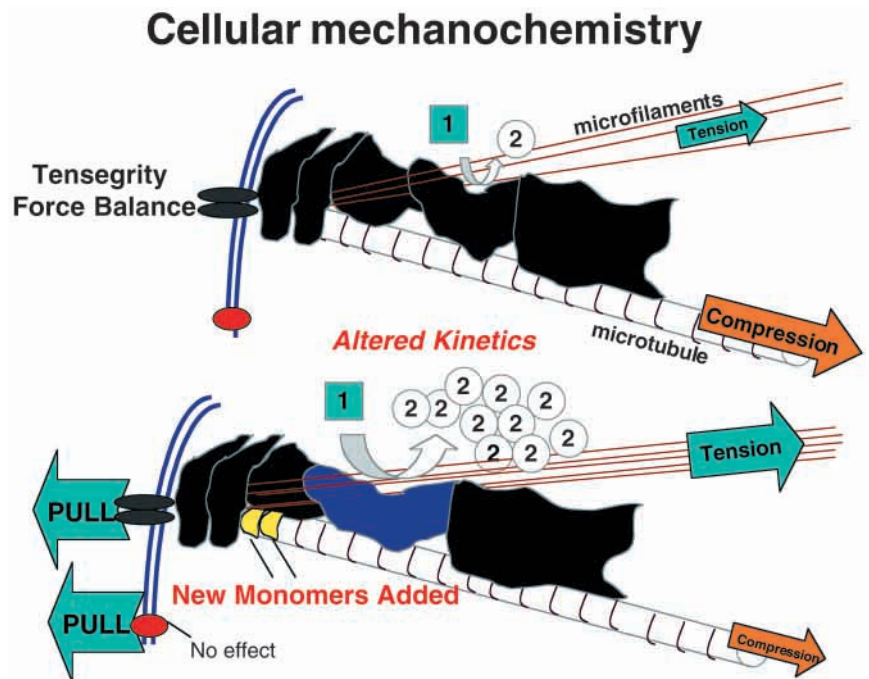


Fig. 3. Contribution of cellular tensegrity to mechanochemical transduction. (Top) A schematic diagram of the tensegrity-based complementary force balance between tensed microfilaments, compressed microtubules and transmembrane integrin receptors (gray oval dimer) in living cells (intermediate filaments are not shown for simplicity) (for details, see Ingber, 2003). Black forms indicate regulatory proteins and enzymes that are physically immobilized on load-bearing cytoskeletal filaments; red oval represents a transmembrane protein that does not link to the internal cytoskeletal lattice. (Bottom) When force is applied to integrins, thermodynamic and kinetic parameters change locally for cytoskeleton-associated molecules that physically experience the mechanical load; when force is applied to non-adhesion receptors that do not link to the cytoskeleton, stress dissipates locally at the cell surface, and the biochemical response is muted. In this diagram, new tubulin monomers add onto the end of a microtubule (yellow symbols) when tension is applied to integrins, and the microtubule is decompressed as a result of a change in the critical concentration of tubulin. The blue form indicates a molecule that is physically distorted by stress transferred from integrins to the cytoskeleton and, as a result, changes its kinetics (increases its rate constant for chemical conversion of substrate 1 into product 2). In this manner, both cytoskeletal structure (architecture) and prestress (tension) in the cytoskeleton may modulate the cellular response to mechanical stress.

scale, resulting in altered chemical reaction rates (Ingber, 1999; Chen and Ingber, 1999).

Tissue morphogenesis in context

How could such mechanical mechanisms contribute to tissue patterning? Again, one must consider molecular regulation in the physical context of the whole living tissue. Analysis of the mechanisms of epithelial morphogenesis and angiogenesis has revealed that tissue remodeling is mediated by local changes in ECM structure and mechanics. Regions of the basement membrane at the tips of growing epithelial buds (Bernfield and Banerjee, 1978) and new capillary sprouts (Ausprunk and Folkman, 1977) become thinner owing to high ECM turnover. Thinning of the ECM scaffold will result in an increase in its mechanical compliance. Because the basement membrane is tensed because of tensegrity and the existence of a stabilizing prestress at the tissue level (Ingber, 2003), this local region may stretch out more than adjacent regions, much like a 'run' in a woman's stocking. Thus, a cell adhering to the thinned (stretched) region of the basement membrane would experience a tug on its cell surface integrins, whereas its neighbors on intact basement membrane would not (Fig. 4). In other words, this local alteration in ECM stiffness would change the cellular force balance and alter internal cytoskeletal structure within a subset of cells. This, in turn, would permit these cells to respond to soluble mitogens and motogens by switching from quiescence to growth and motility locally, and thereby drive tissue patterning (Fig. 4). Reiteration of this process along newly formed buds would then result in formation of fractal-like patterns that characterize all growing tissues (Ingber and Jamieson, 1985; Huang and Ingber, 1999).

Thus, although macroscale forces may be an obvious cause of tissue patterning in bone (Koch, 1917) and large muscles, variations in force distribution on the microscale may similarly guide morphogenesis in other living tissues (Ingber and Jamieson, 1985; Huang and Ingber, 1999). This possibility is supported by the recent finding that epithelial branching morphogenesis in embryonic lung can be selectively inhibited or accelerated by preventing or enhancing tension generation (cytoskeletal prestress), respectively (Moore et al., 2002). Application of tensional forces through the ECM also directly promotes capillary outgrowth (Korff and Augustin, 1999) as well as axon elongation in nerve cells (Bray, 1984). In fact, the tensegrity principle could explain pattern formation in various tissues and organs in species ranging from mammals (Ingber and Jamieson, 1985; Joshi et al., 1985; Van Essen, 1997; Galli-Resta, 2002) to paramecium (Kaczanowska et al., 1995) and fungi (Kaminsky and Heath, 1996), as well as loss of tissue morphology during cancer formation (Ingber et al., 1981; Ingber and Jamieson, 1985; Pienta and Coffey, 1991). It also may provide a molecular basis for gravity sensing (Ingber, 1999; Yoder et al., 2001) and control of circadian rhythmicity (Shweiki, 1999) in both animals and plants. In addition, tensegrity may help to explain why cellular components that are not directly involved in actomyosin-based tension generation, such as microtubules, intermediate filaments and ECM, can contribute significantly to contractile function in various cell types, including cardiac myocytes, vascular smooth muscle and skeletal muscle (Northover and Northover, 1993; Tsutsui et al., 1993; Lee et al., 1997; Tagawa et al., 1997;

