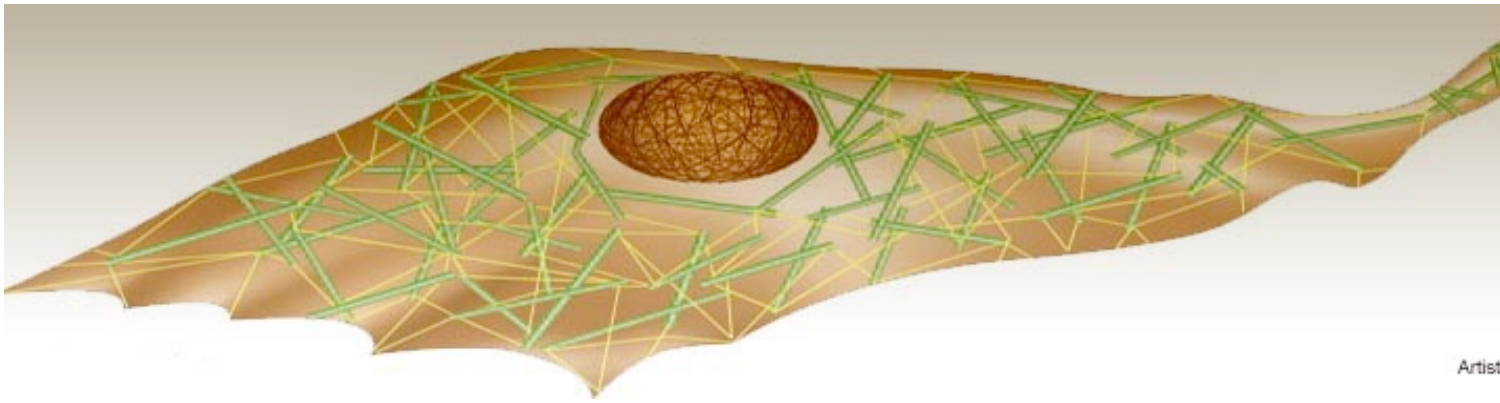

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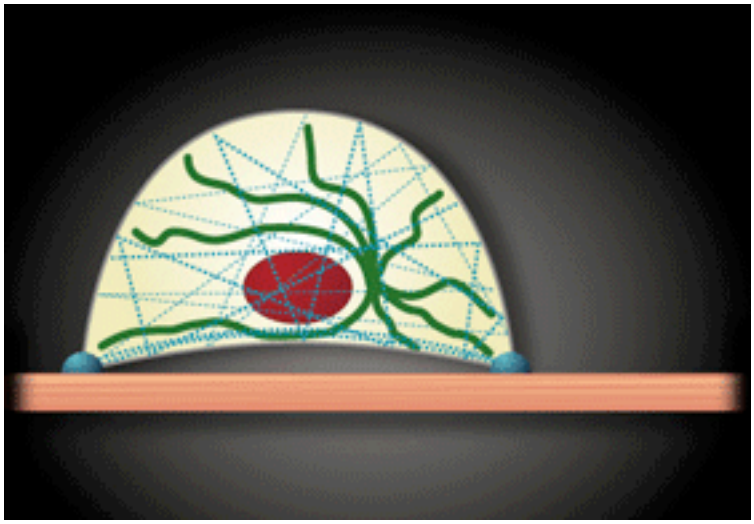


Ingber

Tensegrity and Complex Systems Biology

Living organisms are hierarchical structures that integrate their smallest constituent parts - individual molecules including DNA, proteins and lipids - across multiple levels of organization, from organelles, to cells, tissues, organs, and the organism. Thus, a major challenge in biology and medicine today is to understand how large numbers of different molecular parts interact and self-organize into a whole system that exhibits organic properties that cannot be explained solely in terms of their component properties. A major part of our current effort is therefore to understand how interactions among different molecular components map to system-wide behaviors in living cells and tissues. As cellular biochemistry does not proceed in solution, but rather on insoluble scaffolds and structures within the cytoplasm and nucleus of living cells, we are interested in both cellular hardware (structure and mechanical properties) and software (information processing capabilities), as well as how they interplay to c ontrol cell form and function.

