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Cellular Basis of Mechanotransduction

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Physical forces, such as those due to gravity, are fundamental regulators of tissue development. To influence morphogenesis, mechanical forces must alter growth and function. Yet little is known about how cells convert mechanical signals into a chemical response. This presentation attempts to place the potential molecular mediators of mechanotransduction within the context of the structural complexity of living cells.

Our experimental approach is based on the hypothesis that cells use tensegrity architecture to structure themselves (Ingber, 1993, 1998; Ingber and Jamieson, 1985). Most man-made structures gain their stability through continuous compression; one element weighs down on the element below due to the force of gravity. In contrast, tensegrity structures stabilize themselves through continuous tension that is distributed across all of the structural elements and balanced by a subset of these elements that resist compression locally. These internal struts generate an internal tension or "prestress" that mechanically stabilizes the entire structure. Tensegrity cell models composed of sticks and elastic string (Fig. 1) predict many complex cell behaviors, including how cells change shape when they adhere to rigid or flexible extracellular matrices (Ingber, 1993, 1998; Ingber and Jamieson, 1985). Tensegrity models also predict that cells and nuclei are hard-wired to respond immediately to mechanical stresses transmitted over cell surface receptors that physically couple the cytoskeleton to the extracellular matrix and to other cells.

We recently developed a technique to apply controlled mechanical forces (rotational shear stresses) to cell surface receptors in living cells. In brief, magnetic microspheres are coated with specific receptor ligands and are thus bound to the cell surface. The microspheres are magnetically twisted, and their rotation (angular strain) is simultaneously quantified. Using this method, magnetic twisting cytometry (Wang *et al.*, 1993; Wang and Ingber, 1995), we have been able to confirm that extracellular matrix receptors, such as integrins, and cell-cell adhesion receptors (*e.g.*, E-selectin) provide preferred paths for mechanical signal transfer across the cell surface and to the internal cytoskeleton (Wang *et al.*, 1993; Wang and Ingber, 1995; Yoshida *et al.*, 1996). We also were able to show directly that living cells behave mechanically as if they were tensegrity structures. Our evidence includes a demonstration of linear stiffening behavior; results indicating that cell stiffness depends on internal prestress in the cytoskeleton; and data showing that microtubules resist lateral compression in the cytoplasm (Wang *et al.*, 1993; Wang and Ingber, 1994, 1995; Stamenovic *et al.*, 1996; Maniotis *et al.*, 1997; Tagawa *et al.*, 1997; Lee *et al.*, 1998). In addition, we have been able to demonstrate that pulling on cell surface integrins with matrix-coated micropipettes in living cells results in immediate realignment of cytoskeletal filaments, as well as tension-dependent changes in structure inside the nucleus (Maniotis *et al.*, 1997). This latter finding directly confirms the existence of hard-wiring (mediated by intermediate filaments and actin microfilaments) in cells and emphasizes that conventional biomechanical models of the cell based on a viscous cytosol surrounded by an elastic membrane are not accurate or useful when considering the molecular basis of cell mechanics.

The finding that integrins mediate the transfer of mechanical signals across cellular membranes is important for tissue physiology because integrins also coordinate other forms of signal transduction in the cell. Many sig-