Mechanical control of morphogenesis

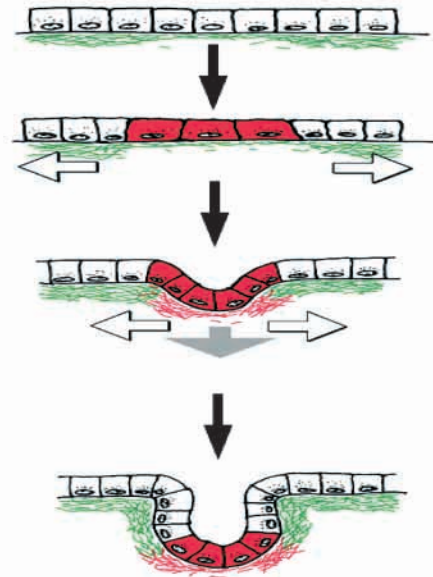


Fig. 4. A schematic showing how local changes in ECM mechanics may guide tissue patterning. The local thinning in the ECM produced by accelerated ECM turnover will increase ECM compliance and result in local cell distortion through the action of tractional forces exerted by surrounding cells. Increased tension transfer across cell surface ECM receptors (integrins) will result in coordinated changes in cell and cytoskeletal form and, thereby, produce changes in cellular biochemistry that result in the localized growth and motility that drive tissue morphogenesis. Thus, in this view, cell growth and migration are constrained to the small group of cells (red) that is underlined by the thinned region of the basement membrane (green). Outward budding results when red cells extend and grow because neighboring cells along the same basement membrane do not experience the stress and, hence, remain quiescent (white cells).

D'Angelo et al., 1997; Eckes et al., 1998; Gillis, 1999; Wang and Stamenovic, 2000; Keller et al., 2001; Balogh et al., 2002; Loufrani et al., 2002) as well as to control of permeability barrier function in endothelia (Moy et al., 1998).

Confronting the biocomplexity problem

At the start of this article, I noted that the major challenge in science today is the problem of biocomplexity. By this rather broad term, I mean the challenge of addressing how specialized behaviors emerge from collective interactions within complex molecular networks. The current approach in the 'complexity' field is to gain insight into the functioning of the whole system by mapping out the nodes in these networks, elucidating the relationships between the different components and determining the dynamics that ensue from their interactions (Kauffman, 1993). Mathematical approaches used in this area have effectively predicted phase transitions such as abrupt changes from randomness to pattern, and from deterministic to chaotic behavior. However, these approaches cannot explain how three-dimensional, hierarchical structures, such as living cells and tissues, generate their specific forms and functions.

Tensegrity similarly teaches us that we cannot consider individual molecules or molecular binding interactions in isolation. Collective behavior within supramolecular assemblies, higher-order architecture and mechanical forces also have to be considered (Ingber, 2003). Moreover, the mathematical tensegrity formulation explains how complex behaviors (in this case mechanical) can emerge through multi-component interactions or, in simple terms, how the whole can indeed be greater than the sum of its parts. Thus, work on cellular tensegrity suggests that existing ‘complexity’ theories may be limited because they fail to consider the physicality of the network (e.g. material properties of the elements that connect interacting nodes, attractive/repulsive interactions, internal force balances and three-dimensional architecture).

Tensegrity also explains how hierarchical structures may form from systems within systems (molecules within cells within tissues within organs) and yet still exhibit integrated mechanical behavior (Ingber, 2003). In addition, it reveals how robust behaviors, such as mechanical stiffness and shape stability, can be generated from ‘sloppy’ parts (e.g. flexible molecular filaments), which is a key feature of both complex networks and living systems (Csete and Doyle, 2002). In fact, tensegrity experts in control theory – a field that focuses on how the design of one component is influenced by the dynamics of all other components to achieve some global property of the system – have identified prestressed tensegrity structures as the ultimate ‘smart’ materials whose shapes can be actively adjusted and controlled (Skelton and Sultan, 1997). Both material architecture and feedback information architecture (control mechanisms) are jointly determined in these tensegrity structures because they are innate in the design, just as they are in living cells. Thus, tensegrity may represent the ‘hardware’ behind living systems.

But what about the software? This leads us to the problem of how structural networks affect information-processing networks at the level of the whole cell, where tensegrity seems to exert its effects on signal integration. Experiments show that, although individual cells can receive multiple simultaneous inputs, they can rapidly integrate these signals to produce just one of a few possible outputs or phenotypes (e.g. growth, quiescence, differentiation or apoptosis). But studies on mechanoregulation raise a fundamental question: how can a gradual change in a physical parameter over a broad range,

such as cell shape (distortion from round to spread), be translated into these distinct cell fates?

Cell biologists tend to view signal transduction in terms of linear signaling pathways that lead to one particular outcome. However, the information conveyed by the signal transduction machinery is often distributed among numerous pathways, and the same stimulus can lead to many different responses. For example, activation of a single signaling receptor can induce scores of genes (Fambrough et al., 1999), and the same signaling molecule may elicit entirely different effects (e.g. growth versus apoptosis), depending on the cell type, the activity state of other regulatory proteins and the physical context in which it acts. Thus, the concept of linear signaling pathways is inappropriate (Strohman, 1997; Coffey, 1998). Instead, these characteristic phenotypes that cells exhibit during development represent emergent behaviors that arise within a complex signaling network comprising many interacting components.