Tensegrity

Our approach to understanding cellular hardware is based on cellular tensegrity theory. **Tensegrity is a building principle that was first described by the architect R. Buckminster Fulle**r and first visualized by the sculptor Kenneth Snelson. Fuller defines tensegrity systems as structures that stabilize their shape by continuous tension or “tensional integrity” rather than by continuous compression (e.g., as used in a stone arch). Tensegrity includes two broad structural classes - prestressed and geodesic -

which would both fail to act like a single entity or to maintain their shape stability when mechanically stressed without continuous transmission of tensional forces. The former hold their joints in position as the result of a “prestress” (pre-existing tensile stress or isometric tension) within a structural network that is tensed because of a subset of elements that resist being compressed. The latter triangulate their structural members and orient them along geodesics (minimal paths) to geometrically constrain movement. Our bodies provide a familiar example of a prestressed tensegrity structure: our bones act like struts to resist the pull of tensile muscles, tendons and ligaments, and the shape stability (stiffness) of our bodies varies depending on the tone (prestress) in our muscles. Examples of geodesic tensegrity structures include Fuller’s geodesic domes, carbon-based buckminsterfullerenes (buckyballs), and tetrahedral space frames, which are of great interest in astronautics because they maintain their stability in the absence of gravity and, hence, without continuous compression.

The cellular tensegrity model proposes that the whole cell is a prestressed tensegrity structure, although geodesic structures are also found in the cell at smaller size scales (e.g. clathrin-coated vesicles, viral capsids). In the model, tensional forces are borne by cytoskeletal microfilaments and intermediate filaments, and these forces are balanced by interconnected structural elements that resist compression, most notably internal microtubule struts and ECM adhesions. However, individual filaments can have dual functions and hence bear either tension or compression in different structural contexts or at different size scales (e.g. contractile microfilaments generate tension, whereas actin microfilament bundles that are rigidified by cross-links bear compression in filopodia). The tensional prestress that stabilizes the whole cell is generated actively by the contractile actomyosin apparatus. Additional passive contributions to this prestress come from cell distension through adhesions to the ECM and other cells, osmotic forces acting on the cell membrane, and forces exerted by filament polymerization. Intermediate filaments that interconnect at many points along microtubules, microfilaments and the nuclear surface provide mechanical stiffness to the cell based on their material properties and on their ability to act as suspensory cables that interconnect and tensionally stiffen the entire cytoskeleton and nuclear lattice.

Tensegrity in a Cell

See how Tensegrity Works in a Cell on the Children's Hospital 'interactive website'. You can change the length or mechanical properties of the different support elements in cells (e.g., microfilaments, microtubules, matrix adhesions) and immediately see how they influence cell shape and cytoskeletal organization.

In addition, the internal cytoskeleton interconnects at the cell periphery with a highly elastic, geodesic cytoskeletal (actin-spectrin-ankyrin) network directly beneath the plasma membrane. The efficiency of mechanical coupling between this submembranous structural network and the internal cytoskeletal lattice depends on the type of molecular adhesion complex that forms on the cell surface. The entire integrated

cytoskeleton is then permeated by a viscous cytosol and enclosed by a differentially permeable surface membrane.

Importantly, working with collaborators, such as Drs. Ning Wang (Dept. of Respiratory Biology, Harvard School of Public Health) and Dimitrije Stamenovic (Dept. of Biomedical Engineering, Boston U.), we have been able to demonstrate that living mammalian cells behave mechanically like tensegrity structures. Moreover, we have developed a theoretical formulation of the tensegrity model starting from first mechanical principles that has yielded accurate qualitative and quantitative predictions of many static and dynamic cell mechanical behaviors. We are currently trying to extend and strengthen this computational approach to explain systems-wide mechanical properties in mammalian cells, and to explore their hierarchical basis.