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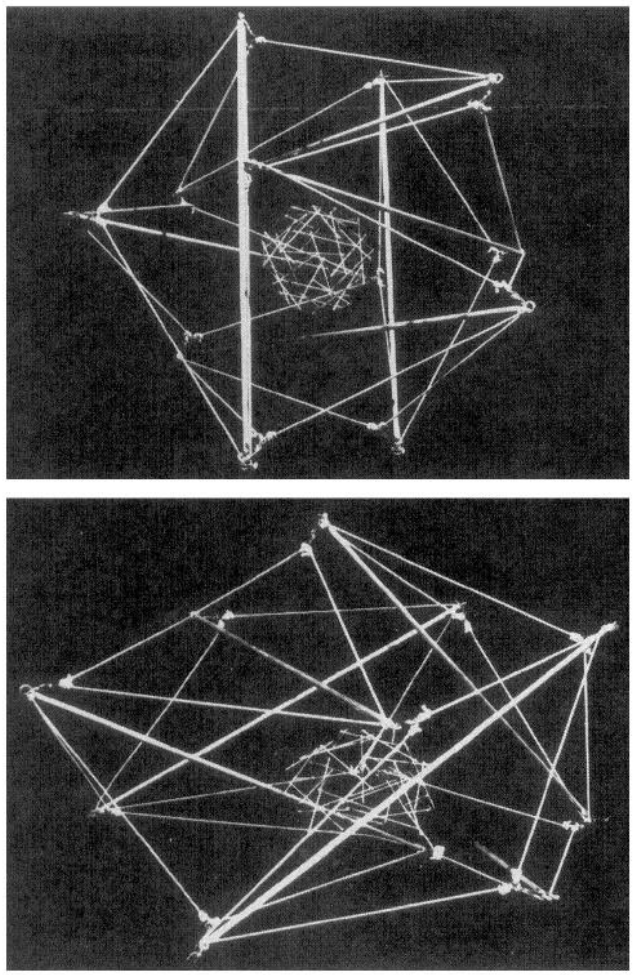

Figure 1. A tensegrity model composed of sticks and elastic strings. This model rounds up on a flexible substrate (upper panel), but it spreads when attached to a rigid foundation (lower panel), much like a living cell (Ingber, 1993, 1998; Ingber and Jamieson, 1985).

nal-transducing molecules associate with cytoskeletal scaffolds within the focal adhesion complex and appear to concentrate at the site of integrin binding (Plopper *et al.,* 1995). Importantly, these same signaling components mediate the cellular effects of soluble growth factors and insoluble extracellular matrix, as well as mechanical forces. Thus, integrins are perfectly poised to mediate mechanochemical transduction. We have found, in fact, that cells can be switched between programs of growth, differentiation, and apoptosis by changing the balance of forces across cell surface integrins and thus altering cell shape (Ingber and Folkman, 1989; Ingber, 1990; Singhvi *et al.,* 1994; Chen *et al.,* 1997). These results demonstrate that while extracellular matrix, growth factors, and mechanical forces all contribute to cellular regulation, mechanical signals are the dominant regulators.

Mechanical stresses may be integrated with other environmental signals and transduced into a biochemical response through force-dependent changes in cytoskeletal scaffold geometry or through local changes in molecular shape that alter chemical potential and thereby influence thermodynamic parameters (Ingber, 1997; Chicurel *et al.,* 1998a). For example, we have recently found that increasing tension across integrins results in cytoskeletal restructuring events that lead to the creation of a cellular microcompartment specialized for local protein synthesis at the site of integrin binding (Chicurel *et al.,* 1998b). Taken together, our results suggest that tensegrity provides a mechanism to focus mechanical energy on critical molecular transducers and to both orchestrate and tune the cellular response to mechanical stress (Ingber, 1993, 1997, 1998; Chicurel *et al.,* 1998a). Tensegrity also may explain how stresses are transmitted through tissues, and how cellular responses are integrated within the hierarchical complexity of living tissues and organs (Ingber and Jamieson, 1985; Ingber, 1993, 1998; Chen and Ingber, 1998).

Acknowledgments

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Discussion

BORISY: How do you imagine that the nucleus is receiving and transmitting the mechanical signal into a chemical signal, especially since there are no intermediate filaments, microtubules, or actin filaments within the nucleus?