The observation that gradual variations in a single control parameter (cell shape) can switch cells between distinct gene programs (cell fates) is reminiscent of a phase transition in physics. Gradual changes in temperature, for example, produce abrupt macroscopic changes between qualitatively discrete stable states (e.g. liquid versus gas or solid). Sui Huang in my group, therefore, explored the possibility that cell fates can be viewed as ‘cellular states’ and that the switches between these states may represent biological phase transitions (Huang, 1999; Huang and Ingber, 2000). To explain this type of qualitative behavior, he viewed the cell’s molecular signaling machinery as a dynamic information-processing network. In this manner, he was able to describe the collective behavior of the cell’s signaling molecules and their relationship to cell fate switching without focusing on the properties of the individual molecular components. This path led to the suggestion that cell fates can be viewed as common end-programs or ‘attractors’ that self-organize within the cell’s dynamic regulatory networks (Huang, 1999). To visualize attractors, think of a ball traveling on a complex landscape, where stable cell states are represented by valleys (‘basins of attraction’) separated by unstable transition regions or ‘mountainous’ terrain (Fig. 5). A ball (or cell) located at the lowermost point in one of these valleys (the attractor) will tend to remain there. Displacement to another part of the landscape will move the ball away from

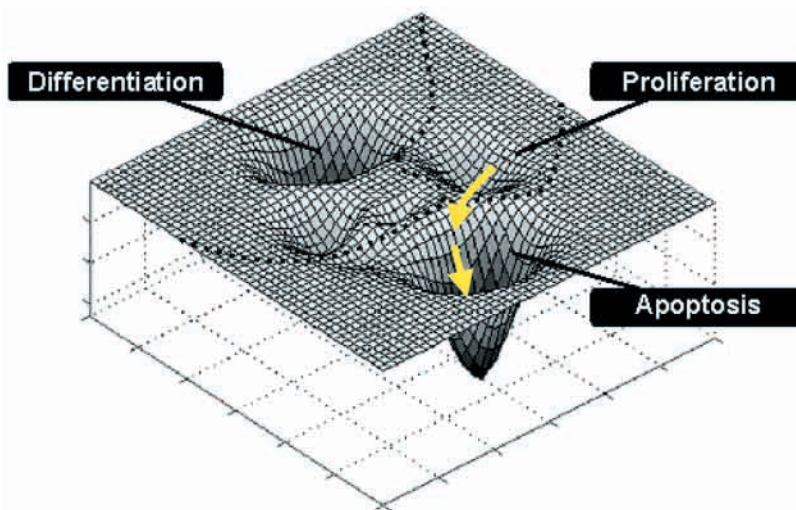


Fig. 5. Attractor landscape representation of cell fate determination. A hypothetical ‘potential landscape’ that represents the n -dimensional state space compressed into two dimensions (XY) for visualization purposes. Every position in the XY plane would correspond to a network state (e.g. expression profile of gene and protein activities). The vertical axis (Z) represents a potential function, an ‘energy equivalent’, representing some distance measure of a network state to the attractor state. Lowest points in the valleys correspond to attractor states that represent cell fates. Yellow arrows indicate a path that takes the cell from growth to apoptosis.

the valley, but small perturbations will generally cause it to roll back down to its own starting point in the same valley. Under the influence of a larger perturbation, however, the ball could move over a mountainous peak in the landscape. At this point it is irrevocably committed to rolling down the other side of the hill until it reaches another attractor in a neighboring valley and, hence, takes on a different stable phenotype. Interestingly, Waddington used this very metaphor over 40 years ago to explain developmental control (Fig. 6), without knowing about genes, interactions or regulatory networks (Waddington, 1956).

The challenge here is that these hills and valleys are not physical structures or energy wells (as in potential energy diagrams), rather they respectively represent unstable and stable states relative to the cell's dynamic information-processing network. The formation of the attractors is an emergent property that depends on the dynamic constraints imposed by the functional interconnections (e.g. gene-gene, gene-protein or protein-protein interactions) in the network. For example, when activated by some stimulus, the internal information state of the cell will shift through various cell states (e.g. gene and protein activation profiles) depending on specific pre-programmed regulatory interactions between the various signaling components that make up its signaling network (e.g. protein A will inhibit B, C will stimulate D, and E will turn on if F and G are both present). Because these interactions are hard-wired, certain states are impossible. For example, A and B cannot both be activated simultaneously if one inhibits the expression of the other; conversely, C and D must both turn on if one activates the other. The key is that these regulatory interactions constrain how these networks change over time. Extensive analysis of theoretical networks has revealed that even highly complex networks will dynamically change until they converge on a limited number of possible common end states – these are the attractors (Kauffmann, 1993).

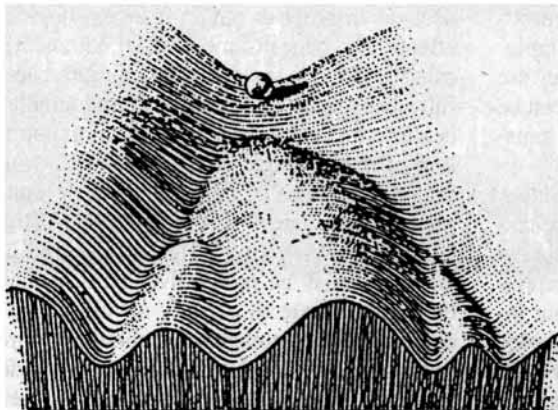


Fig. 6. Cell fate switching in an epigenetic landscape, as portrayed by Waddington (Waddington, 1956). In this view of embryonic regulation, cell fate regulation is based on selection between pre-existing, intrinsically robust fates. The dynamics of this developmental selection is represented as a landscape with hills and valleys. The phenotypic state of a cell at any time is indicated by the position of a marble on that landscape. The marble will spontaneously roll down the valleys (stable developmental paths) leading to a distinct phenotype. The lowest points in the valleys correspond to the distinct, stable phenotypes within a given repertoire of fates that the cell may experience.

Computer simulations of dynamic networks reveal that it is necessary to alter the activities of multiple network elements in order to switch the network between different attractor states (Huang, 2000). Phenotypic transitions in living cells should therefore require simultaneous modification of the activity status of multiple regulatory molecules. This prediction is consistent with the observation that external stimuli that trigger pleiotropic effects, such as soluble mitogens, ECM adhesion and cell distortion, can induce similar cell fate transitions (Huang and Ingber, 1999). It should be noted that the few 'master switch' genes that have been identified, such as MyoD (Walsh and Perlman, 1997) or PPAR γ (Morrison and Farmer, 1999), do not drive standard phenotypic transitions between growth, apoptosis, etc. Rather, they trigger a transition to the differentiation attractor of an entirely different cell type, and even these trigger genes must activate a large set of other genes to produce this effect.