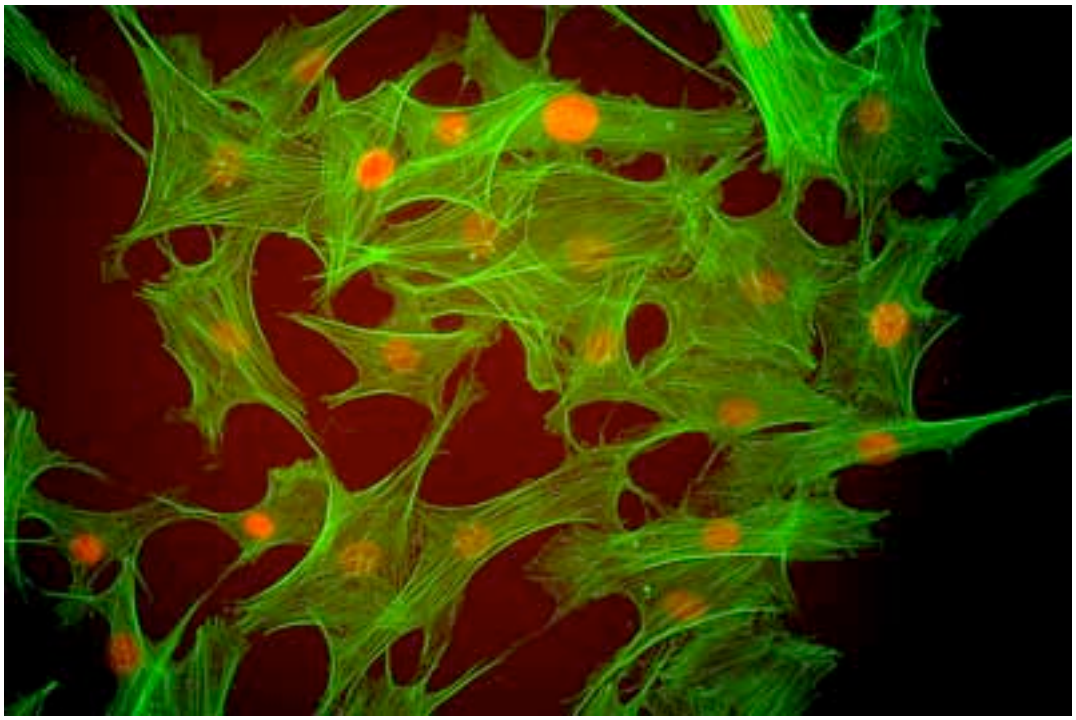
Complex Systems Biology

Systems Biology is a new field that focuses on the problem of how specialized behaviors emerge from collective interactions within complex molecular networks. The common approach is to work from the “bottom-up” by accumulating huge data sets with massively-parallel techniques or molecular analytical approaches, and then use computational modeling to “reverse engineer” network topology and behavior. Our work on tensegrity revealed that great insight could be gained by viewing the system as a whole and working from the “top-down”. Specifically, we found that when trying to understand collective mechanical behavior within supramolecular assemblies, higher-order architecture and physical forces must also be considered. Tensegrity also explains how hierarchical structures may be comprised of systems within systems (molecules within cells within tissues within organs) and yet still exhibit integrated mechanical behavior. In addition, it reveals how robust behaviors, such as persistence, mechanical adaptability, and shape stability, can be generated using “sloppy” parts (e.g., flexible molecular filaments), a key feature of both complex networks and living systems. 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 and the cytoskeleton seem to exert their effects on signal integration. Experiments show that while individual cells may receive multiple simultaneous inputs, they are able to rapidly integrate these signals so as to produce just one of a few possible outputs or phenotypes (e.g., growth, quiescence, differentiation, apoptosis). But studies on mechanoregulation raise a fundamental question: how can a gradual change in a physical parameter over a broad continuum, such as cell shape (distortion from round to spread), be translated into just a few, discrete cell fates?