INGBER: We believe that mechanical connections to the nucleus effect changes in chemical signals at the plasma membrane. There are also data suggesting that nuclear pore size and nuclear transport rates are being affected at the nucleus. Some studies suggest that the nuclear pores are distorted when nuclei spread, affecting the efficiency of nuclear transport. This is of interest to us because we find that cells need to spread late in G1 to get into S phase, and nuclear transport of large enzyme complexes is a requirement for S phase entry late in G1. We can harpoon the nucleus, pull out all of the nucleoplasm on a string in interphase or pull out all the chromosomes on a string in metaphase, then add a small amount of magnesium, and all these structures unwind. After dilution of the magnesium, they all rewind to reconstruct their original form and position (Maniotis *et al.*, 1997. *J. Cellul. Biochem.* **65:** 114–130). This effect is not nonspecific; rather it is DNA-based. The structure of DNA and its nuclear matrix scaffoldings are being affected. The literature tells how DNA is wound on the nuclear matrix, which dictates its regulation. I think that there are things in the nucleus that are load-bearing and by pulling on them we may change their kinetics and thermodynamics. This also may increase the efficiency, possibly allowing specific transcription factors to enter certain sites on parts of transcriptionally active DNA near the nuclear matrix. But it is not going to be a simple on–off process. We need to continue developing the techniques to study these effects.

MACINTOSH: Are there any other ways to look for the development of nonrandom networks of this kind? Obviously one could look for order of some kind. My second question relates to prestressed fibers. How do you visualize prestressed fibers in a network where many of the crosslinks are highly dynamic and transient?

INGBER: Our view of the cytoskeleton is almost exclusively based on the use of immunofluorescence microscopy, which presents a problem of limited resolution. We think that actin stress fibers are "floating" in a black sea of cytoplasm. That sea is filled with a continuous network of actin filaments in loose polygonal arrangements, perhaps more actin than in stress fibers. Dynamic polymerization on a stress fiber can be described in terms of a molecular rope made up of many smaller ropes, with the group in the middle maintaining mechanical connectedness as the outer rope components "polymerize" on and off. In terms of cross-links and dynamics, I believe that the actin cytoskeleton is a tensegrity structure which immediately responds to a quick pull on its attachments to the cell surface by slightly realigning all its elements; through tensegrity you get flexibility out of a structure, even when it contains nonextensible or rigid elements. There also may be some regions that exhibit relatively increased distortion which may change molecular shape and thus alter local thermodynamic parameters and, hence, influence molecular biochemistry (Ingber, 1997. *Annu. Rev. Physiol.* **59:** 575–599). For example, this could influence rates of cross-link breakage and reformation or alter cytoskeletal filament polymerization as has been observed for microtubules. If the mechanical stress is sustained, as might be expected for an adhesive interaction with a substrate, then this process would proceed in an iterative manner and result in progressively greater levels of cytoskeletal restructuring as is observed in spreading cells.

In response to your first question about patterns: there is a lot of order in the cytoskeleton. Mathematical descriptions of our tensegrity model predict the linear stiffening behavior we observe in living cells whether the models incorporate elastic elements and rigid struts or nonextensible tensile elements and buckleable struts. I believe this latter configuration is really how

it works with microtubules or cross-linked bundles of actin filaments acting as the buckleable struts. This is now clear from the work of Andrew Matus (Kaech *et al.*, 1996. *Neuron* **17:** 1189–1199). Intermediate filaments are also coils that are basically extensible structures that can change in length and that mechanically couple the nucleus to cell surface receptors (Maniotis *et al.*, 1997. *Proc. Natl. Acad. Sci. USA* **94:** 849–854). Many cells also contain titin, which is a highly elastic molecule. I think the cell builds hierarchically; it's not just six struts in a cell. That's why after a cell is cut with a microneedle, each piece has the properties of the whole system, such as the ability to move, as shown many years ago by Gunter Albrecht-Buehler.

STEWART: It seems to me that there are two elements in this about the idea of mechanical transduction in the nucleus. What concerns me is that the elements in the nucleus that are involved in gene expression are not likely to be bearing the loads that are going to come down through the nucleus.

INGBER: We have data to show that we reorient the mitotic spindle by pulling on integrins in a mitotic cell. So we are getting force to every chromosome.

STEWART: Yes, but the mitotic spindle is not involved in transcription.