The possibility that attractors exist in cellular information-processing networks is supported by the observation that various stimuli that activate multiple proteins across several signaling pathways often can trigger the same cellular phenotypes. For example, differentiation can be switched on by non-specific agents (e.g. DMSO or ethanol) in many cell types (Sprengelli and Dexter, 1984; Messing, 1993; Yu and Quinn, 1994), and general inhibitors of protein kinases or phosphatases may both induce apoptosis (Jacobson et al., 1993; Hehner et al., 1999). Similarly, growth factors, ECM and cell distortion all regulate the same cell cycle intermediates, such as cyclin D1 (Baldin et al., 1993; Bohmer et al., 1996; Huang et al., 1998). Thus, simultaneous perturbation of multiple targets in different pathways results in the channeling of the biochemical effects into common end-programs and hence expression of the same set of distinct cell fates or attractor states. In fact, mathematical models that incorporate information relating to known regulatory interactions between different growth signaling molecules generate a cell 'cycle' as well as different quiescent (G_0) states that are highly reminiscent of those displayed by living cells (Huang and Ingber, 2000). Data supporting the existence of attractors within the genomic regulatory networks in human cells also have been recently obtained by using massively parallel gene profiling techniques (S. Huang, G. Eichler and D.E.I., unpublished).

Thus, in contrast to existing paradigms that rely on explanations in terms of specific factors and linear signaling pathways, the functional state of the cell appears to 'self-organize' as a result of the architecture and dynamics of its underlying regulatory network. In this context, tensegrity-based changes in cytoskeletal structure may influence cell phenotype switching on the basis of their ability simultaneously to alter the biochemical activities of multiple cytoskeleton-associated signaling components throughout the cell. Because it provides a structural basis for the formation of functionally integrated molecular hierarchies, tensegrity might also have played a central role in the origin of cellular life (Ingber, 2000b). Thus, in the future, it will be interesting to combine the mathematical formulation of the tensegrity theory described in Part I of this article (Ingber, 2003) with dynamic network models to explore how these different types of biological network – one mechanical and the other informational – coevolved so as to allow the cell to function with the incredible efficiency it does.

Conclusion

Tensegrity is a design principle that describes how network structures develop shape stability (Fuller, 1961). Because tensegrity also defines how cells, tissues and organs stabilize themselves mechanically (Ingber, 2003), it has a direct impact on how mechanical stresses applied at the macroscopic level can influence molecular structure and function inside living cells. In fact, the key determinants of tensegrity – architecture and prestress – appear to be critical governors of the cell's biochemical response to stress. Exploration of the potential role of tensegrity in mechanoregulation also led to the discovery that integrins receptors that physically couple the ECM to the internal cytoskeleton play a central role as mechanoreceptors and mediators of mechanochemical transduction. In particular, pursuit of the tensegrity theory has revealed previously unrecognized roles of ECM, cytoskeletal structure and cytoskeletal tension (prestress) in the control of gene expression, growth, differentiation, apoptosis, contractility, directional motility and tissue patterning. These insights may have important ramifications in areas, such as angiogenesis, cardiovascular physiology and cancer, where cytoskeletal elements and cell surface adhesion receptors now offer potential new sites for therapeutic intervention (Ingber, 2002a; Ingber, 2002b). Understanding of the critical roles that ECM compliance and mechanical forces play in cell and tissue regulation is also beginning to impact the design and fabrication of synthetic biomaterials for tissue engineering.

The dominant view in cell biology is that cell behavioral control is governed by soluble factors and insoluble adhesive ligands, which exert their effects by ligating cell surface receptors and thereby activating signal transduction cascades inside the cell. The tensegrity model incorporates this concept but overlays a mechanism whereby changes in the balance of mechanical forces across transmembrane adhesion receptors that link to the cytoskeleton can provide additional regulatory signals to the cell. Moreover, although signal transduction is usually described in terms of linear pathways, the functional state of the cell appears to self-organize as a result of the architecture and dynamics of its underlying gene and protein regulatory networks. Computer simulations of dynamic networks suggest that multiple targets in different pathways must be simultaneously perturbed to switch the network between a limited number of different stable end-programs (attractor states), such as growth, differentiation and apoptosis. Mechanical distortion of living cells (a generalized stimulus) and binding of specific growth factors and ECM proteins to their respective cell surface receptors all switch cells between these same discrete cell fates. The tensegrity model suggests that it is precisely because force-induced changes in cytoskeletal mechanics and chemistry can alter the activities of many signaling components at once that generalized cell distortion can produce these same discrete changes in cellular phenotype. The tensegrity principle also provides another perspective on the complexity problem in that cell mechanical behaviors similarly appear to self-organize through collective network interactions, but in this case through use of mechanical (cytoskeletal) networks, rather than gene or protein signaling networks.

In conclusion, perhaps the greatest impact of the tensegrity model is based on how it has helped to change the frame of reference in cell biology. In the past, we focused exclusively

on the molecular components. In contrast, tensegrity describes how molecules function collectively as components of integrated, hierarchical systems in the physical context of living cells and tissues. It also further expands the frame of reference by adding 'tone' (tension) and 'architecture' (three-dimensional design) into the calculation. This shift in perspective has led to explanations for behaviors that could not be explained with conventional reductionist paradigms. The mathematical formulation of tensegrity theory described in Part I of this Commentary (Ingber, 2003), while rudimentary, also represents a computational approach that can be used to confront the complexity challenge from a structural perspective. It already has been successfully used to explain how complex mechanical behaviors emerge from multi-component interactions within cytoskeletal networks. Mathematical descriptions of dynamic networks similarly provide insights into system-wide information processing behaviors at the genomic and proteomic levels. The challenge now is to use these tools to gain greater insight into the underlying principles that govern cell function and, in the future, to unite these approaches to create a more unified description of biological regulation.