Signal transduction has historically been viewed in terms of linear signaling pathways that lead from a specific input to a particular outcome. However, the information conveyed by the signal transduction machinery is often distributed among numerous pathways, and the same stimulus can generate different responses depending on the setting. Activation of a single signaling receptor can induce scores of genes, 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. For example, we have shown that the soluble angiogenic mitogen, FGF, produces growth in spread endothelial cells, differentiation (capillary tube formation) in partially retracted cells, and death in fully retracted cells. Thus, the concept of linear signaling pathways may be insufficient. Instead, the characteristic phenotypes that cells exhibit during development represent emergent and intrinsically robust behaviors that arise within a complex signaling network comprised of 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, similarly produce abrupt macroscopic changes between qualitatively discrete stable states (e.g., liquid versus gas or solid). Dr. Sui Huang in our group is currently exploring the possibility that cell fates may be viewed as stable high-dimensional “attractor” states in gene activation state space. A natural consequence of this model, is that switches occur between these characteristic states upon changes in environmental conditions or in response to multiple perturbations. Such switches are manifest as abrupt phenotypic changes, and hence, may represent some kind of biological "phase transition”.

But what is the molecular basis for the existence of these discrete, stable phenotypic states and associated all-or-nothing transitions between them? Generic computer models of the dynamics of large systems of interacting genes studied by Stuart Kauffman for almost 30 years revealed that, for a subset of network architectures, a few

stable states spontaneously emerge as a result of the constraints imposed by the regulatory interactions. Most network states are unstable and are "attracted" to the stable states, known as the "attractor" states. To visualize attractors in cell regulatory networks, 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. A ball (or cellular state) located at the nadir of one of these valleys (the attractors) will tend to remain there. Displacement to another part of the landscape will move the ball away from the valley, but with small perturbations it will generally “roll back down” to its own starting point in the same valley. Under the influence of a larger perturbation, however, the ball could traverse 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.

In summary, the dynamics predicted by this "attractor landscape" metaphor is precisely reflected in the cell fate switching behavior that is observed in living cells within multicellular organisms. Unfortunately, the emphasis in the past decades on the characterization of individual pathway modules that were assumed to have specific functions has side-lined the notion that physiologically relevant cell behaviors are regulated at the level of cell-wide regulatory networks. With the advent of genomics and proteomics technologies which can provide specific information on signaling cascades, we can now move from generic network models to studying nominal molecular pathways in the context of complexity and network theories.

Thus, we view the cell’s molecular signaling machinery as a dynamic information processing network, and suggest that cell fates represent common end-programs or "attractors" that self-organize within these networks. In this manner, we are able to describe the collective behavior of the cell's signaling molecules and their relationship to cell fate switching without having to elucidate the functions of individual molecular components. Importantly, recent experimental work from our laboratory based on dynamic whole-genome profiling of cells during the course of a cell fate switch provides direct support for the attractor hypothesis. We have developed Gene Expression Dynamics Inspector (GEDI) software (http://www.childrenshospital.org/research/ingber/GEDI/gedihome.htm) to facilitate analysis of dynamic network behavior, and to relate these changes to specific sets of molecules that contribute to changes in cellular phenotype.

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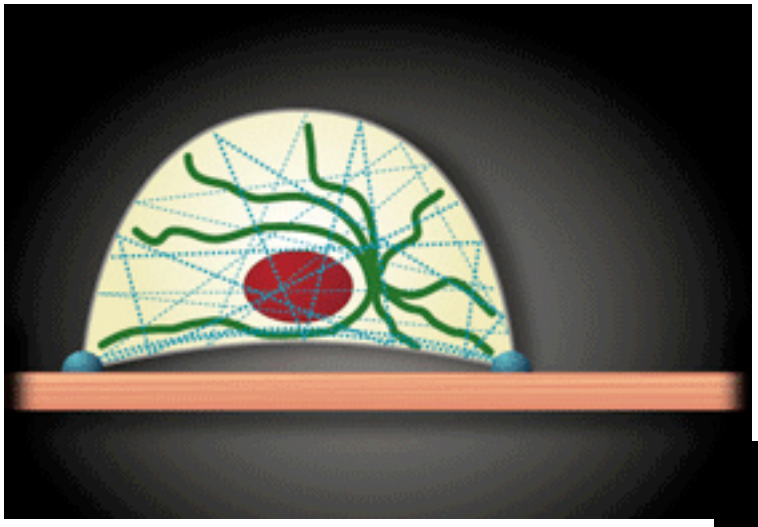
The major challenge in biology today is biocomplexity: the need to explain how cell and tissue behaviors emerge from collective interactions within complex molecular networks. Part I of this two-part article, described a mechanical model of cell structure based on tensegrity architecture that explains 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.