INGBER: I showed pictures where we have analyzed SC35 splicing sites. Don Coffey and co-workers have shown (Pienta *et al.*, 1991. *Crit. Rev. Eukaryot. Gene Expr.* **1:** 355–385) that the parts of the genome actively involved in transcription are on the nuclear matrix. He has mapped the genes on a prostate cell that is sensitive to androgen and finds that they are all at the base of DNA loops on the nuclear matrix, which is probably part of a load-bearing scaffolding. After castration the animal loses androgen sensitivity, becoming estrogen sensitive within hours. Within a matter of hours, those same genes are at the tip of the loop. These genes that have been turned on were at the bottom, in physical interconnection with the load-bearing system. You may have your conception, but I believe that we have actual data to show that it is not correct.

STEWART: How can this happen by just applying a mechanical stress? It seems to me that there are well-documented pathways involving chemical messages that could easily come from the cytoskeleton. One of the principal regulatory roles in terms of communication between the nucleus and the cytoplasm is that elements are immobilized on the cytoskeleton—NFkappaB, for example. You could easily imagine that being released and transported.

INGBER: I completely agree with that. That is why the first thing I said in answer to your question was that the initial effect of mechanical force is to change chemicals in the cytoplasm. It is not one or the other, it is both. I think most cells have specialized structures, like mechanoreceptor cells, to take the load. A pressure-sensitive cell in your skin has lots of matrix so when you press once it feels it, then the stress dissipates. Different cells are structured so that stress may never get to the nucleus, in terms of causing a change. I'm not saying that when you stretch the nucleus you make it grow. In G2 phase the nucleus spreads all the time, you don't get S phase. My point is that if you have all the chemicals coming from the cytoplasm, you don't get the same result; it will depend on the structure of the nucleus. All of these factors are necessary, but not sufficient; and they are all interdependent. This is just another potential way to feed in information. Half of my lab work is based on what you are talking about (that is, chemical signaling) because we think it is equally important.

STEWART: To make it plausible you need to first show that the forces are being distributed to the elements that are working, as opposed to the nucleus itself. You have to provide some sort of mechanism whereby those forces can produce realistic effects. You need to think of the magnitude of the forces compared to the elements that are involved in actually changing the structure of the chromatin. I am concerned that the forces that are involved and are going to produce the regulatory changes of the nucleus are rather large compared to the mechanical forces that you are likely to be able to concentrate.

INGBER: We don't necessarily have to distort anything to affect function. If you have a spring that vibrates and you change the center of gravity of that spring, you change its vibration; you change kinetics. If you slightly distort the spring you can change kinetics without having to distort the whole thing. But I agree with you. It has taken a number of years for me to reach this point. We have had to combat the arguments that you can't get force to the nucleus, by testing it. Right now, I don't know how this works at the level of transcription. However, my only point is that forces applied to the cell surface can get to the nucleus. Just because we can't envision a mechanism doesn't mean that it doesn't happen.

FORGACS: I wish to propose an alternative idea which is based on percolative networks. This is more random than tensegrity. Tensegrity structures appear more ordered than percolative structures. I would like to point out that the linear stress-strain relationship is a generic feature of connective networks. Percolative structures possess exactly the same behavior.

INGBER: Only if they are prestressed, and we are talking about linear stress-stiffness curves here, not linear stress-strain.

FORGACS: Percolative structure can basically produce the same thing once you fix the network somehow, which you may refer to as prestressed. My question concerns how mechanical forces can induce relevant changes. We had the model calculation which shows that mechanical forces of the magnitude that can be produced in percolative networks can really kick molecules bound to the cytoskeleton and bring them from one molecular or quantum energy level to another. I interpret this as going from one conformation to another. It would be nice if someone would design an experiment to test this theoretical possibility.

INGBER: Percolation presents a complementary view to understand the connectivity of these networks: how you go from losing connections to having connections, and how signals transmit over this. However, I don't think that percolation can predict the patterning and mechanical response of these structures in living cells (Ingber, 1998. *Proceedings of the Les Houches Meeting on Dynamical Networks in Physics and Biology.* France, Springer-Verlag. In press).