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References

- Alenghat, F. J. and Ingber, D. E. (2002). Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. *Sci. STKE*. **119**, PE6.
- Ausprunk, D. H. and Folkman, J. (1977). Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvasc. Res.* **14**, 53-65.
- Balaban, N. Q., Schwarz, U. S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L. and Geiger, B. (2001). Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat. Cell Biol.* **3**, 466-472.
- Baldin, V., Lukas, J., Marcote, M. J., Pagano, M. and Draetta, G. (1993). Cyclin D1 is a nuclear protein required for cell cycle progression in G1. *Genes Dev.* **7**, 812-821.
- Balogh, J., Merisckay, M., Li, Z., Paulin, D. and Arner, A. (2002). Hearts from mice lacking desmin have a myopathy with impaired active force generation and unaltered wall compliance. *Cardiovasc. Res.* **53**, 439-450.
- Banerjee, S. D., Cohn, R. H. and Bernfield, M. R. (1977). Basal lamina of embryonic salivary epithelia. Production by the epithelium and role in maintaining lobular morphology. *J. Cell Biol.* **2**, 445-463.
- Ben-Ze'ev, A., Farmer, S. R. and Penman, S. (1980). Protein synthesis requires cell surface contact while nuclear events respond to cell shape in anchorage-dependent fibroblasts. *Cell* **21**, 365-372.
- Ben-Ze'ev, A., Robinson, G. S., Bucher, N. L. and Farmer, S. R. (1988). Cell-cell and cell-matrix interactions differentially regulate the expression of hepatic and cytoskeletal genes in primary cultures of rat hepatocytes. *Proc. Natl. Acad. Sci. USA* **85**, 2161-2165.
- Bernfield, M. R. and Banerjee, S. D. (1978). The basal lamina in epithelial-mesenchymal interactions. In *Biology and Chemistry of Basement Membranes* (ed. N. Kefalides), pp. 137-148. New York: Academic Press.
- Boger, D. L., Goldberg, J., Silletti, S., Kessler, T. and Cheresch, D. A. (2001). Identification of a novel class of small-molecule antiangiogenic agents through the screening of combinatorial libraries which function by inhibiting the binding and localization of proteinase MMP2 to integrin alpha(V)beta(3). *J. Am. Chem. Soc.* **123**, 1280-1288.
- Bohmer, R. M., Scharf, E. and Assoian, R. K. (1996). Cytoskeletal integrity is required throughout the mitogen stimulation phase of the cell cycle and mediates the anchorage-dependent expression of cyclin D1. *Mol. Biol. Cell* **7**, 101-111.