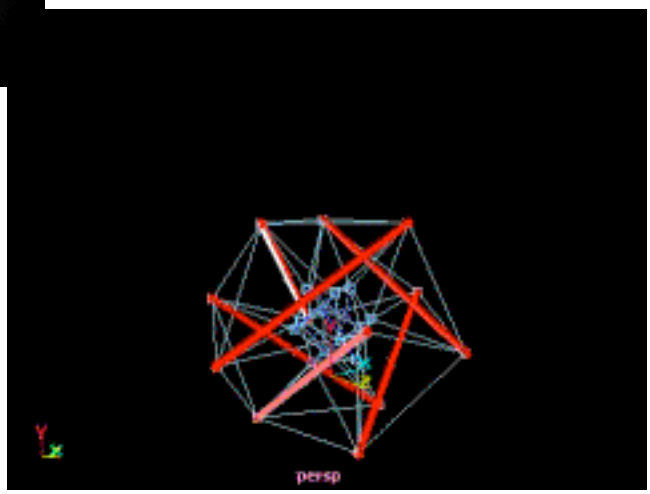
Another driving force behind this paradigm shift is the resurgence of interest in mechanical forces, rather than chemicals cues, as biological regulators. Clinicians have come to recognize the importance of mechanical forces for the development and function of the heart and lung, the growth of skin and 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 matix (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.

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.

Resources

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http://www.childrenshospital.org/research/Site2029/mainpageS2029P23sublevel24.html

Tensegrity in a Cell

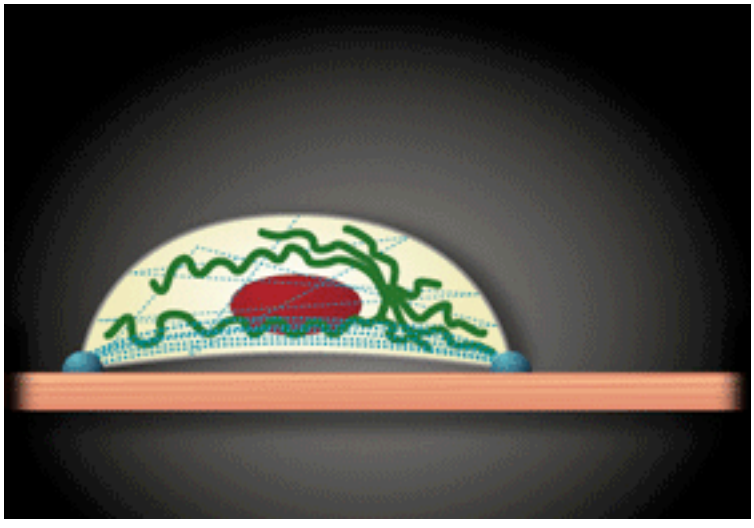
Go to the Tensegrity in a Cell interactive feature. Requires Flash plugin.
What do the human body, a sailboat's rigging and a circus tent have in common? If you happened to catch the title of this page, you won't be surprised at the answer: Tensegrity.

A tensegrity structure is composed of parts, or elements, that are "tensed," working in opposition to other elements that resist being compressed. These elements are balanced in such a way that they tense and stabilize the entire structure. In the human body, bones are compression struts that oppose the tension created by muscles, tendons and ligaments. In a sailboat, the mast, spreaders and hull act as compressional elements working in opposition to wire cables that tense and stabilize the entire structure. Likewise, tensed canvas fabric pulls against rigid pegs in the ground and vertical poles to give the circus tent its shape.