GUNDERSEN: As a biochemist, I believe there are mechanical effects on cells and that this is an important component of how cells respond to their environment. Your tensegrity models seem to predict fairly well some of the basic properties of cells. However, you never labeled your models. What are the struts and what are the elements tying them? I am interested in whether there is a 1:1 correspondence between your rods and the tie elements to some cytoskeletal structure? Is this a good representation of the behavior of those elements in the cell? Is it possible that there may be other things in the cell, for example, the dynamics of the filaments, that contribute to the behavior of your tensegrity models?

INGBER: In the video I showed, our modeled networks of actomyosin and those individual struts are 3.6 μm in length. The geodesic nets and linear stress fibers created by those models exhibit structural features that are exactly those predicted from analysis of the actomyosin network in living cells based on thin section transmission electron microscopy (Lazarides, 1976. *J. Cell Biol.* **68:** 202–219; Osborn *et al.,* 1978. *Cell* **14:** 477–488). Our model is exactly precise, strut for strut, vertex for vertex, at least in this context. In terms of the compression elements, Steve Heidemann and co-workers (Joshi *et al.,* 1985, *J. Cell Biol.* **101:** 697–705) have shown that bundles of microtubules in the neurite act like compression struts. Andrew Matus has recently shown this directly in cells containing microtubules labeled with green fluorescent protein. They are being pulled by actin and balanced by matrix tethers, just as we are saying here. The matrix itself consists of local compression struts, due to the distribution of forces between focal adhesion at either end of the same stress fiber, resulting in the stability of the whole cell, which is globally tensile. Thus the cell is a tensegrity structure, based on definition at the whole cell level. I have shown you that connecting single microtubules with many actomyosin filament nets with dimensions of 3.6 μm creates hierarchical structures, again with tensegrity-based mechanical stability. We are just beginning to develop testable hypotheses. If that is true, we should be able to determine curvature of a microtubule on a specific size scale and ranges of amplitude, and how changing contractility affects that. That is where we are heading.

GUNDERSEN: Do you think you could isolate, in a biochemical sense, something that would behave like your stick and strut models? What I am asking is, can you do the biochemistry behind the tensegrity models? Do you think that is possible?

INGBER: Steve Heidemann has used tensegrity to define a thermodynamic model that explains how microtubule polymerization is regulated. This also explains microtubule polymerization in hepatocytes, as we have published. I must emphasize that as cells stick and spread they go from round to a pancake. We have measured this and find no correlation between the total amount of actin, microtubule or intermediate filaments, and spreading.

GUNDERSEN: Maybe it is in their dynamics. The dynamics of all these filaments is very sensitive to all kinds of different changes.

INGBER: What I am saying is that actin polymerization goes up 20-fold when an isolated hepatocyte attaches to a matrix-coated dish, with no change in shape. It then goes down 20-fold, with no change in shape. When the cell goes from round to a pancake, microtubules are constant; intermediate filaments are constant. You could argue that it is changing in a local domain. This may be so, but it is not a global, viscous polymerization. It does change when the cell is moving and forming ruffling edges; I totally agree with that. However, I don't believe that it is possible to explain mechanotransduction and higher order integration on the basis of a single molecule. If you are asking whether we can identify an assemblage of these elements that have certain mechanical properties, my answer is "I hope so." One possible approach would be to look at self-assembly reactions in whole cell extracts. This has been done with the mitotic spindle, which we believe behaves in similar ways; that is, it is a unit that stiffens by global transmission of tensile forces that are resisted internally by multiple microtubule struts.

BARAKAT: There is evidence that small forces, such as shear stresses over endothelial cells of a magnitude less than 1, and even as low as 0.1 dyne per square centimeter, can elicit biochemical responses. These forces are thought to be significantly smaller than what is required to induce mechanical deformation in certain cytoskeletal elements. Do you think that the fact that these small forces elicit biochemical responses is consistent with the notion of tensegrity?

INGBER: Is there any knowledge about the frequency of those stimulations? One can change the harmonics without changing the deformation and get some of the same things. These structures are coupled harmonic oscillators; by banging the whole cell the nucleus starts moving with the same frequency.