- Bray, D. (1984). Axonal growth in response to experimentally applied mechanical tension. *Dev Biol.* **102**, 379-389.
- Buxbaum, R. E. and Heidemann, S. R. (1988). A thermodynamic model for force integration and microtubule assembly during axonal elongation. *J. Theor. Biol.* **134**, 379-390.
- Chen, B. M. and Grinnell, A. D. (1997). Kinetics, Ca²⁺ dependence, and biophysical properties of integrin-mediated mechanical modulation of transmitter release from frog motor nerve terminals. *J. Neurosci.* **17**, 904-916.
- Chen, C. S. and Ingber, D. E. (1999). Tensegrity and mechanoregulation: from skeleton to cytoskeleton. *Osteoarthritis Cartilage* **7**, 81-94.
- Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. and Ingber, D. E. (1997). Geometric control of cell life and death. *Science* **276**, 1425-1428.
- Chen, J., Fabry, B., Schiffrin, E. L. and Wang, N. (2001). Twisting integrin receptors increases endothelin-1 gene expression in endothelial cells. *Am. J. Physiol. Cell Physiol.* **280**, C1475-C1484.
- Chen, K. D., Li, Y. S., Kim, M., Li, S., Yuan, S., Chien, S. and Shyy, J. Y. (1999). Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. *J. Biol. Chem.* **274**, 18393-183400.
- Chicurel, M., Chen, C. S. and Ingber, D. E. (1998a). Cellular control lies in the balance of forces. *Curr. Opin. Cell Biol.* **10**, 232-239.
- Chicurel, M. E., Singer, R. H., Meyer, C. and Ingber, D. E. (1998b). Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. *Nature* **392**, 730-733.
- Coffey, D. S. (1998). Self-organization, complexity and chaos: the new biology for medicine. *Nat. Med.* **4**, 882-885.
- Csete, M. E. and Doyle, J. C. (2002). Reverse engineering of biological complexity. *Science* **295**, 1664-1669.
- D'Angelo, G., Mogford, J. E., Davis, G. E., Davis, M. J. and Meininger, G. A. (1997). Integrin-mediated reduction in vascular smooth muscle [Ca²⁺]_i induced by RGD-containing peptide. *Am. J. Physiol.* **272**, H2065-H2070.
- Davies, P. F. (1995). Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* **75**, 519-560.
- Dennerll, T. J., Joshi, H. C., Steel, V. L., Buxbaum, R. E. and Heidemann, S. R. (1988). Tension and compression in the cytoskeleton II: quantitative measurements. *J. Cell Biol.* **107**, 665-674.
- Dennerll, T. J., Lamoureux, P., Buxbaum, R. E. and Heidemann, S. R. (1989). The cytomechanics of axonal elongation and retraction. *J. Cell Biol.* **107**, 3073-3083.
- Dike, L., Chen, C. S., Mrksich, M., Tien, J., Whitesides, G. M. and Ingber, D. E. (1999). Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. *In Vitro Cell Dev. Biol. Anim.* **35**, 441-448.
- Eckes, B., Dogic, D., Colucci-Guyon, E., Wang, N., Maniotis, A., Ingber, D. E., Merckling, A., Aumailley, M., Kotliansky, V., Babinet, C., and Krieg, T. (1998). Impaired mechanical stability, migration, and contractile capacity in vimentin-deficient fibroblasts. *J. Cell Sci.* **111**, 1897-1907.
- Fambrough, D., McClure, K., Kazlauskas, A. and Lander, E. S. (1999). Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. *Cell* **97**, 727-741.
- Flusberg, D. A., Numaguchi, Y. and Ingber, D. E. (2001). Cooperative control of Akt phosphorylation bcl-2 expression and apoptosis by cytoskeletal microfilaments and microtubules in capillary endothelial cells. *Mol. Biol. Cell* **12**, 3087-3094.
- Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D. and Vlodavsky, I. (1988). A heparin-binding angiogenic protein—basic fibroblast growth factor—is stored within basement membrane. *Am. J. Pathol.* **130**, 393-400.
- Folkman, J. and Moscona, A. (1978). Role of cell shape in growth control. *Nature* **273**, 345-349.
- Fuller, B. (1961). Tensegrity. *Portfolio Artnews Annual* **4**, 112-127.
- Galbraith, C. G., Yamada, K. M. and Sheetz, M. P. (2002). The relationship between force and focal complex development. *J. Cell Biol.* **159**, 695-705.
- Galli-Resta, L. (2002). Putting neurons in the right places: local interactions in the genesis of retinal architecture. *Trends Neurosci.* **25**, 638-643.
- Gillis, J. M. (1999). Understanding dystrophinopathies: an inventory of the structural and functional consequences of the absence of dystrophin in muscles of the mdx mouse. *J. Muscle Res. Cell Motil.* **20**, 605-625.
- Glogauer, M., Arora, P., Yao, G., Sokholov, I., Ferrier, J. and McCulloch, C. A. (1997). Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching. *J. Cell Sci.* **110**, 11-21.
- Goldschmidt, M. E., McLeod, K. J. and Taylor, W. R. (2001). Integrin-mediated mechanotransduction in vascular smooth muscle cells: frequency and force response characteristics. *Circ. Res.* **88**, 674-680.
- Hegner, S. P., Hofmann, T. G., Droge, W. and Schmitz, M. L. (1999). Inhibition of tyrosine phosphatases induces apoptosis independent from the CD95 system. *Cell Death Differ.* **6**, 833-841.
- Hill, T. L. and Kirschner, M. W. (1982). Bioenergetics and kinetics of microtubule and actin filament assembly-disassembly. *Int. Rev. Cytol.* **78**, 1-125.
- Huang, S. (1999). Gene expression profiling, genetic networks and cellular States: an integrating concept for tumorigenesis and drug discovery. *J. Mol. Med.* **77**, 469-480.
- Huang, S. (2000). Cell state dynamics and tumorigenesis in boolean regulatory networks *InterJ. Complexity*, Article 416 (<http://www.interjournal.org/>).
- Huang, S. and Ingber, D. E. (1999). The structural and mechanical complexity of cell-growth control. *Nat. Cell Biol.* **1**, E131-E138.
- Huang, S. and Ingber, D. E. (2000). Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Exp. Cell Res.* **261**, 91-103.
- Huang, S., Chen, C. S. and Ingber, D. E. (1998). Control of cyclin D1, p27^{Kip1} and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol. Biol. Cell* **9**, 3179-3193.
- Ingber, D. E. (1990). Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Natl. Acad. Sci. USA* **87**, 3579-3583.
- Ingber, D. E. (1991). Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol.* **3**, 841-848.
- Ingber, D. E. (1993). The riddle of morphogenesis: a question of solution chemistry or molecular cell engineering? *Cell* **75**, 1249-1252.
- Ingber, D. E. (1997). Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59**, 575-599.
- Ingber, D. E. (1999). How cells (might) sense microgravity. *FASEB J.* **S1**, 3-15.
- Ingber, D. E. (2000). The origin of cellular life. *Bioessays* **22**, 1160-1170.
- Ingber, D. E. (2002a). Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ. Res.* **91**, 877-887.
- Ingber, D. E. (2002b). Cancer as a disease of epithelial-mesenchymal interactions and extracellular matrix regulation. *Differentiation* **70**, 547-560.
- Ingber, D. E. (2003). Cellular tensegrity I. Cell structure and hierarchical systems biology. *J. Cell Sci.* 1157-1173.
- Ingber, D. E. and Folkman, J. (1989). Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. *J. Cell Biol.* **109**, 317-330.
- Ingber, D. E. and Jamieson, J. D. (1985). Cells as tensegrity structures: architectural regulation of histodifferentiation by physical forces transduced over basement membrane. In *Gene Expression During Normal and Malignant Differentiation* (eds L. C. Andersson, C. G. Gahmberg and P. Ekblom), pp 13-32. Orlando, Florida: Academic Press.
- Ingber, D. E., Madri, J. A. and Jamieson, J. D. (1981). Role of basal lamina in the neoplastic disorganization of tissue architecture. *Proc. Natl. Acad. Sci. USA* **78**, 3901-3905.
- Ingber, D. E., Madri, J. A. and Jamieson, J. D. (1986). Basement membrane as a spatial organizer of polarized epithelia: exogenous basement membrane reorients pancreatic epithelial tumor cells in vitro. *Am. J. Pathol.* **122**, 129-139.
- Iwig, M., Czeslick, E., Muller, A., Gruner, M., Spindler, M. and Glaesser, D. (1995). Growth regulation by cell shape alteration and organization of the cytoskeleton. *Eur. J. Cell Biol.* **67**, 145-157.
- Jacobson, M. D., Burne, J. F., King, M. P., Miyashita, T., Reed, J. C. and Raff, M. C. (1993). Bcl-2b locks apoptosis in cells lacking mitochondrial DNA. *Nature* **361**, 365-369.
- Jalali, S., del Pozo, M. A., Chen, K., Miao, H., Li, Y., Schwartz, M. A., Shyy, J. Y. and Chien, S. (2001). Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. *Proc. Natl. Acad. Sci. USA* **98**, 1042-1046.
- Janmey, P. A. (1998). The cytoskeleton and cell signaling: component localization and mechanical coupling. *Physiol. Rev.* **78**, 763-781.
- Joshi, H. C., Chu, D., Buxbaum, R. E. and Heidemann, S. R. (1985). Tension and compression in the cytoskeleton of PC 12 neurites. *J. Cell Biol.* **101**, 697-705.
- Kaczanoska, J., Iftode, F., Jeanmairewolf, R., Clerot, J. C., Kiersnowska, M. and Adoutte, A. (1995). Tensegrity model of pattern formation during cytokinesis of a ciliate, paramecium – effects of an inhibitor of phosphorylation. *J. Exp. Zool.* **273**, 494-510.