Thirty years ago, while still an undergraduate student at Yale, Donald Ingber, MD, PhD, began to think that cells, too, might be tensegrity structures. In the years since, he and his colleagues at Children's Hospital Boston and Harvard University have gone on to substantiate the idea. They have shown that contractile microfilaments in the cell's molecular skeleton, or cytoskeleton, act like stretched rubber bands as they compress hollow cytoskeletal fibers called microtubules and pull on molecular pegs that anchor the cell to an underlying scaffold -- the extracellular matrix. Moreover, they have found that physical distortion of the cell and cytoskeleton can alter cellular biochemistry and gene expression. This form of "mechanobiology," says Dr. Ingber, is as important to body function as hormones and other chemical interactions. An example of mechanobiology in action occurs after an injury in which cells are removed. Freed from their normally crowded context, the cytoskeletons and nuclei of cells that were adjacent to the removed cells distort, which triggers genes and chemical reactions that cause them to multiply until they get so tightly packed and compressed that they stop growing. This is how tissue regenerates.

Here, you can experiment with the counteracting forces inside the molecular skeleton of a single cell by selectively changing the length or tension of cytoskeletal filaments and by altering the rigidity of the extracellular matrix. See how your actions affect the various structural elements inside the cells, as well as the overall shape of the cell.

Interactive Feature: Tensegrity in a Cell (700 K)
Manipulate the forces, structural restraints and molecular elements of a cell's cytoskeleton and see how your changes affect the cell's shape. Requires Flash plugin.