- Kaminsky, S. G. W. and Heath, I. B.** (1996). Studies on *Saprolegnia-Ferax* suggest the general importance of the cytoplasm in determining hyphal morphology. *Mycologia* **88**, 20-37.
- Kauffman, S. A.** (1993). *The origins of order*. Oxford: Oxford University Press.
- Kaverina, I., Krylyshkina, O., Beningo, K., Anderson, K., Wang, Y. L. and Small, J. V.** (2002). Tensile stress stimulates microtubule outgrowth in living cells. *J. Cell Sci.* **115**, 2283-2291.
- Keller, R. S., Shai, S. Y., Babbitt, C. J., Pham, C. G., Solaro, R. J., Valencik, M. L., Loftus, J. C. and Ross, R. S.** (2001). Disruption of integrin function in the murine myocardium leads to perinatal lethality, fibrosis, and abnormal cardiac performance. *Am. J. Pathol.* **158**, 1079-1090.
- Koch, J. C.** (1917). The laws of bone architecture. *Am. J. Anat.* **21**, 177-298.
- Korff, T. and Augustin, H. G.** (1999). Tensional forces in fibrillar extracellular matrices control directional capillary sprouting. *J. Cell Sci.* **112**, 3249-3258.
- Lee, K.-M., Tsai, K., Wang, N. and Ingber, D. E.** (1997). Extracellular matrix and pulmonary hypertension: control of vascular smooth muscle cell contractility. *Am. J. Physiol.* **274**, H76-H82.
- Lee, H. S., Millward-Sadler, S. J., Wright, M. O., Nuki, G. and Salter, D. M.** (2000). Integrin and mechanosensitive ion channel-dependent tyrosine phosphorylation of focal adhesion proteins and beta-catenin in human articular chondrocytes after mechanical stimulation. *J. Bone Miner. Res.* **15**, 1501-1509.
- Li, M. L., Aggeler, J., Farson, D. A., Hatier, C., Hassell, J. and Bissell, M. J.** (1987). Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proc. Natl. Acad. Sci. USA.* **84**, 136-140.
- Liu, Y., Chen, B. P., Lu, M., Zhu, Y., Stemeran, M. B., Chien, S. and Shyy, J. Y.** (2002). Shear stress activation of SREBP1 in endothelial cells is mediated by integrins. *Arterioscler. Thromb. Vasc. Biol.* **22**, 76-81.
- Loufrani, L., Matrougui, K., Li, Z., Levy, B. I., Lacolley, P., Paulin, D. and Henrion, D.** (2002). Selective microvascular dysfunction in mice lacking the gene encoding for desmin. *FASEB J.* **16**, 117-119.
- Low, S. Y. and Taylor, P. M.** (1998). Integrin and cytoskeletal involvement in signalling cell volume changes to glutamine transport in rat skeletal muscle. *J. Physiol.* **512**, 481-485.
- Lynch, T. M., Lintilhac, P. M. and Domozych, D.** (1998). Mechanotransduction molecules in the plant gravisensory response: amyloplast/statolith membranes contain a beta 1 integrin-like protein. *Protoplasma* **201**, 92-100.
- Maroto, R. and Hamill, O. P.** (2001). Brefeldin A block of integrin-dependent mechanosensitive ATP release from *Xenopus* oocytes reveals a novel mechanism of mechanotransduction. *J. Biol. Chem.* **276**, 23867-23872.
- Mehta, A. D., Rief, M., Spudich, J. A., Smith, D. A. and Simmons, R. M.** (1999). Single-molecule biomechanics with optical methods. *Science* **283**, 1689-1695.
- Messing, R. O.** (1993). Ethanol as an enhancer of neural differentiation. *Alcohol Alcohol Suppl.* **2**, 289-293.
- Meyer, C. J., Alenghat, F. J., Rim, P., Fong, J. H.-J., Fabry, B. and Ingber, D. E.** (2000). Mechanical control of cyclic AMP signaling and gene transcription through activated integrins. *Nat. Cell Biol.* **2**, 666-668.
- Miyamoto, S., Teramoto, H., Coso, O. A., Gutkind, J. S., Burbelo, P. D., Akiyama, S. K. and Yamada, K. M.** (1995). Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. *J. Cell Biol.* **131**, 791-805.
- Mochitate, K., Pawelek, P. and Grinnell, F.** (1991). Stress relaxation of contracted collagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. *Exp. Cell Res.* **193**, 198-207.
- Mooney, D., Hansen, L., Langer, R., Vacanti, J. P. and Ingber, D. E.** (1994). Extracellular matrix controls tubulin monomer levels in hepatocytes by regulating protein turnover. *Mol. Biol. Cell* **5**, 1281-1288.
- Moore, K. A., Huang, S., Kong, Y., Sunday, M. E. and Ingber, D. E.** (2002). Control of embryonic lung branching morphogenesis by the Rho activator, cytotoxic necrotizing factor 1. *J. Surg. Res.* **104**, 95-100.
- Morohashi, M., Winn, A. E., Borisuk, M. T., Bolouri, H., Doyle, J. and Kitano, H.** (2002). Robustness as a measure of plausibility in models of biochemical networks. *J. Theor. Biol.* **216**, 19-30.
- Morrison, R. F. and Farmer, S. R.** (1999). Insights into the transcriptional control of adipocyte differentiation. *J. Cell Biochem. Suppl.* **32-33**, 59-67.
- Moy, A. B., Bodmer, J. E., Blackwell, K., Shasby, S. and Shasby, D. M.** (1998). cAMP protects endothelial barrier function independent of inhibiting MLC20-dependent tension development. *Am. J. Physiol.* **274**, L1024-L1029.
- Northover, A. M. and Northover, B. J.** (1993). Possible involvement of microtubules in platelet-activating factor-induced increases in microvascular permeability in vitro. *Inflammation* **17**, 633-639.
- Opas, M.** (1989). Expression of the differentiated phenotype by epithelial cells in vitro is regulated by both biochemistry and mechanics of the substratum. *Dev. Biol.* **131**, 281-293.
- Parker, K. K., Brock, A. L., Brangwynne, C., Mannix, R. J., Wang, N., Ostuni, E., Geisse, N. A., Adams, J. C., Whitesides, G. M. and Ingber, D. E.** (2002). Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J.* **16**, 1195-1204.
- Pavalko, F. M., Chen, N. X., Turner, C. H., Burr, D. B., Atkinson, S., Hsieh, Y. F., Qiu, J. and Duncan, R. L.** (1998). Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. *Am. J. Physiol.* **275**, C1591-C1601.
- Pienta, K. J. and Coffey, D. S.** (1991). Cell motility as a chemotherapeutic target. *Cancer Surv.* **11**, 255-263.
- Pienta, K. J., Getzenberg, R. H. and Coffey, D. S.** (1991). Cell structure and DNA organization. *Crit. Rev. Eukary. Gene Express* **1**, 355-385.
- Plopper, G., McNamee, H., Dike, L., Bojanowski, K. and Ingber, D. E.** (1995). Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Mol. Biol. Cell* **6**, 1349-1365.
- Potard, U. S., Butler, J. P. and Wang, N.** (1997). Cytoskeletal mechanics in confluent epithelial cells probed through integrins and E-cadherins. *Am. J. Physiol.* **272**, C1654-C1663.
- Putnam, A. J., Cunningham, J. J., Dennis, R. G., Linderman, J. J. and Mooney, D. J.** (1998). Microtubule assembly is regulated by externally applied strain in cultured smooth muscle cells. *J. Cell Sci.* **111**, 3379-3387.
- Putnam, A. J., Schultz, K. and Mooney, D. J.** (2001). Control of microtubule assembly by extracellular matrix and externally applied strain. *Am. J. Physiol. Cell Physiol.* **280**, C556-C564.
- Riveline, D., Zamir, E., Balaban, N. Q., Schwarz, U. S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B. and Bershadsky, A. D.** (2001). Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J. Cell Biol.* **153**, 1175-1186.
- Salter, D. M., Robb, J. E. and Wright, M. O.** (1997). Electrophysiological responses of human bone cells to mechanical stimulation: evidence for specific integrin function in mechanotransduction. *J. Bone Miner. Res.* **12**, 1133-1141.
- Schmidt, C. E., Horwitz, A. F., Lauffenburger, D. A. and Sheetz, M. P.** (1993). Integrin-cytoskeletal interactions in migrating fibroblasts are dynamic, asymmetric, and regulated. *J. Cell Biol.* **123**, 977-991.
- Schwartz, E. A., Bizios, R., Medow, M. S. and Gerritsen, M. E.** (1999). Exposure of human vascular endothelial cells to sustained hydrostatic pressure stimulates proliferation. Involvement of the alphaV integrins. *Circ Res.* **84**, 315-322.
- Schwarzbauer, J. E. and Sechler, J. L.** (1999). Fibronectin fibrillogenesis: a paradigm for extracellular matrix assembly. *Curr. Opin. Cell Biol.* **11**, 622-627.
- Shweiki, D.** (1999). The physical imperative in circadian rhythm: a cytoskeleton-related physically resettable clock mechanism hypothesis. *Medical Hypoth.* **53**, 413-420.
- Singhvi, R., Kumar, A., Lopez, G., Stephanopoulos, G. N., Wang, D. I. C., Whitesides, G. M. and Ingber, D. E.** (1994). Engineering cell shape and function. *Science* **264**, 696-698.
- Skelton, R. E. and Sultan, C.** (1997). Controllable tensegrity, a new class of smart structures. *Proceedings of the SPIE 4th Symposium on Smart Structures and Materials* **3039**, 166-177.
- Spremlui, E. N. and Dexter, D. L.** (1984). Polar solvents: a novel class of antineoplastic agents. *J. Clin. Oncol.* **2**, 227-241.
- Strohman, R. C.** (1997). The coming Kuhnian revolution in biology. *Nat. Biotechnol.* **15**, 194-200.
- Tagawa, H., Wang, N., Narishige, T., Ingber, D. E., Zile, M. R. and Cooper, G.** (1997). Cytoskeletal mechanics in pressure-overload cardiac hypertrophy. *Circ. Res.* **80**, 281-289.
- Thompson, D. W.** (1952). *On Growth and Form* (second edition). Cambridge UK: Cambridge University Press.
- Tsutsui, H., Ishihara, K. and Cooper, G.** (1993). Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. *Science* **260**, 682-687.
- Urbich, C., Dernbach, E., Reissner, A., Vasa, M., Zeiher, A. M. and Dimmeler, S.** (2002). Shear stress-induced endothelial cell migration