http://www.childrenshospital.org/research/cell_tensegrity/index.html

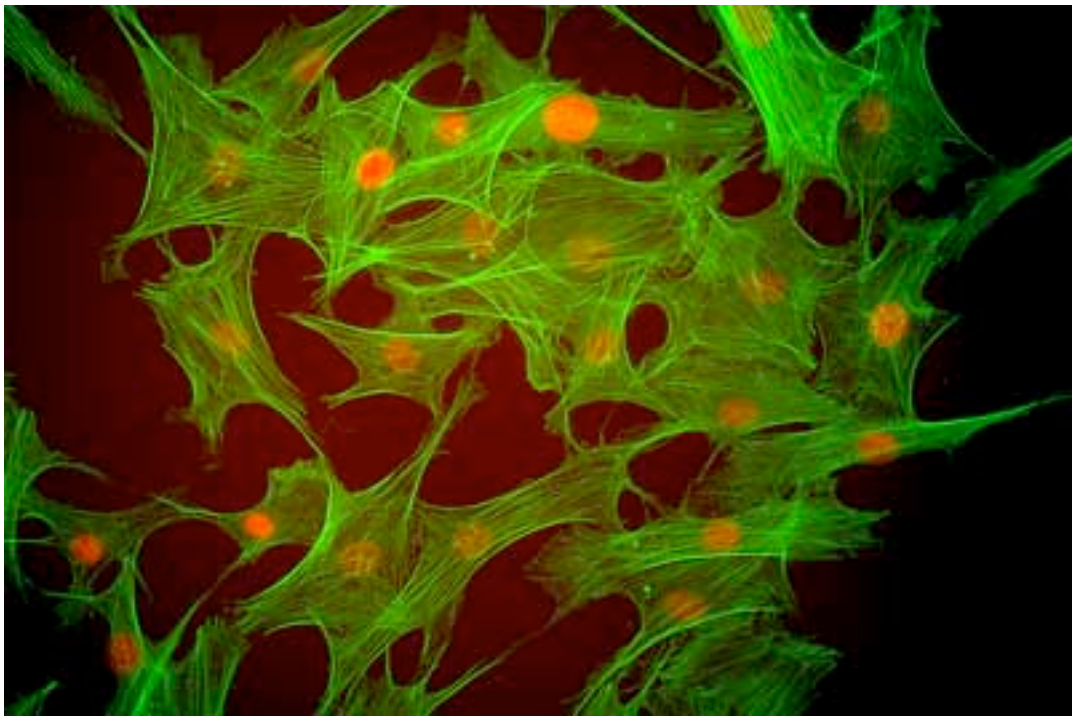
More on Tensegrity

Animations - could try this on ppt

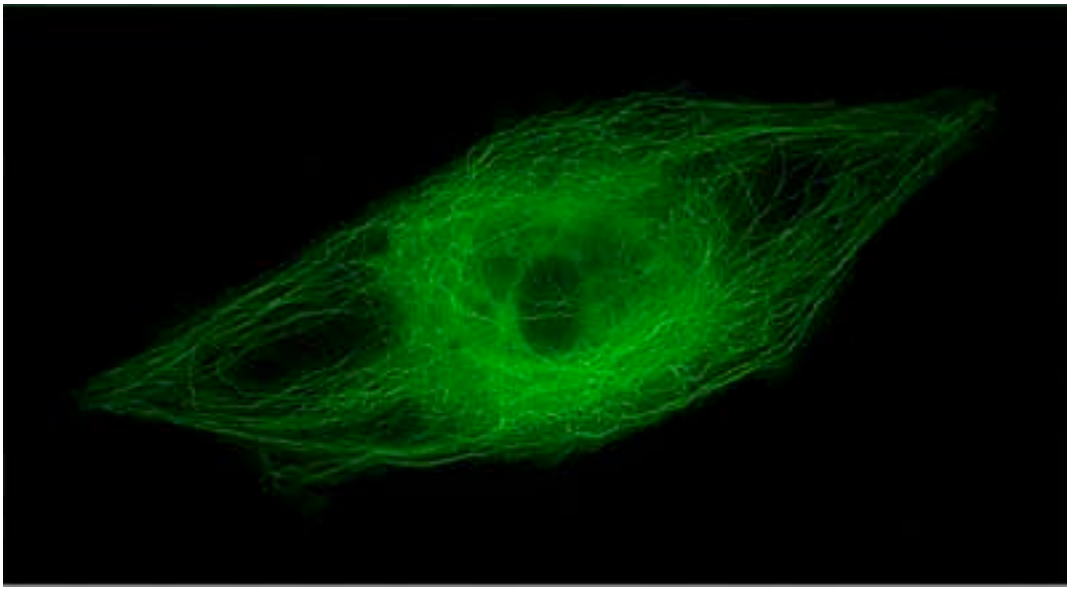
These three animated gifs, provided by Eddy Y. Xuan of Biomedical Communications, University of Toronto, Canada, shows how hierarchical tensegrity structures, such as a cell with a nucleus, behave when pulled, stretched and sheared.

- Tensegrity structure -- pull [632 K]
- Tensegrity structure -- shear [864 K]
- Tensegrity structure -- stretch [484 K]

Articles on the Web



The Mechanical Cell, by Nancy Fliesler From Dream magazine, published by Children's Hospital Boston. A group of cells from capillary blood vessels cultured on an extracellular matrix and stained to visualize their tensed microfilament network (green) and central nuclei (red). (Tom Polte and Don Ingber)



Molecular struts in the cytoskeleton of a living cell

Hollow “microtubule” filaments form a connected array that extends through the cytoplasm and helps support cell shape. (Keiji Naruse and Don Ingber)

The Architecture of Life, by Donald Ingber
From Scientific American, January 1998 [Requires payment to view.]

Tensegrity I. Cell structure and hierarchical systems biology, by Donald E. Ingber.
From the Journal of Cell Science.

Tensegrity II. How structural networks influence cellular information processing networks, by Donald E. Ingber. From the Journal of Cell Science.

Mechanochemical Basis of Cell and Tissue Regulation, Donald E. Ingber
From the National Academy of Engineering, Fall 2004

Related Links

Donald Ingber, MD, PhD

Ingber's Egg Analogy

The Ingber Lab

Scientists Discover Secret Behind Human Red Blood Cell's Amazing Flexibility -- from the UCSD Jacobs School of Engineering

Further Reading

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D'Arcy W. Thompson. On Growth and Form. Revised Edition. Cambridge University Press, 1942 (Reprinted 1992).

Credits -- Interactive Feature

Subject Matter Expert: Donald E. Ingber, MD, PhD, Children's Hospital Boston
Writer/Producer: Rick Groleau, Children's Hospital Boston
Illustrator: Chesley Lowe
Designer: Sonali Patel, WGBH Interactive
Developer: Daniel Bulli, WGBH Interactive

Additional images and movies provided by Sui Huang,
Keiji Naruse, Ben Matthews, and Don Ingber.

http://www.childrenshospital.org/research/cell_tensegrity/index.html

http://www.childrenshospital.org/research/Site2029/Documents/tensegrity_pull.gif