- involves integrin signaling via the fibronectin receptor subunits alpha(5) and beta(1). *Arterioscler. Thromb. Vasc. Biol.* **22**, 69-75.
- Van Essen, D. C.** (1997). A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature* **385**, 313-318.
- Waddington, C. H.** (1956). *Principles of Embryology*. London: Allen & Unwin Ltd.
- Walsh, K. and Perlman, H.** (1997). Cell cycle exit upon myogenic differentiation. *Curr. Opin. Genet. Dev.* **7**, 597-602.
- Wang, N., Butler, J. P. and Ingber, D. E.** (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260**, 1124-1127.
- Wang, N., Naruse, K., Stamenovic, D., Fredberg, J., Mijailovic, S. M., Maksym, G., Polte, T. and Ingber, D. E.** (2001a). Mechanical behavior in living cells consistent with the tensegrity model. *Proc. Natl. Acad. Sci. USA* **98**, 7765-7770.
- Wang, D. S., Proffitt, D. and Tsao, P. S.** (2001b). Mechanotransduction of endothelial oxidative stress induced by cyclic strain. *Endothelium* **8**, 283-291.
- Wang, N. and Stamenovic, D.** (2000). Contribution of intermediate filaments to cell stiffness, stiffening, and growth. *Am. J. Physiol. Cell Physiol.* **279**, C188-C194.
- Wang, J., Tolan, D. R. and Pagliaro, L.** (1997). Metabolic compartmentation in living cells: structural association of aldolase. *Exp. Cell Res.* **237**, 445-451.
- Weaver, V. M., Lelievre, S., Lakins, J. N., Chrenek, M. A., Jones, J. C., Giancotti, F., Werb, Z. and Bissell, M. J.** (2002). Beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell* **2**, 205-216.
- Wilson, E., Sudhir, K. and Ives, H. E.** (1995). Mechanical strain of rat vascular smooth muscle cells is sensed by specific extracellular matrix/integrin interactions. *J. Clin. Invest.* **96**, 2364-2372.
- Wolff, Y.** (1892). *Das Gesetz der Transformation der Knochen*. Berlin, 152.
- Wozniak, M., Fausto, A., Carron, C. P., Meyer, D. M. and Hruska, K. A.** (2000). Mechanically strained cells of the osteoblast lineage organize their extracellular matrix through unique sites of alphavbeta3-integrin expression. *J. Bone Miner. Res.* **15**, 1731-1745.
- Yano, Y., Saito, Y., Narumiya, S. and Sumpio, B. E.** (1996). Involvement of rho p21 in cyclic strain-induced tyrosine phosphorylation of focal adhesion kinase (pp125FAK), morphological changes and migration of endothelial cells. *Biochem. Biophys. Res. Commun.* **224**, 508-515.
- Yoder, T. L., Zheng Hq, H., Todd, P. and Staehelin, L. A.** (2001). Amyloplast sedimentation dynamics in maize columella cells support a new model for the gravity-sensing apparatus of roots. *Plant Physiol.* **125**, 1045-1060.
- Yoshida, M., Westlin, W. F., Wang, N., Ingber, D. E., Rosenweig, A., Resnick, N. and Gimbrone, M.** (1996). Leukocyte adhesion to vascular endothelium induces e-selectin association with the actin cytoskeleton. *J. Cell Biol.* **133**, 445-455.
- Yu, Z. W. and Quinn, P. J.** (1994). Dimethyl sulphoxide: a review of its applications in cell biology. *Biosci. Rep.* **14**, 259-281.